31st Conference of the European Society for Biomaterials
ESB2021

Together with the 43rd Annual Congress of the Iberian Society of Biomechanics and Biomaterials (SIBB)

5 - 9 September, 2021, Porto, Portugal

| Fully Virtual |

Futuring biomaterials

WWW.ESB2021.ORG
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contents</td>
<td>3</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>3</td>
</tr>
<tr>
<td>Welcome Message by the ESB President</td>
<td>8</td>
</tr>
<tr>
<td>Welcome Message by the ESB 2021 Chairs</td>
<td>9</td>
</tr>
<tr>
<td>Welcome Message by the SIBB Chairs</td>
<td>11</td>
</tr>
<tr>
<td>Local Organizing Committee / Program Committee ESB2021</td>
<td>12</td>
</tr>
<tr>
<td>Local Organizing Committee / Program Committee SIBB2021</td>
<td>12</td>
</tr>
<tr>
<td>National Advisory Committee ESB2021</td>
<td>13</td>
</tr>
<tr>
<td>International Advisory Committee ESB2021</td>
<td>16</td>
</tr>
<tr>
<td>Scientific Committee of SIBB2021 Congress - Biomechanics</td>
<td>20</td>
</tr>
<tr>
<td>Sponsors &amp; Partners</td>
<td>21</td>
</tr>
<tr>
<td>Plenary Speakers</td>
<td>23</td>
</tr>
<tr>
<td>Award Winners</td>
<td>25</td>
</tr>
<tr>
<td>Invited Keynote Speakers</td>
<td>32</td>
</tr>
<tr>
<td>Keynote Speakers Symposia</td>
<td>38</td>
</tr>
<tr>
<td>Schedule Sunday, 5 September, 2021</td>
<td>60</td>
</tr>
<tr>
<td>Schedule Monday, 6 September, 2021</td>
<td>61</td>
</tr>
<tr>
<td>Schedule Tuesday, 7 September, 2021</td>
<td>62</td>
</tr>
<tr>
<td>Schedule Wednesday, 8 September, 2021</td>
<td>63</td>
</tr>
<tr>
<td>Schedule Thursday, 9 September, 2021</td>
<td>64</td>
</tr>
<tr>
<td>Program Sunday, 5 September, 2021</td>
<td>65</td>
</tr>
<tr>
<td>Program Monday, 6 September, 2021</td>
<td>68</td>
</tr>
<tr>
<td>OC</td>
<td>Opening Ceremony</td>
</tr>
<tr>
<td>GW AW01</td>
<td>George Winter Award 2020</td>
</tr>
<tr>
<td>JL AW01</td>
<td>Jean Leray Award 2020</td>
</tr>
<tr>
<td>TRS-S01</td>
<td>Translation Research Symposia</td>
</tr>
<tr>
<td>K01</td>
<td>Nanobiomaterials for Biomedical Applications</td>
</tr>
<tr>
<td>S01</td>
<td>Organ-On-a-Chip Technologies Meet Biofabrication: Towards Physiologically Relevant Organ Models</td>
</tr>
<tr>
<td>S02</td>
<td>Supramolecular Peptide-based Biomaterials for Regenerative Medicine</td>
</tr>
<tr>
<td>S03</td>
<td>Bioinspired Antimicrobial Materials: Addressing Infections in Regenerative Medicine</td>
</tr>
<tr>
<td>N01</td>
<td>Biomaterials for Application in Neurosciences</td>
</tr>
<tr>
<td>N02</td>
<td>Biomaterials for Orthopedic Applications I</td>
</tr>
<tr>
<td>YSF GA</td>
<td>YSF General Assembly</td>
</tr>
</tbody>
</table>
## Contents

<table>
<thead>
<tr>
<th>Social Activity 1</th>
<th>Yoga</th>
<th>170</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRS-S02</td>
<td>Translation Research Symposia</td>
<td>171</td>
</tr>
<tr>
<td>K02</td>
<td>Organ-on-a-Chip Systems in Precision Medicine</td>
<td>173</td>
</tr>
<tr>
<td>S04</td>
<td>Smart (Nano)biomaterials and their Applications</td>
<td>188</td>
</tr>
<tr>
<td>ESB-CSBM S</td>
<td>ESB-Chinese Society for Biomaterials (CSBM) Joint Symposium:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frontiers of Materobiology</td>
<td>198</td>
</tr>
<tr>
<td>N03</td>
<td>Surface-Modified Biomaterials</td>
<td>207</td>
</tr>
<tr>
<td>N04</td>
<td>Biomaterials for Orthopedic Applications II</td>
<td>229</td>
</tr>
<tr>
<td>TRS-S03</td>
<td>Translation Research Symposia</td>
<td>249</td>
</tr>
<tr>
<td>K03</td>
<td>Futuring Biomaterials</td>
<td>251</td>
</tr>
<tr>
<td>S05</td>
<td>Advanced Technologies and Cellular Approaches for the Development of</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Precise 3D Tendon and other Musculoskeletal Tissue Substitutes and</td>
<td>265</td>
</tr>
<tr>
<td></td>
<td>Models to Understand Regeneration Mechanisms</td>
<td></td>
</tr>
<tr>
<td>ESB-SLABO S</td>
<td>ESB-Latin American Society for Biomaterials, Tissue Engineering</td>
<td></td>
</tr>
<tr>
<td></td>
<td>and Artificial Organs (SLABO) Joint Symposium: Development of New</td>
<td>280</td>
</tr>
<tr>
<td></td>
<td>Biomaterials with Multifunctional Surfaces</td>
<td></td>
</tr>
<tr>
<td>N05</td>
<td>Hydrogels and Smart Biomaterials II</td>
<td>289</td>
</tr>
<tr>
<td>N06</td>
<td>Biomaterials for Drug Delivery</td>
<td>306</td>
</tr>
<tr>
<td>N07</td>
<td>Biomaterials for Electrical Stimulation</td>
<td>327</td>
</tr>
<tr>
<td>PL1</td>
<td>Plenary Lecture 1</td>
<td>343</td>
</tr>
<tr>
<td>Social Activity 2</td>
<td>YSF Night Out</td>
<td>344</td>
</tr>
<tr>
<td><strong>NANOSTEM Satellite Symposium</strong></td>
<td></td>
<td>345</td>
</tr>
<tr>
<td>NANOSTEM 01</td>
<td>Nanogels for drug delivery</td>
<td>346</td>
</tr>
<tr>
<td>NANOSTEM 02</td>
<td>Investigation of nanocarriers as drug delivery systems</td>
<td>351</td>
</tr>
<tr>
<td>NANOSTEM 03</td>
<td>Nanoparticles and blood-brain barrier - mechanisms of permeation</td>
<td>358</td>
</tr>
<tr>
<td></td>
<td>and permeability for successful drug delivery</td>
<td></td>
</tr>
<tr>
<td>NANOSTEM 04</td>
<td>Nanoparticles for modulation of gene expression</td>
<td>367</td>
</tr>
<tr>
<td><strong>Program Tuesday, 7 September, 2021</strong></td>
<td></td>
<td>373</td>
</tr>
<tr>
<td>KdG AW01</td>
<td>Klaas de Groot Award 2020</td>
<td>374</td>
</tr>
<tr>
<td>JL AW02</td>
<td>Jean Leray Award 2021</td>
<td>376</td>
</tr>
<tr>
<td>K04</td>
<td>Graphene-based Biomaterials</td>
<td>378</td>
</tr>
<tr>
<td>S06</td>
<td>Hybrid Cell-Microbiomaterial 3D Assemblies</td>
<td>392</td>
</tr>
<tr>
<td>S07</td>
<td>Biomaterials: The Roadmap Towards Personalised Strategies in</td>
<td>409</td>
</tr>
<tr>
<td></td>
<td>Musculoskeletal Tissue Engineering</td>
<td></td>
</tr>
<tr>
<td>ESB-CRS S</td>
<td>ESB-Controlled Release Society (CRS) Joint Symposium</td>
<td>420</td>
</tr>
<tr>
<td>ESB-BIOMAT S</td>
<td>ESB-French Society for the Development of Biomaterials (BIOMAT)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Joint Symposium: What Will be the Future Biomaterials Implanted in</td>
<td>431</td>
</tr>
<tr>
<td></td>
<td>Humans?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tribute to Prof. Charles Baque</td>
<td></td>
</tr>
<tr>
<td>N08</td>
<td>Cell-Biomaterial Interactions</td>
<td>449</td>
</tr>
<tr>
<td>N09</td>
<td>Biomaterials for Orthopedic Applications III</td>
<td>468</td>
</tr>
</tbody>
</table>
## Contents

Social Activity 3 | Yoga ................................................................. 486
LS | Lunch Symposium Rousselot: Biomedical Applications of Gelatins ........................................... 487
K05 | Immunoresponse Towards Biomaterials ..................................................................................... 490
S08 | Beyond Bone and Teeth: Bioactive Glasses in Soft Tissue Regeneration ................................... 504
ESB-SIBB S01 | ESB-Iberian Society for Biomechanics and Biomaterials (SIBB) Joint Session I: Antimicrobial Biomaterials ....................................................................................................................... 515
ESB-ISBF S | ESB-International Society for Biofabrication (ISBF) Joint Symposium .................................. 532
ESB-BMJ S | ESB-Biomaterials Journal Joint Session: Meet the Editor Session ......................................... 541
N10 | Skin Regeneration and Wound Healing ......................................................................................... 543
N11 | Biomaterials for Orthopedic Applications IV ................................................................................ 562
PL2 | Plenary Lecture 2 ......................................................................................................................... 583
Social Activity 4 | Discover Portuguese Wine Regions with Sogrape ............................................................ 584

**ESB-AEROgELS COST Action Joint Symposium** ............................................................................ 585
AERO S01 | Wound materials-based dressings and processing ................................................................. 586
AERO PS01 | Coffee-Break & Poster session 01 ....................................................................................... 596
AERO S02 | 3D-scaffolds-based aerogels for tissue regeneration ............................................................... 606
YSF-AEROgELS WS | YSF Educational workshop - “Writing and Publishing” .......................................... 615
YSF & “AEROgELS” Joint COST action event ................................................................................. 615
AERO InvF | Early-career investigators Forum ......................................................................................... 617
AERO S03 | Drug-delivery systems and Nutraceutical/food systems ........................................................... 636
AERO Closing | Closing session ............................................................................................................... 646

**Program Wednesday, 8 September, 2021** .................................................................................... 647
GW AW02 | George Winter Award 2021 .................................................................................................... 648
KdG AW02 | Klaas de Groot Award 2021 ................................................................................................. 649
K06 | Biomimetic Nanomaterials and Biosensing Devices ..................................................................... 650
S09 | Role of Biomaterial Properties on Cell-Scaffold Interplay ......................................................... 663
S10 | Advances in Functionalization and Fabrication of Gelatin Hydrogels for Biomedical Applications ................................................................................................................................. 678
S11 | Nanostructure-based Biomaterials: Design and Biological Interactions ........................................ 694
ESB-SIBB S02 | ESB-Iberian Society for Biomechanics and Biomaterials (SIBB) Joint Session II: Biomaterials for cancer models and treatment ................................................................................................................. 704
N12 | Biomaterials for Cardiovascular Applications and Angiogenesis ................................................ 731
YSF NC | YSF National Chapter Meeting .................................................................................................. 749
Social Activity 5 | Yoga .............................................................................................................................. 750
K07 | Biomaterials for Cardiac Tissue Engineering and Regenerative Medicine ..................................... 751
K08 | Mechanomodulatory Biomaterials and its Impact on Cell Behaviour ........................................... 768
## Contents

**ESB 2021 | Abstract Book**

<table>
<thead>
<tr>
<th>Session</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>S12</td>
<td>Biocompatibility Testing According to ISO 10993: What are the Changes and What can the Biomaterials Community Learn from it?</td>
<td>784</td>
</tr>
<tr>
<td>S13</td>
<td>Biomaterials Informatics and Data-Driven Biomaterials</td>
<td>798</td>
</tr>
<tr>
<td>ESB-SFB S</td>
<td>ESB-Society For Biomaterials (SFB) Joint Symposium: Immunomodulatory Biomaterials</td>
<td>807</td>
</tr>
<tr>
<td>N13</td>
<td>Advances in Additive Manufacturing and Biofabrication</td>
<td>817</td>
</tr>
<tr>
<td>Int AW</td>
<td>International Award 2021</td>
<td>833</td>
</tr>
<tr>
<td>Social Activity 6</td>
<td>Music Evening</td>
<td>834</td>
</tr>
</tbody>
</table>

**Program Thursday, 9 September, 2021** .................................................................................. 835

<table>
<thead>
<tr>
<th>Session</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL3</td>
<td>Plenary Lecture 3</td>
<td>836</td>
</tr>
<tr>
<td>ESB GA</td>
<td>ESB General Assembly</td>
<td>838</td>
</tr>
<tr>
<td>K09</td>
<td>New Tools to Study Physiology and Disease</td>
<td>839</td>
</tr>
<tr>
<td>K10</td>
<td>Supramolecular Peptide Assemblies as a Platform for Antifouling, Antimicrobial and Antiviral Materials</td>
<td>856</td>
</tr>
<tr>
<td>S14</td>
<td>Innovative Approaches to Develop 3D Nanostructured Devices for Bone and Osteochondral Regeneration</td>
<td>871</td>
</tr>
<tr>
<td>S15</td>
<td>Regeneration of the Intervertebral Disc: The Battles that Biomaterials Will Have to Win</td>
<td>886</td>
</tr>
<tr>
<td>N14</td>
<td>Electrospun and Electrowritten Biomaterials</td>
<td>895</td>
</tr>
<tr>
<td>N15</td>
<td>Nanobiomaterials</td>
<td>915</td>
</tr>
<tr>
<td>N16</td>
<td>Hydrogels and Smart Biomaterials I</td>
<td>931</td>
</tr>
<tr>
<td>CC</td>
<td>Awards &amp; Closing Ceremony</td>
<td>951</td>
</tr>
</tbody>
</table>

**Biomechanics by SIBB Symposium** ...................................................................................... 952

<table>
<thead>
<tr>
<th>Session</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomechanics Opening</td>
<td>Welcome Ceremony Biomechanics by SIBB Symposium: Sports biomechanics and biomedicine</td>
<td>953</td>
</tr>
<tr>
<td>Biomechanics 01</td>
<td>Anatomo-functional bases of entensopathies in sports</td>
<td>954</td>
</tr>
<tr>
<td>Biomechanics 02</td>
<td>Swimming Biomechanics</td>
<td>957</td>
</tr>
<tr>
<td>Biomechanics 03</td>
<td>Oral Presentations 01</td>
<td>959</td>
</tr>
<tr>
<td>Biomechanics 04</td>
<td>Oral Presentations 02</td>
<td>973</td>
</tr>
<tr>
<td>Biomechanics 05</td>
<td>SIBB General Assembly and Award Ceremony</td>
<td>991</td>
</tr>
</tbody>
</table>

**PS1 | Poster Sessions 1** ............................................................................................................. 992

<table>
<thead>
<tr>
<th>Session</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS1-01</td>
<td>Biomaterials for Biofabrication</td>
<td>993</td>
</tr>
<tr>
<td>PS1-02</td>
<td>Biomaterials Characterization</td>
<td>1051</td>
</tr>
<tr>
<td>PS1-03</td>
<td>Biomaterials for Tissue Engineering</td>
<td>1093</td>
</tr>
<tr>
<td>PS1-04</td>
<td>Biomaterials for Orthopedic Applications</td>
<td>1118</td>
</tr>
<tr>
<td>PS1-05</td>
<td>Biomaterials Development</td>
<td>1165</td>
</tr>
<tr>
<td>PS1-06</td>
<td>Ceramic Biomaterials</td>
<td>1194</td>
</tr>
<tr>
<td>PS1-07</td>
<td>Other Biomaterials Applications</td>
<td>1210</td>
</tr>
<tr>
<td>PS1-08</td>
<td>Hydrogels and Smart Biomaterials</td>
<td>1250</td>
</tr>
</tbody>
</table>
Welcome Message by the ESB President
Pamela Habibović

Dear members of the European Society for Biomaterials,
Dear colleagues and friends,

It is my pleasure to welcome you to ESB2021, the 31st Annual Conference of our society, to be held 5-9 September, 2021. We had all very much looked forward to spending five days in Porto, Portugal, where the conference was planned to be held, to enjoy one of the most beautiful European cities and the well-known Portuguese hospitality. Unfortunately, the Covid-19 pandemic continues to restrict our freedom to travel and meet at large events. Therefore, the organizing committee, in agreement with the ESB Council, had to take the difficult decision to turn the conference into a virtual event.

This is the second time that an ESB Conference is hosted by a Portuguese team. In 1995, the 12th Annual ESB Conference was also held in Porto, and it was a great success. Portugal has a long and successful track record in biomaterials research, and participation and contribution of Portuguese scientists to the ESB has been significant since the society’s foundation.

Despite the fact that we will not be able to meet in person, I am convinced that the ESB2021 Conference will offer plenty of opportunities to discuss recent exciting research and development achievements in the field of biomaterials. Our hosts, Ana Paula Pêgo, M. Cristina L. Martins and Pedro L. Granja have developed a truly impressive scientific program, with plenary and keynote speakers from all over the world and covering a great range of exciting topics. Moreover, several special symposia will be organized, including the full-day ESB symposium on research translation. The organizers have done their utmost best to accommodate oral and poster presentations of as PhD candidates and post-doctoral fellows and give them the opportunity to showcase their work at one of the most important scientific events in the field of biomaterials.

Together with the rest of the ESB Council, I look forward to meeting you at the virtual ESB2021!

Kind regards,

Pamela Habibović
ESB President
Welcome Message by the ESB 2021 Chairs
Ana Paula Pêgo, M. Cristina L. Martins, Pedro L. Granja

Dear friends and colleagues,

On behalf of the Organizing Committee, we would like to cordially welcome you to the 31st Annual Conference of the European Society for Biomaterials (ESB 2021), taking place virtually from September 5 to 9, 2021. This is the second time this event is organized in Portugal (in 1995, it has been already organized in Porto being chaired by Mário Barbosa). This year it also accommodates the 43rd Annual Congress of the Iberian Society for Biomechanics and Biomaterials (SIBB).

The motto of our Conference is “Futuring Biomaterials”, as a challenge to our community to explore biomaterials in conjunction with advanced technologies, such as Nanotechnology, Biofabrication, Microfluidics and Bioimaging to develop the clinical combination products and functionalities of the future. These conferences, whose first event took place in Strasbourg (France) in 1977, are held annually and are interspersed every 4 years by the World Biomaterials Congress. The ESB Conferences aim to bring together researchers, clinicians and companies for the presentation and discussion of the most recent advances in the areas of Science and Engineering of Biomaterials, both in terms of more fundamental research, as well as clinical and industrial research. It is intended that this multidisciplinary environment can promote scientific and technological knowledge for innovation in health.

As you know, at the beginning of this adventure we have planned to host the ESB 2021 Conference in Porto, as a live and lively event. However, the COVID19 pandemic drastically altered our plans. Despite our massive effort to organize the event live, after numerous discussions and with the support of the ESB Council, we have taken the difficult decision to move ESB 2021 to a fully virtual format. From then on, all our energies were put into making ESB 2021 a VIRTUOUS VIRTUAL successful event!

Despite the current pandemic the support of our community has been massive. We have received 795 abstracts and 39 proposals for symposia. This allowed us to prepare an exciting program that really leaves to our motto!

In quantitative terms, we have 975 registered participants, from 48 countries, including 15 from outside Europe, proving the truly international character of the event. The program includes 10 plenary presentations, including 3 given by invited renowned speakers and 7 by scientists awarded by the ESB in 2020 and in 2021. In addition, it also includes 98 invited lectures and 795 presentations, of which 348 oral and 447 posters (as of August 24, 2021). We really boosted the number of oral presentations to assure that as many teams as possible could see their works discussed in a podium presentation. Best oral and poster presentations made by young researchers, will also be awarded. To promote networking, we will provide access to a very interactive tool – Gathertown. Please, do actively participate in the poster sessions and be prepared to see old friends and meet new ones! As in every Conference, the Translation Symposia will address timely topics. We have a session dedicated to the recent alterations observed in the regulatory framework of medical devices and another that involves Business Angels. Finally, we actively involved the YSF in all aspects of the event and this year you will see the introduction of YSF chairs in all our oral sessions. You will also be able to meet our sponsors during the event.

We really need to leave here a word of thanks to all those that worked hard to make this Conference a reality: speakers, reviewers, special session organizers, sponsors, the local organizing committee, and all the volunteers.
Despite the fact that we will not be able to welcome you live in Porto, we organized a virtual Social Program that will give you a flavor of our city. We have a wine expert explaining what makes our Portuguese wines so special, and we have also a musical show recorded specially for our event. We have also planned yoga sessions during the lunch breaks. So, enjoy!

On behalf of the Organizing Committee, we thank you very much for your participation and wish you a pleasant virtual "stay" in Porto!

Ana Paula Pêgo (Chair), M. Cristina L. Martins (Co-Chair) and Pedro L. Granja (Co-Chair)

Institute for Research and Innovation in Health (i3S) and INEB – Instituto de Engenharia Biomédica, University of Porto, Portugal
Welcome Message by the SIBB Chairs
Enrique Navarro & Inês Gonçalves

On behalf of the organizing committee, we would like to warmly welcome all of you to the 43rd Annual Congress of the Iberian Society for Biomechanics and Biomaterials (SIBB).

In an attempt to bring closer the Portuguese and Spanish scientific communities in our fields, this year we have organized our annual conference in Portugal, embracing the opportunity of joining the 31st Annual Conference of the European Society for Biomaterials (ESB2021), held in Porto, Portugal.

Up to now, we have 270 Iberian participants, including 153 from Portugal and 92 from Spain in the Biomaterials field and 5 from Portugal and 20 from Spain in the Biomechanics field. From these, 161 are young scientists, and whom we expect to join efforts to strengthening this society in the future.

The SIBB biomaterials sessions are fully integrated within the ESB2021, with a huge variety of topics with plenary and keynote speakers. Given the interesting and high number of works from several Portuguese and Spanish groups in the areas of “Biomaterials for Cancer Models and Treatment”, and in “Antimicrobial Biomaterials”, we have dedicated 2 joint symposiums from ESB-SIBB to these topics. The SIBB biomechanics sessions will occur in the last day of the conference (September 9th), being dedicated to “Anatomo-functional bases of entesopathies in sports” and to “Swimming Biomechanics”.

In the very end of the last day, we will have our SIBB general assembly, where we will have a chance to discuss important points regarding our society and have the awards ceremony.

Unfortunately, due to the COVID-19 pandemic that prevented us from having a face-to-face meeting, this year it will not be possible to perform the workshops, which are so typical of our annual congresses.

Despite you were not able to sense the beautiful city of Porto and the Portuguese hospitality, we still hope that after these days of conference, you are able to meet and chat with colleagues and friends during the online sessions and in our virtual “coffee shops”, and that you feel inspired by the scientific atmosphere of the conference.

Abraços,

Enrique Navarro (SIBB President)

Inês Gonçalves (SIBB Board)
Principal Investigator at i3S/INEB, Porto, Portugal
Local Organizing Committee / Program Committee ESB2021

Pêgo, Ana Paula, Chair
Martins, M. Cristina L., Co-Chair
Granja, Pedro L., Co-Chair
Sarmento, Bruno
Barras, Cristina C.
Ribeiro, M. Cristina C.
Monteiro, Fernando Jorge
Gonçalves, Inês C.
Barbosa, Mário A.
Lamghari, Meriem
Gonçalves, Raquel M.

Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal

Local Organizing Committee / Program Committee SIBB2021

Enrique Navarro, President, Chair
Inês Gonçalves, Board, Chair
Jose Campos, Board
Esteban Collell, Board
Luis Rodriguez, Board
Javier Gil, Board
Daniel Rodriguez, Board
Diego Velasco, Board
Antonio Viladot, Board
## National Advisory Committee ESB2021

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almeida, Maria Inês</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB)</td>
</tr>
<tr>
<td></td>
<td>and Instituto de Ciências Biomédicas Abel Salazar da (ICBAS), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Alves, Cecília Juliana</td>
<td>NeuroSketal Circuits Group, Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Portugal</td>
</tr>
<tr>
<td>Albuquerque, João</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Amaral, Isabel Freitas</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Barata, David</td>
<td>Instituto Medicina Molecular (IMM), Portugal</td>
</tr>
<tr>
<td>Barbosa, Mário A.</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Barros, Joana</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Bettencourt, Ana</td>
<td>Faculty of Pharmacy, University of Lisbon, Portugal</td>
</tr>
<tr>
<td>Bidarra, Silvia Joana</td>
<td>Bioengineered 3D Microenvironments, Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Portugal</td>
</tr>
<tr>
<td>Borges, João</td>
<td>Nova University of Lisbon, Portugal</td>
</tr>
<tr>
<td>Caires, Hugo</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S)/Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Portugal</td>
</tr>
<tr>
<td>Caldeira, Joana</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Carvalho, Angela</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Castro, Flávia</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Cunha, Carla</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>das Neves, José</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>dos Santos, Tiago</td>
<td>Biofabrication Group, Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Farinha, José Paulo</td>
<td>Instituto Superior Técnico (IST), University of Lisbon, Portugal</td>
</tr>
<tr>
<td>Fernandes, Maria Helena Vaz</td>
<td>University of Aveiro, Portugal</td>
</tr>
<tr>
<td>Ferraz, Maria Pia</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Ferreira, Lino Silva</td>
<td>University of Coimbra, Portugal</td>
</tr>
<tr>
<td>Freitas, Jaime</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Gama, Miguel</td>
<td>University of Minho, Portugal</td>
</tr>
<tr>
<td>Gomes, Manuela</td>
<td>3B's Research Group/I3Bs Research Institute, University of Minho, Portugal</td>
</tr>
<tr>
<td>Gomes, Paula</td>
<td>University of Porto, Portugal</td>
</tr>
<tr>
<td>Granja, Pedro L.</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Kundu, Subhas C.</td>
<td>3B's Research Group, University of Minho, Portugal</td>
</tr>
<tr>
<td>Leiro, Victoria</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Name</td>
<td>Institution</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>--------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Leite Pereira, Catarina</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Lobato, Claudia</td>
<td>IST, University of Lisbon, Portugal</td>
</tr>
<tr>
<td>Lourenço, Bianca N.</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Mano, João</td>
<td>University of Aveiro, Portugal</td>
</tr>
<tr>
<td>Marques, Alexandra P.</td>
<td>3B's Research Group, University of Minho, Portugal</td>
</tr>
<tr>
<td>Marques, Paula Alexandra</td>
<td>University of Aveiro, Portugal</td>
</tr>
<tr>
<td>Martins, M. Cristina L.</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Monteiro, Claudia</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Moreno, Pedro</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Nascimento, Diana S</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Neto, Estrela</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Novais Barbosa, Judite</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Oliveira, Ana</td>
<td>Catholic University, Portugal</td>
</tr>
<tr>
<td>Oliveira, Miguel</td>
<td>i3Bs Research Institute, University of Minho, Portugal</td>
</tr>
<tr>
<td>Pereira, Maria do Carmo</td>
<td>University of Porto, Portugal</td>
</tr>
<tr>
<td>Pereira, Andreia Trindade</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Pereira, Rúben F.</td>
<td>Biofabrication Group, Instituto de Ciências Biomédicas Abel Salazar da (ICBAS)/Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Pinto, Artur</td>
<td>LEPABE, Dept. Chemical Engineering, Faculty of Engineering, University of Porto, Portugal</td>
</tr>
<tr>
<td>Pinto do Ó, Perpétua</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Pires, Liliana</td>
<td>International Iberian Nanotechnology Laboratory (INL), Portugal</td>
</tr>
<tr>
<td>Rai, Akhilesh</td>
<td>Center for Neuroscience and Cell Biology and Faculty of Medicine, University of Coimbra, Portugal</td>
</tr>
<tr>
<td>Rocha, Luís Augusto</td>
<td>University of Minho, Portugal</td>
</tr>
<tr>
<td>Rodrigues, João</td>
<td>Centro de Química da Madeira (CQM), University of Madeira, Portugal</td>
</tr>
<tr>
<td>Salgado, Christiane</td>
<td>Biocomposites Group, Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Santos, Susana</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Santos, Sofia Duque</td>
<td>nanoBiomaterials for Targeted Therapies Group (nBTT)/ Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Serro, Ana Paula</td>
<td>IST, University of Lisbon, Portugal</td>
</tr>
<tr>
<td>Sousa, Susana</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Sousa, Rui A.</td>
<td>Stemmmatters, Biotecnologia e Medicina Regenerativa S.A., Portugal</td>
</tr>
<tr>
<td>Name</td>
<td>Institution and Location</td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Sousa, Aureliana</td>
<td>Biofabrication Group, Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Tamagnini, Paula</td>
<td>Faculty of Sciences of University of Porto (FCUP), Portugal</td>
</tr>
<tr>
<td>Tomás, Helena</td>
<td>University of Madeira, Portugal</td>
</tr>
<tr>
<td>Torres, Ana L.</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Vasconcelos, Daniela Pereira</td>
<td>Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal</td>
</tr>
<tr>
<td>Xavier, Miguel</td>
<td>International Iberian Nanotechnology Laboratory (INL), Portugal</td>
</tr>
</tbody>
</table>
International Advisory Committee ESB2021

Ahluwalia, Arti  University of Pisa, Italy
Alblas, Jacqueline  Utrecht University, The Netherlands
Alini, Mauro  AO Research Institute, Switzerland
Alsberg, Eben  University of Illinois at Chicago, United States of America
Amedee, Joëlle  University Bordeaux, Tissue bioengineering Laboratory, France
Anselme, Karine  Institute of Materials Science of Mulhouse, France
Aparicio, Conrado  University of Minnesota, Minneapolis, United States of America
Azevedo, Helena Sepulveda  Queen Mary – University of London, United Kingdom
Baker, Matthew  Maastricht University, The Netherlands
Barberis, Fabrizio  Universita' degli Studi di Genova, Department of Civil Chemical and Environmental Engineering, Italy
Barcikowski, Stephan  Univ. Essen-Duisburg, Germany
Barralet, Jake  McGill University, Canada
Bassett, David  University of Birmingham, United Kingdom
Bayon, Yves  Medtronic, France
Benfenati, Valentina  Consiglio Nazionale delle Ricerche, Istituto per la Sintesi Organica e Fotoreattività, Italy
Bergmeister, Helga  Center for Biomedical Research, Medical University of Vienna, Austria
Bernstein, Anke  Uniklinik Freiburg, Germany
Best, Serena  University of Cambridge, United Kingdom
Blanco-Prieto, Maria J.  Universidad de Navarra, Pharmaceutical Technology, Spain
Boccaccini, Aldo R.  University of Erlangen-Nuremberg, Germany
Bohner, Marc  RMS Foundation, Switzerland
Cameron, Neil  Monash University, Australia
Castilho, Miguel  University Medical Center Utrecht, Orthopaedics, The Netherlands
Cervantes-Uc, Jose M.  Yucatan Center for Scientific Research, Mexico
Chatzinikolaidou, Maria  University of Crete, Department of Materials Science and Technology, Greece
Chauduri, Ovit  Stanford University, United States of America
Contessi Negrini, Nicola  Imperial College London, United Kingdom
De Bartolo, Loredana  National Research Council of Italy, Institute on Membrane Technology, Italy
de Bruijn, Joost  Queen Mary University of London, United Kingdom
Deb, Sanjukta  Coventry University, United Kingdom
D’Este, Matteo  AO Research Institute, Davos Regenerative Orthopedics, Switzerland
Doser, Michael  Deutsches Institute für Textil- und Faserforschung, Biomedical Engineering Dept., Germany
Dubruel, Peter  Ghent University, Belgium
Dunne, Nicholas  Dublin City University, Ireland
Dupont-Gillain, Christine  Université catholique de Louvain, Belgium
Eglin, David  Mines Saint Etienne Biomaterials, France
Engel, Elisabeth  Polytechnic University of Catalonia, Spain
Epple, Matthias  University of Duisburg-Essen, Germany
Committees

Faré, Silvia
Politecnico di Milano, Dept Chemistry, Materials and Chemical Engineering, Italy

Fischer, Horst
RWTH Aachen, University Hospital, Dental Materials and Biomaterials Research, Germany

García-González, Carlos A.
University of Santiago de Compostela, Pharmacology, Pharmacy and Pharmaceutical Technology Dept., Spain

Gautrot, Julien
Queen Mary, University of London School of Engineering and Materials Science, United Kingdom

Gbureck, Uwe
University of Wurzburg, Germany

Gelinsky, Michael
Centre for Translational Bone, Joint and Soft Tissue Research, TU Dresden, Germany

Geris, Liesbet
University of Liege, Belgium

Ginebra, Maria-Pau
Technical University of Catalonia, Spain

Giovanni, Vozzi
University of Pisa, Italy

Gomez-Barrena, Enrique
Universitario La Paz, Spain

Gotman, Irena
ORT Braude College, Mechanical Engineering, Israel

Grijpma, Dirk
University of Twente, The Netherlands

Groll, Jürgen
University of Wurzburg, Germany

Groth, Thomas
Martin Luther University Halle-Wittenberg, Biomedical Materials, Germany

Guarino, Vincenzo
National Research Council of Italy, Institute of Polymers Composite and Biomaterials, Italy

Guicheux, Jerome
University of Nantes, France

Gurruchaga, Marilo
University of the Basque Country, Spain

Habibovic, Pamela
Maastricht University, The Netherlands

Hanawa, Takao
Tokyo Medical and Mental University, Japan

N. Hasirci, Vasif
Acibadem Mehmet Ali Aydinlar University, Medical Engineering, Turkey

Haugen, Håvard J
University of Oslo, Norway

Heise, Andreas
RCSI University of Health, Sciences Department of Chemistry, Ireland

Herrero-Vanrell, Rocio
Complutense University of Madrid, Spain

Hunt, John Alan
Nottingham Trent University, United Kingdom

Hutmacher, Dietmar W
Queensland University of Technology, Australia

Im, Gun-II
Dongguk University, Republic of Korea

Jansen, John
Radboud University, Medical Center Dentistry, The Netherlands

Ji, Jian
Zhejiang University, China

Kirkpatrick, Charles James
Johannes Gutenberg University of Mainz, Institute of Pathology, Germany

Kuhn, Liisa
University of Connecticut Health Center, United States of America

Lacroix, Damien
The University of Sheffield, United Kingdom

Leeuwenburgh, Sander
Radboudumc Regenerative Biomaterials, The Netherlands

Leijten, Jeroen
University of Twente, The Netherlands

Letourneur, Didier
Université de Paris, INSERM U1148 LVTS, France

Levato, Riccardo
Utrecht University, The Netherlands

Lewandowska-Szumieł, Małgorzata
Medical University of Warsaw, Poland

Lim, Khoon
University of Otago, Christchurch, New Zealand

Page 17 of 2028
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liverani, Liliana</td>
<td>University of Erlangen-Nuremberg, Institute of Biomaterials, Germany</td>
</tr>
<tr>
<td>Lloyd, Andrew</td>
<td>University of Brighton, United Kingdom</td>
</tr>
<tr>
<td>Malda, Jos</td>
<td>Utrecht University, Netherlands</td>
</tr>
<tr>
<td>Maniura, Katharina</td>
<td>EMPA Biointerfaces, Switzerland</td>
</tr>
<tr>
<td>Mantovani, Diego</td>
<td>Université Laval, Canada</td>
</tr>
<tr>
<td>Maoz, Ben Meir</td>
<td>Tel Aviv University, Biomedical Engineering and Sagol School of Neuroscience, Israel</td>
</tr>
<tr>
<td>Mas-Moruno, Carles</td>
<td>Technical University of Catalonia, Spain</td>
</tr>
<tr>
<td>Massera, Jonathan</td>
<td>Tampere University of Technology, Finland</td>
</tr>
<tr>
<td>Mata, Alvaro</td>
<td>University of Nottingham, United Kingdom</td>
</tr>
<tr>
<td>Meenan, Brian</td>
<td>Ulster University, United Kingdom</td>
</tr>
<tr>
<td>Melchels, Ferry</td>
<td>Heriot Watt University, United Kingdom</td>
</tr>
<tr>
<td>Meyer, Michael</td>
<td>FILK Freiberg, Germany</td>
</tr>
<tr>
<td>Migliaresi, Claudio</td>
<td>University of Trento, Italy</td>
</tr>
<tr>
<td>Mignon, Arn</td>
<td>KU Leuven Engineering Technology, Group T, Belgium</td>
</tr>
<tr>
<td>Missirlis, Yannis</td>
<td>University of Patras, Greece</td>
</tr>
<tr>
<td>Mobini, Sabha</td>
<td>IMN-CNM, CSIC, Madrid, Spain</td>
</tr>
<tr>
<td>Moreira Teixeira, Liliana</td>
<td>University of Twente, Developmental BioEngineering, The Netherlands</td>
</tr>
<tr>
<td>Moroni, Lorenzo</td>
<td>Maastricht University, The Netherlands</td>
</tr>
<tr>
<td>Motta, Antonella</td>
<td>University of Trento, Italy</td>
</tr>
<tr>
<td>Mouzakis, Dionysios E.</td>
<td>Hellenic Army Academy, Military Sciencea, Greece</td>
</tr>
<tr>
<td>OBrien, Fergal</td>
<td>Royal College of Surgeons Ireland, Ireland</td>
</tr>
<tr>
<td>Pamula, Elżbieta</td>
<td>AGH University of Science and Technology, Poland</td>
</tr>
<tr>
<td>Pandit, Abhay</td>
<td>University in Galway, Ireland</td>
</tr>
<tr>
<td>Perez Amodio, Soledad</td>
<td>Institute for Engineering of Catalonia, Spain</td>
</tr>
<tr>
<td>Persson, Cecilia</td>
<td>Uppsala University, Sweden</td>
</tr>
<tr>
<td>Pietzsch, Jens</td>
<td>Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Institute of Radiopharmaceutical Cancer Research, Germany</td>
</tr>
<tr>
<td>Planell, Josep</td>
<td>Open University of Catalonia, Spain</td>
</tr>
<tr>
<td>Ramalingam, Murugan</td>
<td>Tohoku University, Japan</td>
</tr>
<tr>
<td>Ranga, Adrian</td>
<td>Catholic University of Leuven, Belgium</td>
</tr>
<tr>
<td>Ratner, Buddy</td>
<td>University of Washington, Department of Bioengineering, United States of America</td>
</tr>
<tr>
<td>Reches, Meital</td>
<td>The Hebrew University of Jerusalem, Israel</td>
</tr>
<tr>
<td>Redl, Heinz</td>
<td>Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Australia</td>
</tr>
<tr>
<td>Reilly, Gwendolen</td>
<td>University of Sheffield, Germany</td>
</tr>
<tr>
<td>Rhodes, Nick</td>
<td>University of Liverpool, United Kingdom</td>
</tr>
<tr>
<td>Richards, Geoff</td>
<td>AO Foundation, AO Research Institute Davos, Switzerland</td>
</tr>
<tr>
<td>Rimondini, Lia</td>
<td>University of Eastern Piedmont, Italy</td>
</tr>
<tr>
<td>Ritz, Ulrike</td>
<td>Univ. Mainz, Germany</td>
</tr>
<tr>
<td>Roach, Paul</td>
<td>Loughborough University, Department of Chemistry, School of Science, United Kingdom</td>
</tr>
<tr>
<td>Rodriguez Lorenzo, Luis M.</td>
<td>CSIC Polymeric nanomaterials and biomaterials/Biomaterials2/ICTP, Spain</td>
</tr>
<tr>
<td>Name</td>
<td>Institution</td>
</tr>
<tr>
<td>---------------------------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Rodriguez Rius, Daniel</td>
<td>Technical University of Catalonia, Dept. Materials Science and Engineering, Spain</td>
</tr>
<tr>
<td>Rodríguez-Cabello, José Carlos</td>
<td>University of Valladolid, Spain</td>
</tr>
<tr>
<td>Rolauffs, Bernd</td>
<td>Uniklinikum Freiburg, Germany</td>
</tr>
<tr>
<td>Rottmar, Markus</td>
<td>Swiss Federal Laboratories for Materials Science and Technology, Switzerland</td>
</tr>
<tr>
<td>Salmeron Sanchez, Manuel</td>
<td>University of Glasgow, United Kingdom</td>
</tr>
<tr>
<td>Santin, Matteo</td>
<td>University of Brighton, Centre for Regenerative Medicine and Devices, United Kingdom</td>
</tr>
<tr>
<td>Schenke-Layland, Katja</td>
<td>University Tübingen, Dept. of Women’s Health, Germany</td>
</tr>
<tr>
<td>Schnabelrauch, Matthias</td>
<td>Innoveit Jena, Germany</td>
</tr>
<tr>
<td>Shin, Heungsoo</td>
<td>Hanyang University Bioengineering, Republic of Korea</td>
</tr>
<tr>
<td>Sikorski, Pawel</td>
<td>Norwegian University of Science and Technology, NTNU Department of Physics, Norway</td>
</tr>
<tr>
<td>Stancu, Izabela</td>
<td>University Politechnica of Bucharest, Romania</td>
</tr>
<tr>
<td>Tabata, Yasuhiro</td>
<td>Kyoto University, Department of Biomaterials, Japan</td>
</tr>
<tr>
<td>Tanner, Elizabeth</td>
<td>Queen Mary University of London, School of Engineering and Materials Science, United Kingdom</td>
</tr>
<tr>
<td>Tanzi, Maria Cristina</td>
<td>Politecnico di Milano Chemistry, Materials and Chemical Engineering, Italy</td>
</tr>
<tr>
<td>van Rijn, Patrick</td>
<td>University Medical Center Groningen BME, The Netherlands</td>
</tr>
<tr>
<td>Van Vlierberghe, Sandra</td>
<td>Ghent University, Belgium</td>
</tr>
<tr>
<td>Varoni, Elena Maria</td>
<td>University of Milano, Italy</td>
</tr>
<tr>
<td>Velasco Bayón, Diego</td>
<td>University Carlos III of Madrid (UC3M), Bioengineering and Aerospace Engineering, Spain</td>
</tr>
<tr>
<td>Weiss, Anthony</td>
<td>University of Sydney, Australia</td>
</tr>
<tr>
<td>Weiss, Pierre</td>
<td>University of Nantes, France</td>
</tr>
<tr>
<td>Woodfield, Tim</td>
<td>University of Otago, Christchurch, New Zealand</td>
</tr>
<tr>
<td>Xiao, Yin</td>
<td>QUT, Brisbane, Australia</td>
</tr>
<tr>
<td>Zenobi-Wong, Marcy</td>
<td>ETH Zürich, Switzerland</td>
</tr>
<tr>
<td>Zeugolis, Dimitrios</td>
<td>University College Dublin, Ireland</td>
</tr>
</tbody>
</table>
## Scientific Committee of SIBB2021 Congress - Biomechanics

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrantes, João</td>
<td>Universidade Lusofona, Portugal - Chairman</td>
</tr>
<tr>
<td>Gutierrez, Marcos</td>
<td>Universidad de Granada, España - Co-Chairman</td>
</tr>
<tr>
<td>Campos, José</td>
<td>Universidad de Valencia, España</td>
</tr>
<tr>
<td>Faria, Aurélio</td>
<td>Universidade da Beira Interior, Portugal</td>
</tr>
<tr>
<td>Gabriel, Ronaldo</td>
<td>Universidad de Trás-os-Montes e Alto Douro, Portugal</td>
</tr>
<tr>
<td>García, Juan</td>
<td>Universidad de León, España</td>
</tr>
<tr>
<td>Gianikellis, Kostas</td>
<td>Universidad de Cáceres, España</td>
</tr>
<tr>
<td>González Frutos, Pablo</td>
<td>Universidad Francisco de Vitoria, España</td>
</tr>
<tr>
<td>Navandar, Archit</td>
<td>Universidad Europea, España</td>
</tr>
<tr>
<td>Rojas, Javier</td>
<td>Universidad de Granada, España</td>
</tr>
<tr>
<td>Roriz, Paulo</td>
<td>Instituto Universitário da Maia, Portugal</td>
</tr>
<tr>
<td>San Juan, Alejandro</td>
<td>Universidad Politécnica de Madrid, España</td>
</tr>
<tr>
<td>Suarez, Bruno</td>
<td>Universidad Politécnica de Madrid, España</td>
</tr>
<tr>
<td>Veiga, Santiago</td>
<td>Universidad Politécnica de Madrid, España</td>
</tr>
<tr>
<td>Vila-Chã, Carolina</td>
<td>Instituto Politécnico da Guarda, Portugal</td>
</tr>
<tr>
<td>Vilas Boas, João Paulo</td>
<td>Universidade do Porto, Portugal</td>
</tr>
</tbody>
</table>
The organizing committee would like to thank our Sponsors and partners for the support of the conference:

**Gold Sponsor & Exhibitor**

**Rousselot Biomedical**

Rousselot® Biomedical
www.rousselot.com/biomedical

As the most recent strategic segment within Rousselot, we have drawn upon Rousselot’s 125+ years of worldwide expertise and proven track record of pharmaceutical gelatins and collagens to develop X-Pure®, an innovative range of purified gelatins and collagens specifically designed for (bio)medical applications. Offering unique advantages to assure performance, quality and safety, X-Pure is backed by strong scientific data and on-going research. Rousselot Biomedical is committed to facilitating the use of X-Pure in your applications and to help “Advancing medical science”.

**Further Sponsors**

**Elsevier**

https://www.journals.elsevier.com/biomaterials

Biomaterials is an international journal covering the science and clinical application of biomaterials. It is the aim of the journal to provide a peer-reviewed forum for the publication of original papers and authoritative review and opinion papers dealing with the most important issues facing the use of biomaterials in clinical practice. The scope of the journal covers the wide range of physical, biological and chemical sciences that underpin the design of biomaterials and the clinical disciplines in which they are used. These sciences include polymer synthesis and characterization, drug and gene vector design, the biology of the host response, immunology and toxicology and self assembly at the nanoscale. Clinical applications include the therapies of medical technology and regenerative medicine in all clinical disciplines, and diagnostic systems that reply on innovative contrast and sensing agents. The journal is relevant to areas such as cancer diagnosis and therapy, implantable devices, drug delivery systems, gene vectors, bionanotechnology and tissue engineering.

**FLUIDINOVA, S.A.**

www.fluidinova.com/congress

FLUIDINOVA is a specialized manufacturer of synthetic nano-hydroxyapatite and tricalcium phosphate materials marketed as nanoXIM®. FLUIDINOVA provides companies and research institutions around the world with unique and high quality calcium phosphates for the development of medical devices, oral care cosmetics and food products. Particularly, for medical applications, our products are successfully used as bone graft substitutes with improved regenerative performance. Our focus on customer satisfaction and innovation helps us to establish close relationships with clients to develop the best solutions to incorporate our materials in their final products to address a vast number of applications.

**Biomaterials Science, Royal Society of Chemistry**

www.rsc.org/journals-books-databases/about-journals/biomaterials-science/

*Biomaterials Science* is the official journal of the European Society of Biomaterials. Published on a not-for-profit basis by the Royal Society of Chemistry, the journal presents high impact work focused on exploring the design, function, and interactions of biomaterials. Its scope encompasses new concepts in biomaterials design, studies into the interaction of biomaterials with the body, and the use of materials to answer fundamental biological questions. The journal is led by Editor-in-Chief Jianjun Cheng, alongside an international Editorial Board of expert Associate Editors.
Enzifarma S.A. is a leading Portuguese company operating in niche healthcare segments, namely in the import and commercialization of state-of-the-art solutions for the laboratory area, considered a PME Líder '20 with Quality Control in compliance with ISO 9001:2015. Founded in 1996, with headquarters in Lisbon and warehouse operation in the Porto region, where there is also an office that offers administrative support in the commercialization, storage and dispatch processes. We consider our mission to know how to be rigorous and efficient with us and our suppliers, as a way to achieve and maintain a leadership position in the quality of the products and services provided, for a better satisfaction of our customers, shareholders, society and all interested parties, namely the companies we represent. Thus, we strive to understand, satisfy and anticipate customers' needs, building with them a strong and stable relationship. In organizational terms, the company operates according to a business area strategy, and a cross-logic of marketing and product applicability (technical-scientific).

From IOP Publishing, our multidisciplinary journal *Multifunctional Materials* (MFM) serves an emerging field that now connects the materials science, physics, chemistry, bioscience and engineering communities, and translational multifunctional sciences. As a highly selective journal, it’s primary aim is to publish original research of the highest quality, including fundamental concepts to integrate multiple functions, characterisation of functions, novel applications and manufacturing techniques, with the expectation for lasting scientific and technological impact. Discover more about our experienced top-level editorial board, and high-quality online production today. https://iopscience.iop.org/journal/2399-7532

Specanalítica is one of the leading companies in the Portuguese analytical market supported by a motivated and highly qualified team. Focused on the Commercialization of internationally renowned brands in the fields Analytical Chemistry, Material and Life Sciences, customer support and after-sales services are our priorities! We have Accredited Training Certification since 2012 for our customers! Our mission: work in a partnership with our customers offering specialized analytical solutions to meet their analytical needs, keeping leadership as one of the most respected customer partners in the Portuguese industrial, analytical and life science market! Our ambition is to be recognized for the excellent product range we offer and for the technical support provided!

Ceramics (ISSN 2571-6131) is an international peer-reviewed open access journal of ceramics science and engineering published quarterly online by MDPI. It is indexed within Emerging Sources Citation Index (Web of Science), and other relevant databases. It provides a forum for publishing papers which advance the in-depth understanding of the relationship between the structure, the microstructure, the properties and the applications of ceramic materials. It publishes reviews, regular research papers (articles) and short communications. Our aim is to encourage scientists to publish their ex-perimental and theoretical results in as much detail as possible so that the results can be reproduced. Therefore, there is no restriction on the length of the papers. We are committed to drive Ceramics to a position in which it is recognized for its high-quality, cutting-edge research and scientific influence, and strongly encourage and invite your participation and manuscripts.
Brad Nelson has been the Professor of Robotics and Intelligent Systems at ETH Zürich since 2002. He has over thirty years of experience in the field of robotics and has received a number of awards in the fields of robotics, nanotechnology, and biomedicine. He serves on the advisory boards of a number of academic departments and research institutes across North America, Europe, and Asia and is on the editorial boards of several academic journals. Prof. Nelson is the Department Head of Mechanical and Process Engineering at ETH and has been the Chairman of the ETH Electron Microscopy Center and a member of the Research Council of the Swiss National Science Foundation. He also serves on boards of three Swiss companies. Before moving to Europe, Prof. Nelson worked as an engineer at Honeywell and Motorola and served as a United States Peace Corps Volunteer in Botswana, Africa. He has also been a professor at the University of Minnesota and the University of Illinois at Chicago.

Linda Gay Griffith, (BS Georgia Tech, PhD UC Berkeley, Chemical Engineering) is the School of Engineering Teaching Innovation Professor of Biological and Mechanical Engineering and MacVicar Fellow at MIT, where she directs the Center for Gynepathology Research. She led development of the Biological Engineering SB degree program, which was approved in 2005 as MIT’s first new undergraduate major in 39 years. She has pioneered approaches in tissue engineering, including the first tissue-engineered cartilage in the shape of a human ear; commercialization of the 3DP™ Printing Process for manufacture of FDA-approved scaffolds; commercialization of the 3D perfused “LiverChip” for drug development; and synthetic matrices for morphogenesis. A major current focus is gynecology applications, integrating mechanistic disease stratification and tissue-engineered models of lesions for drug development in endometriosis and adenomyosis. She is a member of the National Academy of Engineering, the recipient of a MacArthur Foundation Fellowship, Radcliffe Fellowship and several awards from professional societies. She currently serves on the Advisory Board of the Society for Women’s Health Research and has served on several NIH Advisory Councils including the Advisory Committee to the Director.
Laura De Laporte combines engineering, chemistry and biology to design biomaterials that control and direct the interaction with cells. She is a Chemical Engineer from Ghent, where she got the tissue engineering microbe. To follow her dream, she did her PhD with Lonnie Shea at Northwestern University and engineered guiding implants for nerve regeneration. At EPFL, she learned about hydrogels in Jeffrey Hubbell’s group during her post-doctoral research. Currently, she is a Leibniz Professor at the RWTH University in Aachen, Germany, where she works on Advanced Biomedical Systems at the DWI-Leibniz Institute for Interactive Materials. In the framework of the ERC Starting Grant Anisogel, her team designs low-invasive, polymeric regenerative hydrogel therapies, consisting of nano- and micron-scale building blocks that orient after injection to repair anisotropic tissues. In addition, dynamic hydrogels are created to study mechanobiology.
George Winter Award

The George Winter award is established by the European Society for Biomaterials to recognize, encourage and stimulate outstanding research contributions to the field of biomaterials. The nominee must have contributed significantly to the knowledge in the field of biomaterials through basic, experimental and/or clinical research. The award will be presented annually during the ESB conference.

João Mano

Awardee George Winter Award 2020

University of Aveiro, Department of Chemistry, CICECO – Aveiro Institute of Materials, Aveiro, Portugal

Talk: GW AW-01 | Design of biomaterials/cells structures for bottom-up tissue engineering strategies – less is more
Date: Monday, 6 September, 2021, 9:30 AM
Track: 01

João F. Mano, PhD in Chemistry (1996, Technical Univ. Lisbon), D.Sc. in Tissue Engineering Regenerative Medicine and Stem Cells (2012, Univ. Minho), is a full professor at the Department of Chemistry of the University of Aveiro (Portugal). During his career he has been teaching courses related to biomaterials science and technology, tissue engineering and physical chemistry of polymers and materials, in both undergraduate and graduate levels. He is the director of both Master and Doctoral Degrees in Biotechnology at the Univ. of Aveiro. He has also an appointment as Invited Professor (classe exceptionelle, since 2014) at University of Lorraine (France), and Visiting Professor in KAIST (South Korea) – 2019.

He belongs to the associate laboratory CICECO – Aveiro Institute of Materials where he is directing the COMPASS Research Group, founded in April 2016. His current research interests include the use of biomaterials and cells towards the development of transdisciplinary concepts especially aimed at being used in regenerative and personalised medicine. In particular, biomimetic and nano/micro-technology approaches have been applied to natural-derived biomaterials and surfaces in order to obtain biomedical devices with improved structural and (multi-)functional properties, or in the engineering of microenvironments to control cell behaviour and organization, to be used in therapies or in drug screening.

João F. Mano is author of 630+ original research or review papers in international journals (26000+ citations, h=81 – Web of Science) and about 40 book chapters. João F. Mano co-edited 9 special issues in international journals and 3 books. João F. Mano has been invited to review manuscripts from 275+ different international journals and to routinely evaluate projects from numerous private and state funding agencies from 18 different countries. João F. Mano supervised or co-supervised 60 MSc, 22 PhD students, and 40+ post-doctoral fellows. He filed 6 patents as senior inventor.

He is the Editor-in-Chief of Materials Today Bio (Elsevier). He has been also part of a series of scientific societies and editorial boards of about 10 international journals. He has been coordinating or involved in many national and European research projects and participated in the organization of scientific events in the area of polymer/materials science and biomaterials/tissue engineering.

Professor João F. Mano has been member of scientific committees, organizing committees, referee and chairman in different international meetings. He was invited to present more than 100 invited/keynote/plenary talks in international conferences including: EUROMAT, ESTAC, TERMIS (EU and AP chapters and World conferences), BIOMED, FBPS, NANOMED, COLAOB, ESB, SFB, World Biomaterials Conference, E-MRS, ESAO, EPF, ACS, CBECIMAT, NICE, Inter. HYMA, APME, APCChE, PPM, EPNOE, SELECTBIO.
João F. Mano has received different honours and awards: (i) fellow of the IUPAC (International Union of Pure and Applied Chemistry) since 2004; (ii) the Stimulus to Excellence Award by the Portuguese Minister for Science and Technology in 2005; (iii) the Materials Science and Technology Prize, attributed by the Federation of European Materials Societies (FEMS) in 2007; (iv) UNESCO Chair on Biomaterials attributed in 2008 from the University of Havana (Cuba); (v) the major BES innovation award in 2010 (at that time, one of the most recognised innovation prize in Portugal); (vi) recipient an Advanced Grant from the European Research Council (ERC-AdG), in 2015; (vii) received the title of Professor@Lorraine from the University of Lorraine, France, in 2018; (viii) recipient a Proof of Concept Grant from the European Research Council (ERC-PoC), in 2018; (ix) title of Doctor Honoris Causa, given by University of Lorraine, in 2019; (x) awarded with a Gutenberg Chair, supported by the Great East Region of France, in 2020; (xi) recipient a second ERC-AdG, in 2020; (xii) recipient a second ERC-PoC, in 2020; (xiii) elected fellow of the European Academy of Sciences.

Pekka Kalevi Vallittu

Awardee George Winter Award 2021

University of Turku (UTU), Department of Biomaterials Science, Turku, Finland

Talk: GW AW-02 I Development of fiber-reinforced composites for dental and medical reconstructive biomaterial

Date: Wednesday, 8 September, 2021, 9:30 AM

Track: 01

Pekka Vallittu (www.utu.fi/en/people/pekka-vallittu) has earned his degrees in Dental Technology in 1988, Doctor of Dental Surgery and Doctor of Philosophy in 1994, received Adjunct Professorship in 1995 and specialized in prostodontics and stomatognathic physiology in 2000. Presently, he is a Full Professorship and Chair of Biomaterials Science in the Faculty of Medicine, University of Turku, Finland and works as Dean of the Institute of Dentistry at the University of Turku and as the Director of Turku Clinical Biomaterials Centre. He holds Honorary Professorship at the University of Hong Kong, Pokfulam and Visiting Professorship at the King Saud University in Riayadh, Saudi Arabia.

His predominating research activity on fiber-reinforced composites has lasted over 30 years since 1980’s. The first clinical applications of fiber-reinforced composites were found in clinical dentistry and thereafter in combination with bioactive glass components and surface coatings in bone surgical applications as non-metallic bioactive implants. Pekka Vallittu has over 640 ISI Web of Science Indexed publications, more that 140 granted patents based on 32 inventions and his h-index is 92. He has established two companies (Stick Tech Ltd – GC Group and Skulle Implants Corp.) for getting newly developed composite materials for clinical use.
Jean Leray Award

The Jean Leray award is established by the European Society for Biomaterials to recognize, encourage and stimulate outstanding research contributions to the field of biomaterials by early-career scientists. The nominee must have contributed significantly to the knowledge in the field of biomaterials through basic, experimental and/or clinical research. The award will be presented annually during the ESB conference.

Jeroen Leijten

Awardee Jean Leray Award 2020

Dept. Developmental BioEngineering, Faculty of Science and Technology, Technical Medical Centre, University of Twente, The Netherlands

Talk: JL AW-01 I Cell fate controlling micromaterials for the engineering of multiscale tissues
Date: Monday, 6 September, 2021, 10:15 AM
Track: 01

Jeroen Leijten is currently an associate professor in the Developmental BioEngineering department in the Technical Medical Centre of University Twente. He earned his Master’s degree in Biomedical Sciences at Leiden University in 2007, and his PhD degree in Tissue Engineering with Prof. Blitterswijk at University of Twente in 2012, where he continued to work as a postdoc for an additional year with Prof. Karperien. In 2013, he joined Prometheus, the division of Skeletal Tissue Engineering of the KU Leuven, to work with Prof. Luyten. As a self-funded postdoc, he worked on the development of microtechnological platforms to produce and investigate the potential of stem cell based micro-organoids and smart micromaterials. In 2014, he joined the Khademhosseini lab at Harvard-MIT as senior postdoctoral researcher where he worked on microfluidics, instructive biomaterials, and organ-on-chips. In 2016, he accepted a tenure track position at University Twente. His lab focuses on developing novel microtechnologies with a particular interest in microfluidics, micromaterials, and on-demand tunable biomaterials, which he leverages to create 3D spatiotemporal microenvironments to control (stem) cell behavior. He has published 59 peer reviewed papers in renowned journals including Cell Stem Cell, Nature Communications, Small, Advanced Functional Materials, and PNAS, resulting in >2100 citations and a H-index of 28. He received >7.5 million euro in funding including a Veni award, Vidi award, and ERC Starting grant, multiple presentation awards/honors including the best-idea-of-2018 by the Dutch academy of Engineers, and was twice selected as a top 10 Young Scientific Talent by New Scientist.
Riccardo Levato

Awardee Jean Leray Award 2021

Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University and Department of Orthopedics, University Medical Center Utrecht, the Netherlands

Talk:  JL AW-02 | Driving complex and synthetic tissue development via volumetric biofabrication technologies

Date:  Tuesday, 7 September, 2021, 10:15 AM

Track:  01

Riccardo Levato is currently associate professor of Translational Bioengineering and Biomaterials at the Department of Clinical Sciences (Faculty of Veterinary Medicine, Utrecht University), and a principal investigator both at the Regenerative Medicine Center Utrecht and at the Department of Orthopedics of the University Medical Center Utrecht.

His research interests focus on the development of novel biofabrication strategies and cell-instructive biomaterials to create bioprinted, lab-made tissue models and transplantable engineered grafts, particularly for the regeneration of the musculoskeletal system. Integrating expertise in engineering, materials science and stem cell biology, these efforts aim both to understand and mimic the multifaceted architectural and biochemical structure of living tissues in order to develop effective treatments for human and veterinary healthcare. In 2020 he was awarded a Starting grant from the European Research Council on the development of a novel volumetric bioprinting technology for organoid research and to engineer functional bone marrow analogues in vitro. In addition, since 2021, he is coordinator of a European consortium (ENLIGHT), funded under the Future and Emerging Technologies scheme (European Innovation Council pilot), aiming at developing biofabricated pancreas units to study treatments for diabetes. To date, he has published 49 peer-reviewed articles international journals (H-index=21), co-authored 2 book chapters and 2 patent applications, and he secured > 13 million euros in research funds for his group and related consortia. In total, he has been (co-)supervisor of 13 PhD students (4 completed, 9 ongoing). For his work, he was conferred several awards including a Orthoregeneration Network Fellowship by the International Cartilage Repair Society (ICRS), the Wake Forest Institute for Regenerative Medicine Young Investigator Award, multiple presentation and travel awards, and the Julia Polak doctoral award from the European Society for Biomaterials. Riccardo is an active member of the ESB, TERMIS, ICRS, and he is serving on the Board of Directors of the International Society for Biofabrication. Prior to moving to Utrecht, he worked and received training in several research groups working in the fields of Biomaterials, Regenerative Medicine and Biofabrication: 3Bs, University of Minho (Portugal); BioMatLab, Technical University of Milan (Italy), the Biomaterials for Regenerative Therapies group at the Institute for Bioengineering of Catalonia (IBEC, Spain), and he holds a cum laude PhD in Biomedical Engineering from IBEC and from the Technical University of Catalonia (Barcelona, Spain).
International Award 2021

The International Award is a prestigious recognition by the ESB of scientists who have generally spent their career outside Europe, who have been internationally recognised, have a high scientific profile, and have made major contributions to the field of biomaterials. Strong evidence of collaborations with members of our scientific community in Europe throughout their career is expected.

David Mooney

Awardee International Award 2021

Wyss Institute for Biologically Inspired Engineering and John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, USA

Talk: IntAW-01 | Cells, Viscoelasticity and Medical Devices
Date: Wednesday, 8 September, 2021, 5:45 PM
Track: 01

David Mooney is the Pinkas Family Professor of Bioengineering in the Harvard School of Engineering and Applied Sciences, and a Founding and Core Faculty Member of the Wyss Institute. He earned his B.S. at the University of Wisconsin, Madison, and his Ph.D in Chemical Engineering at the Massachusetts Institute of Technology. His laboratory designs biomaterials to promote tissue regeneration and immunotherapy, and has made significant advances in tissue engineering, drug delivery, and mechanotransduction. The laboratory emphasizes the education of students and fellows, with over 120 Ph.D. students and post-doctoral fellows to date; 40 lab alumni hold faculty positions with the remainder largely in the biotechnology, pharmaceutical, and medical device industries. He has an H-index of 158, with over 100,000 citations to date of his publications. He has won numerous awards, including the Clemson Award from the SFB, MERIT award from the NIH, Distinguished Scientist Award from the IADR, Phi Beta Kappa Prize for Excellence in Undergraduate Teaching, and the Everett Mendelsohn Excellence in Mentoring Award from Harvard College. He is a member of the National Academy of Engineering, the National Academy of Medicine, and the National Academy of Inventors. In 2019 he was named one of the Top 10 translational researchers in biotech by Nature Biotechnology. His inventions have been licensed by over 18 companies, leading to commercialized products and founding of companies, and he is active on industrial scientific advisory boards.
Klaas de Groot Award

The Klaas de Groot award is a prestigious recognition by the European Society for Biomaterials of scientists who have shown a distinct ability to provide excellent mentorship and guidance to young researchers, helping them to establish their own independent career. We look for colleagues who have nurtured young talent, and who have selflessly invested in this talent, creating a next generation biomaterials scientists in Europe. The award will be presented annually during the ESB conference.

Mário Adolfo Barbosa

Awardee Klaas de Groot Award 2020

Instituto de Ciências Biomédicas Abel Salazar (ICBAS), University of Porto, Porto, Portugal; I3S Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal

Talk:  KdG AW-01 | Keep Learning: Advantages of Age in Science (KLAAS)
Date:  Tuesday, 7 September, 2021, 9:30 AM
Track:  01

Mário Barbosa took his PhD at Leeds University (UK). He is Full Professor at the Instituto de Ciências Biomédicas Abel Salazar (ICBAS), University of Porto, where his area of lecturing is bioengineering. From 2015 to December 2019, and following an application led by him, he was the Director of the I3S (Instituto de Investigação e Inovação em Saúde) of Porto University. I3S was evaluated by the FCT (Fundaçãa para a Ciência e a Tecnologia) with the highest marks (Outstanding in 2015 and Excellent in 2019). I3S is the largest R&D research institute in Portugal, with >1300 investigators, 460 of them holding a PhD degree. Mário was a founding member of Instituto de Engenharia Biomédica (INEB) set up in 1989, which is part of the i3S consortium. Mário has taken leading positions in Portugal, namely as President of the national agency for research (1992 to 1994) and Chairman of its Engineering Sciences Scientific Council (2002 to 2005).

The main topic of Mário’s research is the modulation of inflammation in tissue repair/regeneration, namely through the design of immunomodulatory microenvironments. At i3S he leads the Microenvironments for New Therapies (MiNT) group. MiNT bioengineers microenvironments that promote tissue regeneration/functional restoration through modulation of the host response.

At the post-graduate level Mário has taken a leading role in launching undergraduate and post-doctoral programmes (e.g. the Doctoral Programme in Biomedical Engineering, in 1996, one of the first to operate in Portugal). He is the Director of the International Doctoral Programme on Molecular and Cellular Biotechnology Applied to Health Sciences (now in its 7th edition), that provides research training in bioengineering in top level institutions. Mário has been always strongly committed to advanced training in the area of biomaterials. For instance, from 1988 to 1992 he run a series of European Intensive Courses, funded by the European Commission, which resulted in the publication of four books published by Elsevier (1991, 1992, 1993 and 1994). During 10 years in a row (1999-2009), he organized Advanced Summer Course in Cell/Material Interactions, with an important hands-on component and a large number of international participants. After a short break in 2010, the Summer Schools re-emerged under the general topic of Interrogations at the Biointerface with the goal of bringing together different fields around one topic of interest. The 7th edition took place in 2019.

Among several awards is the George Winter Award of the European Society for Biomaterials (ESB), the ESB award for outstanding work of an established researcher, received in 2001. Mário is a Fellow of the International Union of Societies for Biomaterials Science and Engineering. From 2007 to 2016 he was the representative of the ESB for the Acta Biomaterialia Gold Medal award and from 2007 to 2013 the chairman of the awards committee of the ESB. He was a member of the Directive Council of the ESB (1991-1995). Mário organized several international conferences, including the 12th European Conference on Biomaterials (1995) and Bioceramics 16 (2003). Presently, he is the Secretary of the International Union of the Societies of Biomaterials Science and Engineering (IUSBSE). Former postdocs and students have leading positions in research institutes, both in Portugal and abroad (e.g. China, USA, Brazil and Spain). He has published more than 280 papers in refereed journals (>9000 citations; h index 52).

ORCID:  https://orcid.org/0000-0003-3568-7482  / Web page:  https://www.i3s.up.pt/research-group?x=32

Page 30 of 2028
Clemens A. van Blitterswijk

Awardee Klaas de Groot Award 2021

Professor of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, the Netherlands

Talk: KdG AW-02 | Some reflections on scientific progeny
Date: Wednesday, 8 September, 2021, 10:15 AM
Track: 01

Prof. Dr. Clemens A. van Blitterswijk (born 1957, The Hague, the Netherlands) graduated as a cell biologist from Leiden University (Leiden, the Netherlands) in 1982. He defended his PhD thesis in 1985 at the same university on artificial ceramic middle ear implants, for which he was awarded the Jean Leray award (European Society for Biomaterials), the Marie Parijs award (University of Leiden), and the Kleijn award (University of Utrecht) in the following years. Today, most of his research deals with tissue engineering and regenerative medicine and is based on multidisciplinary research between materials and life sciences. He founded the MERLN Institute for Technology-Inspired Regenerative Medicine in 2014 and acted as its Director until 2019. He is currently a Distinguished Professor of Complex Tissue Regeneration at Maastricht University.

Van Blitterswijk is one of the preeminent scientists in the Netherlands and is a leader of his field internationally. With >500 peer-reviewed published papers (h-index 114, >48,000 citations), van Blitterswijk is in the top-ten, most-cited scientists worldwide in the fields of biomaterials and tissue engineering (Web of Science) and holds the top position in Europe. He has published papers with authors from 25 different countries. He has edited four books, including the first dedicated to tissue engineering. He has received over 250 invitations to present at international conferences and workshops. In addition to his scientific achievements, van Blitterswijk has raised large national structural funding for research in biomaterials, tissue engineering, and regenerative medicine. For example, he has been involved in (often leading) the establishment of Translational Excellence in Regenerative Medicine (TeRM; €25M; main applicant), Netherlands Initiative for Regenerative Medicine (NIRM; €84M), and Biomedical Materials (BMM; €90M). He moved to Maastricht University as part of the triple-helix Kennis-As initiative (€25M) and founded the quadruple-helix, international RegMed XB consortium (with a commitment for €250M).

Van Blitterswijk is recognized at the highest level in the Netherlands as a member of the Royal Netherlands Academy of Arts and Sciences (KNAW) since 2012 and the Royal Holland Society of Sciences and Humanities (KHMW) since 2016. He has received many of the prestigious international awards in his field (e.g., the Career Achievement award of the EU-Chapter of TERMIS, appointment as a Fellow of the International Union of Societies for Biomaterials Science and Engineering, and both the Jean Leray and George Winter awards from the European Society for Biomaterials). He won the 2012 award for the most entrepreneurial scientist in the Netherlands and the 2015 Huijbersen award for performing ground-breaking science with societal impact. He has been granted some of the most prestigious research subsidies in Europe (e.g., the ERC Advanced Grant) and in the Netherlands (e.g., the NWO Gravitation). Van Blitterswijk has raised hundreds of millions in total for his research (~€175M), the major consortia he has (co-)founded, and his companies (~€150M).

During his career Prof. van Blitterswijk has also co-founded multiple biomedical companies and held several functions in these organizations. He acted as CEO of IsoTis (a public life sciences company in the Netherlands) from 1996 to 2002, a company that he co-founded and grew to 150 people. As a result of his work, 10 implant technologies have been brought into clinical evaluation in humans. He combines his professorship with being a Founding Partner of the new Health Economics Fund (LSP-HEF) of the European health care investment group Life Sciences Partners (LSP) in Amsterdam. The focus is on investments in mature innovative medical technology companies that can reduce costs in health care while providing high quality health care to patients.
Invited Keynote Speakers

Ovijit Chaudhuri

Associate Professor at the Department of Mechanical Engineering at Stanford University, Stanford, USA

Talk: K08-KL01 | Extracellular matrix viscoelasticity and its impact on cells
Date: Wednesday, 8 September, 2021, 3:45 PM
Track: 02

Ovijit Chaudhuri is an Associate Professor in the Department of Mechanical Engineering at Stanford University. He earned a B.S. in engineering physics with a minor in mathematics at UC Berkeley. Then, he obtained his Ph.D. in bioengineering at UC Berkeley and UC San Francisco, studying force generation and mechanics of actin cytoskeletal networks. From there, he went on to do a postdoctoral fellowship at Harvard University, studying cell mechanotransduction and developing engineered biomaterials for 3D culture. He joined Stanford in 2013. His group's research interests are broadly in cell biophysics and cell-matrix mechanotransduction in 3D microenvironments. More specifically, his group's work has focused on understanding the impact of extracellular matrix viscoelasticity on cell behaviors in the context of cell migration, cell division, breast cancer progression, cartilage tissue engineering, and stem cell differentiation, using engineered biomaterials for 3D cell culture. His honors include a National Science Foundation CAREER award, a DARPA young faculty award, an American Cancer Society research scholar award, and a National Institutes of Health MERIT award. More information about his group's work can be found at http://chaudhurilab.stanford.edu or be followed on twitter @theChaudhurilab.

Luisa De Cola

Professor at the University of Milan, Italy; Head of the unit Materials for Health at the Istituto di Ricerche Farmacologiche Mario Negri, IRCCS, Italy

Talk: K01-KL01 | Hybrid nanoparticles and hydrogels for biomedical applications
Date: Monday, 6 September, 2021, 11:30 AM
Track: 02

Luisa De Cola is since November 2020 Professor at the University of Milan and head of the unit Materials for Health at the Istituto di Ricerche Farmacologiche Mario Negri, IRCCS, Italy. She is also part time scientist at the INT-KIT, Karlsruhe, Germany. Since 2019 Honorary Professor at Tianjin University, China. She was born in Messina, Italy, where she studied chemistry. After a post-doc in USA she was appointed Assistant Professor at the University of Bologna (1990). In 1998 she was appointed Full Professor at the University of Amsterdam, The Netherlands. In 2004 she moved to the University of Muenster, Germany. In 2012 she has been appointed Axa Chair of Supramolecular and Bio-Material Chemistry, at the University of Strasbourg. She is recipients of several awards, the most recent being the ERC advanced grant, the International Prize for Chemistry “L. Tartufani” from Accademia dei Lincei, the Catalan -Sabatier prize from the Spanish Royal Academy of Science (2015), the Izatt–Christensen Award in Macrocyclic and Supramolecular Chemistry (2019), the gold Medal Natta (2020). She is one of the 39 selected women chemists, from all times, at the Science Museum of Valencia (Ciutat de les Arts y de les Ciencies), Spain. She has been Nominated “Chevalier de la Légion d’ Honneur” by the President of the French Republic, François Hollande, and she is a member of the German National Academy of Sciences Leopoldina, of the Istituto Lombardo Accademia di Scienze e Lettere and she has just been nominated fellow of the American Institute For Medical and Biological Engineering (AIMBE).
Her main interests are luminescent and electroluminescent systems and their assemblies and nano- and porous structures for biomedical applications. She has published more than 370 papers (H index =77), and filed 38 patents.
Jennifer H. Elisseeff

Professor and Director, Translational Tissue Engineering Center
Wilmer Eye Institute and Departments of Biomedical Engineering, Orthopedic Surgery, Chemical and Biological Engineering, and Materials Science and Engineering at Johns Hopkins University, Baltimore, USA

Talk: K05-KL01 | Mapping the immune and stromal response to biomaterials
Date: Tuesday, 7 September, 2021, 4:15 PM
Track: 01

Dr. Elisseeff is the Morton Goldberg Professor and Director of the Translational Tissue Engineering Center at Johns Hopkins Department of Biomedical Engineering and the Wilmer Eye Institute with appointments in Chemical and Biological Engineering, Materials Science and Orthopedic Surgery. She was elected a Fellow of the American Institute of Medical and Biological Engineering, the National Academy of Inventors, and a Young Global Leader by World Economic Forum. In 2018, she was elected to the National Academy of Engineering and National Academy of Medicine. Jennifer received a bachelor's degree in chemistry from Carnegie Mellon University and a PhD in medical engineering from the Harvard-MIT Division of Health Sciences and Technology. Later she was a Fellow at the National Institute of General Medical Sciences, Pharmacology Research Associate Program, where she worked in the National Institute of Dental and Craniofacial Research. She has published over 200 papers, book chapters, and patent applications and received a number of awards including the Carnegie Young Alumni Award and in 2002 she was named by MIT Technology Review as a top innovator under 35. Jennifer's research focus is the development of biomaterials for regenerative medicine applications in orthopedics, plastic and reconstructive surgery, and ophthalmology. She is now studying Biomaterials-directed Regenerative Immunology and the role of the adaptive immune system in tissue repair. She is committed to the translation of regenerative biomaterials and has founded several companies and participates in several industry advisory boards.

Peter Ertl

Vienna University of Technology, Vienna, Austria

Talk: K02-KL01 | Application of Organ-on-a-Chip Systems in Precision Medicine
Date: Monday, 6 September, 2021, 2:00 PM
Track: 02

Prof. Ertl holds an engineering degree in Biotechnology (University of Life Sciences, Austria), a PhD in Chemistry (University of Waterloo, Canada) and received his postdoctoral training as a biophysicist at University of California at Berkeley (US). In 2003 Dr. Ertl co-founded a biotech start-up company, where he served a number of years as Director of Product Development in Kitchener (Canada) developing benchtop-sized microbial analyzers. In 2006 Dr. Ertl moved to Austria where he worked as Senior Scientist in the Biosensor Technology unit at the Austrian Institute of Technology (AIT). Dr. Ertl was also granted a Fulbright Visiting Scholarship at UC Berkeley (2012) and conducted visiting scientist positions at Nanyang Technological University, Singapore (2013), the Medical Center of the University of California at San Francisco (2014) and Imperial College London (2019). In 2016 he was appointed Professor of Lab-on-a-Chip Systems for Bioscience Technologies at the Vienna University of Technology (TUW), where his research focuses on the development of advanced in vitro diagnostic microsystems and organ-on-a-chip systems. Dr. Ertl is also speaker of the Austrian Microfluidics Initiative, Editor-in-Chief of the open access journal Organs-on-a-Chip (Elsevier) and Chief Technology Officer (CTO) of SAICO Biosystems.
Gabor Forgacs

University of Missouri-Columbia; Scientific Founder, Organovo; CSO, Modern Meadow, USA

Talk: K03-KL01 | Biofabrication in commercial setting
Date: Monday, 6 September, 2021, 3:45 PM
Track: 02

Gabor Forgacs is a theoretical physicist turned biophysicist turned bioengineer turned innovator and entrepreneur. His academic affiliations include the George Vineyard Chair in Biophysics at the University of Missouri-Columbia and the Chanderna-Stirkey Chair in Theoretical Physics at Clarkson University, where he also served as the Scientific Director of the Shipley Innovation Center. He is the scientific founder of Organovo, Inc., Modern Meadow, Inc. and Fork & Goode, Inc. and is the Chief Scientific Officer of the latter. He was trained as a physicist at the Roland Eotvos University, Budapest, Hungary. He also has a degree in biology. His research interests and contributions span from topics in theoretical physics to physical mechanisms in early embryonic development. He is the author of over 200 scientific publications and 5 books, in particular the co-author of the celebrated text in the field, “Biological Physics of the Developing Embryo” that discusses physical mechanisms that guide embryonic development. He applies these mechanisms to build organ structures using bioprinting, a technology he pioneered. Such structures are already used for drug development and testing. The technology has also been adapted to engineer consumer products of animal origin such as leather and meat in environmentally friendly and ethically conscious manner. Dr. Forgacs has been recognized by numerous prizes and awards. In particular, he is a member of the National Academy of Innovators and was named as one of the “100 most innovative people in business in 2010” by FastCompany.

Ben M. Maoz

Department of Biomedical Engineering, Sagol School of Neuroscience, The Center for Nanoscience and Nanotechnology, Tel Aviv University, Israel

Talk: K09-KL01 | Organs-On-a-Chip: A New Tool for the Study of Human Physiology
Date: Thursday, 9 September, 2021, 11:45 AM
Track: 01

Dr. Ben Maoz is a faculty member in the Department of Biomedical Engineering, and Sagol School of Neuroscience at Tel Aviv University. Dr. Maoz did his Ph.D in Chemistry at Tel Aviv University under the supervision of Prof. Gil Markovich. He returned to Israel after completing his postdoctoral fellowship at the Wyss Institute at Harvard University at the lab of Prof. Don Ingber and Prof. Kit Parker where he did groundbreaking work in the field of Organs-on-a-Chip. Dr. Maoz received number of prestigious awards such as ERC, Harvard-Wyss technology fellow, Azrieli fellowship for excellence science and others. More information on the MaozLab can be found in https://www.maozlab.com/ and his publication list under https://www.maozlab.com/publications.
Massimiliano Papi

Associate Professor of Biophysics at the Università Cattolica del Sc in Rome, Italy

Talk: K04-KL01 | Optical reduction and shaping of graphene-oxide for controlled bone tissue regeneration and bacterial killing

Date: Tuesday, 7 September, 2021, 11:30 AM

Track: 01

Massimiliano Papi is an Associate Professor of Biophysics at the Università Cattolica del Sc in Rome, Italy. His interests lie at the nexus of physics, biology and biotechnology and he carried out research in the area of biophysics, from single molecules to cells and tissues. Current main research interests are: Development of antibacterial and antiviral surfaces and materials. In this field, new materials and biomaterials based on nanoparticles such as graphene, titanium, silver and mxene have been developed to acquire specific antiviral, bacteriocidal and bacteriostatic properties. Various techniques have been developed for the realization of new materials such as 3D printing for the creation of customized antibacterial and antiviral structures. The technology to integrate antimicrobial nanoparticles into tissues has also been optimized. Nanoparticles-based drug delivery. Numerous innovative approaches have been developed in this field for the transport of a pharmaceutical compound in the body to safely obtain the desired therapeutic effect. Several new biotechnological platforms have been created to optimize the achievement of the site within the body and to facilitate systemic pharmacokinetics. Current research activity in drug transportation includes the development of a targeted release in which the drug is active only in the target area of the body (e.g. in cancerous tissues) and prolonged-release formulations, in which the drug is released for a period of time in a controlled way. Discovery of clinical biomarkers. In this activity, different techniques and approaches of both microscopy and spectroscopy have been developed and refined. The new biomarkers developed in recent years are morphological biomarkers, biomechanical biomarkers and molecular biomarkers. 3D-Bioprinting of biological systems. In this field, an innovative facility in the Gemelli Technological Park has been recently realized. Several biological systems for tissue engineering has been developed: 3D scaffolds for bone and muscle reconstruction and regeneration, 3D tumor models for cancer research; 3D biological microenvironment for drug testing.

Milica Radisic

Professor at the Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Canada

Talk: K07-KL01 | Optimizing synergy between materials and cell microenvironments using functional polyesters

Date: Wednesday, 8 September, 2021, 3:45 PM

Track: 01

Dr. Milica Radisic is a Professor at the University of Toronto, Canada Research Chair in Functional Cardiovascular Tissue Engineering and a Senior Scientist at the Toronto General Research Institute. She is also the Associate Chair-Research for the Department of Chemical Engineering and Applied Chemistry at the University of Toronto, Director of the NSERC CREATE Training Program in Organ-on-a-Chip Engineering & Entrepreneurship and Director of Ontario-Quebec Center for Organ-on-a-Cho Engineering. She obtained B.Eng. from McMaster University, and Ph.D. form the Massachusetts Institute of Technology. She is a Fellow of the Royal Society of Canada-Academy of Science, Canadian Academy of Engineering, the American Institute for Medical & Biological Engineering and Tissue Engineering & Regenerative Medicine Society. She received numerous awards and fellowships, including MIT Technology Review Top 35 Innovators under 35. She was a recipient of the Professional Engineers Ontario-Young Engineer Medal in 2011, Engineers Canada Young Engineer Achievement Award in 2012, Queen Elizabeth II Diamond Jubilee Medal in 2013, NSERC E.W.R Steacie Fellowship in 2014, YWC Toronto Woman of Distinction Award in 2018, and OPEA Research & Development Medal in 2019 to name a few. Her research focuses on organ-on-a-chip engineering and development of new biomaterials that promote healing and attenuate scarring. She
developed new methods to mature iPSC derived cardiac tissues using electrical stimulation. Currently, she holds research funding from CIHR, NSERC, CFI, ORF, NIH, and the Heart and Stroke Foundation. She is an Associate Editor for ACS Biomaterials Science & Engineering, a member of the Editorial Board of Tissue Engineering, Advanced Drug Delivery Reviews, Regenerative Biomaterials, Advanced Biosystems, Journal of Molecular and Cellular Cardiology and eLife. She serves on review panels for Canadian Institutes of Health Research and the National Institutes of Health. She is actively involved with BMES (Cardiovascular Track Chair in 2013 and 2104) and TERMIS-AM (Council member, Chair of the Membership Committee). She was a co-organizer of a 2017 Keystone Symposium, “Engineered Cells and Tissues as Platforms for Discovery and Therapy”. She served on the Board of Directors for Ontario Society of Professional Engineers and currently serves on the Board of Directors of Canadian Biomaterials Society. Her research findings were presented in over 200 research papers, reviews and book chapters with h-index of 61 and over 13,000 citations. Her publications appear in prestigious journals such as: Cell, Nature Materials, Nature Methods, Nature Protocols, Nature Communications, PNAS etc. In 2014, she co-founded an award winning company TARA Biosystems that uses matured human engineered heart tissues in drug development. TARA currently tests drugs for major pharmaceutical companies. In 2017, she founded Quthero Inc, a company focused on disrupting the skin regeneration and wound healing market through the use of proprietary Q-gel to promote scar-free wound healing.

Meital Reches

Associate Professor at the Institute of Chemistry and the Center for Nanoscience and Nanotechnology, the Hebrew University of Jerusalem, Israel

Talk:  K10-KL01 | Supramolecular peptide assemblies as a platform for antifouling, antimicrobial and antiviral materials

Date:  Thursday, 9 September, 2021, 11:45 AM
Track:  02

Meital Reches is an Associate Professor at the Institute of Chemistry and the Center for Nanoscience and Nanotechnology, the Hebrew University of Jerusalem. After completing her Ph.D. studies at Tel Aviv University in 2007 (supervisor: Prof. Ehud Gazit) and spending three years as an EMBO and a HFSP postdoctoral research fellow at Harvard University, (supervisor: Prof. George Whitesides), Prof. Reches established her independent research group at the Hebrew University in 2010. Research in the Reches group focuses on understanding the interactions of biological entities such as proteins, bacteria, viruses and cells with surfaces. In addition, her group develops surfaces to control these interactions. One example is a peptide-based coating that prevents bacterial adhesion and attracts mammalian cell. Altogether, Prof. Reches has over 80 papers in peer-reviewed journals (including Science, Nature Nanotechnology, Nature Communications, Advanced Materials, ACS Nano and PNAS), two book chapters and 13 families of patents. For her innovations, she was awarded by the Hebrew University with the prestigious Kaye Award for Innovation, the Marie Currie Alumni Association Best Innovator Award and the Tenne Family Prize in Nanoscale Sciences.
Goreti Sales received a degree in pharmaceutical sciences in 1994 and a PhD in analytical chemistry in 2000, from the Faculty of Pharmacy of Porto University. She is Associate professor in the Department of Chemical Engineering of the Faculty of Sciences and Technology of the University of Coimbra. Her research interests are focused on biomimetic nanomaterials and biosensing devices. She coordinates the research group BioMark@UC, since 2020, and she was the founder (in 2011) of the research group BioMark@ISEP. She was awarded (in 2012) a Starting Grant by the European Research Council, targeting a new technical approach that merges biosensors with solar cells, and she is currently coordinating the FET-Open project (H2020) MindGAP.
Khuloud Al-Jamal is a Chair of Drug Delivery & Nanomedicine and Head of Medicines Developments at the Institute of Pharmaceutical Science, King’s College London. She has developed an extensive experience in designing and developing novel nanoscale delivery systems including dendrimers, liposomes, quantum Dots, polymers, viral vectors, chemically functionalised carbon nanotubes and graphene oxide. Her current work involves pre-clinical translation nanomedicines with special interests in brain diseases and cancer. She received several awards including the Maplethrope Research and Teaching Award, BBSRC New Investigator Award, Royal Pharmaceutical Society of Great Britain Science Award, the Controlled Release Society Nanomedicine and Nanoscale Delivery Focus Group Young Investigator Award and Wellcome Trust Image Awards. She is a member of the General of Pharmaceutical Council. She is a management board member and is on the steering committee of the London Centre of Nanotechnology and the Children Brain Tumour Drug Delivery Consortium. She is on the Editorial Board of Biomaterials Science (RSC), MedBioMed (Wiley), Scientific Reports (Nature Publisher) and Journal of Drug Targeting. She is a member of the General of Pharmaceutical Council and a Fellow of Royal Society of Chemistry.

Ana Paula Rosifini Alves Claro, Ph.D., FSBE, is currently Principal Investigator, Head of the laboratory for Development of New Biomedical Materials, and Full Professor at the São Paulo State University (UNESP). Dr. Alves Claro has over twenty years of experience in the field of Biomaterials. She was elected Vice-president of SLABO in 2018. Dr. Alves Claro is a member of the Editorial Board In vitro Models. In the last years, she supervised Ph.D. and MSc students and is involved in mentoring postdoctoral research fellows.
Luigi Ambrosio

Institute of Polymers, Composites and Biomaterials (IPCB), National Research Council, Naples, Italy

ESB-French Society for the Development of Biomaterials (BIOMAT) Joint Symposium ESB-BIOMAT S-KL01 | Nano-biocomposites: from tissue repair/regeneration to therapeutic behaviour

Talk: What Will be the Future Biomaterials Implanted in Humans? Tribute to Prof. Charles Baquey
Date: Tuesday, 7 September, 2021, 11:30 AM
Track: 05

Luigi Ambrosio is Director of Institute of Polymer, Composites & Biomaterials, National Research Council, Naples, Italy. He received the doctoral degree in Chemical Engineering (1982) from University of Naples „Federico II”. He was Research Associate at University of Naples (1983-1985), Research Associate at University of Connecticut, USA (1985-1986), and Visiting Scientist at Kontron Medical Inc., USA (1986-1988). Adjunct Professor of University of Connecticut, USA (1997-2003) and of University of Naples „Federico II” (1997-2010). Qualified Full Professor in Bioengineering and in Materials Science and Technology. Director of Institute of Composites and Biomedical Materials, National Research Council, Naples, Italy (2008-2012). President of the European Society of Biomaterials (2007-2013), Past President (2013-2017), Honorary Member (since 2018). Director of Chemical Sciences & Materials Technology Department, National Research Council, Rome, Italy (2011-2017). Member of the High Level Group on Key Enabling Technologies, European Commission (2010-2015). Member of the International Advisor Board of Sichuan University and Co-Director of MPBRC, SCU-CNR Joint Research Centre. (since 2016). He is recipient of the „G. Winter Award”, for the high worldwide contribution to the Biomaterials Science, European Society for Biomaterials (March 2015). China-Italy Science and Technology Innovation Cooperation Contribution Award, China-Italy Technology Transfer Centre, Nov. 2017, Beijing, China. Member of the Scientific Committee on Chemical and Biomolecular Research of the Microbiome: Nutritional Applications and Impact on Metabolic Health. Université Laval, Quebec, Canada. (since 2016). Co-Chairmen of the Working group on “Advanced Materials and Nanotechnologies” Italy-USA Cooperation on Science and Technology, 12th Joint Commission Meeting. Italian Ministry of Foreign Affairs and International Cooperation (since 2016). He has been nominated Fellow of American Institute for Medical and Biological Engineering (2001), Fellow of Biomaterials Science and Engineering (2004) and Fellow of the European Alliance for Medical and Biomedical Engineering & Science (2018) and Member of the European Academy of Science (2019). Editor-in-Chief of Journal of Materials Science: Materials in Medicine (since 2017). Research interests include design and characterisation of polymers and composites for medical applications and tissue engineering, structural properties of natural tissue, properties and processing of polymers and composites and nanostructures, hydrogels and biodegradable polymers, additive technologies. Publications include over 320 papers on international scientific journals and book, 18 patents, over 170 invited lectures and over 500 presentations at international and national conferences, (Google Scholar H-Index: 73).
Daniel G. Anderson

Massachusetts Institute of Technology, David H. Koch Institute for Integrative Cancer Research / Institute for Medical Engineering and Science / Harvard and MIT Division of Health Science and Technology / Department of Chemical Engineering, Cambridge, USA

Talk: S11-KL01 | Nucleic acid delivery systems for RNA therapy and genome editing
Date: Wednesday, 8 September, 2021, 11:30 AM
Track: 04

Daniel G. Anderson is a Professor in the Department of Chemical Engineering, the Institute for Medical Engineering and Science, the Koch Institute for Integrative Cancer Research, and the Harvard-MIT Division of Health Science and Technology at MIT. The research done in Prof. Anderson’s laboratory is focused on developing new materials for medicine. He has pioneered the development of smart biomaterials, and his work has led to advances in a range of areas, including medical devices, cell therapy, drug delivery, gene therapy and material science. Prof. Anderson received a B.A. in mathematics and biology from the University of California at Santa Cruz and a Ph.D. in molecular genetics from the University of California at Davis. His work has resulted in the publication of over 400 papers, patents and patent applications. These advances have led products that have been commercialized or are in clinical development, as well as to the foundation of companies in the pharmaceutical, biotechnology, and consumer products space. Dr. Anderson is a founder of Living Proof, Olivo Labs, Crispr Therapeutics (CRSP), Sigilon Therapeutics, Verseau Therapeutics, Orna, and VasoRx.

Nasim Annabi

Department of Chemical and Biomolecular Engineering, University of California, Los Angeles (UCLA), USA

Talk: ESB-Biomaterials Science-KL01 | Multifunctional adhesive biomaterials for medical applications
Date: Wednesday, 8 September, 2021, 11:30 AM
Track: 06

Nasim Annabi is an Assistant Professor in the Department of Chemical and Biomolecular Engineering at University of California, Los Angeles (UCLA). She received a PhD in Chemical Engineering from the University of Sydney (Australia). From 2011-2014, she was a postdoctoral fellow at Harvard Medical School and the Wyss Institute for Biologically Inspired Engineering. Before joining UCLA in 2018, she was an Assistant Professor in the Department of Chemical Engineering at Northeastern University. Dr. Annabi’s group has expertise in the design and engineering of advanced biomaterials for applications in regenerative medicine. In addition, her research team has devised innovative strategies for the development of advanced bioadhesives and surgical sealants with high clinical translation for surgical applications. Dr. Annabi has published over 136 articles in peer-reviewed journals. She has been cited over 13,338 times and her H index is already at 58. Her innovations have resulted in 15 patents and generated significant commercial interest. Dr Annabi has been recognized with several national and international awards including the 2021 Young Investigator Award from the Society for Biomaterials (SFB), the 2020 Nanoscale Science and Engineering Forum (NSEF) Young Investigator Award of American Institute of Chemical Engineers (AIChE), the Australian Prestigious Endeavour Award, and the National Health and Medical Research Council Early Career Award. Her team has received major grants from the National Institutes of Health (NIH), the Department of Defense (DOD) and the American Heart Association (AHA).
Helena S. Azevedo is a Reader in Biomedical Engineering & Biomaterials at the School of Engineering & Materials Science in Queen Mary University of London where she leads her own research group. Her work focuses on self-assembling hyaluronan-peptide biomaterials for cell culture, drug delivery, regenerative medicine, and biosensing. She is author of >100 publications, including papers in Science, Nat Chem, Nat Comm, Adv Funct Mater, Nano Lett, Adv Health Mater, and has edited 3 books on natural-based biomaterials, self-assembling biomaterials and soft matter for biomedical application. She is a Fellow of the Royal Society of Chemistry (FRSC), Member of the Advisory Board of the RSC journal Molecular Systems Design & Engineering and Associate Editor of the Journal of Tissue Engineering and Regenerative Medicine (Wiley).

Aldo R. Boccaccini is Professor and Head of the Institute of Biomaterials at University of Erlangen-Nuremberg, Germany. He is also visiting Professor at Imperial College London, UK. He holds a nuclear engineering degree from Instituto Balseiro (Argentina), Dr.-Ing. (PhD) from RWTH Aachen University (Germany) and Habilitation from Technical University of Ilmenau (Germany). Prior to his current position, he spent 10 years at Imperial College London, Department of Materials, as Lecturer, Reader and Professor of Materials Science. He has held post-doctoral positions at University of Birmingham (UK) and University of California, San Diego (USA). He was Head of the Department of Materials Science and Engineering in Erlangen (2017-2019). The research activities of Prof. Boccaccini are in the field of ceramics, glasses and composites for biomedical, functional and/or structural applications. He is the author or co-author of more than 900 scientific papers and 25 book chapters. His work has been cited more than 46,000 times (h = 94, Scopus, “Highly cited researcher” 2014 and 2018). He is the Editor-in-Chief of the journal “Materials Letters” (Elsevier). He serves in the editorial board of more than 10 international journals. Boccaccini has been a visiting professor at different universities around the world. He is a Fellow of the Institute of Materials, Minerals and Mining (IOM3) (UK), the American Ceramic Society, the Society of Glass Technology (UK) and the European Ceramic Society. His achievements have been recognized with several awards including the Materials Prize of the German Materials Society (DGM) in 2015, the Turner Award of International Commission on Glass (2016) and Friedberg Lecture Award (2016) of American Ceramic Society. Boccaccini is also an elected member of the World Academy of Ceramics and of the National Academy of Engineering and Applied Sciences of Germany (acatech) and advisor to the Science and Technology Ministry of Argentina. Boccaccini serves in the Executive Committee of the Federation of European Materials Societies (FEMS) representing the German Materials Society and he has been a member of the Council of the European Society for Biomaterials (ESB) since 2015. He has served in several review panels of the European Research Council (ERC) and of the German Research Foundation (DFG).
Jan de Boer

Institute for Complex Molecular Systems and department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, the Netherlands

Talk: S13-KL01 | Biomaterial surface topography induces cell signaling events that control cell phenotype: how does it work and how can we use it?

Date: Wednesday, 8 September, 2021, 3:45 PM
Track: 04

Jan de Boer is chair of the BioInterface Science lab at the Institute for Complex Molecular Systems and department of Biomedical Engineering at Eindhoven University of Technology. He studied biology at the University of Utrecht and obtained his PhD in the lab of Jan Hoeijmakers at the Erasmus MC Rotterdam on mouse models for premature ageing in 1999. After a postdoc at the MRC Laboratory of Molecular Biology in Cambridge, UK, he started as a research associate at IsoTis B.V. where his research focused on bone tissue engineering, which was continued as associate professor at the University of Twente and as full professor at the University of Maastricht.

Jan studies the interplay between cells biomaterial, and how to use molecular circuits to control cell and tissue function. With a background in mouse and Drosophila genetics, he entered the field of biomedical engineering in 2002 and has since focused on understanding and implementing molecular biology in the field of tissue engineering, regenerative medicine and medical devices interface biology. His research is characterized by a holistic approach to both discovery and application, aiming at combining high throughput technologies, computational modeling and experimental cell biology to streamline the wealth of biological knowledge to real clinical applications.

He is former chair of the Netherlands Society for Biomaterials and Tissue Engineering, CEO and co-founder of Materiomics b.v., co-founder of the Platform for Therapeutic Biomaterials Discovery and co-founder of the Merln Institute for Technology-inspired Regenerative Medicine.

Horacio Cabral

Department of Bioengineering, Graduate School of Engineering, The University of Tokyo, Japan

Talk: ESB-CRS S-KL02 | Tumor-Targeted Nanomedicine Approaches for Enhanced Immunotherapy

Date: Tuesday, 7 September, 2021, 12:00 PM
Track: 04

Horacio Cabral is an Associate Professor in the Department of Bioengineering, Graduate School of Engineering, The University of Tokyo. He received his Ph.D. in Materials Engineering from The University of Tokyo in 2007 under the supervision of Prof. Kazunori Kataoka. Dr. Cabral was an Assistant Professor at the Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, The University of Tokyo, until 2010, when he joined the Department of Bioengineering of The University of Tokyo as a Lecturer. In 2014, he was promoted to his current position.

Dr. Cabral’s major research interests relate to the development of nanomedicines for diagnosis and therapy, particularly systems directed to intractable cancers. He has made major influential achievements in the development of translatable polymeric nanocarriers. Particularly, his polymeric micelles targeting anticancer drugs to tumors have proceeded into human clinical studies, improving patients’ survival and reducing side effects. Moreover, he has accomplished seminal contributions in the understanding of the effect of nanocarrier design on activity, particularly through intratumoral and intracellular navigation. He has published more than 100 articles in refereed journals, including Nature Nanotechnology, Nature Reviews Clinical Oncology, Science Translational Medicine, PNAS, etc., and he is inventor of more than 30 patents.

Dr. Cabral has been recipient of several awards, such as the Asahi Glass Foundation and the Mochida Memorial Foundation for Medical and Pharmaceutical Research. He currently serves on the editorial board of Science and Technology of Advanced Materials, and Nanomaterials, and the advisory board of Macromolecular Bioscience and MedChem.

Page 42 of 2028
Gianni Ciofani
Senior Researcher Tenured, Istituto Italiano di Tecnologia, Smart Bio-Interfaces, Principal Investigator Center for Materials Interfaces, Coordinator, Pontedera (Pisa), Italy

Talk: S04-KL02 | Piezoelectric materials for biomedical applications: From tissue engineering to cancer nanomedicine
Date: Monday, 6 September, 2021, 2:30 PM
Track: 03

Gianni Ciofani is Senior Researcher Tenured at the Istituto Italiano di Tecnologia (Italian Institute of Technology, IIT, Italy), where he is Principal Investigator of the Smart Bio-Interfaces Research Line (https://www.iit.it/research/lines/smart-bio-interfaces) and Coordinator of the Center for Materials Interfaces (https://www.iit.it/centers/cmi-sssa).
He received his Ph.D. in Innovative Technologies (with honors) from the Scuola Superiore Sant'Anna (Italy) in 2010. From January 2010 to August 2013 he was Post-Doc at the IIT, Center for Micro-BioRobotics, where, from September 2013 to October 2015, he was Researcher in the framework of the Smart Materials Platform. From October 2015 to October 2019 he was Associate Professor at the Polytechnic University of Torino (Italy), maintaining at the same time his research activity in IIT, where he is Senior Research Tenured since November 2019. His main research interests are in the field of smart nanomaterials for nanomedicine, bio/non-bio interactions, and biology in altered gravity conditions. He is coordinator or unit leader of many grants/projects, and he was awarded an ERC Starting Grant and an ERC Proof-of-Concept Grant in 2016 and 2018, respectively. Gianni Ciofani is author of more than 140 papers on international journals (H-index 38), 3 edited books, and 16 book chapters. He serves as Reviewer for more than 180 international journals, and as Editorial Board Member of Nanomedicine UK, Scientific Reports, International Journal of Nanomedicine, Journal of Physics: Materials, Bioactive Materials, and Advances in Nano Research; he is Specialty Chief Editor (Nanobiotechnology) for Frontiers in Bioengineering and Biotechnology.

Matthew Dalby
Centre for the Cellular Microenvironment, Institute of Molecular, Cell & Systems Biology, MVLS, University of Glasgow, Glasgow, Scotland

Talk: ESB-CSBM S-KL02 | Nanoscale control by materials of mesenchymal stem cells for identification of bioactive metabolites
Date: Monday, 6 September, 2021, 2:30 PM
Track: 04

After a PhD at Queen Mary University of London on osteoblast response to bioactive composites. I moved to Glasgow to study cell-nanoscale interactions. In 2003 I became an independent researcher securing a BBSRC David Phillips Fellowship to explore mesenchymal stem cell response to nanotopography. Appointed to a lecturership in 2008 and a Readership in 2010, I became Professor of Cell Engineering at the University of Glasgow in 2014. I hold grants from EPSRC, MRC, BBSRC, Leverhulme Trust and Sir Bobby Charlton Foundation. I am director of the EPSRC-SFI IiETIME centre for doctoral training.
My research has focussed on developing insight into MSC differentiation and self-renewal using materials and mechanotransductive cues, making contributions in journals such as Nature Materials, Nature Biomedical Engineering, Advanced Materials, Chem, Science Advances etc (>200 papers). More recently I have become interested in using materials to find activity metabolites that can be used to control MSC phenotype. As well as basic science, I am interested in translational science and have been involved in veterinary bone regeneration trials and am working now towards a human bone regeneration trial.
In 2016 I was elected a Fellow of the Royal Society of Edinburgh and have won a number of awards – most recently the Biochemical Society Industrial-Academic Collaboration Award.
Paul Dalton

University of Oregon, USA

Talk: ESB-ISBF S-KL01 | Hybrid Biofabrication using Nano-, Micro- and Macro-scale Features

Date: Tuesday, 7 September, 2021, 4:15 PM

Track: 04

Paul Dalton is an Associate Professor at the University of Oregon who specializes in manufacturing technologies for biomedical applications. An early adopter of melt electrospinning and pioneer of melt electrowriting, his research targets advanced biomaterials that can translate to the clinic. Graduating from Curtin University in Perth, Australia, he was part of a bioengineering team at the Lions Eye Institute that successfully took an artificial cornea from concept to the clinic. His academic career has an international perspective, with post-docs at the University of Toronto and RWTH-Aachen followed by positions at the University of Southampton, Shanghai Jiao Tong University and University of Würzburg. He invented and developed melt electrowriting as a distinct class within 3D printing with the Queensland University of Technology and has over 25 years’ experience across several disciplines.

Nicola Döbelin

RMS Foundation, Bettlach, Switzerland

Talk: S12-KL01 | Biocompatibility assessment of medical devices according to ISO 10993

Date: Wednesday, 8 September, 2021, 3:45 PM

Track: 03

Nicola Döbelin studied Earth Sciences at the University of Bern, Switzerland, from 1998 to 2002. In 2000 he spent three months in South Africa for a field work project before receiving his Master’s degree in 2002 for a thesis on the thermal stability of ion-exchanged natural zeolites studied by single-crystal X-ray diffraction. From 2002 to 2006 he worked on his PhD thesis on ion exchange in synthetic titanosilicate phases under supervision of Prof. Thomas Armbruster at the University of Bern. During this time, he became an expert on X-ray powder diffraction and Rietveld refinement. After receiving his PhD in 2006, Nicola started working as a senior scientist at RMS Foundation in Bettlach, Switzerland, with a focus on research, development, and characterization of bioceramics, specifically of calcium phosphate bone-graft substitutes. In 2010 he became deputy quality manager at RMS and specialized on validation of analytical methods. From 2015 to 2019 he took a part-time position as a senior lecturer at the Institute of Geological Sciences, University of Bern. Since 2016, Nicola leads the bioceramics team at RMS. Thanks to his many years of research on synthetic bone graft substitutes, he is now a recognized expert for the synthesis and characterization of calcium phosphate bioceramics.
Silvia Farè

Department of Chemistry, Materials and Chemical Engineering “G. Natta”, Politecnico di Milano, Milan, Italy

Talk: S07-KL02 | Biomimetic 3D scaffold-based model for in vitro investigation of bone pathologies
Date: Tuesday, 7 September, 2021, 12:00 PM
Track: 03

Silvia Farè obtained her Master of Science degree in Management and Industrial Engineering, Politecnico di Milano, in July 1994. In May 1998, she obtained her PhD degree in Biomaterials (X^ cycle), Politecnico di Milano, with a thesis on the investigation of the mechanisms related to the in vitro oxidative degradation of medical-grade polyurethanes. Silvia Farè obtained a Post-doc research Fellowship (1998 –1999) within the project “Third generation artificial heart” and she was Research Assistant from 1999 to 2003, at the Bioengineering Department, Politecnico di Milano. From 2005 to 2014, she was Assistant Professor at the Bioengineering Department (2005-2012), and at the Department of Chemistry, Materials, and Chemical Engineering “G. Natta” (2013-2014), Politecnico di Milano. From December 2014 to March 2021, she was Associate Professor in Industrial Bioengineering at the Chemistry, Materials and Chemical Engineering, Politecnico di Milano. Starting from April 2021 she is Full Professor at the Department of Chemistry, Materials, and Chemical Engineering “G. Natta”, Politecnico di Milano. From February 1993 to February 1996, she was visiting Master and PhD student at the Joint Research Centre, Institute for Advanced Materials, Ispra (VA), Italy. From March 1996 to September 1996 and from January 1997 to March 1997, she was visiting PhD student at the University of Laval and Quebec Biomaterials Institute, Quebec, Canada under the supervision of Gaetan Laroche and Diego Mantovani. She was Member of National and International Congress Committee. From 2012 she is Associate Editor for the Journal of Applied Biomaterials and Functional Materials (JAB-FM) Sage Ed. In 2005 she was elected as member of the Council of the Italian Society for Biomaterials (SIB), in charge as treasurer, and now she is in charge as President. From 2017 she is one of the members of the Council of the European Society for Biomaterials. From 2001 and up to the current academic year Silvia Farè has been in charge of different courses in biomaterials and regenerative medicine fields at Master and Bachelor in Biomedical Engineering, Politecnico di Milano. Silvia Farè is author and co-authors of one hundred international and national scientific publications related to materials science, in particular to biomaterial and regenerative medicine fields.

Lino Silva Ferreira

CNC | Centro de Neurociências e Biologia Celular, CIBB | Centro de Inovação em Biomedicina e Biotecnologia, University of Coimbra, Coimbra, Portugal;
Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Talk: NANOSTEM 01-KL01 | Modulation of stem cells using nanotechnologies
Date: Monday, 6 September, 2021, 10:40 AM
Track: 08

Lino Silva Ferreira holds a Ph.D. in Biotechnology from the University of Coimbra (Portugal). He did postdoctoral work at INEB (Portugal) and MIT (USA) in the group of Robert Langer in the areas of stem cells and nanotechnologies. He established his research group in 2008 at the University of Coimbra. Since then is the director of the Biomaterials and Stem Cell-Based Therapeutics research group, CNC coordinator of the MIT-Portugal Program and the founder of the biotech company Matera. He is also the associate editor of Biomaterials Science Journal (RSC). In 2012, he was awarded with a prestigious European Research Council starting grant and in 2016 a prestigious ERA Chair position at the University of Coimbra. His group has interest in the development of tissue models from stem cells and in the use of nanomedicine platforms to modulate the activity of endogenous (stem) cells.
Andrés J. Garcia

Executive Director, Parker H. Petit Institute for Bioengineering and Bioscience; Regents' Professor, George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA, U.S.A.

Talk: ESB-SFB S-KL01 | Biosynthetic Hydrogels for Islet Engraftment and Immune Acceptance
Date: Wednesday, 8 September, 2021, 3:45 PM
Track: 05

My research program centers on integrating innovative engineering, materials science, and cell biology concepts and technologies to generate (i) novel insights into the regulation of adhesive forces and mechanotransduction, and (ii) biofunctional materials (hydrogels, coatings, and nanoparticles) to control protein/cell delivery and tissue repair. This cross-disciplinary effort has resulted in new biomaterial platforms that elicit targeted cellular responses and tissue repair in various applications (bone repair, therapeutic vascularization, pancreatic islet delivery, and infection), innovative technologies to exploit cell adhesive interactions, and new mechanistic insights into the interplay of mechanics and cell biology. My research has been funded by the NIH, NSF, Coulter Foundation, Arthritis Foundation and Juvenile Diabetes Research Foundation. I have supervised 28 Ph.D. graduates and 13 postdoctoral fellows who have continued onto successful careers in academia, industry and government agencies. I currently supervise 11 Ph.D. students and 4 postdoctoral fellows. I am a co-inventor on 5 issued and 8 pending U.S patents and a co-founder of 3 companies (CellectCell, CorAmi Therapeutics, iTolerance). I am a Fellow of Biomaterials Science and Engineering of the International Union of Societies of Biomaterials Science and Engineering, AIMBE, ASME, and AAAS. I served as President for the Society for Biomaterials for 2018-2019. I am an elected member of the National Academy of Engineering and the National Academy of Inventors.

Maria Pau Ginebra

Universitat Politècnica de Catalunya, BarcelonaTech, Department of Materials Science and Engineering, Barcelona, Spain

Talk: S14-KL01 | 3D printing synthetic bone grafts: challenges and opportunities
Date: Thursday, 9 September, 2021, 11:45 AM
Track: 03

Maria-Pau Ginebra is Professor and Head of the Department of Materials Science and Engineering at the Universitat Politècnica de Catalunya (UPC) in Barcelona, Director of the Biomaterials Division of the Biomedical Engineering Research Center of UPC (CREB) and Associate Researcher at the Institute of Bioengineering of Catalonia (IBEC). She is the author of more than 200 articles published in indexed international journals and 10 patents. In 2013 she founded the company Mimetis Biomaterials, a spin-off of the UPC. Her research lines include the design and development of new biomaterials for bone regeneration, tissue engineering and drug delivery, and the fundamental study of the biological mechanisms that control the interactions of biomaterials with cells and tissues. Her research group has made relevant contributions in the development and characterization of a new generation of biomimetic calcium phosphates, capable of mimicking the extracellular matrix of bone, which may incorporate synthetic or natural polymers, and / or bioactive molecules. She is also working on new biofabrication strategies, including injectable foams for bone regeneration and 3D printing of implants for regenerative medicine, as well as on the design of multifunctional surfaces with bioactive and antimicrobial properties. In 2013 she received the Racquel LeGeros Award from the International Society for Ceramics in Medicine for her contribution to the research in the field of calcium phosphates and in 2019 the Klaas de Groot Prize of the European Society for Biomaterials in recognition of her mentoring of young scientists.
Manuela E. Gomes

3B’s Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Portugal

Talk: S05-KL01 | Magnetic Assisted 3D bioprinting and mechanoactuation technologies for achieving advanced biofunctionality of 3D constructs and precise models to engineer the regeneration of tendons

Date: Monday, 6 September, 2021, 3:45 PM
Track: 05

Manuela E. Gomes is Associate Professor and President of the I3Bs Research Institute of the University of Minho, Portugal. She graduated in Metallurgical and Materials Engineering, University of Porto, Portugal in 1997, obtained a MSc in Polymer Engineering, Univ. of Minho in cooperation with the company Isotis, Bilthoven (Holland), and a PhD in Materials Science and Engineering – Tissue Engineering/Hybrid Materials in collaboration with the Rice University (USA), in 2005. She was a co-founder of the 3B’s Research Group and one of the vice-directors of the group since 2011. Her research interests currently focus on tendon tissue engineering strategies, namely in the development of scaffold materials and bioinks based on biodegradable natural origin polymers, stem cells sourcing and differentiation (using biochemical and physical methods). Manuela Gomes has been involved in numerous European and national/regional projects as PI or team member. She currently coordinates a Consolidator Grant from the European Research Council (ERC CoG) to develop magnetically assisted tissue engineering technologies for tendon regeneration and with an EC funded Twinning Project – “Achilles: Overcoming specific weaknesses in tendon biology to design advanced regenerative therapies”, among others. Manuela Gomes is editor 2 books and co-editor of the Tissue Engineering Encyclopaedia (Elsevier, 2019) and author of 44 book chapters, over 200 full papers published in international refereed journals, and more than 300 communications in international conferences (7.900 citations, H-index of 47). She has received several awards, including the 2013 Young Investigator Award of the Tissue Engineering and Regenerative Medicine International Society – European Chapter (TERMIS-EU). Manuela Gomes is an active member of several international scientific organizations, being currently a member-at-large and chair of the membership committee of TERMIS, and Portugal Ambassador for the EORS.

Jinlian Hu

Director of Laboratory of Wearable Materials for Healthcare; Joint Professor of Materials Science and Engineering, Department of Biomedical Engineering, City University of Hong Kong, HKSAR, China

Talk: S04-KL01 | Shape Memory Biomaterials and Their Applications

Date: Monday, 6 September, 2021, 2:00 PM
Track: 03

Prof. Jinlian HU is a shape memory polymers, textiles and biomaterials scientist. Graduated from Donghua University in textile materials and received a PhD from Manchester University, she recently joined the Department of Biomedical engineering, City University of Hong Kong where she established a Laboratory of Wearable Materials for Healthcare. The laboratory focuses on unearthing scientific principles and providing solutions to key problems in Healthcare of Wearable Materials in three major areas: Traditional Chinese medical therapies and their materials, energy materials and healthcare as well as spider silks and their relatives as biomaterials as well as personal protective integration. As such, we apply advanced methods including custom-made wearable electronics as basic tools to examine materials, their application methods, and particularly their interactions with human body in terms of physical, chemical, biological, philological and informational relations. From the discoveries and models of basic research, applied investigation, product developments and standardization are envisaged, which can produce societal as well economic impact in addition to scientific advances. Before CityU, professor Hu worked at Hong Kong Polytechnic University for more than 20 years. Professor Jinlian HU is a Fellow of the Royal Society of Chemistry, Hong Kong Institution of Textile and Apparel and the British Textile Institute. She is the founding chairman of the Hong Kong Health Science and Technology Park, the executive vice chairman of the Hong Kong Invention and Innovation Federation and a Council member of the Hong Kong Far Infrared Association.

Page 47 of 2028
Sang Jin Lee
Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston-Salem, USA

Talk: **S07-KL01 | 3D Bioprinting Strategies to Bioengineer Skeletal Muscle Constructs that Accelerate Muscle Function Restoration**

**Date:** Tuesday, 7 September, 2021, 11:30 AM
**Track:** 03

Sang Jin Lee, Ph.D. is currently a tenured Associate Professor at Wake Forest Institute for Regenerative Medicine (WFIRM), Wake Forest School of Medicine. Dr. Lee received his Ph.D. in Chemical Engineering at Hanyang University, Seoul, Korea in 2003 and took a postdoctoral fellowship in the Laboratories for Tissue Engineering and Cellular Therapeutics at Harvard Medical School and Children’s Hospital Boston and the WFIRM where he is currently a faculty member. He is also cross-appointed to the Virginia Tech-WFU Biomedical Engineering and Science. Dr. Lee has authored more than 150 scientific publications and reviews, has edited 2 textbooks, and has written 34 chapters in several books. Dr. Lee has extensive knowledge and experience in biomaterials science, especially, biodegradable polymers and tunable hydrogels, with specific training and expertise in key research areas for tissue engineering and regenerative medicine. His research team has developed various biomaterial systems that improve cellular interactions by providing appropriate environmental cues. These biomaterial systems consist of drug/protein delivery systems, nano/micro-scaled topographical features, and hybrid materials that can actively participate in functional tissue regeneration. Recently, his team is utilizing automated 3D bioprinting technology to manufacture complex, multi-cellular living tissue constructs that mimic the structure of native tissues. This can be accomplished by optimizing the formulation of biomaterials to serve as bioinks for 3D bioprinting, and by providing the biological microenvironment needed for the successful delivery of cells and biomaterials to discrete locations within the 3D structure.

Kam W. Leong
Columbia University, Department of Biomedical Engineering, New York, USA

Talk: **ESB-BMJ S-03 | ESB-Biomaterials Journal Joint Session: Meet the Editor Session**

**Date:** Tuesday, 7 September, 2021, 04:15 PM
**Track:** 05

Kam W. Leong holds the James B. Duke Professor of Biomedical Engineering in the Pratt School of Engineering of Duke University, at which he also holds a joint appointment in the Department of Surgery of the School of Medicine. He received his undergraduate degree in Chemical Engineering from the University of California at Santa Barbara and his PhD, also in Chemical Engineering, from the University of Pennsylvania. He then ventured into drug delivery research as a postdoctoral fellow in the laboratory of Professor Robert Langer at MIT, where he helped develop the biodegradable polyanhydrides as a drug carrier for brain tumor therapy. After his training at MIT, he joined the faculty in the Department of Biomedical Engineering at The Johns Hopkins School of Medicine, and rose through the rank to become a full professor in 1998. While based at Johns Hopkins, he served as a Program Director in the Institute of Materials Research and Engineering in Singapore to help develop biomaterials research in the Institute from 1998-2004. Around the same time, he also directed a lab in Johns Hopkins Singapore on Therapeutics and Tissue Engineering. He moved to Duke University in 2006 to join the Department of Biomedical Engineering, focusing on the development of nanotherapeutics. In particular, his research concentrates on understanding and exploiting the interactions of cells with nanostructures for therapeutic applications. Discrete nanostructures in the form of multi-functional nanoparticles are applied to deliver drugs, antigen, protein, siRNA, and...
DNA to cells for drug, gene, and immunotherapy. Continuous nanostructures in the form of electrospun nanofiber and imprinted nanopattern are applied to influence cellular behavior, including expansion of various stem cells. The major research projects of his lab are linked by innovative design of polymeric biomaterials and an effort to understand the mechanism of cellular interaction with nanomaterials.

Kam W. Leong serves on the editorial boards of major journals in the fields of biomaterials, drug delivery, nanomedicine, and gene and cell therapy. He owns more than 40 issued patents, and has published over 230 peer-reviewed research manuscripts. His research was recognized by the Young Investigator Research Achievement Award of Controlled Release Society in 1994, and several awards by the same Society on Excellence in Guidance of Graduate Student Research. He also received the Stansell Family Distinguished Research Award from Duke University in 2010.

Changsheng Liu  
Shanghai University, Academician of Chinese Academy of Sciences, Shanghai, China  

**Talk:** ESB-CSBM S-KL01 | Decoding the materiobiology: multiple roles of biomaterials in participating life activities  
**Date:** Monday, 6 September, 2021, 2:00 PM  
**Track:** 04

Changsheng Liu, PhD, Professor of Shanghai University, Academician of Chinese Academy of Sciences. Dr. Liu is director of Engineering Research Center for Biomaterial Materials of Ministry of Education, director of Key Laboratory for Ultrafine Materials of Ministry of Education etc.  
Dr. Liu has interdisciplinary academic background of biology and materials engineering. He has devoted for decades into the fundamental and application research of biological materials. His major research areas include biomaterials and tissue regeneration; fabrication of inductive growth factors and designing of bioactive skeletal substitutes for bone repair; and nano-biomaterials as well. Dr. Liu has obtained a few outstanding achievements in the field of biomaterials. He has published 5 books and more than 290 papers, including the TOP journal of the field, such as Chem Rev, Sci Adv, Biomaterials, Adv Mater, Adv Func Mater, and Acta Biomater, etc. Moreover, he has also applied 65 invention patents including 4 US patents. He developed a few novel biomaterials and got the approval from CFDA, and now widely used in clinic application. He achieved international Fellow of Biomaterials Science and Engineering (2012), American Institute for Medical and Biological Engineering (AIMBE) Fellow (2018), and Ho Leung Ho Lee Foundation-Prize for Scientific and Technological Innovation Award (2007). Furthermore, he won the Second-prize of National Award for Natural Science in 2014, the Second-prize of National Award for Science and Technology Progress in 2003.

Hai-Quan Mao  
Johns Hopkins University, Baltimore, USA  

**Talk:** ESB-BMJ S-03 | ESB-Biomaterials Journal Joint Session: Meet the Editor Session  
**Date:** Tuesday, 7 September, 2021, 04:15 PM  
**Track:** 05

Hai-Quan Mao is the associate director of the Institute of NanoBioTechnology (INBT) and a professor of materials science and engineering at Johns Hopkins University. He holds a joint appointment in the Translational Tissue Engineering Center and the Department of Biomedical Engineering at the School of Medicine. Prof. Mao’s research focuses on engineering novel nanomaterials for regenerative medicine and therapy delivery applications. He has developed nanofiber scaffolds from synthetic and natural biomaterials for liver tissue engineering; stem cell expansion and differentiation; and soft tissue regeneration. He also has discovered a synergistic effect between nanofiber topography and biochemical cues on the proliferation of human hematopoietic stem and progenitor cells.
(HSCs); invented a more efficient HSC expansion method that can enable various HSC-based cellular therapies; engineered different methods for promoting lineage-specific differentiation of neural (crest) stem cells; and developed tailored, nanofiber-based scaffolds for vascular engineering, skeletal muscle regeneration, spinal cord repair and peripheral nerve regeneration. His contributions in therapeutic engineering include understanding the assembly mechanism of nanoparticles from the polyelectrolyte complex of plasmid DNA, RNA or protein therapeutics with charged polymer carriers; engineering DNA nanoparticles with tunable shape and uniformity that mimic natural viral particles; the development of scalable production methods for these therapeutic nanoparticles; and their applications in local and systemic delivery of macromolecular therapeutics and vaccines.

Prof. Mao also serves on the editorial board of ACS Biomaterials Science & Engineering and Journal of Materials Chemistry B. He holds 28 U.S. patents and has published more than 190 peer-reviewed research manuscripts. He has been elected a Fellow of the Royal Society of Chemistry and the American Institute for Medical and Biological Engineering, and a senior member of the National Academy of Inventors. He was the recipient of the Young Investigator Award at the National University of Singapore in 2002 and the National Science Foundation faculty CAREER Award in 2008.

Jesús Martínez de la Fuente

Consejo Superior de Investigaciones Científicas, Instituto de Nanociencia y Materiales de Aragón, Zaragoza, Spain

Talk: ESB-CRS S-KL01 | Nanoactuators for Therapy and Diagnosis
Date: Tuesday, 7 September, 2021, 11:30 AM
Track: 04

Prof. Jesús Martínez de la Fuente (Barakaldo, 1975) created his own research group (BIONANOSURF Group) at the Univ of Zaragoza in 2007, becoming internationally recognized in nanomaterials and biofunctionalization. The multidisciplinary nature of the group facilitates research and development in numerous areas, including biosensors, gene therapy, magnetism, photochemistry, surface chemistry and molecular metal oxides, among others. He has extensive experience in the synthesis and characterization of novel nanomaterials and their biofunctionalization for the use and development of the next generation of nanobiosensors and nanotherapeutics. In 2009, he founded the spin-off Nanoimmunotech SL. He has also been a pioneer in the application of gold nanoparticles in gene therapy and he has developed a methodology for the use of gold nanoparticles functionalized with carbohydrates (glycan nanoparticles) for the study of biological processes (embryogenesis, cancer, inflammation, etc.). He has been PI of research projects with a total budget of more than 6 M€. 75% of this budget is derived from European projects (1 ERANET (Coord); 1 ERC-StG (Coord), 1 ERC-POC (Coord), 7 MSCA-IOF/IEF/IF (Coord), 1 ENMII, 1 TRANSCANII (Coord), 1 FP7, 3 H2020-NMBP); 10% comes from collaborations with companies (CASEN-FLEET, ORPHAN DRUG-RECORDATI, MECWINS, NB, NIT, PROTEOMIKA, BSH, VIRBAC); and the remaining 15% comes from research projects of national calls. In 2010 he was awarded the Aragón Investiga prize „Young Researchers”. In 2013 he was awarded by the Shanghai Administration with the 1000 Talent Plan program to be a visiting professor at the Jiao Tong University of Shanghai. Since 2014, he is a permanent researcher at the Instituto de Nanociencia y Materiales de Aragon-CSIC.
Jonathan Massera

Tampere University, Faculty of Medicine and Health Technology, Tampere, Finland

Talk:  S08-KL01 | Bioactive glasses-based biomaterials with potential in soft tissue engineering

Date:  Tuesday, 7 September, 2021, 4:15 PM

Track:  02

Jonathan Massera is Professor at Tampere University, Finland, in the Faculty of Medicine and Health Technology. He graduated his BSc in Materials Science and Engineering, from the university of Grenoble. He then received a double MSc degree from Polytech’Montpellier (France) and Politecnico di Torino (Italy). He obtained his PhD from Clemson University (SC, USA), in 2009. During his PhD he studied tellurite based fibers for Mid-IR applications. He then received a scholarship to perform his post-doctoral research on the crystallization of bioactive glasses and its impact on bioactivity at Åbo Akademi, Finland. He then was granted a post-doctoral researcher grant from the Academy of Finland. The aim of the project was to combine photonic and bioactive glasses for sensing application. Finally, he became assistant researcher (tenure) at Tampere University, in 2014, where he was also granted the Academy Research Fellow from the academy of Finland. He became full professor in 2021. As of today, Jonathan Massera is head of the bioceramics, bio-glasses and bio-composites research group and head of the international MSC degree programme in Biomedical Sciences and Engineering. His research group focuses on inorganic biomaterials development and their composites/hybrids as well as cell/materiels and protein/materials interactions. Another axis of research deals with combining photonic into bioactive glasses to develop new biophotonic materials for low light therapy, sensing and imaging. Currently his research is funded by the Academy of Finland, The Jane and Aatos Erkko Foundation and EU-H2020.

Clara Mattu

PolitoBIOMed Lab, Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Torino, Italy

Talk:  S11-KL02 | Leveraging polymer biomaterials to improve the design of nanomedicines

Date:  Wednesday, 8 September, 2021, 12:00 PM

Track:  04

Dr. Clara Mattu is Assistant Professor in the Department of Mechanical and Aerospace Engineering at Politecnico di Torino. She completed her Ph.D. in the group of Industrial Bioengineering at Politecnico di Torino in 2012. She has been visiting scientist at the Royal College of Surgeons in Ireland in 2011, visiting postdoctoral fellow in the department of Translational Imaging (2014) and in the department of Nanomedicine (2016-2018) at the Houston Methodist Research Institute, and co-founder of the spin-off Company Geltis. In 2012, she was awarded the Italian Working Capital National Innovation grant (30.000 €) to perform research on injectable hydrogels for drug delivery. In 2015, she received the prestigious Marie Curie International Outgoing Fellowship from the European Commission to design hybrid drug delivery systems for intra-cranial treatment of brain tumors. Her research interests are highly super-disciplinary, and span from biomedical polymer synthesis to cancer nanomedicine.
Lorenzo Moroni

Maastricht University, MERLN Institute, Dept. of Complex Tissue Regeneration, The Netherlands

Talk: S01-KL01 | Integrating biofabrication with organ-on-chip technologies: marrying micro with macro for improved 3D in vitro models.

Date: Monday, 6 September, 2021, 11:30 AM

Prof. Dr. Lorenzo Moroni studied Biomedical Engineering at Polytechnic University of Milan, Italy, and Nanoscale Sciences at Chalmers Technical University, Sweden. He received his Ph.D. cum laude in 2006 at University of Twente on 3D scaffolds for osteochondral regeneration, for which he was awarded the European doctorate award in Biomaterials and Tissue Engineering from the European Society of Biomaterials (ESB). In 2007, he worked at Johns Hopkins University as a post-doctoral fellow in the Elisseeff lab, focusing on hydrogels and stem cells. In 2008, he was appointed the R&D director of the Musculoskeletal Tissue Bank of Rizzoli Orthopedic Institute, where he investigated the use of stem cells from alternative sources for cell banking, and the development of novel bioactive scaffolds for skeletal regeneration. From 2009 till 2014, he joined again University of Twente, where he got tenured in the Tissue Regeneration department.

Since 2014 he works at Maastricht University, where he is a founding member of the MERLN Institute for Technology-Inspired Regenerative Medicine. In 2016, he became full professor in biofabrication for regenerative medicine. His research group interests aim at developing biofabrication technologies to generate libraries of 3D scaffolds able to control cell fate, with applications spanning from skeletal to vascular, neural, and organ regeneration.

In 2014, he received the prestigious Jean Leray award for outstanding young principal investigators from the ESB and the ERC starting grant. In 2016, he also received the prestigious Young Scientist Award for outstanding principal investigators from TERMIS. In 2017, he was elected as faculty of the Young Academy of Europe and in the top 100 Italian scientists within 40 worldwide by the European Institute of Italian Culture. Since 2019, he is chair of the Complex Tissue Regeneration department and vice-director of MERLN. From his research efforts, 3 products have already reached the market.

website: http://merln.maastrichtuniversity.nl/content/lorenzo-moroni / http://www.moronilab.org

Willem J. M. Mulder

Department of Internal Medicine, Radboud Institute of Molecular Life Sciences (RiMLS) and Radboud Center for Infectious Diseases (RCI), Radboud University Nijmegen Medical Center, Netherlands;

Laboratory of Chemical Biology, Department of Biomedical Engineering and Institute for Complex Molecular Systems, Eindhoven University of Technology, Netherlands;

CSO, Trained Therapeutix Discovery; CTO, BIOTRIP.nl.

Talk: ESB-SFB S-KL02 | Taking control of innate immunity with nanobiologic therapeutics

Date: Wednesday, 8 September, 2021, 4:15 PM

Willem Mulder, Ph.D., a biomedical engineer with a background in chemistry, is Professor of Precision Medicine at the Radboud University Medical Center (Radboudumc) and the Eindhoven University of Technology (TU/e), The Netherlands. Mulder obtained an M.Sc. in Chemistry from the Utrecht University (2001) and a Ph.D. in Biomedical Engineering from TU/e (2006). He was professor and founding director of the Nanomedicine Lab at Icahn School of Medicine at Mount Sinai in New York from 2006 to 2020.

Mulder’s research focus is on nano-immunotherapy and precision imaging in cardiovascular diseases, cancer, infectious diseases and transplantation. He pioneered the exploitation of nanomaterials as highly tunable immunotherapeutics. Mulder established proprietary technology encompassing nanomaterials devised from natural apolipoproteins named nanobiologics. Using modular nanobiologic functionalization approaches and through in vivo
screening and immuno-imaging, his team meticulously builds precision immunotherapies. When appropriately designed, such nanobiologic immunotherapeutics can be applied to empower the immune system's ability to fight disease by targeting myeloid cell dynamics, by promoting or inhibiting molecular programs that regulate immune responses, or by 'training' macrophage function.

https://www.mulderlab.com

Mulder has published more than 175 scientific publications in top scientific journals, including Science Translational Medicine, Nature Nanotechnology, Nature Reviews Drug Discovery, Nature Biomedical Engineering, Immunity and Cell. His H-index is 73 and his work has been cited nearly 15,000 times. Mulder was the principal investigator of multiple National Institutes of Health grants. In 2013, Mulder received a Vidi grant and in 2018 he was awarded a Vici grant, both from the Dutch Research Council (NWO). In 2021, he won the prestigious ERC Advanced Grant. Mulder is a Founder and Chief Scientific Officer of Trained Therapeutix Discovery (https://ttxdiscovery.com) and a Founder and Chief Technology Officer of the Radboudumc-TU/e biotech incubator BIOTRIP (https://biotrip.nl)

Mariana B. Oliveira

CICECO – Aveiro Institute of Materials, Department of Chemistry. University of Aveiro, Portugal

Talk: S06-KL01 | Designing (almost) all-cellular living materials
Date: Tuesday, 7 September, 2021, 11:30 AM
Track: 02

Mariana B. Oliveira is a Junior Researcher at the COMPASS Research Group, part of CICECO – Aveiro Institute of Materials of the University of Aveiro, Portugal. She studied Biomedical Engineering at the University of Minho (Portugal), and obtained a PhD in 2014, from the same institution. Mariana Oliveira has focused her research on the development of biomaterials for the modulation of cellular response. She is the principal investigator and coordinator of two active research projects (>480 kEuros) focused on the exploitation of mesenchymal stem cells as scaffold-free and low-biomaterial structures to tailor pancreatic islet acceptance on transplantation scenarios, and for the promotion of tissue regeneration. She authors or co-authors 52 research and review publications, which include papers in Advanced Materials, Biomaterials, Trends in Biotechnology, and Advanced Healthcare Materials. She serves in the Young Career Board of ACS Biomaterials Sci & Eng, and is an Associate Editor of Frontiers in Bioengineering and Biotechnology and Frontiers in Materials.

Abhay Pandit

Scientific Director, CÚRAM, Centre for Research in Medical Devices, National University of Ireland, Ireland

Talk 1: S15-KL01 | Understanding the Regenerative Response Induced by Biomaterials Systems: Insight into the Role of Glycosylation
Date: Thursday, 9 September, 2021, 11:45 AM / Track: 04

Talk 2: ESB-BMJ S-01/S-02 | ESB-Biomaterials Journal Joint Session: Meet the Editor Session
Date: Tuesday, 7 September, 2021, 04:15 PM / Track: 05

Professor Abhay Pandit is an Established Professor in Biomaterials and Scientific Director of CÚRAM, the Science Foundation Ireland (SFI), funded Research Centre for Medical Devices at NUI Galway, Ireland. Prof. Pandit has over thirty years of experience in the field of Biomaterials. After a seven-year stint in industry, he has worked in academia for the last twenty-three years. Prof. Pandit's research has been funded by Science Foundation Ireland, the 7th EU Framework programme, Enterprise Ireland, Health Research Board, the AO Foundation and industry sources to the tune of €150 million. Prof Pandit built a critical mass of biomaterial expertise in Ireland by establishing the Network of Excellence for Functional Biomaterials (NFB). Later the centre was incorporated into a
Strategic Research Cluster (SRC) in 2007 with funding from Science Foundation Ireland (SFI) to establish collaborative partnerships with national and international universities and industries. Building on this critical mass of expertise and enormous success of CÚRAM Phase I, Prof. Pandit now leads phase II of CÚRAM. CÚRAM brings together more than 520 researchers with synergistic expertise in biomaterials, biomechanics, regenerative medicine, glycobiology, drug delivery and medical implant design, in addition to 50 plus industry partners.

Prof. Pandit has developed next-generation reservoir delivery vehicles with high payload capacity, programmable degradation profiles and inbuilt gradients of physical, chemotropic and protective cues, facilitating spatiotemporal localised sustained delivery of multiple biomolecules to target injury mechanisms at the molecular and cellular levels. His research integrates material science and biological paradigms in developing solutions for chronic diseases.

Prof. Pandit was elected to the American Institute of Medical and Biological Engineering (AIMBE) College of Fellows to recognise his outstanding contributions in creating a national centre to develop innovative device-based solutions to treat global chronic diseases. He is the first Irish academic to earn this distinction. He was inducted as an International Fellow in Biomaterials Science and Engineering by the International Union of Societies for Biomaterials Science and Engineering (IUSBSE) and Fellow of the Tissue Engineering Regenerative Medicine International Society. Prof Pandit is the first Irish academic to receive these honours. He was also inducted as a Fellow of the Irish Academy of Engineering.

Prof Pandit is the author of 27 patents and has licensed three technologies to medical device companies. Prof Pandit has published >305 papers in peer-reviewed high impact journals, >700 conference abstracts, and has over 15K citations. Prof. Pandit has successfully supervised 36 PhD students, 24 postdoctoral researchers with a current cohort of 8 Postdoctoral researchers, 20 PhD students and three research associates.

Ana Paula Pêgo

University of Porto, Instituto de Investigação e Inovação em Saúde (i3S), Porto, Portugal

Talk: ESB-BMJ S-03 | ESB-Biomaterials Journal Joint Session: Meet the Editor Session
Date: Tuesday, 7 September, 2021, 04:15 PM
Track: 05

Ana Paula Pêgo got her Ph.D. in Polymer Chemistry and Biomaterials from the University of Twente, the Netherlands, in 2002. In 2003 she moved to INEB – Instituto de Engenharia Biomédica, where she became a Principal Investigator in 2012. In 2015, INEB joined the i3S – Instituto de Investigação e Inovação em Saúde (Universidade do Porto), where Ana Paula Pêgo leads the nanoBiomaterials for Targeted Therapies (nBTT) Group.

By using nanomedicine strategies the nBTT Group, aims at providing in situ and in a targeted manner the required signals to promote nervous tissue regeneration. The research on new biomaterials for application in neurosciences includes the development of new polymers for the design of alternative vectors to viruses for efficient nucleic acid delivery, the preparation of nerve grafts for spinal cord injury treatment and the design of brain tissue engineered platforms. Societal and ethical issues that concern Regenerative Medicine and NanoMedicine are also topics in which Ana Paula Pêgo is involved.

She has been appointed the Scientific Director of the Bioimaging Centre for Biomaterials and Regenerative Therapies of INEB and she is an Invited Associate Professor at the Instituto de Ciências Biomédicas Abel Salazar (ICBAS) and at the Faculty of Engineering (FEUP) of the University of Porto.

Currently Ana Paula Pêgo is a member of the Board of Directors of i3S, being the Head of Strategy & Creation of Value Unit, is the Secretary of the Council of the European Society for Biomaterials (ESB), serves as an Associate Editor of Biomaterials (Elsevier journal) and is part of the Board of Reviewing Editors of Science (AAAS).

She received several distinctions that include the Neuroscience Awards by SCML (the most prestigious national award in the field) and the Young Scientist Award 2015 at the 5th China-Europe Symposium on Biomaterials and Regenerative Medicine (Hangzhou, China).

Dr. Pêgo has published more that 80 peer-reviewed articles in leading international journals in the biomaterials, nanotechnology and nanomedicine fields; authored 7 book chapters and is an inventor in 3 active patents (one licensed to Pharma) and 1 patent.

She is very proud of her mentoring/supervision duties actively contributing to the training of the next generation of bioengineers. To date, 22 PhD students, 7 post-doc and 47 MSc students are or have been supervised by her.
Julietta V. Rau

Institute of the Structure of Matter of the Italian National Research Council (ISM-CNR), Rome, Italy; Sechenov First Moscow State Medical University, Moscow, Russia

Talk: ESB-SLABO S-KL01 | New trends in multifunctional surface modification of stable and biodegradable biomedical implants
Date: Monday, 6 September, 2021, 3:45 PM
Track: 04

Julietta V. Rau (Dr, PhD, Prof.) is currently Principal Investigator, Head of the laboratory and research group at the Institute of the Structure of Matter of the Italian National Research Council (ISM-CNR, Rome, Italy) and Associate Professor at the Sechenov First Moscow State Medical University (Moscow, Russia). Dr. Rau has authored +160 international peer-reviewed journal publications and presented her research as Invited and Plenary talks at +30 International Conferences. She supervised PhD and MSc students and is involved in mentoring of postdoctoral research fellows. Dr. Rau received several international awards for her research achievements. She is the CHAIR and organizer of the biennial BioMaH “Biomaterials and Novel Technologies for Healthcare” International Conference (https://biomah.ism.cnr.it) and the Member of the International Scientific Committees of various International Conferences in the field of Materials Science, Nanoscience, Biomaterials and Medical devices. Dr. Rau is Associate Editor of the Bioactive Materials journal and the Member of the Editorial Boards of numerous scientific journals. Her present research interests regard biomaterials and novel technologies for healthcare, innovative biomaterials for regenerative medicine, among them calcium phosphates and glass-ceramic based materials for tissue engineering applications. One of her research topics is focussed on coatings for biomedical implant applications and, namely, on nanostructured coatings for orthopaedic and dental implants, which are developed via an interdisciplinary international collaborative network team.

https://www.researchgate.net/profile/Julietta_Rau
http://orcid.org/0000-0002-7953-1853

Marina Resmini

Dept. of Chemistry, Queen Mary University of London, London, UK

Talk: NANOSTEM 02-KL01 | How do we create an inclusive and diverse research culture
Date: Monday, 6 September, 2021, 1:00 PM
Track: 08

Prof Resmini is Professor of Materials Chemistry in the Chemistry Department at Queen Mary University of London. Her research interests are focused in the area of functional nanomaterials with applications at the interface between physical and bio/medical sciences, particularly as sensors and drug delivery systems. She has a strong track record of assembling successful multidisciplinary consortia with academic teams both in the UK and Europe as well as with industrial partners. She has a distinguished publication record in the area of nanomaterials, more recently focusing on imprinted polymers, nanogels and understanding the relationship between structure and functional properties. She is passionate about equality and positive actions that can drive changes towards a more diverse and inclusive environment in the Chemical Sciences. In 2011 she was elected Fellow of the Royal Society of Chemistry and since 2019 she has been the Chair of the RSC Inclusion and Diversity committee and also a member of the RSC’s Board of Trustees.
Alberto Saiani

The University of Manchester, Department of Materials & Manchester Institute of Biotechnology, Manchester, UK

Talk: **S02-KL01 | Exploiting β-sheet peptides self-assembly to design hydrogels for biomedical applications**

Date: Monday, 6 September, 2021, 11:30 AM

Track: 04

Alberto Saiani is currently Professor of Molecular Materials at the University of Manchester (ORCID: 0000-0002-5504-8306). He graduated PhD in Polymer Physics from the University Louis Pasteur, Strasbourg, France. Following his PhD he held several post-doctoral positions in Japan, UK and Belgium before joining in 2002 the University of Manchester where he established the Polymers & Peptides Research Group (polymersandpeptides.co.uk). His work includes fundamental, industrial, and translational research and spans a wide area of experimental polymer and biopolymer science. In 2014 he co-founded a start-up company, Manchester BIOGEL (manchesterbiogel.com), which develops and commercialises advanced gel-based combination products for the life science and biomedical sector.

Manuel Salmeron-Sanchez

James Watt School of Engineering, University of Glasgow, Glasgow, UK

Talk: **S09-KL01 | Mechanochemical processes at the cell/material interface**

Date: Wednesday, 8 September, 2021, 11:30 AM

Track: 02

Prof Manuel Salmeron-Sanchez, FRSE is Head of Biomedical Engineering and co-director of the Centre for the Cellular Microenvironment at the University of Glasgow. He did a PhD in Valencia and postdoctoral work at the Institute for Macromolecular Chemistry in Prague and the Katholieke Universiteit Leuven. In 2005 he was appointed as Assistant Professor at Universitat Politècnica de València, promoted to Associate Professor in 2008 and Full Professor in 2010. He did a sabbatical year at the Georgia Institute of Technology and moved to the University of Glasgow in 2013 as the Chair of Biomedical Engineering. Manuel is an ERC (European Research Council) investigator. He was elected Fellow of the Royal Society of Edinburg, Scotland national academy of science and letters, in 2017. He is visiting Professor in Valencia (Spain) and Kyushu University (Japan). His work on materials for growth factor delivery has set up the basis for a programme of research to help civilians affected by landmines, funded by the Sir Bobby Charlton Foundation. Overall, his work spans fundamental mechanisms at the cell/material interface as well as translational research that saved from amputation the leg of a first veterinary patient, a dog called Eva (https://goo.gl/1Z3r8t). He has authored more than 160 papers in major journals including PNAS, Science Advances, Nature Biomedical Engineering, Nature Metabolism and Advanced Materials. He has had his research featured in newspapers, websites and TV channels around the world. He is editor-in-chief of Materials Science and Engineering C: Materials for Biological Applications.
Ankur Singh
Georgia Institute of Technology Wallace H Coulter, Department of Biomedical Engineering,, Atlanta, USA

Talk: ESB-BMJ S-03 l ESB-Biomaterials Journal Joint Session: Meet the Editor Session
Date: Tuesday, 7 September, 2021, 04:15 PM
Track: 05

Ankur Singh is a Woodruff Faculty Fellow and an Associate Professor of George W. Woodruff School of Mechanical Engineering at Georgia Institute of Technology and Wallace H. Coulter Department of Biomedical Engineering at Georgia Tech and Emory University. Before Georgia Tech, he was an Associate Professor at Cornell University, where he served on the Center for Immunology’s Executive Council and Cornell’s academic integration advisory council. He was the Associate Director of Cornell’s NIH Immunoengineering training grant. His laboratory develops immune organoids and enabling technologies to understand healthy and diseased immune cells and translate therapeutics. He has received funding from the National Institute of Health, National Science Foundation, Wellcome Leap HOPE, Department of Defense, Defense Threat Reduction Agency, and Lymphoma and Leukemia Society. He has published >60 articles in peer-reviewed journals, including Nature Methods, Nature Materials, Nature Nanotechnology, Science Advances, Nature Communications, Nature Reviews Materials, Nature Protocols, PNAS, Blood, Advanced Functional Materials, and Advanced Materials. He has written multiple editorials for Science Translational Medicine. He is a recipient of the NSF CAREER, Society for Biomaterials Young Investigator Award, CMBE Young Innovator Award, CMBE Rising Star Award, 3M Faculty Award, DoD Career award, Cornell’s Teaching Excellence Award, and Cornell’s Research Excellence Award. His immune organoids were identified among the Top 100 Discoveries of 2015 by Discover Magazine. He is the Founder and past-Chair of the Immune Engineering SIG at the Society for Biomaterials and Controlled Release Society. He serves on the editorial board of Biomaterials, Advanced NanoBiomed Research, Cellular and Molecular bioengineering, Scientific Reports, and J of Immunology and Regenerative Medicine journals.

Federico Soria Gálvez

ENIUS Chairman European Cooperation in Science & Technology Endoscopy Coordinator Centro de Cirugía de Mínima Invasión Jesús Usón, Cáceres, Spain

Talk: ESB-SIBB S01-KL01 l Urinary stents. Side effects in patients. Why do we need to improve them? Biofilm, Coatings and new stent designs.
Date: Tuesday, 7 September, 2021, 4:15 PM - 5:45 PM
Track: 03

Dr. Federico Soria is the European Network of Multidisciplinary Research to Improve the Urinary Stents (ENIUS) Chairman, European Cooperation in Science & Technology (COST Actions, CA16217) and Endoscopy Coordinator in Jesus Uson Minimally Invasive Surgery Centre (Cáceres-Spain). He leads a multidisciplinary research group focus in Biomaterials and new design in ureteral stents. Research topic: Ureteral stents, Biodegradable Ureteral stents and Ureteral Drug Eluting Stents. Principal investigator in more than 20 Research Grants in the area of Ureteral Stents and Translational Research (1999-2021). He is editor in two books and author of over 130 peer-reviewed scientific papers. He serves on the Editorial Board in BMC Urology and Act Urol Esp. Inventor of three patents in Urology medical devices. 12 Master’s Thesis Director and 4 PhD Thesis Director.
**Marianna Tryfonidou**

RMCU, Utrecht University, Utrecht, The Netherlands  
**Talk:** S15-KL02 | The iPSpine project: smart biomaterials escorting iPS cells to rejuvenate the degenerate disc  
**Date:** Thursday, 9 September, 2021, 12:15 PM  
**Track:** 04

Professor Tryfonidou (1973) joined the department of Clinical Sciences of Companion of the Faculty of Veterinary Medicine (1998), followed an annual Internship and finished thereafter her PhD (2002) Cum Laude. In 2007 she certified as a veterinary surgeon (European College of Veterinary Surgeons (ECVS)), in 2015 was appointed as Associate Professor and as off February 2018 appointed as Professor Regenerative Orthopedics. The focus of her research is on understanding the underlying pathophysiology, unravelling cellular communications and on developing treatment strategies for musculoskeletal diseases, including back pain and osteoarthritis. Challenges are addressed by combining their multidisciplinary clinical background, involving unique spontaneous diseased canine models, with cutting edge biomolecular techniques. Results of in vitro and in vivo studies are interpreted with a clinical directive; experiments are designed with the translation from bench to bed in mind.  
Her work is embedded in international scientific networks and the “Dutch Arthritis Society” (ReumaNederland) recognized the Tryfonidou lab as a Research Centre of Excellence. She has participated in large public-private partnerships (e.g. BioMedical Materials IDiDAS, LSH ArlADNE, AO Spine Research Spine Network) where she coordinated translation of therapeutic strategies, being successful at the cross road of industry, academia, clinicians and patients. She leads the Horizon 2020 consortium (iPSpine; 2019-2023) bringing a transdisciplinary team of 21 partners together to address the challenges and bottlenecks of iPS-based advanced therapies towards their transition to the clinic. Here, chronic back pain due to intervertebral disc degeneration is employed as a show case.

**Sandra Van Vlierberghe**

Polymer Chemistry & Biomaterials Group, Centre of Macromolecular Chemistry, Ghent University, Belgium; Brussels Photonics, Vrije Universiteit Brussel, Belgium  
**Talk:** S10-KL01 | Crosslinkable gelatins - From Innovative Chemistry and Valorization of Bioinks towards Human Trials: Utopia or Reality?  
**Date:** Wednesday, 8 September, 2021, 11:30 AM  
**Track:** 03

Prof. Dr. Sandra Van Vlierberghe holds a 10% professorship at Vrije Universiteit Brussel (VUB) and a full professorship (100%) at Ghent University (Polymer Chemistry & Biomaterials Group, Belgium). She has acquired expertise related to the synthesis, the modification and the processing of (bio)polymers including thermoplasts (e.g. polyesters) and hydrogels (e.g. proteins and polysaccharides) for a variety of tissue engineering applications. She is experienced in the field of polymer processing using 3D printing, electrospinning and two-photon polymerization (2PP). She received her PhD in Sciences in 2008 at Ghent University. She authored >170 Web of Science Core Collection cited papers, she has a h-index of 34 (WoS), is promoter of 20 PhD students (in addition to 12 defended PhDs) and she edited three books, authored 7 chapters in books of which 5 invited. She is treasurer of the Belgian Polymer Group (BPG), former spokesperson of the ‘Young Scientist Forum (YSF)’, TERMIS-EU council member and member of the independent ESB Awards Committee. She serves on the editorial board of several journals focussing on tissue engineering. In 2017, she received the Jean Leray award from the European Society for Biomaterials. She is also very active in research valorization as reflected by two ongoing spin-off projects (XpectINX (Xpect-Inx | Your Support in Bioprinting (xpect-inx.com) which will be launched in September 2021 and GelGraft Medical which is focussing on the translation of an injectable biomaterial for breast reconstruction from in vivo animal testing towards first in-human trials).
Dr. S.A.J. Zaat is Principal Investigator at the Amsterdam UMC, University of Amsterdam, Department of Medical Microbiology and Infection Prevention. His research line „Biomaterial-associated infection and novel antimicrobial strategies”, is focused on pathogenesis, prevention and treatment of biomaterial-associated infections, both in soft tissue (catheters, surgical meshes) and bone (implants, fixation devices). Based on research on the pathogenesis involving the (molecular) interactions between the pathogen, the host response and the biomaterial, novel preventive strategies are being developed. These include anti-infective coatings not subject to bacterial resistance development, prevention of biofilm formation, influencing the host immune response and enhancement of killing of bacteria hiding in tissue surrounding inserted or implanted medical devices. These approaches are aimed at maximally reducing the risk of biomaterial-associated infection for patients.

Dr. Zaat has been work package leader in the BMM NANTICO “Non-adherent ANTImicrobial Coatings” project, Principal Investigator of the BMM IBIZA “Imaging of Biomaterial-associated Infection using Zebrafish Analysis” project, coordinator of the EU FP7 BALI “Biofilm Alliance” project developing novel Synthetic Antimicrobial and Antibiofilm Peptides, and vice Working Group Chair of the EU COST Consortium IPROMEDAI “Improved PROtection Of Medical Devices Against Infection”. Currently he is leading or involved in a number of National and international consortium projects, including EU Horizon2020 Marie Curie training networks PRINT-AID and STIMULUS, EU Horizon 2020 Twinning project CEMBO, and Dutch National consortia SUPER-ACTIVE, PHOTO-TREAT, GDST, NEDSAP and DARTBAC.

Marcy Zenobi-Wong is a tenured Full Professor of Tissue Engineering and Biofabrication and Director of the Institute for Biomechanics at ETH Zürich in Switzerland. She is a Mechanical Engineer by training, and received her Bachelor degree from MIT and Master/PhD from Stanford University. She leads a multidisciplinary team with strong focus on biofabrication technologies including bioprinting, two-photon polymerization, and casting, and on the development of advanced biomaterials for tissue regeneration. She is the author of over 100 peer-reviewed publications (cited over 5000 times) and co-inventor on several licensed patents. She is currently President of the Swiss Society for Biomaterials and Regenerative Medicine (SSB+RM) and General Secretary for the International Society of Biofabrication (ISBF). She serves on the editorial board for Biofabrication and Advanced Healthcare Materials. In 2019 she was elected as Fellow to the European Alliance for Medical & Biological Engineering and Science (EAMBES).
# Schedule Sunday, 5 September, 2021

<table>
<thead>
<tr>
<th>Time</th>
<th>Session/Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00</td>
<td></td>
</tr>
<tr>
<td>9:30</td>
<td></td>
</tr>
<tr>
<td>10:00</td>
<td></td>
</tr>
<tr>
<td>10:30</td>
<td></td>
</tr>
<tr>
<td>11:00</td>
<td></td>
</tr>
<tr>
<td>11:30</td>
<td></td>
</tr>
<tr>
<td>12:00</td>
<td></td>
</tr>
<tr>
<td>12:30</td>
<td></td>
</tr>
<tr>
<td>13:00</td>
<td></td>
</tr>
<tr>
<td>13:30</td>
<td></td>
</tr>
<tr>
<td>14:00</td>
<td></td>
</tr>
<tr>
<td>14:30</td>
<td></td>
</tr>
<tr>
<td>15:00</td>
<td></td>
</tr>
<tr>
<td>15:30</td>
<td><strong>YSF WS</strong>&lt;br&gt;YSF Opening Workshop – Green Lab sustainability &amp; Communication skills</td>
</tr>
<tr>
<td>16:00</td>
<td></td>
</tr>
<tr>
<td>16:30</td>
<td></td>
</tr>
<tr>
<td>17:00</td>
<td></td>
</tr>
<tr>
<td>17:30</td>
<td></td>
</tr>
<tr>
<td>18:00</td>
<td></td>
</tr>
<tr>
<td>18:30</td>
<td></td>
</tr>
<tr>
<td>19:00</td>
<td></td>
</tr>
<tr>
<td>19:30</td>
<td></td>
</tr>
</tbody>
</table>

**Caption**

- **AW**: Award Lecture
- **AERO**: EFS-AERoGELS COST Action Joint Symposium Biomechanics/SiBB, Biomechanics Session by SIBB
- **GA**: General Assembly
- **K**: Keynote Lecture
- **LS**: Lunch Symposium
- **S**: Symposium
- **PL**: Plenary Lecture
- **N**: Oral Session
- **NANOSTEM**: NANOSTEM Project Satellite Symposium
- **PS**: Poster Session
- **TRS**: ES02D21- Translation Research Symposia
- **WS**: Workshop
- **YSF**: Young Scientist Forum
  - **Plenary**: Plenary
  - **Keynote**: Keynote
  - **Symposia**: Symposia
  - **Oral**: Oral
  - **NANOSTEM**: NANOSTEM | COST AEroGELS | Biomechanics/SiBB
  - **General Assembly**: General Assembly
  - **Poster Session**: Poster Session
## Schedule Monday, 6 September, 2021

<table>
<thead>
<tr>
<th>Time</th>
<th>Track01</th>
<th>Track02</th>
<th>Track03</th>
<th>Track04</th>
<th>Track05</th>
<th>Track06</th>
<th>Track07</th>
<th>Track08</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00</td>
<td>QC Opening Ceremony</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:30</td>
<td>GW AW01 Geong Winter Award</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:30</td>
<td>JL AW01 Jean Leray Award 2020</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:00</td>
<td>COFFEEBREAK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NANOSTEM 01 Biopolymers for Drug Delivery</td>
</tr>
<tr>
<td>11:30</td>
<td>TRS-501 Translation Research Symposia</td>
<td>K01 NanoBio-materials for Biomedical Applications</td>
<td>S01 Organ-on-a-Chip Technologies Meet Biofabrication</td>
<td>S02 Supramolecular Peptide-based Biomaterials for Regenerative Medicine</td>
<td>S03 Biomaterials for Application in Neurosciences</td>
<td>N01 Biopolymers for Orthopedic Applications</td>
<td>N02 Biopolymers for Orthopedic Applications</td>
<td>NANOSTEM 02 Investigation of nanocarriers as drug delivery systems</td>
</tr>
<tr>
<td>12:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:00</td>
<td>YSF GA YSF General Assembly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:30</td>
<td>LUNCH BREAK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:30</td>
<td>TRS-502 Translation Research Symposia</td>
<td>K02 Organ-on-a-Chip Systems in Precision Medicine</td>
<td>S04 Smart (Nano)biomaterials and their Applications</td>
<td>ESBL-CSBM 5 Chinese Society for Biomaterials Joint Symposia: Frontiers of Materials Research</td>
<td>N03 Surface Modified Biomaterials</td>
<td>N04 Biopolymers for Orthopedic Applications</td>
<td>N05 Biopolymers for Drug Delivery</td>
<td>N06 Biopolymers for Electrical Stimulation</td>
</tr>
<tr>
<td>15:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:00</td>
<td>TRS-503 Translation Research Symposia</td>
<td>K03 Futuring Biomaterials</td>
<td>S05 Advanced Technologies and Cellular Approaches for the Development of Precise 3D Tendon and other Musculoskeletal Tissue Substitutes and Models to Understand Regeneration Mechanisms</td>
<td>ESBL-SLABA 5 Latin American Society for Biomaterials, Tissue Engineering and Artificial Organs - Joint Symposia: Development of New Biomaterials with Multifunctional Surfaces</td>
<td>N05 Hydrogels and Smart Biomaterials</td>
<td>N06 Biopolymers for Drug Delivery</td>
<td>N07 Biopolymers for Electrical Stimulation</td>
<td>NANOSTEM 04 Nanoparticles for modulation of gene expression</td>
</tr>
<tr>
<td>16:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Track01</td>
<td>Track02</td>
<td>Track03</td>
<td>Track04</td>
<td>Track05</td>
<td>Track06</td>
<td>Track07</td>
<td>Track08</td>
</tr>
<tr>
<td>--------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>9:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:30</td>
<td><strong>KdG AW01</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>AERO S01</strong></td>
</tr>
<tr>
<td></td>
<td>Klaas de Groot Award 2020</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Wound materials-based dressings and aerogels processing</td>
</tr>
<tr>
<td>10:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>AERO S02</strong></td>
</tr>
<tr>
<td>10:30</td>
<td><strong>JL AW02</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3D-scaffolds-based aerogels for tissue regeneration</td>
</tr>
<tr>
<td>11:00</td>
<td>COFFEE BREAK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:30</td>
<td><strong>K04</strong></td>
<td><strong>S06</strong></td>
<td><strong>S07</strong></td>
<td><strong>ESB-CRS S</strong></td>
<td><strong>ESB-BIOMAT S</strong></td>
<td><strong>N08</strong></td>
<td><strong>N09</strong></td>
<td><strong>AERO P501</strong></td>
</tr>
<tr>
<td>12:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>AERO InvF</strong></td>
</tr>
<tr>
<td>12:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Early-career investigators Forum</td>
</tr>
<tr>
<td>13:00</td>
<td><strong>L5</strong></td>
<td>Social 3</td>
<td>Yoga</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>YSF-AeRoGELS WS</strong></td>
</tr>
<tr>
<td></td>
<td>Lunch Symposium Rousselot: Biomedical Applications of Gelatins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>YSF-Educational workshop - “Writing and Publishing”, YSF &amp; “AeRoGELS” Joint COST action event</td>
</tr>
<tr>
<td>13:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>LUNCH BREAK</strong></td>
</tr>
<tr>
<td>14:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>PS 1</strong></td>
</tr>
<tr>
<td>15:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Poster Sessions 1</td>
</tr>
<tr>
<td>16:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>BRAKE</strong></td>
</tr>
<tr>
<td>16:30</td>
<td><strong>K05</strong></td>
<td><strong>S08</strong></td>
<td><strong>ESB-SIBB S01</strong></td>
<td><strong>ESB-ISBF S</strong></td>
<td><strong>ESB-BMRS S</strong></td>
<td><strong>N10</strong></td>
<td><strong>N11</strong></td>
<td><strong>AERO P502</strong></td>
</tr>
<tr>
<td></td>
<td>Immunoresponse Towards Biomaterials</td>
<td>Beyond Bone and Teeth: Bioactive Glasses in Soft Tissue Regeneration</td>
<td>Iberian Society for Biomechanics and Biomaterials-Joint Session II: Antimicrobial Biomaterials</td>
<td>International Society for Biodegradation-Joint Symposium</td>
<td>ESB-Biomaterials Journal Joint Session: Meet the Editor Session</td>
<td>Skin Regeneration and Wound Healing</td>
<td>Biomaterials for Orthopedic Applications IV</td>
<td>Coffee-Break &amp; Poster session 02</td>
</tr>
<tr>
<td>17:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>AERO S03</strong></td>
</tr>
<tr>
<td>17:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Drug-delivery systems and Nutraceutical/food systems</td>
</tr>
<tr>
<td>18:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>AERO Closing</strong></td>
</tr>
<tr>
<td>18:30</td>
<td><strong>PL2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Closing session</td>
</tr>
<tr>
<td>19:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Social 4</td>
</tr>
<tr>
<td>19:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Discover Portuguese Wine Regions with Sogrape</td>
</tr>
<tr>
<td>20:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Track01</td>
<td>Track02</td>
<td>Track03</td>
<td>Track04</td>
<td>Track05</td>
<td>Track06</td>
<td>Track07</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>---------------------------------------------</td>
<td>---------------------------------------------</td>
<td>---------------------------------------------</td>
<td>---------------------------------------------</td>
<td>---------------------------------------------</td>
<td>---------------------------------------------</td>
<td>---------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>9:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:30</td>
<td>GW AW02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:00</td>
<td>George Winter Award 2021</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:30</td>
<td>KdG AW02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:00</td>
<td>COFFEEBREAK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:30</td>
<td></td>
<td>K06</td>
<td>S09</td>
<td>S10</td>
<td>S11</td>
<td>ESB-SIBB S02</td>
<td>N12</td>
<td></td>
</tr>
<tr>
<td>12:00</td>
<td>Biomimetic Nanomaterials and Biosensing Devices</td>
<td>Role of Biomaterial Properties on Cell-Scaffold Interplay</td>
<td>Advances in Functionalisation and Fabrication of Gelatin Hydrogels for Biomedical Applications</td>
<td>Nanostructure-based Biomaterials Design and Biological Interactions</td>
<td>Iberian Society for Biomechanics and Biomaterials Joint Session II: Biomaterials for cancer models and treatment</td>
<td>ESB-BM5 Joint Session: Biomaterials Science Journal Lectureship Symposium</td>
<td>Biomaterials for Cardiovascular Applications and Angiogenesis</td>
<td></td>
</tr>
<tr>
<td>12:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:00</td>
<td>LUNCH BREAK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:30</td>
<td>YSF NC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>YSF National Chapter Meeting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Schedule Wednesday, 8 September, 2021**

- **9:30 AM**: GW AW02 - George Winter Award 2021
- **10:00 AM**: KdG AW02 - Klaas de Groot Award 2021
- **11:00 AM**: COFFEEBREAK
- **11:30 AM**: K06 - Biomimetic Nanomaterials and Biosensing Devices
  - S09: Role of Biomaterial Properties on Cell-Scaffold Interplay
  - S10: Advances in Functionalisation and Fabrication of Gelatin Hydrogels for Biomedical Applications
  - S11: Nanostructure-based Biomaterials Design and Biological Interactions
- **12:00 PM**: ESB-SIBB S02 - Iberian Society for Biomechanics and Biomaterials Joint Session II: Biomaterials for cancer models and treatment
- **12:30 PM**: ESB-BM5 Joint Session: Biomaterials Science Journal Lectureship Symposium
- **1:00 PM**: N12 - Biomaterials for Cardiovascular Applications and Angiogenesis
- **2:00 PM**: YSF NC - YSF National Chapter Meeting
- **2:30 PM**: Social 5 - Yoga
- **4:00 PM**: PS 2 - Poster Sessions 2
- **4:30 PM**: BREAK
- **5:00 PM**: K07 - Biomaterials for Cardiac Tissue Engineering and Regenerative Medicine
  - K08 - Mechanomodulatory Biomaterials and its Impact on Cell Behaviour
  - S12 - Biocompatibility Testing According to ISO 10993: What are the Changes and What can the Biomaterials Community Learn from it?
  - S13 - Biocompatibility Testing and Data-Driven Biomaterials
- **6:00 PM**: ESB-SFB S - Society for Biomaterials Joint Symposium: Immunomodulatory Biomaterials
- **6:30 PM**: N13 - Advances in Additive Manufacturing and Biofabrication
- **7:00 PM**: Int AW - International Award 2021
- **8:00 PM**: Social 6 - Music Evening - Performance by a University of Porto "Tuna"
<table>
<thead>
<tr>
<th>Time</th>
<th>Track 01</th>
<th>Track 02</th>
<th>Track 03</th>
<th>Track 04</th>
<th>Track 05</th>
<th>Track 06</th>
<th>Track 07</th>
<th>Track 08</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:30</td>
<td>PL3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Opening</td>
</tr>
<tr>
<td>10:00</td>
<td>ESB GA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Biomechanics 01</td>
</tr>
<tr>
<td></td>
<td>ESB General Assembly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Anatomofunctional bases of entesopathies in sports</td>
</tr>
<tr>
<td>11:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:30</td>
<td>BREAK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:00</td>
<td>K09</td>
<td>K10</td>
<td>S14</td>
<td>S15</td>
<td>N14</td>
<td>N15</td>
<td>N16</td>
<td>Biomechanics 02</td>
</tr>
<tr>
<td></td>
<td>New Tools to Study Physiology and Disease</td>
<td>Supramolecular Peptide Assemblies as a Platform for Antifouling, Antimicrobial and Antiviral Materials</td>
<td>Innovative Approaches to Develop 3D Nanostructured Devices for Bone and Osteochondral Regeneration</td>
<td>Regeneration of the Intervertebral Disc: The Battles that Biomaterials Will Have to Win</td>
<td>Electrospun and Electrowritten Biomaterials</td>
<td>Nanobiomaterials</td>
<td>Hydrogels and Smart Biomaterials I</td>
<td>Swimming Biomechanics</td>
</tr>
<tr>
<td>12:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:00</td>
<td>CC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Biomechanics 03</td>
</tr>
<tr>
<td></td>
<td>Awards &amp; Closing Ceremony</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Oral Presentations 01</td>
</tr>
<tr>
<td>13:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Biomechanics 04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Oral Presentations 02</td>
</tr>
<tr>
<td>14:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Biomechanics 05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Oral Presentations 03</td>
</tr>
<tr>
<td>15:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3:00 p.m. – 6:00 p.m.

Track01

**YSF WS | YSF Opening Workshop – Green Lab sustainability & Communication skills**

**Chairs**

**David Barata**
Instituto Medicina Molecular, Lisboa, PT

**Liliana Liverani**
University of Erlangen-Nuremberg, Institute of Biomaterials, Erlangen, DE

**Arn Mignon**
KU Leuven, Engineering Technology, Group T, Leuven, BE

**Liliana Moreira Teixeira**
University of Twente, Developmental BioEngineering, Enschede, NL

**Elena Maria Varoni**
University of Milano, IT

*In this workshop, we will briefly explain the YSF’s “Green Lab sustainability” Initiative, and Pedro Alves (Instituto de Investigação e Inovação em Saúde - U. Porto (i3s), Portugal) will explain and share his “Greenlab experience”. Afterwards, the session on “Communication skills” will include insights on how to “Boost your Communication skills & How to do a good pitch” by Jane Bennett-Powell (freelance journalist and science communication trainer), followed by the important advices on “Pitching to Investors” from Sonia Hallen (LSP connecting investors to inventors, The Netherlands). During the same session, Jasper Van Hoorick (Ghent University & Xpect-INX, Belgium) will share his experience on the steps “From Academic research to spin-off company: the story of XPECT INX”. This session will end with an interactive round table with the audience.*
YSF Opening

YSF Workshop opening by YSF ESB Board officers

Elena Maria Varoni¹, Liliana Liverani²

¹ University of Milano, Milano, IT; ² FAU, Nürnberg-Erlangen, DE

YSF WS-02

YSF “Green Lab sustainability” Initiative

YSF WS-03

Greenlab experience

Pedro M. Alves

i3s, University of Porto, INEB, Porto, PT

YSF WS-04

Open discussion

Coffee Break

YSF WS-05

“Communication skills” session opening

Arn Mignon¹, David Barata², Liliana Teixeira³

¹ KU Leuven, Engineering Technology, Group T, Leuven, BE; ² Maastricht University, Maastricht, NL; ³ University of Twente, Enschede, NL
YSF WS-06

Boost your Communication skills & How to do a good pitch

Jane Bennett-Powell

freelance journalist and science communication trainer, London, GB

YSF WS-07

Pitching to Investors

Sonia Hallen

LSP connecting investors to inventors, Amsterdam, NL

YSF WS-08

From Academic research to spin-off company: the story of XPECT INX

Jasper Van Hoorick

XPECT INX / Ghent University, Department of Organic and macromolecular Chemistry, Ghent, BE

YSF WS-09

Interactive round table with the audience

YSF ESB Board Officers, Elena Varoni, Liliana Liverani, Jane Bennett-Powell, Sonia Hallen, Jasper Van Hoorick
<table>
<thead>
<tr>
<th>Time</th>
<th>Track01</th>
<th>Track02</th>
<th>Track03</th>
<th>Track04</th>
<th>Track05</th>
<th>Track06</th>
<th>Track07</th>
<th>Track08</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00</td>
<td>OC Opening Ceremony</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:30</td>
<td>GW AWD1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Georg Winter Award 2020</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:30</td>
<td>JL AWD1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jean Leray Award 2020</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>COFFEEBREAK</td>
</tr>
<tr>
<td>11:30</td>
<td>TRS-501</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Translation Research Symposium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K01</td>
<td>Nano-biomaterials for Biomedical Applications</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S01</td>
<td>Organ-On-a-Chip Technologies Meet Biolabfabrication</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S02</td>
<td>Supramolecular Peptide-based Biomaterials for Regenerative Medicine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S03</td>
<td>Biocompatible and Bioactive Materials: Addressing Infections in Regenerative Medicine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N01</td>
<td>Biomaterials for Application in Neurosciences</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N02</td>
<td>Biomaterials for Orthopedic Applications I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LUNCH BREAK</td>
</tr>
<tr>
<td>13:30</td>
<td>YSF GA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>YSF General Assembly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LUNCH BREAK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:00</td>
<td>TRS-502</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Translation Research Symposium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K02</td>
<td>Organ-on-a-Chip Systems in Precision Medicine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S04</td>
<td>Smart (Nano)biomaterials and their Applications</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ESB-CSBM 5</td>
<td>Chinese Society for Biomaterials: Joint Symposium: Frontiers of Biomaterials</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N03</td>
<td>Surface-Modified Biomaterials</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N04</td>
<td>Biomaterials for Orthopedic Applications II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N05</td>
<td>Nanoparticles and Blood-Brain Barrier: Mechanisms of Permeation and Permeability for Successful Drug Delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N06</td>
<td>Biomaterials for Drug Delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N07</td>
<td>Biomaterials for Electrical Stimulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BREAK</td>
</tr>
<tr>
<td>16:00</td>
<td>TRS-503</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Translation Research Symposium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K03</td>
<td>Futuring Biomaterials</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S05</td>
<td>Advanced Technologies and Cellular Approaches for the Development of Precise 3D Tissue and other Musculoskeletal Tissue Substitutes and Models to Understanding Regeneration Mechanisms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ESB-SLABO 5</td>
<td>Latin American Society for Biomaterials, Tissue Engineering and Artificial Organs Joint Symposium: Development of New Biomaterials with Multifunctional Surfaces</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N05</td>
<td>Hydrogels and Smart Biomaterials II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N06</td>
<td>Biomaterials for Drug Delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N07</td>
<td>Biomaterials for Electrical Stimulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BREAK</td>
</tr>
<tr>
<td>18:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Social 2</td>
</tr>
<tr>
<td>20:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>YSF Night Out</td>
</tr>
<tr>
<td>20:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
9:00 a.m. – 9:30 a.m.

Track01

**OC | Opening Ceremony**

**Chairs**
Ana Paula Pêgo  
M. Cristina L. Martins  
Pedro L. Granja

Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal

**Program**

Welcome address by Ana Paula Pêgo on behalf of the ESB2021 Conference Chairs
Welcome address by Pamela Habibović, President of ESB
Welcome address by Inês Gonçalves on behalf of the SIBB Congress Chairs
Welcome address by Manuel Heitor, Minister for Science, Technology and Higher Education
Welcome address by Pedro Rodrigues, Vice-Rector for Research, Innovation and Internationalization of the University of Porto

ESB Conferences @Porto. An historical commitment.
9:30 a.m. – 10:15 a.m.

Track01

GW AW01 | George Winter Award 2020

Chair
Pamela Habibovic
Univ. Maastricht, NL

GW AW01-01

Design of biomaterials/cells structures for bottom-up tissue engineering strategies – less is more

João F. Mano

University of Aveiro, CICECO - Aveiro Institute of Materials, Department of Chemistry, Aveiro, PT

The control of cell microenvironment is of crucial importance for the ability to direct cell behavior in 3-dimensional tissue engineering structures. Bioinspired engineered microenvironments provide cells with a holistic “instructive niche” that harbors signals for cellular control both in both space and time. We explore this problem using reductionistic approaches, in which cells are exposed to substrates exhibiting controlled geometries, surface, (bio)chemical compositions and mechanical properties. Miniaturized supports can be then assembled into larger constructs using bottom-up tissue engineering tools. Examples from our group are presented covering alternative approaches of assembly cells with precise spatial arrangement, including cell encapsulation in (i) microgels or in (ii) liquified capsules, (iii) 1D/2D cell-rich objects, and (iv) combinations of cell and biodegradable polymeric microparticles. Non-bulk properties of biomaterials are highlighted towards minimalistic strategies involving low amounts of biomaterials.

Acknowledgement
This work was supported by the European Research Council grant agreement ERC-2014-ADG-669858 for project “ATLAS”. It was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 and UIDP/50011/2020, financed by national funds through the FCT/MEC and when appropriate cofinanced by FEDER under the PT2020 Partnership Agreement.
10:15 a.m. – 11:00 a.m.

Track01

**JL AW01 | Jean Leray Award 2020**

Chair
**Peter Dubruel**
Ghent University, BE

**JL AW01-01**

**Cell fate controlling micromaterials for the engineering of multiscale tissues**

**Jeroen Leijten**

*University Twente, Developmental BioEngineering, Enschede, NL*

The modular design of tissues is of indispensable importance for proper organ function. Yet, most tissue engineering strategies are based on creating homogeneous tissues, which has limited are capacity to create viable and functional tissues. We have developed several novel micromaterials that allow for the facile creation of tissues with modular designs, which allow for unprecedented control over cell fate and drive the engineering of functional multiscale tissues. Specifically, we used microfluidic droplet generation to fabricate 3D single microgels with on-demand tunable biophysical and biochemical properties to controllably program stem cell differentiation along chosen lineages. These microgels were combined with distinct biomaterials to create modular bioinks that offer uncoupled control over the engineered tissues pericellular and extracellular environments. Similarly, we have used our microtechnology to create hollow microgels that create, protect, and instruct organoids, which was explored for various biomedical applications including production of cardioids and immunoprotected islet-mimics. In addition, we have used microfluidic technology to produce oxygen generating micromaterials to enable the engineering of viable clinically sized tissues. Lastly, to endow our tissues with macroscale properties such as tissue shape, we have used our modular bioinks in combination with various biofabrication techniques such as injection molding, photolithography, and 3D printing to engineered multiscale hierarchical living implants with unprecedented design and function. In short, I will here present several of our microfluidic micromaterial-based concepts that are focused on advancing the engineering of multiscale hierarchical tissues constructs.
11:30 a.m. – 1:00 p.m.

Track01

TRS-S01 | Translation Research Symposia

Chairs
**Fabrizio Barberis**
Universita' degli Studi di Genova, Department of Civil Chemical and Environmental Engineering, Genova, IT
**Yves Bayon**
Medtronic, Trévoux, FR

The session will share an overview on the Medical Device Regulation (MDR), starting to be fully in force in May 2021. It will illustrate the importance of the regulation throughout the life cycle of medical devices from their development to their market approval in Europe. A focus will be made on implants and biomaterials.

Lectures will be given by speakers with different background, representatives of start-up companies, large corporate companies and MedTech Europe, the European trade association of MedTech companies.
TR-S01-01

Stemmatters (Portugal)

Rui A. Sousa

Stemmatters, Biotecnologia e Medicina Regenerativa S.A., BARCO GMR, PT

TR-S01-02

Lymphatica (Switzerland)

Valentina Triacca

Lymphatica Medtech SA, Lausanne, CH

TR-S01-03

MedTech Europe (Belgium)

Merlin Rieschel

MedTech Europe, Brussels, BE

TR-S01-04

Medtronic – Sofradim Production (France)

Yves Bayon, Jhony Mallet

Medtronic, Trévoux, FR
11:30 a.m. – 1:00 p.m.

Track02

K01 | Nanobiomaterials for Biomedical Applications

Chairs

**Maria Rosa Aguilar**
Consejo Superior de Investigaciones Científicas, Institute of Polymer Science and Technology, Madrid, Spain

**Bruno Sarmento**
i3S/Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, PT

**Giorgia Cerqueni (YSF)**
Università Politecnica delle Marche, Department of Clinical and Molecular Sciences, ANCONA, IT
Hybrid nanoparticles and hydrogels for biomedical applications

Luisa De Cola

University of Milano, Department DISFARM and Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milano, IT

Advancements in the use of nanoparticles for biomedical applications have clearly shown their potential for the preparation of improved imaging and drug-delivery systems. However, only a few successfully translate into clinical practice, because, a common “barrier” preventing nanoparticles from delivering efficiently their payload to the target site after administration, is related to the nanoparticle uptake by macrophages. We have recently reported disulfide-bridged organosilica nanoparticles with cage-like morphology, and assessed in detail their bioaccumulation in vivo. [1] The fate of intravenously injected 20 nm nanocages was investigated in both healthy and tumor bearing mice. Interestingly, the nanoparticles exclusively co-localize with hepatic sinusoidal endothelial cells (LSECs), while avoiding Kupffer-cell uptake (less than 6%), in both physiological and pathological condition. Our findings suggest that organosilica nanocages hold the potential to be used as nanotools for LSECs modulation, potentially impacting key biological processes such as tumor cell extravasation and hepatic immunity to invading metastatic cells or a tolerogenic state in intrahepatic immune cells in autoimmune diseases.

Recently we have also shown that nanoparticles can be an interesting component for hybrid hydrogels. [2] We have shown that injectable nanocomposite hydrogel able to form in situ a tissue mimicking matrix as an innovative material can be employed for the treatment of esophageal fistulas. [3] The hydrogel is based on hyaluronic acid (HA), the cross-linking process occurs at physiological conditions leading to a hydrogel made of >96% by water and with a large-pore microarchitecture. The material, easily injectable with an endoscopic needle, is formed in a time compatible with the surgical procedure and has final mechanical properties suitable for cell proliferation. The in vivo experiments (porcine model) on esophageal-cutaneous fistulas, showed improved healing in the animals treated with the hydrogel compared with the control group.

References
The combination of Superparamagnetic Iron Oxide Nanoparticles and Type I collagen to design 3D Electrospun Scaffolds for bone regeneration

Giorgia Montalbano1, Manuel Estevez Amado2, Clarissa Tomasina4, Sonia Fiorilli1, Blanca González2,3, Isabel Izquierdo-Barba2,3, Sandra Camarero Espinosa5, Lorenzo Moroni4, Maria Vallet-Regi2,3, Chiara Vitale Brovarone1

1 POLITECNICO DI TORINO, DEPARTMENT OF APPLIED SCIENCE AND TECHNOLOGY (DISAT), TORINO, IT; 2 Universidad Complutense de Madrid, Departamento de Química en Ciencias Farmacéuticas, Facultad de Farmacia, MADRID, ES; 3 Biomateriales y Nanomedicina (CIBER-BBN), Centro de Investigación Biomédica en Red de Bioingeniería, MADRID, ES; 4 Maastricht University, MERLN Institute for for Technology-Inspired Regenerative Medicine, MAASTRICHT, NL; 5 University of the Basque Country UPV/EHU, Polymat, Donostia-San Sebastián, ES

Introduction

Nowadays there is an increasing need for engineered biomaterials in the field of bone tissue regeneration due to some limitations presented by the current pharmacological and surgical strategies. Especially in pathological conditions such as osteoporosis, constructs able to guide and rebalance cell activities are highly demanded [1]. In this context, three dimensional (3D) nanofibrous scaffolds fabricated by means of electrospinning represent promising tools for bone tissue engineering, mimicking the chemical and morphological features of the native tissue [2]. Moreover, the use of superparamagnetic iron oxide nanoparticles (SPIONs) have demonstrated their potential in the design of responsive biomaterials for different tissue engineering applications, exploiting the well-known healing effect of the magnetic field on different tissues, including bone [3].

In this study, SPIONs were produced by a thermal decomposition process. Subsequently, type I collagen, which is the main organic phase of bone, was combined with SPIONs to design magnetically responsive nanofibrous composite scaffolds by means of electrospinning. The resulting mats were characterized in terms of physicochemical, magnetic, and biological properties to explore their potential for bone tissue regeneration applications.

Experimental Methods

SPIONs were synthesized by thermal decomposition of iron (III) oleate in octadecene at 315 °C. The particles were further processed exploiting a ligand exchange of the oleic acid surface layer with dimercaptosuccinic acid to obtain a stable suspension in water. The nanoparticles were characterized by Transmission Electron Microscopy (TEM), X-ray diffraction (XRD), Dynamic Light Scattering (DLS) and Fourier Transform Infrared measurements (FT-IR). Concentration in Fe2O3 was determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). Magnetic characterization was carried out in a vibrating sample magnetometer (VSM). A novel hybrid formulation containing about 0.4 wt% SPIONs was obtained by adding the calculated volume of a stable aqueous suspension of magnetic particles into a 20 wt% type I collagen solution, prepared by dissolving lyophilized rat tail collagen in 40% acetic acid. After the rheological characterization of the suspensions, 3D fibrous scaffolds were fabricated using a Fluidnatek LE-50 electrospinning machine (Bioinicia, Spain) equipped with a plate collector, at a distance of 10 cm with voltage and flow rate of 20 kV and 1 mL/h, respectively. The morphological features of the scaffolds were investigated by Scanning Electron Microscopy (SEM) and TEM. The magnetization of the composite electrospun matrix was studied as function of the applied magnetic field, while their biocompatibility was preliminary evaluated in vitro by using mesenchymal stem cells derived from human bone marrow.

Results and Discussion
SPIONs measuring 12 nm in diameter have been successfully synthetized and subsequently coated to obtain a stable colloidal suspension in water over time. The SPION suspension was subsequently combined with a type I collagen solution, obtaining a final homogeneous hybrid suspension containing 0.4 wt% of magnetic particles. After optimization of the process parameters, magnetically responsive composite scaffolds were obtained by means of electrospinning, where morphological analyses confirmed the formation of fibers in the range of 100-500 nm as well as the uniform distribution and embedding of SPIONs (Figure). Finally, a preliminary biological assessment was exploited to confirm the biocompatibility of the developed constructs and their potential as bone regenerative scaffolds.

**Conclusion**

Magnetically responsive nanostructured scaffolds were successfully obtained combining type I collagen and superparamagnetic iron oxide nanoparticles (SPIONs) after the optimization of new synthesis strategies. The comprehensive characterization of the resulting scaffolds demonstrated their potential for bone tissue regeneration applications.

**Acknowledgement**

This project has received funding from the European Union’s Horizon 2020 research and innovation program under grant agreement No. 814410. The Authors acknowledge Novaicos s.r.l.s for the supply of rat tail collagen used for the study.

*References*

Peptide conjugated light-responsive NPs to target blood-brain barrier

Akhilesh Rai¹,², Rafaela Ferrão¹,²

¹ University of Coimbra, Faculty of Medicine, and Center for Neuroscience and Cell Biology, Coimbra, PT; ² Center for Neuroscience and Cell Biology, Center for Neuroscience and Cell Biology, Coimbra, PT

Introduction

No therapies are currently available to completely reboot the brain function after stroke [1]. One major drawback for the treatment of these diseases is the incapacity of drugs/carriers to cross efficiently the blood-brain barrier (BBB) [2]. BBB is a bottleneck in the development of drugs or drug carriers for the treatment of brain diseases. Studies have demonstrated that nanoparticles (NPs), upon an intracerebroventricular administration, can deliver active molecules at the SVZ region, triggering the neurogenic process [3]. Nevertheless, this type of administration is very invasive and requires specific medical facilities. Here, we report that near-infrared (NIR) light-responsive polydopamine (PDA) NPs can transiently open the BBB and facilitate their crossing across in vitro BBB model. Moreover, we demonstrate the paracrine effect (cytokines secreted by macrophages after the exposure of PDA NPs and NIR light) on the integrity of the BBB model.

Experimental Methods

PDA NPs (100 nm) were synthesized using dopamine as a precursor under the alkaline condition. Different densities of transferrin (Tf) peptides were covalently conjugated on PDA NPs using amine-PEG-maleimide (5kDa) linker. In vitro Mouse BBB model was used to analyze the permeability of Tf conjugated PDA NPs. Mouse brain endothelial cells (bEND.3) was used to prepare monoculture BBB model. ICP-MS measurement was done to quantify the amount of NPs, which transcytosed across in vitro BBB model. Moreover, the integrity of BBB after exposure of NPs and NIR light was evaluated by analysis of permeability of Lucifer yellow and electrical resistance (TEER) measurement. To study the paracrine effect, the conditional media (CM) were collected after the incubation of PDA-Tf NPs with mouse macrophages and the exposure of NIR light. CM was added in the mouse BBB model for 24 h and the integrity of BBB was evaluated by permeability and TEER measurement.

Results and Discussion

NIR light-responsive PDA NPs are synthesized using dopamine as a precursor under the alkaline condition. TEM measurement indicates the size of PDA NPs is 100 nm. We have prepared PDA-Tf₅₀/₁₀₀ NPs by conjugating 50 and 100 Tf-maleimide complexes per PDA NP. TEM measurement indicates that there is no significant increase in the size of PDA NPs after conjugation with Tf peptide. Zeta potential measurement shows that PDA-Tf₅₀/₁₀₀ NPs are positively charged as compared to PDA NPs. Importantly, PDA-Tf₅₀/₁₀₀ NPs are stable in EGM2 media for 24 h while NPs aggregates in EBM (without FBS) media. Photothermal measurement shows that 10 and 50 μg/mL of PDA NPs increase the temperature of solution to 2 and 6°C respectively after the exposure of NIR light (wavelength 785 nm, power density 2W/cm²) for 5 min. Results obtained from in vitro BBB model showed that 50 μg/mL of PDA-Tf₁₀₀ NPs crossed the BBB more efficiently than 50 μg/mL of PDA-Tf₅₀ NPs and the bare PDA NPs after the exposure of NIR light for 5 min. 50 μg/mL of PDA-Tf₁₀₀ NPs and bare PDA NPs do not affect the integrity of the BBB after the exposure of NIR light. The transcytosis of PDA-Tf₅₀ NPs and PDA-Tf₁₀₀ NPs through the BBB depend on their avidity to Tf receptors of bEND.3 cells. Importantly, 50 and 100 μg/mL PDA-Tf₁₀₀ NPs and the bare PDA NPs do not induce cytotoxicity to bEND.3 cells with or without exposure of NIR light. We further show that there is no significant change in the integrity of in vitro BBB model after incubation with the conditional media (cytokines secreted by mouse macrophages after incubation of 50 μg/mL of PDA-Tf₁₀₀ NPs and the exposure of NIR light).
Conclusion
We show that light-responsive PDA-Tf NPs can transiently open the BBB upon the exposure of NIR light and thereby promoting greater crossing of NPs. There is no harmful effect of cytokines secreted by macrophages after the NIR light exposure on the integrity of in vitro BBB model. Overall, our results open the possibility of targeting effectively the brain by modulating the physicochemical property of NPs.

Acknowledgement
The authors would like to thank the support of FCT (IF/00539/2015) grant.

References
[Ru(η⁵-C₅H₅)(PPh₃)₂]-PAMAM metalloendrimers as anticancer candidates able of triggering the "stress-induced premature senescence" pathway

Nádia Sofia H. Nunes¹, Helena Tomás¹, João Rodrigues¹²

¹ CQM – Centro de Química da Madeira, MMRG, Universidade da Madeira, Funchal, PT; ² School of Materials Science and Engineering/Center for Nano Energy Materials, Northwestern Polytechnical University, Xi’an, CN

Introduction
The research interest in metallodrugs was promoted by the successful clinical application of platinum anticancer drugs; nevertheless, the side effects and drug resistance constitute a severe problem. As a result, ruthenium complexes have emerged as a promising alternative because, i.e., of their possibility to kill cancer cells with different mechanisms of action, their ability to bind to albumin and transferrin, which facilitate their uptake into tumor cells, and related lower toxicity towards normal tissues[1]. Furthermore, their therapeutic activity, solubility, and selectivity can be enhanced when incorporated onto dendritic scaffolds, producing metalloendrimers[2]. These macromolecules have attractive three-dimensional and hyperbranched globular nanopolymeric architectures with a variable number of tailor-made terminal groups. They can incorporate metal-based drugs on their periphery, be scattered all over the framework, or encapsulated in their internal cavities[2]. Specifically, PAMAM dendrimers have an ethylenediamine core, polyamide branches, and tertiary amines as branching points[3].

Experimental Methods
Based on our knowledge of the field of dendrimers as anticancer drugs[4], we herein present the synthesis and characterization (by NMR, FTIR, EA, MS, and DLS techniques) of a new family of G0 to G3-ruthenium(II)-metalloendrimers with 4 to 32 Ru-moieties, respectively, based on polynitrile PAMAM dendrimers quantitatively coordinated to the metallofragment [Ru(η⁵-C₅H₅)(PPh₃)₂]⁺ (RuCp). Its in vitro cytotoxicity, hemolytic activity, DNA binding, and mechanism of action were also studied.

Results and Discussion
Our results have shown that the acquired IC₅₀ values for the selected set of human cancer cell lines (A2780, A2780cisR, and MCF-7) were generation dependent and comprised within a nanomolar range when compared with RuCp and cisplatin, which suggests the superior anticancer activity of the ruthenium(II)-based metalloendrimers vs. platinum-based systems. They were also less toxic towards human primary fibroblasts (BJ cell line); they exhibit nontoxicity for the human red blood cells and strong interactions with calf thymus DNA. The mechanism of action of the G3-metalloendrimer (G3-RuCp), when compared to RuCp and cisplatin, was investigated in the breast cancer cell line MCF-7 revealing that the cancer cell death stimulated by this metalloendrimer could be caused by "stress-induced premature senescence" triggered by high ROS levels and cell cycle arrest in the G0/G1 phase. This compound does not trigger cancer cell death by apoptosis, necrosis, or autophagy (fig. 1). Such mechanism of action is totally different from the one developed after the treatment of cisplatin (DNA damage response that involves cell cycle arrest in the G2/M phase and induced apoptosis[5], which tells us that the G3-RuCp could overcome the cisplatin resistance phenomena.

Conclusion
To conclude, the abovementioned results validate that these novel metalloendrimers are quite promising for anticancer research. In this sense, in vivo studies are ongoing.

Acknowledgement
This research was supported by FCT - Fundação para a Ciência e a Tecnologia - through the CQM Base Fund - UIDB/00674/2020, and Programmatic Fund - UIDP/00674/2020. The authors also acknowledge ARDITI - Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação, for the support through the project M1420-01-0145-FEDER-000005 - CQM* (Madeira 14-20 Program), and FCT for the PhD grant of Nádia Nunes (2020.04679.BD).

G3-RuCp triggers the "stress-induced premature senescence" pathway in MCF-7 cells.

Fig. 1: Proposed mechanism of action of G3-RuCp.

References
K01-05

Core-shell PLA nanofibers@SPIONs nanocomposites with enhanced properties for biomedical imaging and magnetic applications

Benjamin Nottelet¹, Hussein Awada¹-², Danielle Laurencin², Ryan Gilbert³, Jessica Funnell², Florence Franconi⁴, Joulia Larionov², Gautier Félix², Laurent Lemaire⁴, Yannick Guari²

¹ University of Montpellier, Department of Polymer for Health and Biomaterials - IBMM, Montpellier, FR; ² University of Montpellier, Ingénierie Moléculaire et Nano Objets - ICGM, Montpellier, FR; ³ Rensselaer Polytechnic Institute, The Gilbert Lab: Biomaterials for Spinal Cord Injury, Troy, US; ⁴ University of Angers, Micro et Nanomédecines Biomimétiques, Angers, FR

Introduction

Composites combining superparamagnetic iron oxide nanoparticles (SPIONs) and polymers are largely present in modern (bio)materials [1,2]. However, while SPIONs embedded in polymer matrices are classically reported, this approach fails in the case of electrospun nanocomposites where the incorporation of SPIONs results not only in beaded defects and a larger distribution in the diameter of the fibers, but also strongly impacts their mechanical and degradation properties, as well as the magnetic properties. Therefore, the controlled anchoring of SPIONs on polymer surfaces is still a major challenge. Herein, we propose an efficient strategy for the direct and uniform anchoring of SPIONs on the surface of functionalized-poly(l-lactide) (PLA) nanofibers via a simple free ligand exchange procedure to design PLA@SPIONs core@shell nanocomposites to be used as multifunctional scaffolds with magnetic and imaging properties [3]. A long term in vivo evaluation of the magnetic nanocomposites in terms of MRI visualization and tissue integration is discussed.

Experimental Methods

Electrospun PLA nanofibers are chemically modified to generate alkyne surface groups [4]. Bifunctional thiol-phosphonic ligand is covalently bound to the surface by thiol-yne photoaddition. Finally, SPIONs are anchored on the PLA fibers via a free ligand exchange procedure. PLA@SPIONs hybrid biomaterials are characterized by electron microscopy (SEM and TEM) and EDXS analysis, to probe the morphology and detect elements present at the organic/inorganic interface, respectively. Magnetic properties are assessed in vitro by using a Quantum Design MPMS-XL SQUID magnetometer working in the 1.8–350 K temperature range with the applied magnetic field up to 7 T. MR imaging was performed on a Bruker Biospec 70/20 system operating at a magnetic field of 7T (Bruker, Wissembourg, France) using a 72-mm diameter birdcage resonator and a respiratory triggered RARE sequence (effective repetition time TR ~ 2000 ms; rat breathing rate 40-50/min; effective echo time TEeff = 23 ms; RARE factor = 8; FOV = 55mm x 55mm; matrix 256 x 256, slice thickness= 1 mm and 8 accumulations). Host-tissue morphologic response to the presence of the scaffolds was assessed by histopathology with hematoxylin-eosin-suffran (HES) and Perls staining.

Results and Discussion

The morphology and core-shell structure of the PLA@SPIONs nanocomposites were fully characterized by electron microscopy (SEM and TEM) and EDXS, revealing the homogeneous coverage of the nanofiber surface by a quasi-monolayer of SPIONs corresponding to 8 wt% of SPIONs. In contrast to classic magnetic composites, magnetization experiments proved that the magnetic properties of the SPIONs are well-preserved after their anchoring on the PLA fibers and that no aggregation occurred. Following an initial cytotoxicity study showing no toxicity of the PLA@SPIONs nanocomposites, their implantation in rats was performed to confirm the high sensitivity of detection obtained in vitro using standard T2-weighted spin echo sequence. PLA@SPIONs were easily detected using the same sequence in a pre-clinical MR scanner with a clear hyposignal due to the SPIONs anchored at the surface of
the PLA fibers and acting as superparamagnetic contrast agents. Even at 6 months post-implantation, the PLA@SPIONs were easily detected and were still clearly distinct from the surrounding tissues. Histological data highlighted a moderate chronic inflammatory reaction that was similar for both PLA and PLA@SPIONs nanofibers. Thanks to Perls staining, the role of macrophages in the degradation of the scaffolds and in the elimination of the SPIONs was clarified. Finally, it also showed similar degradation/integration behavior for the PLA@SPIONs nanofibers compared to PLA fibers thus confirming our initial hypothesis that the herein proposed strategy allows obtaining magnetic nanocomposites without alteration of the degradation properties of the nanofibers.

**Conclusion**

This set of data confirms the soundness of the proposed strategy that, through an efficient anchoring of SPIONs at the surface of PLA nanofibers, yields magnetic core-shell nanocomposites with i) maintained magnetic properties compared to free SPIONs, ii) that allow a clear and long-term MRI visualization despite low SPIONs content, iii) whose tissue integration and degradation are similar to the ones of pure PLA. Overall it confirms the potential of this new class of core-shell magnetic nanocomposites that opens new opportunities in the field of magneto-scaffolds.

**Acknowledgement**

Funding for this work was provided by the following sources: France Life Imaging FLI (ANR-11-INBS-0006 grant from the French “Investissements d’Avenir” program) and LabEx CheMISyst (ANR-10-LABX-05-01 grant from the French “Investissements d’Avenir” program).

---

**References**

Page 83 of 2028


An in-depth analysis of the effect of polydopamine nanoparticle size on their interaction with glioblastoma multiforme cells and their photo-thermal conversion abilities

Matteo Battaglini¹, Alessio Carmignani¹,², Gianni Ciofani¹

¹ Italian institute of technology, Smart Bio-Interfaces, Pontedera, IT; ² Scuola Superiore Sant'Anna, The Biorobotics Institute, Pontedera, IT

Introduction
Polydopamine nanoparticles (PDNPs) have drawn the attention of the scientific community thanks to their properties, in particular, owing to their antioxidant capacities and their near-infrared (NIR) photo-thermal conversion abilities.¹⁻³ PDNPs have been exploited as a mean to promote the thermal ablation of various forms of cancers ranging from cervix tumor to brain cancer.⁴⁻⁵ PDNPs have one key advantage over inorganic materials due to their organic nature that grants them the ability to be degraded avoiding potential accumulation inside tissues with potentially adverse effects.² However, how the characteristics of the PDNPs, in particular their size, affect their photothermal conversion abilities and their interaction with cancer cells is still largely unknown. In this work, we tried to fill this knowledge gap by investigating how PDNPs of 8 different sizes respond to NIR stimulation in terms of heat generation and interaction with glioblastoma multiforme cells.

Experimental Methods
PDNPs were prepared using a Stöber reaction protocol with different ratios between reagents to obtain particles at different sizes. Obtained PDNPs were characterized in terms of size (dynamic light scattering measurements), surface charge (Z-potential analysis), morphology (scanning electron microscopy –SEM–), and NIR absorption. Then the photo-thermal conversion ability of the 8 different batches of PDNPs was tested both in water and after injection inside an explant of cow brain tissue. Lastly, the interaction of the 8 different batches of PDNPs with U87 glioblastoma multiforme cells (both wild type and transfected for GFP expression) was assessed in terms of internalization (confocal, flow cytometry, and SEM), and ability to elicit cellular thermal ablation.

Results and Discussion
Characterization of the PDNP dispersions demonstrates the presence of well-dispersed and homogeneous structures at 8 different sizes (ranging from 145±13 nm to 710±57 nm). We demonstrated how the increment of PDNPs size caused an increment in NIR (808 nm) absorption and a decrement in U87 cellular uptake. Photo-thermal conversion abilities were affected by the size of PDNPs bigger PDNPs were able to stimulate a greater heat generation upon NIR stimulation (figure 1). PDNPs also showed the ability to induce cellular apoptosis on U87 cells when combined with NIR irradiation.

Conclusion
Size is a key element in PDNP properties. Our work showed how PDNP size can affect both cellular uptake and photo-thermal conversion properties of the nanoparticles. Smaller particles are internalized by cells at a higher extent but have poorer photo-thermal conversion abilities compared to bigger nanostructures. These data were confirmed both in water dispersions and on an explant of cow brain tissue. Obtained data could be used as a guide to optimize PDNP size to obtain the optimal efficiency in terms of cellular uptake, heat generation upon NIR irradiation, and subsequent thermal ablation of cancer cells.

Acknowledgement
This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant number N°709613, SLaMM).
Figure 1
From top to bottom, representative thermal images of Wilko Petri dishes filled with 100 µg/ml of PDNPs of 145 nm average diameter, and with 100 µg/ml of 710 nm average diameter. The water dispersions are shown at three different time points during NIR irradiation (0, 60, and 120 s). As shown by the images, bigger PDNPs were able to elicit a greater increment in temperature compared to smaller PDNPs after NIR irradiation.

References
11:30 a.m. – 1:00 p.m.

Track03

**S01 | Organ-On-a-Chip Technologies Meet Biofabrication: Towards Physiologically Relevant Organ Models**

**Chairs**

Francesca Gervaso  
CNR NANOTEC - Institute of Nanotechnology, National Research Council, Lecce, IT

Alessandro Polini  
CNR NANOTEC - Institute of Nanotechnology, National Research Council, Lecce, IT

Víctor S. Rosales (YSF)  
Universidade Santiago de Compostela, Pharmacology, Pharmacy and Pharmaceutical Technology, Santiago de Compostela, ES

Biofabrication, including bioinks development and bioprinting technologies, can emulate living organs with a high complexity level. OoCs have a tremendous potential in spatiotemporally recreating physical forces and (bio-)chemical concentrations present in the human body. Integrating such technologies rationally would lead to highly physiologically relevant organs and diseases in vitro models.
S01-KL01

Integrating biofabrication with organ-on-chip technologies: marrying micro with macro for improved 3D in vitro models.

Lorenzo Moroni\textsuperscript{1,2}

\textsuperscript{1} Maastricht University, Complex Tissue Regeneration/MERLN, Maastricht, NL; \textsuperscript{2} CNR Nanotec, Lecce, IT

Organ-on-a-chips (OoCs) are being developed as highly innovative, predictive tools with limitless potential for organ function modelling, drug discovery and testing. OoCs are miniaturized \textit{in vitro} systems that can recapitulate the microenvironment and key functions of different organs with a high degree of fidelity, facilitating the study of complex cell-cell interaction and regulation. They integrate relevant cell types, physical forces, (bio-)chemical concentration and gradients with unprecedented confidence, spatiotemporally recreating physical, biological and chemical features of the target microenvironment. By mimicking human key organ functions \textit{in vitro}, they are proposed as models for studying physiological processes as well as disease-related mechanisms to elucidate pathological pathways and test the safety and efficacy of potential drug candidates. Despite numerous efforts to integrate different OoC platforms into a multi-organ system, current set-ups are still limited by recapitulating only a specific function of targeted tissues and organs in a two-dimensional configuration.

On the other hand, an increasing level of emulation of living organ architectures has been recently grasped by exploiting the latest achievements in the field of biofabrication, especially considering bioinks development and bioprinting technologies. The fabrication of biomimetic 3D organ structures, displaying highly defined deposition and assembly of several materials and cell types, is more and more widespread, allowing accurate spatiotemporal control over cell–cell and cell–extracellular matrix interactions, but their combination in OoC is still in its infancy. Integrating such technologies rationally, exploiting their individual advantages in an orchestrated manner, would lead to \textit{in vitro} models of organs and diseases with a high degree of fidelity to the real \textit{in vivo} counterpart in terms of cell positioning, cell-cell and cell-matrix communication, taking into account physical and chemical properties of the microenvironment at the cell scale in an unprecedented manner.

In this direction, here I will be presenting some of the recent advances in the field aiming at developing 3D in vitro microphysiological models integrating OoC and biofabrication principles, with a particular focus on neurodegenerative disease models.

\textbf{Acknowledgement}

We are grateful to the "Tecnopolo per la medicina di precisione" (TecnoMed Puglia)—Regione Puglia: DGR n.2117 del 21/11/2018, CUP: B84I18000540002 and "Tecnopolo di Nanotecnologia e Fotonica per la medicina di precisione" (TECNOmed)—FISR/MIUR-CNR: delibera CIPE n.3449 del 7-08-2017, CUP: B83B17000010001.
S01-02

Freestanding magnetic microtissues for tissue engineering applications

Lúcia F. Santos, Sónia G. Patrício, Ana S. Silva, João F. Mano

University of Aveiro, CICECO - Aveiro Institute of Materials, Aveiro, PT

Introduction
The major challenge of tissue engineering (TE) remains in the fabrication of functional tissue-like constructs able to recapitulate the well-organized and hierarchical architecture of native tissues composed by well-defined microscale subunits [1]. Regarding “bottom-up” principles, microscale tissue building blocks have been engineered with diverse architectures and then assembled in larger tissue constructs. Cell sheet (CS) engineering has been showing great potential for the construction of such building blocks. In fact, Magnetic-based force TE (Mag-TE) has been explored for the fabrication of complex-shaped and robust tissues [2]. The magnetically controlled strategy has the unique ability to manipulate multiple cell types to form complex tissue-like constructs. In this sense, we herein propose the fabrication of freestanding complex-microtissues by Mag-TE that could act as building blocks for the construction of complex and hierarchical tissue-like constructs.

Experimental Methods
Firstly, Fe$_3$O$_4$ nanoparticles were synthetized and incubated with human adipose-derived stromal cells (hASCs). Then, the magnetically-labelled cells were used to create magnetic microtissues with defined sizes and shapes using superhydrophobic surfaces patterned with wettable superhydrophilic domains [3]. Briefly, the cells were seeded on the superhydrophilic domains, cultured for pre-determined periods and then detached with the aid of a magnet. After 7 days, the cell viability and proliferation of the magnetic microtissues was accessed. Moreover, the cell-cell interactions and the presence of a collagenous matrix was also investigated. Finally, the ability of the microtissues to invade neighbouring tissue models were evaluated along the time using methacrylated platelet lysates and methacylated laminarin hydrogels. To recreate the dynamic environment of native tissues, both static and dynamic conditions were explored. As hypothesized, rapidly sprouting of microtissues in hydrogel was visualized, losing their shape and providing trophic and immunomodulatory factors crucial to proper regeneration of tissues.

Results and Discussion
We herein report a simple, one-pot and high-throughput strategy to obtain microtissues with geometrically controlled shapes by using superhydrophobic surfaces patterned with wettable superhydrophilic domains with specific designs. For the first time, such platform was exploited to produce cell-based tissue constructs, mimicking the structure of native tissues. Microtissues with round, square and fiber-like shapes were successfully engineered, showing great robustness and cell-cell interactions within the construct. Freestanding microtissues showed the remarkable ability to preserve the geometry without the presence of a subtract or a magnetic field with great cell survival, overcoming the current issues of scaffold-free approaches. The ability of such constructs to integrate in vitro tissue models was herein accessed using methacylated platelet lysates and methacylated laminarin hydrogels. To recreate the dynamic environment of native tissues, both static and dynamic conditions were explored. As hypothesized, rapidly sprouting of microtissues in hydrogel was visualized, losing their shape and providing trophic and immunomodulatory factors crucial to proper regeneration of tissues.

Conclusion
Such microtissues can be employed to fill defects with particular architecture and high complexity, addressing the challenge of TE in fabricating functional tissue-like constructs able to recapitulate the well-organized and hierarchical architecture of native tissues. We hypothesise that such structures could be also used as high-retention cell delivery vehicles for regenerative therapies by providing cells and adequate trophic factor to the targeted tissue.

Acknowledgement
Page 89 of 2028
We acknowledge the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement and PROMENADE (Ref. PTDC/BTM-MAT/29830/2017). This work was also supported by the project ATLAS (ref.ERC-2014-ADG-669858) and through the doctoral grant SFRH/BD/141523/2018 (Lúcia F. Santos).

The invasion ability of freestanding magnetic microtissues

A. Schematic representation of freestanding magnetic microtissues ability to fill defects using LAM and PLMA hydrogels as in vitro tissue models in static in dynamic; B. Evaluation of the invasion and sprouting of the freestanding microtissues in the in vitro tissue models after 7, 14 and 21 days. For each condition, the brightfield image of the construct (microtissue and hydrogel) is displayed in left panel. The cell survival in the hydrogels was also evaluated along the time for all conditions (in right panel)- living cells (green) and dead cells (red).
Fabrication of freestanding magnetic microtissues by Mag-TE.

A. Fluorescence images of the produced freestanding microtissues with circle, square and fiber shapes after magnetic harvesting. These images revealed a preserved structure with the presence of F-actin filaments (red) and nuclei (blue); B. The freestanding magnetic microtissues cultured during 7 days. The cell viability was accessed by the live-dead fluorescence assay at days 1, 3 and 7 of culture of freestanding microtissues after short-term culture (2 days) and long-term culture (7 days). Living cells were stained with calcein (green) and dead cells by propidium iodide (red).

References
Cell-Assembled extracellular Matrix (CAM): an effective biopaper for the biofabrication of human pre-vascularized and perfusable tissues

Hugo Oliveira, Chantal Medina, Nathalie Dusserre, Charles Handschin, Marie-Laure Stachowicz, Jean-Christophe Fricain, Nicolas L’Heureux

Inserm U1026 BioTis: Bioingénierie Tissulaire, Université Bordeaux, Bordeaux, FR

Introduction
The vascular system plays an essential role in maintaining the homeostasis and function of the great majority of tissues in the human body. When considering regenerative approaches, the efficient formation of a functional vasculature that can support the metabolic needs of bioengineered tissues is essential for their survival after implantation. However, it is widely recognized that the post-implantation microenvironment of the engineered tissues is often hypoxic due to insufficient vascularization, resulting in ischemia injury and necrosis. This is one of the main limitations of current tissue engineering applications aiming at replacing significant tissue volumes.

Here, we hypothesize that fabricated tissue constructs containing pre-organized and more mature vasculature structures can lead to improved graft integration. We have previously shown that cell-assembled extracellular matrix (CAM), synthesized by normal human skin fibroblasts in vitro, could be assembled in a completely biological vascular graft, with sufficient mechanical properties to warrant its testing in a clinic setting. Physiological mechanical strength could be achieved without the need for synthetic materials avoiding the release of the inflammatory response associated with foreign body reaction to permanent synthetic scaffolds, or the harmful degradation products of resorbable polymers. Here, we exploit the inherent qualities of this human biomaterial for the creation of a pre-vascularized network, by using CAM sheets as a biopaper, combined with 3D bioprinting for the controlled deposition of endothelial cells.

Experimental Methods
For the production of cell-assembled extracellular matrix (CAM) sheets, human skin fibroblast (HSFs) were seeded in standard 6-well plates and cultured for 8 weeks with DMEM/F-12 supplemented with 20% FBS. Laser microdissection (30 µm diameter round perforations, spaced every 300 µm, to a final 1.5 x 1.5 cm square, with 50 by 50 perforations) was used to make precise and reproducible perforations within the sheets to enable passive perfusion of the culture medium and oxygen necessary to sustain cell viability and proliferation. Then, HSF were seeded on the surface before bioprinting RFP+ human umbilical vein endothelial cells (HUVECs) in 1.5 cm long lines, spaced by 4 mm, using the microvalve bioprinting function of the 3D Discovery platform (RegenHU, Switzerland). The bioprinted sheets were assembled and cultured in a transwell® system for a total of 26 days.

HUVEC organization was evaluated using confocal imaging. To test the inflammatory response and the capacity of the bioprinted constructs to integrate in an animal host, materials were implanted into a dorsal subcutaneous site of 9 weeks old male NOD Scid Gamma (NSG) mice for 3 weeks. Following in vitro maturation or/and subcutaneous implantation, tissue samples were harvested, fixed, processed for paraffin sectioning and immunostained for CD31, VWF, alpha-SM actin, COL4A, Human Nucleolar and Ulex Europaeus: agglutinin I, and analyzed by microscopy.

Results and Discussion
We demonstrated, for the first time, the use of this unique human biomaterial based on unprocessed ECM, as a microperforated biopaper. Using microvalve dispensing bioprinting, concentrated human endothelial cells (30 million/ml) were deposited in a controlled geometry and cocultured with human skin fibroblasts. Following multilayer assembly, HSF enabled the fusion of the different layers, generating a thick ECM-based construct that supported the survival and maturation of capillary-like structures for up to 26 days of culture. After 3 weeks in the subcutaneous

Page 92 of 2028
space of an immunocompromised mice model, this construct triggered no inflammatory/ degradative response and the pre-formed vasculature successfully connected with the host circulatory system to establish active perfusion.

Conclusion
Here, by combining cellular assembly, laser microdissection and bioprinting, we biofabricated a completely human 3D model of capillary networks and demonstrated its capacity to integrate subcutaneously in an immunodeficient mice model. This mechanically resilient tissue-equivalent has great potential as a base scaffold for the creation of more complex implantable tissues, where rapid anastomosis is sine qua non for cell survival and efficient tissue integration.

Acknowledgement
Authors thank the financial support of “L’Institut national de la santé et de la recherche médicale” Inserm, France, in the framework of the “Accélérateur de recherche technologique” ART on Bioprinting (ART BioPrint).

References
S01-04

ECM-microfiber laden bioinks enable spatial control of capillary formation in 3D bioprinted constructs

Margo L. Terpstra1, Jinyu Li2, Anneloes Mensinga3, Mylène de Ruijter1, Michiya Matsusaki2, Jos Malda1,3, Riccardo Levato1,3

1 University Medical Center Utrecht, Department of Orthopaedics, Utrecht, NL; 2 Osaka University, Division of Applied Chemistry, Graduate School of Engineering, Osaka, JP; 3 Utrecht University, Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht, NL

Introduction
Most tissues in our body are fully vascularized, to facilitate gas and nutrient exchange from the blood circulation. Menisci, however, consist of a vascularized and non-vascularized region. In tissue engineering, mimicking the spatial distribution of vascular components is paramount. In fact, capillary ingrowth into non-vascularized tissues can lead to tissue matrix alterations and subsequent pathology. Three-dimensional (3D) bioprinting has the potential to reproduce tissues with anisotropic features. Nevertheless, recreating a construct with stable vascularized and non-vascularized regions has not been achieved to date. In the current study we present the development of two 3D printable bioinks, that can be used and co-printed to recapitulate distinct vascular and avascular zones of the meniscus.

Experimental Methods
Extrusion based bioprinting was performed using two fibrin-gelatin-based bioinks (figure 1). The bioink for the vascularized red meniscal zone was supplemented with type I collagen microfibers (length of 212 ± 72 µm, and diameter of 27 ± 8 µm)(1), HUVECs and MSCs. The bioink for the white avascular meniscal zone were supplemented with cartilage decellularized extracellular matrix microfibers, developed for this study, and meniscus cells. An anatomical meniscus-like construct was 3D bioprinted into a sacrificial suspension bath composed of gellan gum microgels (average maximum diameter of 21 ± 9 µm), using a microCT scan as blueprint.

Results and Discussion
Previously, it was shown that fibrin bioinks with type I collagen microfibers facilitate capillary and lumen formation of HUVECs (1). We developed microfibers derived from cartilage extracellular matrix extracts, bearing components normally involved in preserving the avascular nature of cartilage (2,3). We demonstrate that such microfibers successfully inhibited HUVEC capillary formation (figure 2A), compared to type I collagen microfibers (figure 2B). Through supplementation of the two fibrin-based bioinks with type I collagen or cartilage matrix microfibers, we 3D bioprinted complex structures with distinct regions that facilitate or inhibit vascularization. Within the bath, filament had a thickness of 350 µm (figure 2D), and construct maintained their shape and geometry upon removal of the supporting gellan gum microgels. Four days after printing, the HUVECs showed confined capillary network formation, in the bioink regions printed with collagen microfiber-supplemented bioinks.

Conclusion
This study demonstrates the spatial-specific formation of capillaries in a 3D bioprinted construct. With this, we contribute to the development of biomimetic meniscal grafts. Moreover, this setup can serve as a platform for generating tissue constructs with vascularized and non-vascularized regions. These bioinks could be utilized for in vitro models for studying effects of new drugs, or mechanisms related to localized and anisotropic vascularization.

Acknowledgement
We acknowledge Liu Hao, Leanne de Silva, and Margot Rikkers, and Jasmijn Korpershoek for their help with the HUVEC co-culture and for providing MSCs and MCs. We thank Yang Li for his help with setting up the migration
system. This project received funding from the European Research Council (3D-JOINT, 647426), European Union’s Horizon 2020 research and innovation programme (MEFISTO, 814444) and ReumaNederland (LLP-12, 22).

Figure 1: Bioprinting of a zonal meniscus construct

A meniscus construct was three-dimensional (3D) bioprinted with two fibrin-based bioinks, using a g-code generated from an equine meniscus CT scan. The bioink representing the red zone, normally vascular, included GFP-HUVECs, MSCs, and type I collagen micro-bers. The bioink representing the white zone, normally avascular, included meniscus cells, and cartilage matrix derived micro-bers. Bioprinting was done into a gellan gum microparticle support bath.

Figure 2: Cartilage matrix microfibers inhibit HUVEC network formation

GFP-HUVECs formed a network, supported by MSC-derived pericytes (α-SMA) (C) in presence of type I collagen micro-bers (B), yet this effect was reduced in presence of cartilage matrix derived micro-bers (A). Constructs 3D-printed in a gellan gum microparticle-based suspension bath showed a -filament thickness of 350 micron (D).

References
Ultra-fast volumetric bioprinting technique for biofabrication of large, complex tissue structures

Paulina Nunez Bernal1, Paul Delrot2, Damien Loterie2, Yang Li1, Jos Malda1,3, Christophe Moser2, Riccardo Levato1,3

1 University Medical Center Utrecht, Department of Orthopaedics, Utrecht, NL; 2 École Polytechnique Fédérale Lausanne, Laboratory of Applied Photonics Devices, Lausanne, CH; 3 Utrecht University, Department of Clinical Sciences, Utrecht, NL

Introduction
Developing complex biological structures of clinically relevant sizes that can guide cell behavior remains a major challenge in tissue engineering. 3D bioprinting is a promising approach that allows for precise patterning of cell-laden biomaterials into tissue-mimetic constructs. Widely used bioprinting techniques like extrusion-based bioprinting (EBB) and digital light processing (DLP) employ a layer-by-layer fabrication strategy, which results in extended printing times for large structures and can detrimentally affect printed cells.[1] Moreover, bottom-up approaches cannot fully capture the convoluted porosity typical of many native tissues and certain complex anatomical features necessary for patient-specific grafts. Overcoming these challenges, a novel optical tomography-inspired printing approach in which spatially controlled visible light projections of a 3D object are used to rapidly fabricate large-scale structures in a single step has been developed.[2] Herein, the concept of volumetric bioprinting (VBP) is introduced alongside a hydrogel bio-resin, demonstrating the fabrication of complex, cell-laden biological structures within seconds.

Experimental Methods
A cell-laden, photosensitive gelatin methacryloyl formulation supplemented with the visible-light photoinitiator lithium phenyl-2,4,6-trimethylbenzoyl-phosphinate (LAP) was developed as bio-resin for VBP. Printing time of centimeter-scale constructs was compared to conventional bioprinting strategies (EBB and DLP). Viability (LIVE/DEAD assay) and metabolic activity (resazurin assay) of bioprinted mesenchymal stromal cells (MSCs) and articular cartilage-derived progenitor cells (ACPCs) was assessed. Mid- and long-term cell functionality post-printing was assessed through the fabrication of an MSC-laden trabecular bone model subsequently seeded with endothelial cells to assess neo-vascularization in vitro and an ACPC-laden meniscus model to evaluate biochemical and mechanical development over 28 days.[3]

Results and Discussion
The gelatin-based bio-resin was printed into human auricle constructs from anatomical scans in 22.7s with high volume accuracy (5.71±2.31%). Printing time remained constant for printing samples scaled to 1.23 and 4.14 cm³ through light dose increments. The same designs resulted in extended printing times for EBB (~30-90min) and DLP (~20-30min). Cells printed via VBP maintained high viability (>80%) and showed increasing metabolic activity over short- and long-term culture, as did EBB and DLP prints and cast control samples. The MSC-laden trabecular bone model presented the smallest resolved feature measuring 144.69±13.55 μm and exhibited a complex, interconnected porous network. After endothelial cell seeding in these pores, constructs exhibited enhanced neo-vessel formation compared to cast controls, as well as capillary infiltration into the printed hydrogel. Finally, meniscus constructs cultured for 28 days produced significant amounts of fibrocartilage-like matrix components and consequently exhibited increasing compressive properties over time, approaching values comparable to native meniscal fibrocartilage (~300kPa).[4] (Figure 1).

Conclusion
Page 96 of 2028
This study established a novel bioprinting approach for shaping hydrogels into complex, tissue-like architectures within seconds. Short printing times and freedom of design shown by VBP compared to conventional bioprinting methods make the technique appealing for biomedical applications, including creating patient-specific grafts and developing more complex in vitro models for disease modelling and drug screening. The use of this technique with a cell-laden, photosensitive hydrogel did not affect cell viability and behavior. Complex biological structures were successfully printed and cells in these printed constructs exhibited salient features post-printing and long-term biochemical and mechanical maturation. These findings open new avenues for designing the next generation of biomaterial-based bioprinted constructs of clinically-relevant size, a necessary step towards future clinical applications.

Acknowledgement

P.N.B, J.M., and R.L. acknowledge funding from ReumaNederland (LLP-12 and LLP-22), the European Research Council (Grant Agreement No. 647426, 3DJOINT), and the Horizon 2020 research and innovation program under the Grant Agreement No. 814444 (MEFISTO). R.L. acknowledges the Materials-Driven Regeneration Young Talent Incentives Program. P.D., D.L., and C.M. acknowledge funding from the Gerbert Rüf Stiftung grant “Flexprint” (GRS-057/18, Pilot Projects track). P.D. and D.L. acknowledge funding from EPFL through the Innogrant 17-16 and the Enable grant for the “Holoprint” project. P.D acknowledges the support of the SNSF and Innosuisse via the Bridge PoC grant 20B1-1_184178.

Figure 1
A) Graphical overview of the VBP process components (rotating reservoir, light source and bioresin). B) Comparison of printing times of constructs with increasing volumes using different bioprinting techniques: VBP, EBB and DLP. VBP shows great potential for biomedical applications due to its versatility in fabricating C) complex cell-laden anatomical models that can be seeded with other cell types post-printing (trabecular bone) and D) biochemically and mechanically mature tissue-like structures (meniscus). Scale bars = 2mm.

References
Ultra-High Throughput Production of Perfusable Microporous Annealed Particle Scaffolds

Maik R. Schot, Malin L. Becker, Carlo A. Paggi, Jeroen Leijten

University of Twente, Developmental Bioengineering, ENSCHEDE, NL

Introduction
Microporous annealed particle scaffolds (MAPs) offer numerous advantages owing to their microporous nature, which associates with improved perfusability, cell migration, implant survival, and tissue integration. However, the low throughputs of current microfluidic strategies has limited the translation of this technology into a clinical setting. We hypothesized that in-air microfluidics (IAMF), which was recently invented by our lab, would allow for the ultra-high throughput production of monodisperse cell-laden microgels to enable the fabrication of clinically sized MAPs. To allow the formation of inter-particle bonds, we produced microgels composed of a dual-crosslinking biomaterial based on tyramine-conjugated alginate (ATA). We here combined this material with IAMF to, for the first time, enable ultra-high throughput production of MAPs.

Experimental Methods
Alginate (80-120cP) was modified with tyramine moieties using DMTMM-based coupling. The degree of substitution was quantified using UV-Vis spectroscopy and vial-tilting was used to verify the sol-gel phase transition of the material following ionic crosslinking using calcium chloride and/or covalent crosslinking using Ruthenium/Sodium Persulfate (Ru/SPS). To generate microgels, a piezo-actuated microjet composed of 0.5% (w/v) ATA was collided with a continuous microjet of 0.1mM CaCl$_2$. Upon impact, calcium chloride solution was wrapped around the alginate drops via Marangoni flow, and microgels are formed via outside-in diffusion of calcium ions (Figure 1A). Cell encapsulation in microgels was achieved by dissolving cells in ATA at cell concentrations ranging from 1x10$^6$ cells/mL to 10x10$^6$ cells/mL. Microgel size, sphericity, monodispersity and cell encapsulation were characterized using fluorescent and phase contrast microscopy. MAPs were created by packing microgels using suction-assisted filtration and spiking the packed constructs with Ru/SPS solutions before applying visible light for three minutes. Construct stability was validated via agitation analysis. Construct perfusability was verified by a hydraulic conductivity assay and by performing micro-particle image velocimetry in a MAP on chip.

Results and Discussion
ATA was successfully functionalized with a degree of substitution of ~4% as determined with UV-Vis spectroscopy. ATA samples were able to form crosslinks using ionic as well as enzymatic and light-based covalent crosslinking strategies. Using IAMF, microparticles of controlled sizes ranging from 50 µm to 350 µm with a CV below 5% could be produced with flow rates ranging from 0.9 mL/min to 3.5 mL/min depending on nozzle size offering microgel production rates of up to 4.57 kHz (Figure 1B/C). Cells could be successfully encapsulated at varying amounts (Figure 1D) and with >80% viability for a wide variety of cell types including hepatocytes, stellate cells and endothelial cells (Figure 1E). After particle packing using injection molding, MAP formation was achieved by forming inter-particle crosslinks using visible light in the presence of Ru/SPS (Figure 2A). Upon exposure to hydrodynamic stress, the packed microgel construct without inter-particle crosslinks (blue) dissolved rapidly, whereas the dual-crosslinked construct (orange) remained fully intact even when vigorously agitated, which indicated successful MAP formation (Figure 2A). Confocal microscopy was used to visualize the internal structure of the MAP (Figure 2B) and to quantify the internal architecture of its integrated interconnected microporous network. Figure 2C and 2D show a MAP on chip and a 3D visualization of a MAP on chip perfused with fluorescent particles, showing the interconnected pore network in the MAP and demonstrating its functionality.
Conclusion
Using IAMF and ATA, we are able to produce microgels of controlled sizes which allows for the production of voluminous MAPs using a second, visible light based crosslinking system resulting in a highly porous, interconnected and perfusable matrix of connected microgels. Additionally, cells show high viability upon encapsulation inside microgels.

Acknowledgement
Financial support was received from the European Research Council (ERC, Starting Grant, #759425).

Figure 1
A. Schematic representation of microgel production using in-air microfluidics followed by inter-particle bond formation using covalent crosslinking. B. Microgel size distribution per nozzle size. C. Microgel morphology as imaged with confocal microscopy. D. Number of encapsulated cells for various cell concentrations. E. Viability plots of several cell types encapsulated in 100µm microgels, insets show cell viability and proliferation from day 1 to 7 for HepG2 cells.
Figure 2

A. Left: Microgels injected into a PDMS mold with Ru/SPS (orange) or food coloring (blue). (scalebar 1cm). Right: Upon agitation, MAPs stay intact (orange) whereas the packed microgels without inter-particle bonds rapidly dissolved into individual particles. B. Internal structure of a MAP as imaged with confocal microscopy (scalebar 100 µm). C. Microfluidic chip trapping microgels for MAP formation on chip (scalebar 500µm). D. 3D visualization of MAP on chip perfused with 1 µm fluorescent particles.

References


Photocurable Manuka Honey-enriched gellan gum-based bioink for engineered articular cartilage constructs

Annachiara Scalzone1, Giorgia Cerqueni2, Michele Pistillo1, Ada Bonifacio3, Stefania Cometa4, Monica Mattioli-Belmonte2, Kenny Dalgarno1, Xiao N. Wang5, Ana M. Ferreira-Duarte1, Elvira De Giglio3, Piergiorgio Gentile1

1 Newcastle University, School of Engineering, Newcastle Upon Tyne, GB; 2 Universita’ Politecnica delle Marche, Department of Clinical and Molecular Sciences – DISCLIMO, Ancona, IT; 3 Universita’ di Bari Aldo Moro, Department of Chemistry, Bari, IT; 4 Jaber Innovation s.r.l., Rome, IT; 5 Newcastle University, Translational and Clinical Research Institute, Newcastle Upon Tyne, GB

Introduction

Bioprinting technology is radically changing regenerative medicine, allowing the manufacturing of tissue-engineered constructs with appropriate control over spatial variations and the capability of precise deposition of biomaterials, cells and biological molecules.1 A great challenge is the identification of bioinks able to fulfill the requirements for reproducible additive manufacturing and biological properties for the type of tissue required.2 Hydrogel-based bioinks are the main formulations used for articular cartilage (AC) biofabrication, due to their similarity to the chondral tissue in terms of morphological and mechanical properties. In this work, we proposed an innovative photocurable gellan gum methacrylated (GGMA) based bioink suitable for extrusion-based bioprinting technology to obtain bioprinted AC-like constructs of clinically relevant sizes. In particular, we exploited a nature-inspired strategy, adding Manuka Honey (MH) to the GGMA composition (GGMA-MH).3

Experimental Methods

H-NMR, XPS and FTIR were performed to assess the methacrylation efficiency. Then, the bioinks formulations composed of GGMA or GGMA-MH and mesenchymal stem cells differentiated in chondrocytes (Y201-Cs) were extruded to obtain a grid-shaped construct with Rokit INVIVO bioprinter (Figure 1). SEM analysis was performed on both compositions without cells to assess the porosity and with cells (at day 1 and day 21 of culture) to evaluate their morphology and organisation. Physical properties as water uptake capability and mechanical properties via a compression test and rheological analyses were assessed. Cells viability was monitored straight after the printing process (3h), and during 7 days of culture via Live/Dead, Immunostaining of Nuclei (DAPI) and Cytoskeleton (Phalloidin Rhodamine) and ATP production (MTS). Gene expression analysis (ACAN, COL2A1 and SOX9), mucopolysaccharides quantification and histological analyses (H&E, Picrosirius Red, Alcian Blue) were performed to evaluate the chondrogenic potential of the bioinks formulation (Figure 2).

Results and Discussion

The methacrylation efficiency of GGMA was chemically assessed by means of H-NMR, XPS and FTIR. GGMA/Y201-C and GGMA-MH/Y201-C bioinks printability, defined by the shapes of the bioprinted fibers, were satisfying, as we obtained bioinks extruded to continuous smooth and homogeneous strands, achieving a 5-layers grid (6 mm x 6 mm x 2 mm) via a multilayered deposition process. The chemical crosslinking process occurred via UV irradiation, while the filament was being deposited on the printing bed. Furthermore, the presence of DMEM/F12 onto the printing bed helped the gelation process, via physical crosslinking (Figure 1). SEM investigation demonstrated that both compositions (GGMA and GGMA-MH) showed porous interconnected structure, with pore size in the range 50-250 μm, optimal for nutrition supply and diffusion (Figure 1). GGMA and GGMA-MH hydrogels showed high hydrophilicity as demonstrated by water uptake analysis and suitable mechanical properties (26.3 ± 3.0 kPa for the GGMA and 23.6 ± 5.0 kPa for the GGMA-MH) for AC regeneration. The beneficial effect of Manuka Honey incorporation on
printability and cell viability and fate was evaluated through cell viability assays (Live and Dead, Nuclei and Cytoskeleton staining (Figure 2) and metabolic activity). The chondrogenic potential of the cells laden GGMA-based bioinks was confirmed over 21 days of culture with collagen and proteoglycans histological analyses, gene expression assessment of main chondrogenic genes (SOX9, ACAN and COL2A1) and glycosaminoglycans quantification. Overall Manuka-honey enriched bioink was found to induce a higher expression of COL2A1 and ACAN in Y201-Cs encapsulated cells at 21 days.

Conclusion

Both GGMA and GGMA-MH, represent potential biomaterials for bioink formulations loaded with mesenchymal stem cells differentiated in chondrocytes to obtain a cartilage-like construct. The bioprinting process, with the proper choice of parameters, enabled us to print these bioinks with spatial and microscale resolution in a rapid and continuous manner, without affecting cell viability. Importantly, the addition of MH led to the obtainment of an antimicrobial environment, more viscous formulation, and an optimal cells distribution pattern, inducing the Y201-C to exhibit maximum biosynthetic ability. Finally, these structurally biomimetic cartilage-like bioprinted constructs, with a cell density of ~ 7 million/mL and Young's module of ~25 kPa could be suitable platforms for future investigations on AC disease modelling and therapy.

Acknowledgement

Annachiara Scalzone acknowledges support from EPSRC for her Ph.D. studentship (EPSRC Grant EP/R51309X/1)

References

11:30 a.m. – 1:00 p.m.

Track04

S02 | Supramolecular Peptide-based Biomaterials for Regenerative Medicine

Chairs

Mariana B. Oliveira
University of Aveiro, Department of Chemistry, CICECO - Aveiro Institute of Materials, Aveiro, PT

Jacek K. Wychowaniec
University College Dublin, School of Chemistry, Dublin, IE

María V. López (YSF)
Universidade de Santiago de Compostela, Departamento de Farmacología, Farmacia y Tecnología Farmacéutica I+D Farma, Santiago de Compostela, ES

Nature has inspired the rational design and development of supramolecular biomaterials exhibiting sophisticated structures, properties and functions at the nanoscale by controlling the self-assembly of its basic molecular components. This symposium will highlight several examples on the supramolecular design of peptide-based biomaterials displaying tunable features and multifunctionalities for regenerative medicine.
Exploiting β-sheet peptides self-assembly to design hydrogels for biomedical applications

Alberto Saiani

University of Manchester, Department of Materials & Manchester Institute of Biotechnology, Manchester, GB

The use of non-covalent self-assembly has become a prominent strategy in material science offering practical routes for the construction of increasingly functional materials for a variety of applications ranging from electronic to biotechnology. A variety of molecular building blocks can be used for this purpose, one such block that has attracted considerable attention in the last 20 years is de-novo designed peptides. Our group work focusses on the development of a technological platform for the design of novel biofunctional hydrogels exploiting the self-assembly of so-called b-sheet forming peptides. These hydrogels can be easily functionalised using specific biological signals and can also be made responsive through the use of enzymatic catalysis and/or conjugation with responsive polymers. Through the fundamental understanding of the self-assembly and gelation processes of these peptides across length scales we have been able to design hydrogels with tailored properties for a range of applications from tissue engineering, cell culture and drug delivery to 3D bioprinting and biosensing [1-5].

Acknowledgement

Acknowledgment of financial support: EPSRC (Fellowship Grant n °: EP/K016210/1); MRC Acellular/Smart Materials –3D Architecture: UK RMP Hub (Grant n °: MR/R015651/1) and Manchester BIOGEL (www.ManchesterBIOGEL.com)

References

Molecular Biomaterials for Regenerative Medicine

Helena S. Azevedo

Queen Mary University of London, School of Engineering & Materials Science, London, GB

The extracellular matrix of tissues contains specialized fibrous proteins and polysaccharides that provide much more than mechanical support for cells. Mimicking this intricate and dynamic protein/polymer network has been a major goal in biomaterials engineering. Using self-assembly as fabrication method, biomaterials can be made with nanoscale precision and inbuilt biophysical and chemical cues. In this bottom-up framework, peptides are obvious supramolecular building blocks as they can be designed to self-assemble into nanostructures with defined shape (e.g. nanofibers) and display desired functionality to control cell functions (e.g. cell adhesion, proliferation, differentiation). Our group has been exploiting the peptide and polymer landscape (polymer composition, architecture, molecular weight, viscosity; peptide sequence, charge, conformation) to develop new self-assembling peptide/polymer biomaterials with properties suitable for applications in drug delivery and regenerative medicine. Through careful selection of the building blocks, and their optimal combinations (peptide/polymer ratio, mixing order), we have realised the formation of self-assembling membranes and hydrogels with diverse and tuneable properties. This talk will cover our work on applying relatively simple (macro)molecular engineering approaches to unveil new features in self-assembling biomaterials.

Acknowledgement

Our work has received support through funds from the European Union, Wellcome Trust and Royal Society.

References

[1] Radvar, E, Azevedo, HS 2018, Supramolecular Peptide/Polymer Hybrid Hydrogels for Biomedical Applications, Macromol Biosci, 1800221
Soft Peptide-based Bioinstructive Supramolecular Multilayered Patches for Spinal Cord Repair

Maria C. Lopes¹, Cristiana F. Sousa¹, Irene D. Dio¹,², Marília Torrado³, Sónia G. Patrício¹, Goksu Cinar⁴, Mustafa O. Guler⁵, Laura Cipolla², Ana P. Pêgo³, João Borges¹, João F. Mano¹

¹ University of Aveiro, Department of Chemistry, CICECO – Aveiro Institute of Materials, Aveiro, PT; ² University of Milano-Bicocca, Department of Biotechnology and Biosciences, Milano, IT; ³ Instituto de Investigação e Inovação em Saúde, Universidade do Porto, INEB – Instituto de Engenharia Biomédica & i3S, Porto, PT; ⁴ Bilkent University, Institute of Materials Science and Nanotechnology - National Nanotechnology Research Center (UNAM), Ankara, TR; ⁵ University of Chicago, Institute for Molecular Engineering, Chicago, US

Introduction

Spinal cord injury (SCI) is a severe neurological disorder arising from traumatic damages inflicted on the spinal cord structure. It represents one of the leading causes of disability worldwide [1], inducing sensory and motor dysfunctions owing to the complex lesion environment and limited ability of the central nervous system (CNS) to self-regenerate [2]. Despite the increasing knowledge in understanding the mechanisms and addressing the lesion pathophysiology, there is still not an effective therapy to promote functional recovery following SCI.

Recently, the advances in supramolecular chemistry, tissue engineering and regenerative medicine, have opened up new avenues in the molecular design and development of functional biomaterial architectures aimed at building up a pro-regenerative microenvironment at the lesion site [3]. To achieve an efficient therapeutic solution, multiple aspects of the spinal lesion environment should be addressed by pursuing a multicomponent biomaterial approach [4]. However, to date, the incorporation of multiple signals in the same system is far from being accomplished, which limits its effectiveness in recreating the complexity, fibrous architecture, functionality and dynamic nature of the neural extracellular matrix (ECM). In this regard, the development of ECM-mimetic biofunctional scaffolds that recreate the native neural ECM and modulate cell functions at the nano/microscale level are of utmost importance. Herein, we spotlight the supramolecular design and development of soft biomimetic multitactical patches endowed with topographical, biomechanical and biochemical cues to recapitulate the diversity of signals in the CNS environment, improve cell-biomaterial interaction and control axonal growth (Figure 1).

Experimental Methods

The patches encompass biocompatible polymers ubiquitous in neural ECM (i.e., hyaluronic acid (HA)), poly(L-lysine), widely used for engineering bioinstructive matrices to support neural cell functions, and laminin-mimetic self-assembling peptide (SAP) recreating the neural ECM and promoting neurite outgrowth [5]. Firstly, the secondary structure of the individual and co-assembled systems was assessed by circular dichroism, ATR-FTIR spectroscopy, wide-angle X-ray scattering, and scanning electron microscopy (SEM). Then, the build-up of soft bioactive supramolecular multilayered thin films in a Layer-by-Layer (LbL) fashion was monitored in situ by quartz crystal microbalance with dissipation monitoring. The multilayered thin films were translated into robust free-standing multilayered patches assembled on templates exhibiting distinct nanotopographies (i.e., flat and nanopatterned substrates) to study their influence on cell functions. The developed patches were further crosslinked via carbodiimide chemistry to enhance their mechanical properties. The physicochemical, morphological and mechanical properties were studied by water contact angle measurements, SEM and Universal Mechanical Testing Machine, respectively. The in vitro biological performance of the native and crosslinked patches was assessed using primary neuronal cortical cells.

Results and Discussion
Secondary structure analysis revealed the ability of HA to trigger the self-assembling capability of peptides, leading to the formation of β-sheet structures with a high degree of supramolecular order. Robust free-standing multilayered patches displaying flat and nanopatterned topographical features were successfully produced and characterized. The presence of a nanopatterned topography enhanced the surface hydrophobicity of the patches when compared with the flat ones. The presence of self-assembling peptides improved hydrophilicity when compared to the biopolymer ending-layers.

The influence of the multilayered patches’ crosslinking and nanopatterned features on the behavior of primary neuronal cortical cells was investigated. At 4 days of culture in vitro, we could see obvious differences between the developed multilayered patches. Crosslinked nanopatterned multilayered patches stimulated the outgrowth of neurites, in all conditions, contrarily to uncrosslinked ones. The incorporation of the SAP, as the outermost layer, revealed the formation of aligned neurites along the nanopatterned direction.

Overall, we confirm the synergistic effects of stiffness, induced by the chemical crosslinking, the presence of the bioactive peptide and the aligned nanopattern, in the orientation of neurite outgrowth of neuronal cortical cells.

**Conclusion**

Robust bioactive flat and nanopatterned free-standing multilayered patches were successfully produced by combining molecular self-assembly with the LbL assembly technology, revealing their ability to tune primary neuronal cortical cells behavior. The gathered results highlight the relevance of the synergistic action of topographical, mechanical and biochemical cues in building complex pro-regenerative environments to instruct neuronal cell functions and be effectively used in spinal cord regeneration.

**Acknowledgement**

This work was supported by Programa Operacional Regional do Centro – Centro 2020, in the component FEDER, and by national funds (OE) through FCT/MCTES, in the scope of the project “SUPRASORT” (PTDC/QUI-OUT/30658/2017, CENTRO-01-0145-FEDER-030658). M. Lopes, C.F.V. Sousa, M. Torrado and J. Borges acknowledge FCT for the individual PhD grants (2020.05210.BD, 2020.04408.BD, SFRH/BD/146754/2019) and individual Assistant Researcher contract (2020.00758.CEECIND), respectively. This work was developed within the scope of the project CICECO – Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through FCT/MCTES.

**References**

S02-04

Accelerating drug development with human protein based platforms for in vitro 3D cell culture and disease modelling

Catarina A. Custódio, Sara C. Santos, Cátia F. Monteiro, Inês A. Deus, João F. Mano

Universidade de Aveiro, Departamento de Química/ CICECO- Aveiro Institute of Materials, Aveiro, PT

Introduction

Data from Humane Society International reveal that every year more than 115 million animals are used in laboratories throughout the world, moreover the National Institute of Health (NIH) has noted that 90% of all drugs tested on animals fail in humans. Beyond the ethical concerns, the use of animal models results in an inefficient, long and expensive drug discovery process. Regarding in vitro tests, 2D cell cultures are a practical option for screening assays, still they do not adequately mimic the complex and 3D organized cell native microenvironment. For that reason 3D models are being adopted as platforms for cell culture and in the drug discovery process.

We have developed a technology to synthesize human based cell culture platforms that overcome major issues in drug development and tissue engineering by using ethically sourced human proteins. The proposed materials have tunable mechanical properties, being versatile platforms for cell culture and microtissue development that will increase the accuracy of in vitro studies by providing realistic microenvironments to cells. These novel materials are easily processed in multiple geometries and in microarrays amenable to ‘organ-on-a-chip’ systems and for instrumentation used in high-throughput (HTS) that are a need for pharma companies. They can find also applications as bioinks for bioprinting and tissue engineering applications.

Experimental Methods

The human based materials were produced from human proteins with origin in the extra cellular matrix (ECM) from amniotic membrane (AM) and platelet lysates (PL) from blood plasma. Placenta was manually dissected and separated in the amnion and the chorion membrane. The peeled membranes were decellularized and solubilized, lyophilized, and processed in a cryogenic grinder. Platelet lysates (PLs) were prepared by exposing pooled PRP to a series of freeze-thaw cycles. The obtained ECM proteins and PLs were chemically modified using a methacrylation protocol well established in our group resulting in methacrylated PL (PLMA) and amniotic membrane (AMMA). Degree and local of modification were evaluated by mass spectrometry. The methacrylated proteins were processed in the form of a hydrogel upon irradiation with UV light. Their structural and mechanical properties were evaluated. Total protein content and the release of specific bioactive molecules was done using Micro BCA and ELISA assay respectively. Cell culture was performed using endothelial cells, stem cells and multiple cancer cell lines either dispersed or in the form of spheroids. The biological performance of the humanized hydrogels was assessed by measuring cell viability and proliferation. At pre-determined time points samples were fixed and immuno-stained.

Results and Discussion

The mechanical properties of the developed hydrogel networks, with Young’s modulus values between 2.5 and 25 kPa, showed to be easily adjusted by tuning degree of methacrylation and concentration of proteins in solution. Protein release profile of shows an overall sustained release. It is also important to refer that the hydrogels maintain their shape during the time release tests were performed. The synthesized gels have proven to support distinct human derived cell cultures (Fig 1).

We have demonstrated the superior bioperformance of such hydrogels compared to classical materials, and their versatility for spheroid invasion, endothelial cell alignment and stem cell culture. Cells could perform important biological processes such as growth, sprouting and migration, displaying a good cell-matrix interaction. Moreover patterned hydrogels were able to provide a support for aligned cell growth. These hydrogels demonstrated to support...
cellular proliferation and formation of a necrotic core in spheroids of stem cells and distinct tumor cell lines. The innovation potential of our materials is based on the fact that contain human biochemical cues, is a complete xenofree solution for human cell culture and easy to manipulate. Preliminary results also suggest the ability of the human derived platforms to support cell culture in the absence of animal derived serum.

Conclusion
This is a novel platform technology that is easy to manipulate, cost effective, reproducible and robust that will accelerate research and drug discovery by providing more realistic results. The benefits of using this next-generation of cell culture materials are both, economic and social, being mainly related to the 1) achievement of more accurate and clinical translatable results, 2) faster drug screening and development processes, 3) reduction in animal-model using and costs associated to late stage drug failures.

Acknowledgement
This work was supported by the European Research Council Proof-of-Concept grant agreement ERC-2020-PoC-957585for the project Amniogel and the Portuguese Foundation for Science and Technology (FCT) through the project BEAT (POCI-01-0145-FEDER-030869). Catarina A. Custódio also acknowledges the FCT for the individual contract CEECIND/02713/2017. This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES.

Figure 1

References
Porous yet dense, shaping biomimetic materials for 3D cell culture applications using ice

Francisco M. Fernandes, Cleo Parisi, Isabelle Martinier, Léa Trichet
Sorbonne Université, LCMCP, Paris, FR

Introduction
Three dimensional cell culture scaffolds are expected to bridge the gap between traditional in vitro cell culture and in vivo animal models. The impact of minimizing in vivo experiments is expected to be outstanding, with consequences both in the ethical and economical aspects in numerous fields. But, despite the promise of increased physiological relevance, 3D cell culture systems are still far from being the norm. In particular, the extensive set of requirements necessary to fabricate effective 3D cell culture materials is far from trivial and few sound solutions are available. Conditions such as non-cytotoxicity, adequate elastic moduli and suitable macroporosity are often difficult to match into a single material. Here, we report recent results in the development of new 3D cell culture systems based on ice templating of highly concentrated type I collagen solutions that are later transformed into macroporous fibrillar collagen gels. Ice templating relies on the use of controlled ice crystal growth to create porosity. The process is easy to implement, cost effective and applicable to a wide range of materials. Since it relies on the use of low temperatures to shape materials, ice templating has sparked growing interest to shape biological matter which is particularly prone to thermal denaturation, ranging from biomolecules up to living cells.

Experimental Methods
Collagen solutions were initially analyzed using DSC to establish the water-collagen phase diagram in the cold 'regime' and define the optimal ice templating conditions. Macroporous fibrillar materials were prepared using a home-built ice templating device. Upon freezing the 40 mg.mL⁻¹ acid soluble type I collagen matrices with controlled unidirectional macroporosity were fibrillated via topotactic stabilization. Obtained matrices were thoroughly characterized using SEM, TEM, PLOM, confocal microscopy (Second Harmonic Generation) as well as tensile testing. Macroporous collagen materials were used as 3D cell culture matrices up to 28 days in presence of human fibroblasts (NHDF) and C2C12 murine myoblasts. During cell culture materials were systematically analyzed (1, 7, 14, 21 and 28 days) by confocal microscopy to extract the morphological response of cultured cells, the colonization dynamics as well as the expression of hypoxic markers. Control experiments using equally concentrated matrices without macroporosity were performed to specifically assess the role of macroporosity in the cellular local environment.

Results and Discussion
We demonstrate that ice templating can be modulated to induce the supramolecular assembly of type I collagen—exhibiting the textural and mechanical features of the extracellular matrix (ECM)—while imposing a controlled macroporous structure that favors cell colonization, as well as nutrient and gas diffusion. Locally, the materials walls present collagen concentration superior to 200 mg.mL⁻¹, due to the concentration effect induced by the freezing front progression. In parallel, the collagen materials display a global elastic modulus in traction superior to 30 kPa. We will discuss how the motifs developed during freezing enable successful migration and colonization of NHDF and C2C12 across the 3D biomimetic collagen scaffolds. Moreover we will discuss recent results showing how these porous matrices compare with equivalent non-porous collagen matrices in terms of kinetics and extent of colonization, opens an exciting pathway to achieve new biomimetic 3D cell culture materials systems with enhanced physiological relevance.

Conclusion
Page 110 of 2028
The new collagen-based materials discussed here combine macroporosity, that enables cell migration as well as gas and nutrient diffusion, and local high concentration developed during ice templating, which leads to a biomimetic model recapitulating the architectural features of the ECM. These characteristics constitute a long sought-after compromise that gathers the advantages of dense collagen matrices without presenting their common limitations such as hypoxic zones due to limited gas diffusion. Porous, yet dense!

Multiscale organization of collagen matrices shaped by ice used as 3D cell culture systems. A) Macroporous collagen matrix obtained by ice templating observed under SEM. B) Fibrillar motifs of macroporous collagen matrix observed under TEM. B’) 2D FFT of dashed yellow region highlighting the fibrillar motifs and orientation. C) NHDF colonization of ice templated collagen scaffold. SHG and fluorescence 3D reconstitution from confocal microscopy. Magenta codes for collagen SHG signal and cyan codes for actin.

References
11:30 a.m. – 1:00 p.m.

Track05

**S03 | Bioinspired Antimicrobial Materials: Addressing Infections in Regenerative Medicine**

**Chairs**
- **Conrado Aparicio**
  University of Minnesota, Minneapolis, Minneapolis, US
- **Carles Mas-Moruno**
  Technical University of Catalonia, ES
- **Paula Parreira (YSF)**
  i3S, Porto, PT

This symposium focuses on a major topic of research for the biomaterials community, which will attract interest from young and experienced researchers alike. It aims to provide a comprehensive overview of the most advanced trends in the development of bioinspired antimicrobial materials.

We plan to cover a wide range of advanced strategies to design novel bioinspired antimicrobial materials, with a special focus on antimicrobial peptides (AMPs), but including also bactericidal topographies, dual-action coatings (combining antibacterial and cell instructive potential), smart stimuli-responsive materials, and strategies to fight antibiotic-resistant bacteria, among others.
Bioinspired strategies to combat multi-drug resistant biomaterial-associated infection

Sebastian A. Zaat

Amsterdam UMC, Medical Microbiology and Infection Prevention, Amsterdam, NL

Biomaterial-associated infections are a main cause of failure of inserted or implanted medical devices, and multidrug resistant bacteria are increasingly prevalent, making treatment complicated (1). Therefore, there is an urgent need for novel antimicrobial solutions capable of also neutralising multi drug resistant pathogens. For such solutions, bioinspired strategies are a promising and expanding field of research. Inserted or implanted biomaterials predispose to infection because they provide pathogens a surface to adhere and to grow out to form biofilms. Moreover, their presence provokes a “Foreign body response”, a cascade of host inflammatory and tissue regenerative responses which, although aimed at proper integration of the foreign material, may increase susceptibility to infection of the tissue surrounding the biomaterial. Due to local immune suppressive effects this may even result in intracellular survival of bacteria within professional phagocytes (2).

We have used biology as an inspiration to develop novel antimicrobials. Thrombocidins, the microbicidal proteins from our own blood platelets (3) have been the design template for our TC-peptides. These peptides have potent microbicidal and anti-biofilm activity, and low toxicity, and are active in animal models of biomaterial-associated infection (4). LL-37, the human cathelicidin with antimicrobial activity produced by human neutrophils and epithelial cells, was the design template for a second family of AMPs, called “SAAPs”, for Synthetic Antimicrobial and Antibiofilm Peptides (5). The most promising SAAPs have been incorporated in PLEX Polymer Lipid Encapsulation Matrix systems for orthopedic implants, allowing their controlled release. A PLEX coating releasing the SAAP OP-145 prevented infection of an implant-associated S. aureus infection in rabbits (5).

We are incorporating these peptides in novel antimicrobial materials for additive manufacturing to construct personalized implants, meeting patient-specific needs and designs. One strategy is to couple the antimicrobial peptides to self-assembling polymers, which may be utilized to coat materials but also for use in electrospinning to construct safe, antimicrobial matrices for in situ tissue engineering, e.g. for prosthetic heart valves or surgical implants (6).

In order to protect the tissue around biomaterials from colonization, it is important to maintain a proper immune condition despite the presence of the implant. To this aim, we study the host immune responses to biomaterials without and with infection, to identify possible pathways associated with increase in susceptibility to infection (7). This research aims to identify to host response-inspired targets to optimize response to biomaterials, and conversely to design materials capable of inducing “the right response”. Moreover, in order to treat infection, antimicrobials need to reach intracellular bacteria. Many antibiotics fail to enter host cells. We therefore have utilized photosensitizers to achieve intracellular delivery of antibiotics, and eradication of intracellular S. aureus infection (8).

Thus, in the triangle of biomaterial – pathogen – biomaterial, bio-inspired solutions are a promising approach to provide new ways to prevent and treat biomaterial-associated infections, even by multidrug resistant bacteria, and to manufacture better and safer biomaterials.

References
The quest for survival in the highly proteolytic environment of a skin chronic wound: a case for efficient antimicrobial peptide conjugation

Pedro M. Alves\textsuperscript{1,2,3}, Rúben F. Pereira\textsuperscript{1,2,5}, Beatriz Costa\textsuperscript{1,2,6}, Natália Tassi\textsuperscript{4}, Cátia Teixeira\textsuperscript{4}, Victoria Leiro\textsuperscript{1,2}, Paula Gomes\textsuperscript{4}, Fabiola Costa\textsuperscript{1,2}, Maria Cristina Lopes Martins\textsuperscript{1,2,5}

\textsuperscript{1} i3s, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, PT; \textsuperscript{2} INEB, Instituto de Engenharia Biomédica, Universidade do Porto, Porto, PT; \textsuperscript{3} Faculdade de Engenharia, Universidade do Porto, Porto, PT; \textsuperscript{4} LAQV-REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Porto, PT; \textsuperscript{5} ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, PT; \textsuperscript{6} Faculdade de Ciências e Tecnologia, FCT, Universidade Nova de Lisboa, Caparica, PT

Introduction

Skin chronic wounds arise when a wound gets arrested in a state of persistent inflammation. Chronic wounds are characterized by an intense inflammatory state that precludes the transition to the proliferative and remodeling stages of wound healing [1], [2]. As the wound bed is often unable to properly close and, therefore, remains exposed to the external environment, microorganisms are able to colonize the wound and frequently establish an infection that further impairs healing [3]. Bioactive molecules are often compromised by the highly proteolytic environment in the wound, if applied freely in solution [4]. Conversely, covalent conjugation onto biomaterials may protect them from such enzymatic degradation, prolonging their therapeutic effect. The use of AMPs is a promising alternative to traditional antimicrobial agents, as the former possess a reduced likelihood of inducing bacterial resistance [5]. Thus, herein, the covalent conjugation of an antimicrobial peptide (AMP), Dhvar5, onto chitosan was explored as an efficient strategy to develop a novel biomaterial that can be used, in the future, to produce improved wound dressings with sustained action against bacterial infection.

Experimental Methods

Conjugation of Dhvar5 onto chitosan was carried out through thiol-norbornene photoclick chemistry (TNPC; Figure 1). To this end, Dhvar5 (LLLFLKLRKKRKY) was modified with a flexible spacer plus a cysteine and the chitosan backbone was modified with norbornene groups in a co-solvent (aqueous/organic) system. Then, upon exposure to UV light in the presence of a photoinitiator, the thiol group of the peptide's cysteine readily reacted with the carbon-carbon double bond in norbornene groups, resulting in the covalent conjugation of Dhvar5 onto chitosan.

Results and Discussion

Despite the high molecular weight chitosan (355 ± 22 kDa) used in the present work, which may difficult the access of the AMP to the norbornene groups, up to 18.5% of initial AMP were present in the chitosan-norbornene-Dhvar5 (NorChit-Dhvar5) conjugate, which corresponds to nearly 34 μmol of AMP per gram of NorChit-Dhvar5. Subsequently, thin films of this conjugate were produced by spin-coating to assess their antimicrobial potential against S. epidermidis (ATCC 35984). A significant reduction in the number of total adhered bacteria (~40%) and of viable adhered bacteria (~15%), relatively to chitosan, was observed for NorChit-Dhvar5 conjugates, when AMP conjugation occurred through the N-terminus (Figure 2). Of note, the exposure of the AMP to UV radiation during TNPC reaction led to no adverse effect on the antimicrobial effect of the AMP, as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were not altered (1 and 2 μg/mL, respectively).

Conclusion

Overall, the results obtained in the present study suggest that covalent conjugation of Dhvar5 onto chitosan by TNPC enhances chitosan’s antimicrobial effect. Moreover, we believe NorChit-Dhvar5 may be a promising biomaterial for the future development of wound dressings. The protective effect of the covalent conjugation of Dhvar5 onto chitosan...
will be evaluated, as it is expected to prolong the residence time and, possibly, the half-life of the AMP in the highly proteolytic environment of chronic infected skin wounds.

**Acknowledgement**

Work financially supported by Portuguese funds through FCT-MCTES as part of project “AntiINFECT” (POCI-01-0145-FEDER-031781, PTDC/NAN-MAT/31781/2017) and through Norte Portugal Regional Operational Programme (NORTE 2020) as part of project NORTE-01-0247-FEDER-033399, funded by FEDER funds through the Sistema de Incentivos à Investigação e Desenvolvimento Tecnológico (SI I&DT). FCT-MCTES is further acknowledged for funding LAQV-REQUIMTE Research Unit (UIDB/50006/2020). Bioimaging, member of PPBI - Portuguese Platform of Bioimaging (PPBI-POCI-01-0145-FEDER-022122), is supported by Portuguese funds - FCT - UID/BIM/04293/2019. P.A. acknowledges the doctoral grant SFRH/BD/145471/2019, F.C. acknowledges the Junior Researcher contract (CEECIND/01921/2017) and V.L. acknowledges her contract in the framework of the projects PTDC/NAN-MAT/30898/2017 financially supported by national (Norte 2020 Framework) and/or European Union funds (ESF – European Social Fund).

**Figure 1**

Reaction scheme of the introduction of norbornene groups onto chitosan (A) and subsequent thiol-norbornene photoclick chemistry to conjugate the antimicrobial peptide Dhvar5 (B).

**Figure 2**

Inverted fluorescence microscopy micrographs highlighting the reduction in viable and total *S. epidermidis* bacteria in NorChit-Dhvar5 (amplification: 630x; staining: Baclight™ Syto9®/PI) and respective quantification. One-way ANOVA (Live: *p<0.05, **p<0.01; Total: •p>0.05).

**References**


Evaluation of anti-Herpes efficacy of biopolymer-based patches enriched with HelixComplex® snail mucus

Maria Francesca Di Filippo¹, Silvia Panzavolta¹, Claudio Trapella²,³, Roberta Rizzo²,³, Andrea Alogna²,³, Valentina Gentili²,³, Luisa Stella Dolci⁴, Nadia Passerini⁴, Beatrice Albertini⁴

¹ University of Bologna, Department of Chemistry ‘G. Ciamician’, Bologna, IT; ² University of Ferrara, Department of Chemical and Pharmaceutical Sciences, Ferrara, IT; ³ HelixPharma srl, Ferrara, IT; ⁴ University of Bologna, Department of Pharmacy and BioTechnology, Bologna, IT

Introduction
In Europe, more than 200 million women (69%) and 187 million men (61%) suffer from cold sores, a widespread and annoying disease caused by the Herpes Simplex Virus type 1 (HSV-1). Cold sore (Herpes labialis) is very contagious and spreads from person to person through close contact. Taking action at the early stage is the most effective way to contain the virus and promote fast healing. Transparent patches are a widespread remedy as they make cold sores less visible and also act as an antivirus shield, reducing the risk of infection. The HelixComplex® snail mucus is a set of molecules with bioactive properties: anti-microbial, protective, regenerative, antioxidant and moisturizing [1-2]. The anti-viral efficacy against HSV-1 of the snail slime extracted from Helix Aspersa has not been demonstrated before. In this work, biopolymer-based patches enriched with snail slime have been developed and characterized in terms of mechanical, structural and biological properties and proposed for the treatment of cold sores.

Experimental Methods
Sodium carboxymethylcellulose (Mw=250 kDa, ACEF, Italy) and sodium hyaluronate were selected for patches formulation. Patches were prepared by the solvent casting technique by dissolving different relative amounts of these natural polymers directly into the snail slime (HelixComplex®). In particular, a 2% w/V of cellulose and two different amounts of hyaluronate (0.5 and 1 % w/V) were tested. Glycerol (Fagron, Italy) was added to the formulations as a humectant, in two different amounts, 5 and 10% w/V. Tensile tests were performed on films immediately after drying using a 4465 Instron dynamometer equipped with a 100N load cell and the Series IX software package. Stress-strain curves were collected and the Young’s modulus, the stress at break and the strain at break were evaluated. The test was performed at a crosshead speed of 5 mm/min on strip-shaped samples (40 mm long and 4 mm width) on at least 10 specimens for each composition. All films have been subjected to structural investigation: FTIR spectra were recorded in ATR mode while X-ray diffraction patterns were recorded in the 2θ range from 4° to 40° by means of a Philips X’Celerator diffractometer. Water vapor permeability (WVP) studies, were performed using the ASTM E96-93 method, slightly modified as reported in literature [3]. The patches morphology was investigated by scanning electron microscopy (SEM), covering the samples with a layer of gold before analysis. The films bioadhesion was evaluated by means of AntonPaar modular compact rheometer MCR102 and the RheoCompass Software using pig ear skin, as reported in literature [4]. The biocompatibility has been evaluated via MTT assay on human fibroblasts cells (MRC-5). Then, the effectiveness of patches enriched with the snail mucus was tested on in vitro experimental models of Herpes Simplex 1 virus (HSV-1) infections (0.01 PFU/cell) on keratinocytes (HaCaT) cell lines.

Results and Discussion
All the obtained films were transparent and easy to handle (Fig.1a). From SEM images they all appeared smooth and without superficial defects. Moreover, all the obtained films showed good adhesion to the skin: once adhered to the skin, they remained attached for hours without drying out or detaching from the edges. These features are of primary importance for a labial patch as they ensure discretion and good compliance of the patient, as it can be inferred from Fig.1b. All the films showed suitable mechanical properties, as they behave flexible, elastic and with...
maximum deformation values reaching even 200%. The ability of the films to provide a humid environment capable of preventing crusts and letting the wound breathe has been verified by WVP tests. In vitro cytotoxicity assay was performed on all the films compositions and the results expressed as cells viability (%). All the samples displayed a promising safety profile. All samples were then tested against HSV-1, evaluating their efficacy pre-infection, post infection and both pre and post infection. Only the labial patches enriched with HelixComplex® were able to reduce the infection, as reported in Fig.2, suggesting that its use could be very effective in the prevention and in the reduction of the disease, avoiding the infection of neighboring cells.

Conclusion
In conclusion, these films represent a sustainable alternative to the commercial plastic patches. Thanks to their adhesiveness and biocompatibility, films enriched with snail mucus extract demonstrated to be very good candidates to act as a labial patch for the treatment of Herpes labialis.

Acknowledgement
Thanks to the HelixPharma Company which gently provided the HelixComplex® snail mucus.

References
Using Lattices to Control the Release of Antibiotics and Growth Factors from a Spinal Cage

Sophie E. Louth\textsuperscript{1}, Luke N. Carter\textsuperscript{1}, Kenneth Nai\textsuperscript{3}, Moataz M. Attallah\textsuperscript{2}, Liam M. Grover\textsuperscript{1}, Sophie C. Cox\textsuperscript{1}

\textsuperscript{1} University of Birmingham, School of Chemical Engineering, Birmingham, GB; \textsuperscript{2} University of Birmingham, School of Metallurgy and Materials, Birmingham, GB; \textsuperscript{3} Renishaw PLC, Stroud, GB

Introduction
Spinal cages are implanted between vertebrae to fuse a section of the spine. This procedure carries an infection rate of around 1% which is higher than infection rates for spinal procedures which do not involve hardware [1,2]. Both early infections, within months of surgery, and late infections can occur [3].

The current treatment for infection includes oral and intravenous antibiotics, debridement of the infected tissues, and irrigation of the site. Most commonly hardware is removed, with new hardware implanted if needed. Antibiotic loaded cement beads can be packed into the region to deliver local antibiotics to increase the chance of hardware retention, this requires a subsequent surgery for removal [4].

Temporary antibiotic loaded cement spacers, that are common in orthopaedic infection treatment, are not currently used in spinal infections. This study aims to investigate whether additive manufacture of Ti-6Al-4V can be used to create a new spinal cage which can be loaded with antibiotics and growth factors in order to clear infection and promote bone growth. A lattice structure will be used to provide mechanical support and control the release of the actives. A polycaprolactone coating loaded with growth factors will cover the lattice, and the voids will be filled with an antibiotic loaded brushite cement (Figure 1).

Experimental Methods
Lattice designs were generated using nTopology Platform (nTopology Inc., USA), and printed on a Ren AM 500M (Renishaw PLC., UK) from gas atomised Ti-6Al-4V powder using optimised in-house parameters. Cylindrical samples (6mm radius, 15mm height) were produced for compression testing, with testing performed in accordance with ISO 13314:2011. Cylindrical samples (10mm radius, 20mm height) with porosity ranges of 20-60% for elution studies were produced in the same way. To test antibiotic release these samples were filled with brushite cement containing 2.5\% w/w gentamicin sulphate (Figure 2A). Release into Phosphate Buffered Saline solution was fluoroscopically measured over eight weeks using o-phthalaldehyde derivatizing agent and a Spark Platereader (Tecan, Switzerland).

Bacterial inhibition was investigated for \textit{Staphylococcus Aureus} and \textit{Pseudomonas Aeruginosa} using zones of inhibition. For growth factor release studies a polycaprolactone coating was loaded with 5\%w/w Albumin as an analogue molecule for growth factors. The released Albumin was fluoroscopically measured using an Albumin protein assay kit and a Spark Platereader (Tecan, Switzerland).

Results and Discussion
Compressions testing showed that a number of lattice designs had compressive yield strengths higher than that of bone making them suitable for this application. Different lattice designs could be used to alter the release profiles of the actives by changing the lattice surface area, cement volume, exposed cement surface area and the loading of the actives (Figure 2B). Bacterial testing found sufficient gentamicin sulphate could be released to kill both \textit{Pseudomonas Aeruginosa} and \textit{Staphylococcus Aureus} (Figure 2C and D).

Conclusion
This work has shown that additive manufacturing can be used to create novel medical devices, and exploited for functions beyond anatomical customisation. More specifically the design freedoms of the technique allow the manufacture of lattices which can provide mechanical support, and control the release of actives in a spinal cage.
device. By controlling the release of the antibiotic the infection can be optimally treated minimising the drug loading which reduces the risk of creating antibiotic resistance.

Future work will investigate whether the presence of the biomaterials will chemically effect the lattice structure. The mechanical properties of the composite of the lattice, polymer and cement will also be investigated both as cylindrical samples, and in the full spinal cage.

Figure 1
Layered schematic of the spinal cage showing the additively manufactured Ti-6Al-4V lattice structure, the polycaprolactone coating loaded with growth factors, and the brushite cement containing gentamicin sulphate antibiotic.

Figure 2
A) BCCZ and Gyroid lattice designs, unit cells, as printed lattices, and lattices filled with brushite cement. B) Release profiles for the release of gentamicin sulphate from BCCZ and Gyroid lattices with the same porosity. C&D) Agar plates after overnight culture showing zones of bacterial inhibition for BCCZ lattices filled with gentamicin sulphate loaded brushite cement, C) Pseudomonas Aeruginosa, and D) Staphylococcus Aureus.

References
Multifunctional bioinspired melanin-CeO₂ hybrid nanozymes for regenerative medicine

Giulio Pota¹, Anna Cuna¹, Giuseppe Vitiello¹,⁴, Anna Zanfardino³, Michela Di Napoli³, Alessandro Pezzella²,⁵,⁶, Giuseppina Luciani¹

¹ University of Naples Federico II, Department of Chemical, Materials and Production Engineering, Naples, IT; ² University of Naples Federico II, Department of Physics, Naples, IT; ³ University of Naples Federico II, Department of Biology, Naples, IT; ⁴ Center for Colloid and Surface Science (CGSI), Florence, IT; ⁵ National Research Council (CNR), Institute for Polymers Composites and Biomaterials (IPCB), Napoli, IT; ⁶ National Interuniversity Consortium of Materials Science and Technology (INSTM), Florence, IT

Introduction

Chemical designed nanozymes has emerged as a next-generation nanomedicine, due to their ability to combine enzyme-like features with high catalytic stability, multiple functions, low cost as well as great design versatility [1]. Moreover, surface modification of nanozymes, appears as an effective strategy to achieve superior catalytic activity, high selectivity and controllable performance for biomedical applications. In particular, nanosized CeO₂ has been rising great interest for its biocompatibility and biological activity, related to the presence of a dual surface oxidation state (Ce³⁺/Ce⁴⁺) caused by oxygen vacancies in the fluorite lattice [2]. This feature makes it a red-ox active material, able to quench or generate ROS, exerting antioxidant and antimicrobial activity. Similar properties are shown by melanin, a class of hydrophobic natural pigments with intrinsic multifunctional properties such as free radical homeostasis as well as anti-inflammatory, antioxidant action and potent antimicrobial activity [3,4]. In this study, CeO₂ and melanin are combined at the molecular scale into a core-shell architecture. To this purpose, CeO₂ nanoparticles were produced by hydrothermal route and subsequently coated with melanin, which was obtained by in-situ 5,6-dihydroxyindole-2-carboxylic acid (DHICA) oxidative polymerization, mediated by Ce⁴⁺ species. Hybrid nanozymes were submitted to physico-chemical characterization to investigate the nanoparticle-biomolecule interactions. Finally, antioxidant and antimicrobial assays were carried out to prove the efficacy of the proposed strategy in boosting intrinsic biocide and antioxidant properties of organic and inorganic components.

Experimental Methods

CeO₂ nanoparticles were produced following a one-step hydrothermal synthesis, starting from Ce(NO₃)₂·6H₂O and NaOH as precursor and morphologic agent, respectively [2]. Melanin coatings were produced by dropwise addition of DHICA-in-ethanol solutions, at different concentrations, to a CeO₂ water suspension. The coated samples were named as CeO₂-DHICA, CeO₂-DHICA 2, CeO₂-DHICA 10. The interactions between the inorganic core and the organic shell were investigated through Fourier Transform Infrared Spectroscopy (FTIR), UV-vis, Electron Paramagnetic Resonance (EPR) spectroscopy. The antioxidant properties of all the samples were tested through DPPH assay. Finally, the antimicrobial power of the nanosystems was assessed towards two bacterial strains, Escherichia Coli (Gram (-)) and Stafilococcus Aureus (Gram (+)).

Results and Discussion

The hydrothermal synthesis produced polyhedral nanoparticles with average size in the range 10-25 nm (Fig. 1a). The adsorption of DHICA onto nanoceria was achieved thanks to a Ce(IV)-catechol complex in the form of a bidentate bridge, as unveiled by FTIR and UV-Vis spectra. The coated sample at the highest DHICA content exhibit higher absorbance in the visible region than bare CeO₂ as well as red-shifted spectrum, suggesting melanin formation. The presence of a melanin shell in CeO₂-DHICA 10 was confirmed by EPR spectroscopy, showing one asymmetric peak (Fig. 1b), with a g-factor of 2.0032 ± 0.0002 and a ΔB of 4.4 ± 0.2, typical values of carbon-centred radicals of melanin.
chains generated through polymerization via ligand-to-metal charge transfer mechanism [5] between DHICA and CeO2. Bare CeO2 nanoparticles exhibited mild antimicrobial activity (Fig. 2a) limited to Gram (+) bacteria probably due to a mechanical damage of the bacterial cell wall, while melanin-coated CeO2 nanozymes experienced a remarkable boost in the biocide power which was more evident against Gram (-) strains and could be determined by a peculiar mechanism of action based on melanin-promoted ROS production and membrane destabilization [4]. Finally, all the nanosystems exhibited DPPH radical scavenging activity, which was higher than bare CeO2 and markedly increased with DHICA content (Fig. 2c). Among all the samples, melanin-coated CeO2-DHICA 10 sample exhibited the best performance. Indeed, DHICA melanin is composed of poly hydroxyindole planar structure with extended resonance which must help stabilize the catechol phenoxy radicals generated during the DPPH radical quenching through a Hydrogen Atom Transfer (HAT) mechanism. Moreover, the sinergistic CeO2-melanin interplay enhanced the antioxidant power exhibited by the bare pigment, stressing the efficiency of the surface combination.

Conclusion

Melanin coating onto CeO2 nanoparticles was successfully obtained exploiting DHICA interaction with Ce4+ ions and its polymerization via ligand-to-metal charge transfer mechanism. Moreover, the conjugation on the molecular scale between the redox-active organic and inorganic moieties resulted in a significant boost of intrinsic antimicrobial and antioxidant activity, related to ROS buffering behaviour, involving both scavenging and production. Obtained nanozymes hold huge promise in regenerative medicine.

References

Cetylpyridinium chloride loaded MBGNs coating on ZrO2 dental abutment for the prevention of peri-implantitis

Zeqian Xu¹,², Kai Zheng³, Sebastian Spintzyk², Stefanie Krajewski², Aldo R. Boccaccini³, Xinquan Jiang¹, Frank Rupp²

¹ Shanghai Jiao Tong University, Department of Prosthodontics, Shanghai Ninth People’s Hospital, Shanghai, CN; ² University Hospital Tübingen, Section Medical Materials Science and Technology, Tübingen, DE; ³ University of Erlangen-Nuremberg, Institute of Biomaterials, Erlangen, DE

Introduction
Dental implants have been widely applied in supporting the dental prosthesis for the patients. Dental abutments, which connect dental implants and dental prosthesis, are partly exposed to the oral cavity. ZrO₂ is widely used as an abutment material due to its mechanical properties and esthetic characteristics. However, peri-implantitis, the destructive inflammatory process around dental implants contributing to supporting bone loss, has become an important issue for implant failure. Bacterial colonization and subsequent biofilm (plaque) formation on the dental abutment and implant surface is one of the essential cause and effect factors for peri-implantitis. Plaque control and tissue regeneration have become the main target in preventing peri-implantitis. Numerous studies have been carried out focusing on antibacterial coatings on the implant surface. However, most effective bactericidal or antibacterial coatings are accompanied by influenced biocompatibility and limited antibacterial duration. Cetylpyridinium chloride (CPC), as a cationic quaternary ammonium compound, is clinically widely used as an antibacterial agent in mouthwash to prevent dental plaque formation. Bioglass exhibits great osteoconductive and osteoinductive function, showing excellent capability of binding to bone tissue as well as to soft tissue through forming a carbonated hydroxyapatite layer (HCA) when exposed to biological fluid. Additionally, mesoporous bioactive glass nanoparticles (MBGNs) developed by the Sol-Gel technology exhibited great drug loading and drug-releasing capability, functional duration, and satisfying biological outcomes. This study aims at applying MBGNs nanoparticles loaded with CPC as an antibacterial coating on ZrO₂ surface and investigating the antibacterial capability and biocompatibility of the coatings, in order to prepare a kind of novel antibacterial coating for ZrO₂ dental abutment to minimize the risk of peri-implantitis.

Experimental Methods
The ZrO₂ blanks (Ivoclar Vivadent) were CAD-CAM into round disks through the dry milling machine K5+ (VHF) and sintered by VITA ZYrcomat sintering oven. After being ultrasonically cleaned with ethanol (99%) and dried with N₂, ZrO₂ round disks (diameter 13 mm, thickness 2 mm) were coated with PDA in a stirring system overnight (19 h) and ultrasonically cleaned with ddH₂O. Meanwhile, the MBGNs were loaded with 0.01%, 0.0.25%, 0.05% CPC for 24 h hours, washed with ddH₂O for 3 times, and harvested through centrifugation (CPC-MBGNs). Afterward, 1 mg/ml CPC-MBGNs loaded with different concentrations were dissolved in PDA and coated under stirring for 4 h. The disks were harvested and dry in 60°C incubator. The ZrO₂ disks (n=3/group, 0.01CPC-MBGNs, 0.025CPC-MBGNs, 0.05CPC-MBGNs, MBGNs, PDA) with and without coating are incubated with Streptococcus gordonii (S. gordonii ) in Schaedler medium (start OD₆ₓ= 0.54) for 6 h, 24 h, 72 h respectively. The OD value of the medium was measured and the samples were harvested for LIVE/DEAD staining. After 3d pre-treatment, 24 h extraction medium in McCoy’s medium were harvested and incubated with L929, HGF, and Saos-2, respectively, the cytotoxicity was evaluated by CCK-8. One-way ANOVA was used for the statistical analysis.

Results and Discussion
CPC-MBGNs nanoparticles were observed on the surface of ZrO₂ disks after the coating process. The ZrO₂ disks coated with CPC-MBGNs have shown significant contact killing effects towards the adhered S. gordonii after 6 h incubation (Fig. 1A). A lower amount of S. gordonii adhesion on the CPC-MBGNs coated ZrO₂ disks was found compared with the control group, which means CPC-MBGNs coating played a role in contact killing and inhibiting primary colonization towards the primary colonizer of oral biofilm formation (S. gordonii). When comparing with the uncoated group, a significant lower OD value was observed after 6 h, 24 h, 72 h incubation with S. gordonii (Fig. 1B), which indicates that the antibacterial coating could inhibit the proliferation of the planktonic bacteria. No significant cytotoxicity was observed towards L929, HGF, Saos-2 (Fig. 1C), which indicates low toxicity towards the soft and hard tissue surrounding the antibacterial coating.

**Conclusion**

The CPC-MBGNs drug-loaded nanoparticle coating could significantly inhibit the oral biofilm formation on the ZrO₂ abutment through contact killing, inhibiting bacterial primary colonization and the proliferation of the planktonic bacteria, without causing significant cytotoxicity towards the surrounding soft and hard tissue. Therefore, the coating has been proven antibacterial. This might offer the possibility for the peri-implant soft tissue to form a tight epithelial seal and to prevent bacterial infiltration. Therefore, the CPC-MBGNs antibacterial coatings on ZrO₂ abutment surfaces showed great potential for preventing bacterial-caused infections, such as peri-implantitis.

**Acknowledgement**

Zeqian Xu is supported by the Forschungsgemeinschaft Dental (FGD), Germany (2019/04). Zeqian Xu would like to acknowledge Ivoclar Vivadent for the material support of IPS e.max ZirCAD LT A2, Ivoclar Vivadent AG Zirconia blank and VHF company for providing the milling machine. This work is partly financially supported by the National Natural Science Foundation of China (Grant No.81921002).

---

**Fig. 1:** Antibacterial function and cytocompatibility of CPC-MBGNs antibacterial coating.

A. Live/Dead staining of bacterial adhered on sample surface after 6 h incubation with S. gordonii;
B. OD value change of the S. gordonii growing in Schaedler medium after 6 h, 24 h, 72 h incubation;
C. Cytotoxicity of sample extraction solution toward L929, HGF and Saos.

---

**References**


Development of Multifunctional Antimicrobial Supramolecular Biomaterials

Martijn Riool1,2, Moniek G. Schmitz2,3, Leonie de Boer1, Patricia Y. Dankers3, Sebastian A. Zaat1

1 Amsterdam UMC (location AMC), Medical Microbiology and Infection Prevention, Amsterdam, NL; 2 Dutch Polymer Institute (DPI), Eindhoven, NL; 3 Eindhoven University of Technology, Department of Biomedical Engineering, Eindhoven, NL

Introduction
The use of biomaterials inside the body always entails the risk of infection. This risk might even be higher in *in situ* tissue engineering applications. Since the porous scaffold materials can form a niche for invading bacteria, the intended *in situ* production of novel tissue may be severely compromised by infection. Therefore, we aim to develop a new polymeric supramolecular scaffold material, exerting two important functions: preventing microbial adhesion and thereby preventing biofilm formation, and inducing endogenous (eukaryotic) cells to regenerate the body.

Experimental Methods
In our research, supramolecular contact-killing materials based on antimicrobial peptides (AMP) are developed. A special class of supramolecular biomaterials are based on fourfold hydrogen bonding 2-ureido-4(1H)-pyrimidinone (UPy) moieties. The supramolecular base material consists of an UPy end-functionalization polycaprolactone (*i.e.* PCLdiUPy). These UPy-materials can be functionalized with bioactive compounds, either via a modular approach in which the UPy-base material is mixed with UPy-modified additives1, or via a post-modification strategy to specifically functionalize the surface of the biomaterial using click chemistry2. The antimicrobial activity is introduced via UPy-functionalized AMPs, using SAAP-148 or TC84, synthetic derivatives of LL-373 and thrombocidin-14, respectively. The regenerative activity is introduced via an UPy-functionalized heparin binding peptide (UPy-HBP). The peptides were synthesized by manual Fmoc-based solid phase peptide synthesis. Solid polymer films were prepared by drop-casting PCLdiUPy with UPy-SAAP-148 or UPy-TC84 on glass coverslips. The antimicrobial activity of the UPy-AMPs in solution and when incorporated in the drop-casted samples was evaluated against *Escherichia coli* ESBL and *Staphylococcus aureus* JAR060131 and LUH14616 (MRSA) and *Acinetobacter baumannii* RUH875 using the LC99.9 (*i.e.* the lowest concentration killing at least 99.9% of the inoculum) and the JISZ2801 surface antimicrobial assay, respectively. Moreover, the cytotoxicity of these AMPs was tested against human dermal fibroblasts.

Results and Discussion
Coupling of the UPy-linker to SAAP-148 did not influence its antimicrobial activity in solution. For the solid drop-casted materials, incorporation of 5 mol% UPy-SAAP-148 is sufficient for killing all 4 bacterial strains tested. This indicates that the peptide remains active after immobilization in the materials. Unfortunately, TC84 loses its antimicrobial activity upon UPy-coupling, both in solution and as a solid. QCM-D adsorption studies revealed that heparin adsorbed to spin coated material films of PCLdiUPy with 5 mol% UPy-HBP mixed via the modular strategy. Current studies focus on characterization of the UPy-SAAP-148/TC84 and multifunctional biomaterial with XPS, AFM, WCA, zeta potential and leakage experiments to investigate the material properties. Moreover, we assess the *in vivo* efficacy of dip-coated titanium implants with 5% UPy-SAAP-148 in the experimental biomaterial-associated infection mouse model.

Conclusion
In conclusion, this modular approach will enable a stable but dynamic incorporation of AMPs, and control of cell adhesion by using cell-adhesive peptides. Ultimately, we aim to use such materials for *in situ* infection-free tissue engineering.

Page 125 of 2028
Acknowledgement
This work is supported by NWO NEWPOL grant SuperActive (Project No. 731.015.505) in collaboration with the Dutch Polymer Institute (DPI, P.O. Box 902, 5600 AX Eindhoven, the Netherlands).

References
11:30 a.m. – 1:00 p.m.

Track06

N01 | Biomaterials for Application in Neurosciences

Chairs

Maria C. Serrano
Instituto de Ciencia de Materiales de Madrid (ICMM-CSIC), Dpto. Materials for Medicine and Biotechnology, Madrid, ES

Paul Wieringa
MERLN, Maastricht University, Complex Tissue Regeneration, Maastricht, NL

Cédric Vranckx (YSF)
Université catholique de Louvain, Institute of Condensed Matter and Nanosciences (IMCN), Louvain-la-Neuve, BE
Tuning the Biomolecular Content of Biosynthetic Hydrogels for 3D Neural Tissue Interfaces

Martina Genta, Catalina Vallejo-Giraldo, Roberto Portillo Lara, Josef Goding, Rylie Green

Imperial College London, Bioengineering, London, GB

Introduction
Material designs and approaches have been explored to mimic native tissues and improve the device-tissue interface of bionic devices. While conductive coatings and biomimetic hydrogels have been used to improve the electrochemical properties and reduce the mechanical mismatch, neural interfaces are still affected by foreign body responses that hinder their performance over time [1]. Current research in this space aims to create a platform able to support neural tissue growth and foster the formation of natural synaptic connections between the device and the host tissues. To reach this objective it is critical to understand the interplay between mechano-biological cues and spatial constraints in 3D environments. In this study, the material design relies on tailoring the hydrogel biomolecular content to generate spatial cues and create a permissive hydrogel for encapsulated cells. In particular, the incorporation of gelatin into poly(vinyl alcohol)-norbornene hydrogels (PVA-Gel-NB) was evaluated to engineer constructs that provide biochemical cues and a supportive environment for encapsulated cells. It was hypothesised that RGD motifs in gelatin chains enhance cell adhesion to the polymer network, while matrix metalloproteinase 2 (MMP-2)-sensitive domains distinctive of gelatin enable enzymatic remodelling of the hydrogels by migratory developing cells.

Experimental Methods
PVA-NB was synthesised as described by Qin et al [2] and Gel-NB synthesis was adapted from Koshy et al [3]. Hydrogels with different polymer ratios, namely 100:0, 50:50, 25:75, and 0:100 (PVA:Gel), were fabricated by visible light photopolymerisation using Eosin Y as initiator and DTT as crosslinker. Compression testing was performed to assess the mechanical properties of the hydrogels, while mass swelling ratio and mass loss were monitored over time to evaluate the physical characteristics of the hydrogels and their hydrolytic degradation behaviour. Cytocompatibility of PVA-Gel-NB hydrogels was evaluated by encapsulating primary P4 Sprague-Dawley rat hippocampus astrocytes. The growth and migratory ability of astrocytes were assessed using an Alamar Blue assay and immunostaining against GFAP to evaluate astrocytes development, MMP-2 to identify gelatinase localisation, as well as paxillin to target focal adhesions.

Results and Discussion
PVA-Gel-NB hydrogels were successfully polymerised with the exception of pure gelatin hydrogels (0:100 PVA:Gel) that did not form a hydrogel but rather remained a viscous liquid (Fig. 1A). This first observation underlined the importance of having a synthetic component as structural support when designing biomimetic hydrogels. The addition of gelatin to the PVA hydrogel network affected the mass swelling behaviour and the mass loss trend, especially in 25:75 (PVA:Gel) hydrogels (Fig. 1B-C). Young’s moduli ranged between 30 to 0.3 kPa over a period of 28 days. Despite the changes caused by the incorporation of gelatin, mechanical and physical properties were in line with common hydrogel systems used for neural tissue engineering [4]. Cytocompatibility was evaluated by assessing the development of primary astrocytes after seven days in culture. Immunostaining with GFAP showed limited development of astrocytes encapsulated in pure synthetic PVA-NB hydrogels (100:0 PVA:Gel), while increasing the gelatin percentage led to a higher cytoplasmic development (Fig. 2B). This behaviour was also confirmed by an increase in metabolic activity (Fig. 1D). In addition to cell spreading, hydrogels with the highest amount of gelatin (25:75 PVA:Gel) supported cell adhesion and the development of mature focal adhesions (Fig. 2C-F). The ability of
cells to spread and anchor to the polymer network indicated that 25:75 (PVA:Gel) hydrogels were able to provide a 3D supportive environment removing any spatial constraints. This was further supported by the translocation of MMP-2 to the cytoplasm, confirmation of the active state of astrocytes, as opposed to nuclear localization indicative of pathological or apoptotic states (Fig. 2D-G) [5].

**Conclusion**

The incorporation of gelatin to PVA hydrogel networks supported cell development by providing both biological and spatial cues necessary for cell interaction and growth. While this is normally achieved by the addition of MMP-degradable crosslinkers and adhesion motifs to the polymer network, the combination of synthetic and natural polymers enabled a simple and scalable fabrication method towards biomimetic bionic interfaces. Moreover, this study showed that primary astrocytes were able to spread and establish mature focal adhesions, which was indicative of their active involvement in network remodelling. These results emphasised the importance of understanding cell-material interactions when designing hydrogels for tissue engineering. Future work will focus on establishing primary co-cultures of astrocytes and neurons in PVA-Gel-NB hydrogels to assess their ability to support the formation of functional neural networks.

**Acknowledgement**

This work was funded by H2020 ERC-COG Living Bionics (Project No. 771985).
Figure 2: Confocal images of encapsulated astrocytes in PVA-Gel-NB hydrogels. Representative images of Hoechst (A), GFAP (B), paxillin (C), MMP-2 (D) staining and merged channels (E) of encapsulated primary astrocytes in PVA-Gel-NB hydrogels with different polymer ratios. F) Mature focal adhesions present at the edges of astrocyte processes in 25:75 PVA-Gel-NB hydrogels. E) Presence of MMP-2 enzyme in the cytoplasm of elongated astrocytes in 25:75 PVA-Gel-NB hydrogels. Scale bars = 30 μm (63x).

References
Polyphenol Rich Extracts-Loaded Liposomes against Neurodegenerative Diseases

Attilio Marino¹, Matteo Battaglini¹, Andrea Desii¹, Gianni Ciofani¹

¹ Istituto italiano di tecnologia, Smart Bio-Interfaces / Center for Materials Interfaces, Pontedera, IT; ² Istituto Giannina Gaslini, Core Facilities-Proteomics Laboratory, Genova, IT

Introduction
Parkinson’s, Alzheimer’s and Huntington’s disease represent devastating neurodegenerative conditions with no satisfactory therapy options. Robust evidence of the role of the reactive oxygen species in inducing the pathogenesis and in sustaining the neurodegenerative processes has been shown in the literature [1], and many preclinical studies and clinical trials involved the use of organic antioxidants, such as polyphenols, for the treatment of these diseases [2]. Results are quite promising but the limited blood-brain barrier (BBB) crossing of these molecules limits their application in vivo. The realization of efficient and safe nano-formulations for brain delivery, such as liposomes, promises to improve brain delivery and treatment efficacy. In this work, selected polyphenol-rich vegetal extracts were used for the fabrication of therapeutic liposomes with elevated BBB crossing capabilities and remarkable protective effects on a drug-induced in vitro model of Parkinson’s disease.

Experimental Methods
Pomaces from white and black grape varieties cultured in the Cinque Terre territories (La Spezia, Liguria, Italy) were used as a natural source of polyphenols. Hydroalcoholic extraction has been performed as indicated elsewhere [3]. Characterization of the extracts was carried out by performing total antioxidant capacity (TAC), Folin-Ciocalteu phenol quantification, and high-performance liquid chromatography (HPLC). Extracts displaying higher antioxidant activity were selected for liposome preparation. Liposomes were prepared by using an Avanti® Mini-Extruder (from Avanti Polar Lipids Inc.) with 100 nm pore membrane and then functionalized with the anti-transferrin receptor (TfR) antibody for improving BBB crossing efficiency [4]. Transmission electron microscopy (TEM) imaging and dynamic light scattering (DLS) measurements have been performed to characterize liposome morphology, size, and z-potential. Biocompatibility studies were performed on brain endothelial cells (HCMEC/D3) and SH-SY5Y-derived neurons by using the WST-1 viability assay at 72 h of liposome incubation. Results were compared to empty liposome-treated cultures and non-treated controls. The protective effects of the extract-loaded liposomes were then investigated in rotenone-treated cells by flow cytometry after FITC-annexin / propidium iodide double staining. BBB crossing was assessed on in vitro static 2D multicellular models by measuring the concentrations of the extract-loaded liposomes in the abluminal compartment at different time points (8, 24, 48, and 72 h).

Results and Discussion
Higher total antioxidant capacity (TAC) was found in extracts from white grape (WG) compared to black grape (BG) pomace varieties. The higher TAC was associated with higher polyphenol content. Specifically, an increased amount of catechines (16% increase) and epicatechines (42% increase) were found in WG compared to BG pomace extracts. Unilamellar liposomes showed a spheroidal shape and a 118 ± 24 nm diameter size with a z-potential of -25 ± 4 mV. A schematic representation of the extract-loaded liposomes and the TEM imaging are shown in Figure 1a and 1b, respectively. The liposomes (both empty and loaded) showed high biocompatibility. Significant toxicity was found only for relatively high concentrations (≥ 800 µg/ml in both neurons and endothelial cells). Interestingly, 400 µg/ml extract-loaded liposomes were able to significantly reduce rotenone-induced cell death in neurons (58% decreased necrosis, 73% decreased early apoptosis, and 73% decreased late apoptosis; Figure 2). The incubation with extract-loaded liposomes in rotenone-treated cells was able to rescue the physiologic cell death levels observed in non-
treated controls (corresponding to ~15% in differentiated SH-SY5Y neurons). Finally, extract-loaded liposomes were able to efficiently cross the BBB, with abluminal concentrations reaching ~50% µg/ml at 72h of incubation.

**Conclusion**
We report on the successful development of a safe and efficient antioxidant nano-formulation able to rescue the apoptotic and necrotic conditions in the rotenone-based Parkinson model. The excellent BBB crossing capability of the proposed nanoformulation paves the way for future preclinical investigations on relevant in vivo models. The potential exploitation of extract-loaded liposomes for the treatment of different neurodegenerative conditions characterized by high ROS levels will be investigated. Finally, antioxidants were extracted from grape pomaces (i.e., a byproduct of wine production) following a “green” sustainable approach. A reduced environmental impact for nanomaterial preparation is therefore guaranteed.

**Acknowledgement**
We kindly acknowledge Mr. Mirco Busco for donating the grape pomaces.

---

**References**


Novel hybrid nanoparticles for Alzheimer’s disease theranostic: efficient overcoming of nanomaterials across the blood-brain-barrier

Catarina I.P. Chaparro1,2, Vera L.S. Neves2, João P. Borges1, Paula I.P. Soares1

1 NOVA School of Science and Technology (FCT-NOVA), CENIMAT/i3N (Centro de Investigação em Matérias), Almada, PT; 2 Faculdade de Medicina da Universidade de Lisboa, Instituto de Medicina Molecular, Lisboa, PT

Introduction
Alzheimer’s disease (AD) is a chronic and progressive brain disease that leads to deterioration of cognitive function [1]. The distinguishing pathological hallmark of AD is the build-up of cortical and cerebrovascular deposits of amyloid-β (Aβ) peptide. The development of novel nanomaterials as imaging contrast agents and drug delivery systems for early detection and treatment of AD is a recent and promising strategy [2]. Nanotechnology-based approaches such as nanoparticles (NPs) have provided enormous hope by overcoming issues regarding poor blood-brain-barrier BBB permeability and drug delivery applications. Magnetic nanoparticles (MNPs) have become an appealing strategy for AD theranostic since they can act as a multifunctional platform. Superparamagnetic iron oxide nanoparticles (SPIONs) are particularly used as contrast agents for magnetic resonance image (MRI), as cell labeling agents for cell tracking and to induce magnetic hyperthermia (MH) [3]. Polymer’s biodegradability facilitates clearance of the nanocarrier and can be exploited to trigger drug release. Poly(lactic-co-glycolic) acid (PLGA) nanoparticles enhance drug pharmacodynamic and bioavailability and, in the present work they are used to incorporate magnetic cores within their nanostructure. The present study aims to develop a nanoparticulate system that translocates the BBB and actively targets Aβ peptides to promote their disaggregation. The work comprises the conjugation of a peptide that reversibly crosses the BBB (PepH3) [4], with a tailored antibody fragment for Aβ (VL2), which allows clearance of the toxic Aβ deposits from the brain to the blood.

Experimental Methods
In this work, SPIONs were synthesized by thermal decomposition technique and PLGA-NPs were prepared with those previously stabilized SPIONs through simple-emulsion solvent evaporation according to a modified procedure reported by Kandasamy et al. [5]. Different parameters such as SPIONs content, polymer weight, and surfactant concentration were varied to optimize the size and encapsulation efficiency of SPIONs. The size of the nanoparticulate system was confirmed by DLS, TEM, and SEM techniques, while the iron content of SPIONs encapsulated in PLGA-NPs was determined using 1,10-phenanthroline colorimetric method through UV/VIS spectrometry. Magnetic hyperthermia was performed to evaluate the temperature variation of all formulations. At last, the functionalization of nanoparticles’ formulations with the construct VL2-PepH3 is being tested for further in vitro assays which comprises translocation across a BBB model and its integrity assessment.

Results and Discussion
SPIONs highly monodispersed with an average core diameter of 6 nm were encapsulated in PLGA-NPs and after the encapsulation process, the formulations tested were within the size range of 212–340 nm (Figure 1 and 2). The encapsulation efficiency (EE) was calculated through the iron content of SPIONs. These values fluctuate between 30 and 60% and are directly related to SPIONs concentration, PLGA mass, and surfactant agents’ concentration. The sizes of nanoparticles are as well dependent on these features. Smaller nanoparticle sizes and higher EE values are achieved when using the following conditions in the preparation of the nanoparticles: 5 mg of SPIONs; 10 mg of PLGA and 0.5% of poly(vinyl alcohol) (PVA) as a surfactant agent. Magnetic hyperthermia studies suggest that both formulations with higher SPIONs EE values are capable of increasing their temperature of up to 7°C.

Conclusion
Page 133 of 2028
We have initially synthesized hydrophobic SPIONs, which are successfully encapsulated inside the PLGA-NPs. Herein, the above-described parameters are varied during the encapsulation process to obtain the most suitable PLGA-NPs for in vitro evaluation. We are studying the BBB interaction with the developed nanosystem with the purpose of supporting those novel hybrid nanoparticles as a new theranostic strategy to overcome the challenges in current therapies of AD.

Acknowledgement
This work is funded by FEDER funds through the COMPETE 2020 Program and National Funds through FCT—Portuguese Foundation for Science and Technology under the project POCI-01-0145-FEDER-007688 (Reference UID/CTM/50025). C. I. P. Chaparro is supported by the grant SFRH/BD/148588/2019 from the Portuguese Foundation for Science and Technology (FCT).

Figure 1
TEM image of iron oxide nanoparticles encapsulated in PLGA-NPs.

Figure 2
SEM image of iron oxide nanoparticles encapsulated in PLGA-NPs.

References

Development of a neurotrophic factor delivery and sustained release system to innervate biomanufactured skeletal muscle constructs

Aurelia Poerio\textsuperscript{1}, Vladimir Mashanov\textsuperscript{2}, Dehui Lai\textsuperscript{2}, Michael Kim\textsuperscript{2}, Young Min Ju\textsuperscript{2}, Ji Hyun Kim\textsuperscript{2}, Anthony Atala\textsuperscript{2}, James Yoo\textsuperscript{2}, Joao Mano\textsuperscript{3}, Franck Cleymand\textsuperscript{1}

\textsuperscript{1} University of Lorraine, Institut Jean Lamour, Nancy, FR; \textsuperscript{2} Wake Forest School of Medicine, Wake Forest Institute for Regenerative Medicine, Winston Salem, US; \textsuperscript{3} University of Aveiro, Department of Chemistry, CICECO, Aveiro, PT

Introduction
The skeletal muscle has limited capacity to regenerate even small defects. Surgical implantation of biomanufactured skeletal muscle constructs has recently emerged as a promising strategy to treat volumetric muscle defects [1]. However, timely innervation of the implanted constructs with the host peripheral nerves remains an unresolved challenge. The objective of this study was to build a 3D bioprinted skeletal muscle construct with a neurotrophic factor (NF) delivery system to accelerate peripheral nerve regeneration and construct innervation.

Experimental Methods
Ciliary neurotrophic factor (CNTF) and glial cell line-derived neurotrophic factor (GDNF) were encapsulated within Poly (lactic-co-glycolic acid) (PLGA) microspheres. The influence of the microspheres on the viscoelastic properties of the bioink was assessed as well as their compatibility with the bioprinting process and viability of human muscle progenitor cells (hMPCs). The release kinetic of the encapsulated factors from 3D bioprinted construct was evaluated with ELISA. The bioactivity and the effect of the sustained release of CNTF and GDNF were evaluated by their ability to enhance neurite outgrowth in an \textit{in vitro} assay with embryonic chick dorsal root ganglia (DRGs).

Results and Discussion
The developed CNTF/GDNF-loaded microspheres have been efficiently and homogeneously incorporated into a fibrin-based bioink without interfering with bioprinting process nor with the viability of hMPCs. Furthermore, we showed that the encapsulated CNTF and GDNF retain their biological activity over at least 14 days and that their sustained release significantly enhanced the neurite growth at the later time points.

Conclusion
In conclusion, we showed the feasibility of PLGA microspheres as a vehicle for the delivery and sustained release of NFs in biomanufactured skeletal muscle constructs demonstrating their potential for further \textit{in vivo} applications.

Acknowledgement
This work was supported by the Medical Technology Enterprise Consortium (MTEC) under Contract #W81XWH-15-9-0001 (grant no. 2017-614-002), by the French PIA project "Lorraine Universite d'excellence" (ANR-15-IDEX-04-LUE).

References
Hydrogel Mediated Delivery of Cerebral Dopamine Neurotrophic Factor Modulates N-glycosylation and Improves Motor Function in a Pre-Clinical Model of Parkinson's Disease

Ana L. Rebelo¹, Sunny Abbah¹, Sergio M. Saldaña¹, Stefan Kirnbauer³, Alexandre Trotier¹, Manus Biggs¹, Richard Drake², Martina Marchetti-Deschmann³, Abhay Pandit¹

¹ National University of Ireland Galway, CÚRAM Research Centre for Medical Devices, Galway, IE; ² Medical University of South Carolina, Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Charleston, US; ³ Technical University of Vienna, Institute of Chemical Technologies and Analytics, Vienna, AT

Introduction
Parkinson's Disease (PD) is a neurodegenerative disorder mainly characterised by the death of dopaminergic neurons in the substantia nigra pars compacta¹, related to different motor symptoms. Currently in the clinic there are only symptomatic therapies available, which fail to address the specific pathophysiology of the disease². However, as an attempt to prolong the remaining neurons' survival, the administration of neurotrophic factors (NTF) has also been heavily investigated. Cerebral dopaminergic neurotrophic factor (CDNF) is an NTF selective for dopaminergic neurons that acts on the endoplasmic reticulum (ER), improving the unfolded protein response (UPR)³,⁴. As ER stress is present in PD pathophysiology and CDNF targets the ER in dopaminergic neurons, its use becomes attractive in this scenario. Additionally, ER stress is intimately associated with dysregulation of N-glycosylation (which is seen in PD), so the improvement of ER homeostasis is likely to regulate N-glycosylation, recovering the healthy brain glycome. With that aim, we have explored the effect of CDNF on the brain N-glycome. Furthermore, as bolus-delivery has some disadvantages, encapsulating CDNF in a hydrogel system for sustained delivery in the target-site to prevent its rapid elimination is of interest. Therefore, this project aims to optimise a collagen-based hydrogel to deliver CDNF into a PD model's brain and assess its effect in modulating the animals' behaviour and the brain's intrinsic N-glycome.

Experimental Methods
In this study, collagen hydrogels with different concentrations of collagen and 4S-StarPEG (crosslinker) were fabricated, and their mechanical and chemical properties studied through stability/degradation assays and rheology. The optimised hydrogel was then used for an in vivo study with 66 male Sprague-Dawley rats. A PD model was created by unilateral stereotactic injection of 6-hydroxydopamine (6OHDA, a neurotoxin that targets dopaminergic neurons) on the medial forebrain bundle. Two weeks post 6OHDA injection, rats were randomly assigned to different groups, including different doses of CDNF (1, 5 and 10 ug), delivered either as a bolus or encapsulated in collagen hydrogels; negative controls injected with collagen hydrogels only or saline were also included. Animals were sacrificed at one and eight weeks post-injection, and tissue collected for immunohistochemistry (against glial and dopaminergic neuronal markers) and N-glycomic analysis (through matrix-assisted laser/desorption ionisation-mass spectrometry imaging (MALDI-MSI)). Throughout the study, every two weeks, the animals' behaviour was assessed through the cylinder test and apomorphine-induced rotations.

Results and Discussion
Upon optimisation, 2mg/ml collagen with 0.4mM 4S-StarPEG hydrogels were found to be the most suitable for intracerebral injection, since these had the storage modulus of 234.08 ± 9.67 Pa, and loss modulus of 21.96 ± 2.27 Pa, and a reduction of free amines to 65%, displaying an excellent crosslinking efficiency. Once injected into the brain, the hydrogels loaded with CDNF improved significantly the behaviour of the 6OHDA injected animals (reduction in the number of apomorphine-induced rotations and enhanced use of both limbs on cylinder test). The animals'
brains were also taken for N-glycomic profiling, where healthy brains revealed a high spatial expression of oligomannose and core-fucosylated glycans (Figure 1), as was previously reported by ultra-performance liquid chromatography\(^5\). These were also modulated upon biomaterial-injection.

**Conclusion**

This study showed the potential of delivering CDNF to the brain using a biomaterial system and its impact on modulating the N-glycomic signature seen upon disease.

**Acknowledgement**

EU Horizon 2020 Programme (H2020-MSCA-ITN-2015) under the Marie Skłodowska-Curie Innovative Training Network and Grant Agreement No. 676408; SFI, co-funded under the European Regional Development Fund, Grant Number 13/RC/2073, and HEA COVID19 Costed Extensions (Ireland)

![Figure 1. N-glycomic profiling of the rat brain using MALDI imaging.](image)

**References**

Exploring a biodegradable dendrimer as carrier of neuroprotective mRNA to reach the ischemic brain

Marília Torrado¹,², Victoria Leiro¹, Pedro Mota¹, Sara C. Silva-Reis¹, Ana Jesus¹, Sofia D. Santos¹, Ana P. Pêgo¹,²,³

¹ i3S/INEB - Institute for Research and Innovation in Health/Institute of Biomedical Engineering, University of Porto, Porto, PT; ² ICBAS - Abel Salazar Biomedical Sciences Institute, University of Porto, Porto, PT; ³ FEUP - Faculty of Engineering, University of Porto, Porto, PT

Introduction

According to the World Health Organization, Stroke is the 2nd cause of death worldwide and the 1st in Europe. 15 million people suffer a stroke per year, of which 5 million are left permanently disabled. Besides the evident social impact, this high morbidity represents an annual economic burden of over $72 billion (US).

Ischemic stroke, accounting for 82-92% of stroke cases, occurs when blood supply to the brain is occluded, resulting in a cascade of events that leads to neuronal death. Yet, the only FDA approved therapy (tissue plasminogen activator) is not applicable to most patients (<10% of cases) [1,2]. Also, this therapy only mitigates the effects of occlusion by restoring the blood flow, lacking neuroprotective and/or neuroregenerative properties.

Along the years, brain-derived neurotrophic factor (BDNF) has been put forward as a promising neuroprotective approach. However, its reduced plasma half-life and the poor blood-brain-barrier (BBB) permeation have limited its therapeutic administration. Additionally, the uncontrollable delivery of BDNF can lead to deleterious side effects [3]. Gene therapy has been explored as a solution for effective BDNF expression at the lesion site. Nevertheless, a biocompatible vector, capable of protecting the neuroprotective nucleic acid from endonuclease degradation and of reaching the ischemic brain in an efficient way remains an unmet challenge.

Herein, we propose the use of biodegradable dendrimers as delivery vectors of mRNA encoding for BDNF (BDNFmRNA) as a novel neuroprotective strategy. These cationic dendrimers, recently developed and patented in our group [4], are endowed with the ability to complex mRNA, shielding it after intravenous administration (Figure 1). Also, their nanosize, full-biodegradability, multivalency and the possibility of fine-tuning their properties make them promising candidates to a successful therapy.

Experimental Methods

The explored dendrimers rely on a gallic acid-triethylene glycol-ester (GATGE) repeating unit and a poly(ethylene glycol) (PEG) chain. Dendriplexes with BDNFmRNA were prepared at different N/P ratios (moles of dendrimer amines/moles of mRNA phosphate groups) and characterized in terms of size and polydispersity index (PdI) by dynamic-light scattering, and Zeta-potential by laser doppler electrophoresis. Additionally, transmission electron microscopy was used for morphological analysis. mRNA complexation was evaluated with gel retardation and SYBRGold exclusion assays. Dendriplexes internalization and cytotoxicity were assessed in mice cortical neuronal cultures, using Lipofectamine-2000 (L2k) as control. In order to evaluate safety for intravenous administration, haemolysis and clotting assays were performed, incubating dendriplexes with purified human red blood cells and recalcified human plasma, respectively. Finally, dendritic nanoparticles biodistribution was evaluated in mice, after systemic administration of Cy5.5-labeled dendriplexes or vehicle.

Results and Discussion

Dendriplexes showed appropriate characteristics for the proposed strategy as they present sizes <100 nm, which is essential for BBB permeation, PdI values <0.3, positive net charges, spherical shape and mRNA complexation efficiencies higher than 90%. The internalization studies revealed that our dendriplexes are internalized by virtually
all neuronal cells in culture, with higher accumulation in cytoplasm and perinuclear regions, contrary to L2k, which internalization was reduced. Also, no cytotoxicity was obtained with our nanoparticles at the used N/Ps, whereas only less than 50% of cells were viable with L2k. Also, haemolytic studies revealed that dendriplexes are non-thrombogenic and coagulation assays further confirmed the hemocompatibility of our system. In vivo, the labelled dendriplexes were detected abundantly in the liver and kidneys, without histological changes. We were also able to observe dendriplexes in the brain, and an increased BDNF expression was verified in all the analysed organs of animals injected with dendriplexes. This demonstrates the great ability of the dendrimer to protect and deliver effectively the BDNFmRNA after systemic administration. Further studies will be performed in a mice model of stroke (pMCAO) to evaluate the neuroprotection effect, where it is expected to obtain a significantly increased accumulation of dendriplexes in the brain, due to the stroke-compromised BBB.

Conclusion
Together, these results point to a novel biocompatible and biodegradable tool, able to reach neurons and to transport potential neuroprotective nucleic acids in a safe and controllable way. Also, with the emerging field of mRNA therapeutics, these vectors represent a valuable asset to be explored to further applications.

Acknowledgement
This study has been funded by the projects NORTE-01-0145-FEDER-000008, INFARMED (FIS-2015-01_CCV_20150630-88), BaiTS (PTDC/CTM-NAN/3547/2014), i3S (POCI-01-0145-FEDER-007274), siRNA (NORTE-01-0247-FEDER-033399), and by FCT DL57/2016/CP1360/CT0013 and SFRH/BD/146754/2019.

The authors would like to acknowledge the Bioimaging, member of the national infrastructure PPBI - Portuguese Platform of Bioimaging (PPBI-POCI-01-0145-FEDER-022122), Histology and Electron Microscopy and Biosciences Screening technicians for the support at i3S Scientific Platforms.

References
Tissue engineered brain-machine interfaces fabricated by two-photon induced degradation of photoresponsive hydrogels

Augusto V. Loffredo1, Lana Van Damme2, Jasper Van Hoorick2, Sandra Van Vlierberghe2, Abhay Pandit1, Manus Biggs1

1 National University of Ireland Galway, CÚRAM - SFI Research Centre for Medical Devices, Galway, IE; 2 Ghent University, Polymer Chemistry & Biomaterials Group (PBM-UGent), Ghent, BE

Introduction
The brain-computer interface and neuromodulation devices provide a means to record and stimulate the nervous system to provide a communication platform to drive peripheral devices/prosthetics and/or mitigate neurological deficits. Indeed, future initiatives to treat and correct myriad neurological conditions rely on a precise interface with the nervous system for optimal monitoring and modulation. There has been substantial progress using penetrating, inorganic microelectrode arrays and optically based methods to record and stimulate from the central nervous system (CNS). However, conventional microelectrodes produce a chronic foreign body response with concomitant signal degradation over time. Moreover, electrical stimulation and recording currently lack specificity in targeting specific neuronal subtypes (e.g. excitatory versus inhibitory) and/or compartments (e.g. dendritic/somatic versus axonal)1. As such, there is currently a need for a chronically stable and highly specific modality for input to and output from the CNS. The goal of this work is to develop tissue engineered ‘living electrodes’ to provide high fidelity connectivity (synaptic integration) with endogenous neural networks to allow biologically based neuromodulation while mitigating the chronic foreign body response that currently limits conventional penetrating electrodes. To fabricate encapsulating neuron hydrogels, we have developed a novel system of 3D printing by multiphoton degradation of a photoresponsive gelatine hydrogel. By patterning the hydrogel to possess microscale voids via two-photon (2P) initiation of DAS4, we demonstrate that hierarchical tissue engineered constructs with controlled neural polarity can be fabricated, and integrated with a micro-electrode array to produce a living neural interface.

Experimental Methods
Methacrylamide-functionalised gelatine (GelMA) was synthesised as previously described2. A degree of substitution of 39% was obtained as confirmed by NMR spectroscopy (data not shown). A 5% w/v of GelMA solution was prepared containing a 0.1% w/v lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) photo-initiator in PBS and crosslinked using UV light at 365 nm for 10 minutes to form a hydrogel. To induce the formation of localised microregions of degradation, the hydrogel was processed using a commercially available two-photon lithography system (Nanoscribe® Photonic Professional GT) in the presence of a biocompatible two-photon initiator (DAS), synthesised as described by Tromayer et al3. An array of 10 µm diameter micro-tunnels (Figure 1A) was developed through free-radical-induced degradation of the gelatine hydrogel by two-photon initiation of DAS4. Nanoindentation and RAMAN microscopy were used to evaluate the impact of 2P exposure, under different laser doses, on the scaffolds mechanical and chemical properties respectively.

In vitro analysis of cell viability and the axonal guidance capability of the morphologically modified hydrogels was evaluated using primary cortical neural population isolated from E17 Sprague dawley pups as previously described8. Neuron viability and polarity were assessed via a LIVE/DEAD assay and immunofluorescent imaging respectively.

Results and Discussion
Van Hoorick et al have recently observed photocleavage in gelatine-based hydrogels when using high laser light intensity4. It was hypothesized that this photocleavage is due to the formation of a very high number of radicals generated under 2P initiation that results in cleavage of the amide bonds, resulting in localised degradation.

Page 141 of 2028
phenomenon was further analysed in the present work using Raman microscopy and nanoindentation that confirmed the profile of the localised degradation under the different laser doses tested. Furthermore, LIVE/DEAD assay indicated that cell viability was not significantly reduced when cultured within the photoresponsive hydrogel. Confocal microscopy of cortical neural cultures after 14 days seeded onto the GelMA hydrogels indicated that the cell axons infiltrated the hydrogel matrix only through the degraded micro-tunnel regions, with the soma restricted to the hydrogel periphery (Figure 1B).

**Conclusion**

By creating degradable gelatine 3D interspaces between the cell layer and the substrate, it was possible to induce neural cell guidance by controlling the dimensions of those spaces generated on the hydrogel layer. We hypothesize that the combination of photocrosslinking and two-photon induced degradation in gelatine-based materials can be used to rapid prototype 3D neural constructs with controlled axonal orientation.

**Acknowledgement**

We would like to acknowledge COST (European Cooperation in Science and Technology) Action 16122 Short Term Scientific Mission grant. This research was supported in part by a grant from Science Foundation Ireland (SFI) and the European Regional Development Fund (ERDF) under grant number 13/RC/2073_2, and by the College of Engineering and Informatics Postgraduate Research Scholarship (NUI Galway).

![Figure 1](image-url)

**(A) An array of cylinder micro-tunnels with 10 µm diameter and 600 µm in length. (B) Confocal images of primary cortical neurons infiltrating the GelMA hydrogel through micro-tunnel structures. The somas of neurons were located at the hydrogel periphery. Nuclei are stained using DAPI (blue), axons with β-tubulin (red) and astrocytes with GFAP (green). Scale bar: 40 µm.**

**References**

Multi-target Eumelanin decorated Electrospun substrates as novel approach to treating Neurodegenerative disorders

Ines Fasolino1, Maria Grazia Raucci1, Eva Carvalho2, Irene Bonadies1, Alessandra Soriente1, Alessandro Pezzella3, Ana P. Pêgo2, Luigi Ambrosio1

1 National Research Council of Italy (CNR), Institute of Polymers, Composites and Biomaterials (IPCB), Naples, IT; 2 Instituto de Engenharia Biomédica (INEB) and i3S-Instituto de Investigação e Inovação em Saúde, nBTT - nanoBiomaterials for Targeted Therapies Group, Porto, PT; 3 University of Naples “Federico II”, Department of Chemical Sciences, Naples, IT

Introduction
Neurodegenerative disorders (NDs) constitute one of the most devastating types of chronic diseases and lead to a crucial medical burden on society. Although, many novel pharmaceuticals have been develop to treat neurological diseases such as Parkinson’s disease (PD) and spinal cord injury (SCI). NDs’ pathogenesis of is marked by an initial toxic insult and a resulting secondary chronic pathological damage. Specifically, early and later stages of NDs involve a decreased neurotrasmission, and an overexpression of inflammatory genes related to specific receptors activation including Toll-like receptor-4 (TLR-4) and Transient potential receptor-4 (TRPV-4) [1]. Indeed, recent findings have identified the neuroinflammation as the major cause of neurodegeneration [2]. Current studies have been reported antioxidant, anti-inflammatory, immunomodulatory, and radioprotective properties of natural and synthetic melanins [3]. Here, the inhibition of neurodegenerative processes by 3D substrates based on coated PLA aligned microfibers functionalized with eumelanin and combined with other therapeutic molecules were investigated.

Experimental Methods
3D electrospun substrates were prepared combining electrospinning, spin coating and solid-state polymerization processes [3]. Physico-chemical and morphological investigations (ATR-FTIR and SEM analyses) were performed to study eumelanin coated microfibers orientation and integrity. In order to evaluate neuroregenerative properties of microfibers, a pre-neuronal human derived cell line from neuroblastoma (SHSY5Y) was cultured onto the 3D electrospun substrates with or without TRPV-4 antagonists and later stimulated with 1-Methyl-4-phenylpyridinium iodide (MPP+), in order to reproduce an in vitro PD model. Cell differentiation and neurodegeneration inhibition were detected through cell viability assay and neuronal marker expression (GAP-43 and TRPV-4 expression) by using confocal analysis. Additionally, to investigate antioxidant and antiinflammatory potential of microfibers, microglial cells were stimulated with lipopolysaccharide (LPS), in order to reproduce an in vitro model of neuroinflammation. Hence, cell morphological changes and substrates mechanism of action on neuroinflammation were investigated by measuring nitrite production, reactive oxygen species levels and inflammatory markers expression through quantitative and qualitative methods (q-PCR, confocal and spectrophotometric analyses).

Results and Discussion
The SEM and ATR measurements on 3D electrospun substrates revealed any chemical degradation process ascribable to the polymer matrix over time. Thus, our substrates represent a valid tool for reproducing more realistically 3D in vivo microenvironments thus promoting cell-cell interaction and biological response in terms of viability and differentiation. Indeed, biological results showed that eumelanin microfibers support pre-neuronal cell survival, adhesion and the formation of neuritic processes (GAP-43 expression) over culture time and inhibited neurodegenerative activities on an in vitro model of PD induced by MPP+ (TRPV-4 modulation). Additionally, 3D electrospun substrates were able to decrease neuroinflammatory response in terms of nitrite production, ROS levels, NF-κB expression and IL-6 secretion induced by LPS in microglial cells through the inhibition of TLR-4 signal. Finally,
morphological studies (SEM and confocal microscopy) suggested that substrates were able to counteract LPS induced cell morphological changes.

Conclusion
Our results validate the promising features of 3D electrospun substrates functionalized with eumelanin as new therapeutic tools for promoting neuroregeneration and controlling neuroinflammation in NDs (as shown in Figure 1).

Acknowledgement
Short Term Mobility (CNR) Program 2018 and project MECHANO (FCT, Portugal). The authors also thank Mrs. Cristina Del Barone of LAMEST laboratory for SEM investigations, Mariarosaria Bonetti for lab technical support & data elaboration and Dr. Roberta Marzella for support to project management.

References
Can 3D graphene oxide scaffolds prompt neural regeneration? Lessons learned *in vivo* at the rat injured spinal cord

**Maria C. Serrano**

*Consejo Superior de Investigaciones Científicas (CSIC), Instituto de Ciencia de Materiales de Madrid, Madrid, ES*

**Introduction**

Since the early 2000's, graphene-based materials (GBMs) have arisen as promising nano- and microplatforms for boosting a plethora of biological phenomena, both *in vitro* and *in vivo*. Within their most remarkable properties with interest in biomedicine, GBMs show customizable nanosized features, mechanical compliance with soft tissues and electrical conductivity. Although their biocompatibility continues to be an open debate for scientists in the field, outstanding efforts are prompting their entrance in the biomedical arena [1]. In neural cultures *in vitro*, GBMs have already proved capacity to modulate neural cell survival, neurite growth, differentiation [2], electrical signaling and ion channel function [3], to cite a few. Nonetheless, progress in preclinical models *in vivo* are still limited. For almost a decade, our group has focused on the exploration of 3D reduced graphene oxide (rGO) biomaterials as supportive platforms for neural repair at the rat injured spinal cord. Specifically, we have explored the capacity of both rGO microfibers [4] and porous foams [5] to boost neural regenerative features at both subacute and chronic stages. More recently, we have incorporated motor training routines mimicking those applied to spinal cord injured patients in the clinic, for who an effective cure remains a therapeutic challenge to date, to prompt their regenerative potential.

**Experimental Methods**

GO slurry was purchased from Graphenea, S.A. Chemical reagents were purchased from Sigma-Aldrich. Adult male Wistar rats were provided by the animal facilities at the Hospital National Hospital for Paraplegics in Spain. 3D porous rGO foams were fabricated by a freeze-casting methodology and rGO microfibers by a hydrothermal process. Results were collected from two different lesion models: a cervical right hemisection at C6 level and a complete transection at T9 level. Additionally, treadmill-based motor training was applied to some of the groups. Animals were sacrificed at 10 (subacute), 30 (early chronic) and 120 (late chronic) days by using a standard perfusion-fixation protocol. Organs were stained with hematoxylin-eosin. Spinal cord samples were examined by immunofluorescence for the presence of neural, glial, inflammatory, and vascular markers. Fluorescence images were collected with a Leica TCS SP5 microscope.

**Results and Discussion**

From the early time points investigated, rGO biomaterials seemed to play a favorable role in the stabilization of the lesion site, evidencing a remarkable infiltration of the totality of their 3D structure by a diversity of cells. In early chronic stages, the formation of vascular structures to nourish the lesion site was supported inside the 3D rGO foams, accompanied by neurites growth and immunomodulatory effects on macrophages. Lesions in late chronic animals receiving 3D rGO foams were largely colonized by excitatory neurites and functional blood vessels. Finally, the combination of these 3D rGO biomaterials with a motor training routine significantly impacted immune, vascular and neural features at the injured spinal cord.

**Conclusion**

Since 2014, our group has provided significant evidences of the beneficial effects that rGO-based 3D biomaterials sustain at the injured spinal cord in rat models. Favorable results in combination with motor training routines bring insights into the prominent field of regenerative rehabilitation. Based on these findings, further work is encouraged to take full advantage of the potential of GBMs in neural tissue engineering applications *in vivo*.

**Acknowledgement**

Page 145 of 2028
This work was funded by the Agencia Estatal de Investigación and the Fondo Europeo de Desarrollo Regional (MAT2016-78857-R, AEI/FEDER, UE).

References
11:30 a.m. – 1:00 p.m.

Track07

N02 | Biomaterials for Orthopedic Applications I

Chairs
David Eglin
Mines Saint Etienne, Biomaterials, Saint ETienne, FR
Elisabeth Engel
Polytechnic University of Catalonia, ES
Elena Maria Varoni (YSF)
University of Milano, IT
3D-printed scaffold combined to 2D osteoinductive coatings to repair a critical-size mandibular bone defect

Charlotte Garot¹,², Michael Bouyer¹,², Paul Machillot¹,², Julien Vollaire³,⁴, Vincent Fitzpatrick², Sanela Morand¹,², Jean Boutonnat⁵,⁶, Véronique Josserand³,⁴, Georges Bettega³,⁷, Catherine Picart¹,²

¹ CEA, CNRS, Université Grenoble Alpes, IRIG Institute, ERL5000 BRM, Grenoble, FR; ² CNRS and Grenoble Institute of Engineering, LMGP UMR5628, Grenoble, FR; ³ INSERM U1209, Institut Albert Bonniot, Grenoble, FR; ⁴ Université Grenoble Alpes, Institut Albert Bonniot, Grenoble, FR; ⁵ Université Joseph Fourier, Faculté de Médecine, Unité médico-technique d'Histologie et Cytologie expérimentale, La Tronche, FR; ⁶ Centre Hospitalier Universitaire de Grenoble, Département d'Anatomie et Cytologie Pathologique, Institut de biomédecine et de pathologie, La Tronche, FR; ⁷ Centre Hospitalier Annecy Genevois, Service de chirurgie maxillo-faciale, Epagny Mets-Tessy, FR

Introduction
The reconstruction of large bone defects (> 5 cm³) remains a challenge for clinicians. Indeed, the gold standard solution to treat such defects is the bone autograft. However, this solution has many disadvantages: it increases the number of surgeries required, thus increasing risks for the patient, it induces pain and morbidity risks at the donor site, and it is only available in a small quantity, making it impossible to repair too large defects [1]. To address this challenge, we developed a new critical-size mandibular bone defect model on mini-pigs (12 cm³), close to human clinical issues, and treated it using a 3D-printed scaffold coated with a 2D bioactive coating (Figure 1).

Experimental Methods
A full-thickness 12 cm³ bone defect, including the periosteum, was created in the mandibular bodies of nine mini-pigs (both sides). Scaffolds made of clinical-grade poly-L-lactic acid (PLLA) were 3D-printed by fused deposition modeling (FDM) and coated with a polyelectrolyte film crosslinked at two different levels (called EDC30 and EDC70, EDC30 films being softer than EDC70 films) delivering an osteogenic bioactive molecule (BMP-2) at different doses (20, 50, and 110 µg/cm³ of scaffold, corresponding to 0.02-0.08 mg/mL) [2]. A preliminary experiment was led on three mini-pigs to optimize bone regeneration and the operating techniques. One animal received two negative controls: an empty defect and a film-coated scaffold at EDC70 without BMP-2; one animal received two film-coated implants, one at EDC30 and one at EDC70, both loaded with BMP-2 at 20 µg/cm³; and one animal received two film-coated implants, one at EDC30 and one at EDC70, both loaded with BMP-2 at 110 µg/cm³. A second main experiment was further led in larger groups to perform statistical analysis. In this experiment, only EDC30 films were used. There was a negative control consisting of a film-coated scaffold without BMP-2, six film-coated scaffolds loaded with BMP-2 at 50 µg/cm³, five film-coated scaffolds loaded with BMP-2 at 110 µg/cm³, and four iliac bone autografts added as a positive control. In both experiments, bone regeneration was assessed qualitatively and quantitatively using CT scans, µCT scans, and histology. CT scans were acquired at days 16, 30, 51, and 91. A CT-scan score was defined by the authors considering four criteria: the percentage of filling of the porous implant (F), the homogeneity of the newly formed bone (H), the ability to distinguish between cortical and cancellous bones (D), and the amount of "ectopic" bone (E). Each criterion was evaluated in a blind manner by four clinicians with a score between 0 and 4. Then, the global score (S) was calculated by each clinician and for each CT-scan:

\[
S = (2F+2H+D-E)/4
\] (1)

µCT scans using a Sanco Medical VivaCT40 scanner were acquired at the endpoint of the study (day 91). Histology was performed by sectioning the samples using a laser microtome (LLS Rowiak) and staining with Sanderson’s rapid stain and Van Gieson’s staining. The histomorphometry provided bone area over total area inside each of the three sections of the implant.

Results and Discussion
Page 148 of 2028
For both experiments, there were no surgical or healing complications. No general or local inflammation occurred during the study. The preliminary experiment allowed optimizing the film coating parameters: no BMP-2 dose-dependence was found for the EDC70 films loaded at two doses while a difference was seen for the EDC30 films, which were selected for the second main experiment. For EDC 30 films, the BMP-2 dose delivered from the scaffold through the polyelectrolyte film significantly influenced the amount of regenerated bone, its mineralization, and the repair kinetics, with a clear BMP-2 dose-dependence (Figure 2). Furthermore, the analysis of µCT scans showed that bone was homogeneously formed inside the scaffolds with little ectopic bone formation, independently of the BMP-2 dose used. Scaffolds loaded with BMP-2 at 110 µg/cm³ showed extensive bone repair, with a fully filled bone defect. In this case, bone repair was as good as for the bone autograft. The BMP-2 doses applied in our study were reduced 20 to 75-fold compared to the commercial collagen sponges currently used in the clinical applications (dose of 1.5 mg/mL), without any adverse effects.

**Conclusion**

3D-printed PLLA scaffolds combined with 2D biomimetic polyelectrolyte films and loaded with reduced doses of BMP-2 thus open perspectives in personalized medicine, since 3D printing allows the customization of the scaffolds shape and the biomimetic film allows the controlled delivery of BMP-2 in space and time.

**Acknowledgement**

We thank the following for their contribution: Isabelle Paintrand (CNRS), Jie Liu (Grenoble Institute of Technology) for help in the preparation of the samples; Jean-Luc Coll (Institute of Advanced Biosciences) and Remy Gerez (Institut Claude Bourgelat) for fruitful discussions, Heiko Richter and Birgitta Stolze (LLS Rowiak) for technical discussions, R Lartizien (Annecy Genenevois hospital) for help in the clinical score, Sebastien Schoumacker for help in 3D printing, Dorothée Palluy (Stryker) for providing the reconstruction plates and screws, Sylvie Berthier (CHU-Grenoble Alpes) for her help in the quantitative histological analysis.

*Figure 1*

Scheme of the approach used to repair mini-pigs mandibles
Figure 2
Quantitative analysis of CT scans for EDC30 film-coated implants loaded with two BMP-2 doses. (A-C) Box plot representations of the total bone volume (A), poorly mineralized (B) and highly mineralized bone volumes (C) as a function of the BMP-2 dose in comparison to BG. (D) CT-scan scores as a function of time and corresponding exponential fits to the data for EDC30 films; corresponding plateau value (Bmax), characteristic time (t) deduced from the fits, and fit quality R² are given in the table. (E) Amount of bone ectopic bone as a function of time for BMP conditions. * p < 0.05; ** p < 0.01.

References
N02-02

Surfactants – undervalued calcium phosphate bone cement additives

Ewelina Cichoń¹, Karolina Stępień², Anna Ślósarczyk¹, Joanna Czechowska¹, Aneta Zima¹

¹ AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Krakow, PL; ² Medical University of Warsaw, Department of Pharmaceutical Microbiology, Warszawa, PL

Introduction
Surfactants - surface active agents have been quite poorly studied as additives to calcium phosphate bone cements (CPCs). In general, surfactants stabilise the foam at the water-air interface, reducing the surface energy required to maintain a larger interphase area associated with the formation of bubbles [1]. This property can be used to obtain macroporous CPCs [2]. What is more, the lowering of surface tension by surfactants usually leads to improvement of cement paste injectability. It should be highlighted that surface active agents in bone cement formulations do not only affect their porosity and injectability, but also other physicochemical properties of the final materials such as setting time, compressive strength, microstructure as well as hydrolysis and degradation rates [3]. Moreover, many surfactants additionally possess interesting biological properties (such as antibacterial activity) that can be transferred to the materials in which they are added.

The aim of this study was the evaluation of the highly porous chemically bonded alpha tricalcium phosphate (α-TCP) based cements designed for bone tissue engineering. The influence of three types of surfactants (Tween 20, Tween 80 and Tetronic 90R4) on the physicochemical and biological properties of foamed calcium phosphate cements (fCPCs) has been investigated.

Experimental Methods
Before cements preparation, surfactants critical micelle concentration (CMC) values in the 2% w/v aqueous solution of Na₂HPO₄ were specified by the pendant drop method. Then, the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of studied surfactants for Staphylococcus aureus NCTC 4163, Staphylococcus epidermidis wild-type 1457, Escherichia coli ATCC 25922 and Pseudomonas aeruginosa PAO1 were determined. The foamed cements were fabricated by the surfactant-assisted process. Three different materials (fTW20, fTW80 and f90R4) were prepared using different nonionic surfactants. The concentration of each surfactant in the cements liquid phases was 1.25 g·L⁻¹. The liquid to powder ratio was equal to 0.7 g·g⁻¹. The control material (fCTRL) was without the addition of any surfactant. Physicochemical and biological properties in vitro of the prepared foamed cements were investigated.

Results and Discussion
The studies revealed that the amount of a particular surfactant should be precisely determined taking into account its critical micelle concentration in the liquid phase of the cement. Measured CMC for Tween 20, Tween 80 and Tetronic 90R4 were approximately 250 mg·L⁻¹, 50 mg·L⁻¹ and 25 mg·L⁻¹, respectively. The foamability of a surfactant solution is maximum at concentrations around and above its CMC [4], therefore the higher concentration of surfactants in the cements liquid phases was applied to cement foaming. MIC and MBC for surfactant solutions are presented in figure 1.

The diffractograms of all prepared cements revealed the presence of two crystalline phases α-TCP and hydroxyapatite (HAp). After 7 days of setting and hardening in air cements fTW20 and fTW80 revealed approximately twofold lower (13 and 15%) amount of HAp phase than material without any surfactant – fCTRL (32%), meanwhile result for f90R4 cement was comparable to it (27%). The porosity of fCTRL reached 54.8 ± 0.5 vol%. For the f90R4 material, it was slightly higher i.e. 57.5 ± 0.8 vol%. Cements with the polysorbates addition possessed higher porosity 72.9 ± 0.8 and 78.1 ± 2.3 vol%, for fTW20 and fTW80, respectively. Compressive strength of the foamed cements...
was relatively low due to their high porosity (>50 vol%) connected to the high L/P ratio of 0.7 g·g\(^{-1}\) and foaming procedure. For the control material – fCTRL it was equal 2.37 ± 0.50 MPa. The surfactant addition caused a further decrease of compressive strengths, which were 1.13 ± 0.35 MPa, 1.79 ± 0.48 MPa, and 1.43 ± 0.43 MPa for fTW20, fTW80 and f90R4, respectively. Investigated foamed cements were chemically stable in vitro (figure 2). After incubation in SBF, numerous apatite-like forms were visible on the materials' surfaces.

**Conclusion**

To conclude, surfactants should not only be considered as foaming agents or binders but also deserve more attention as modifiers affecting the physicochemical and biological properties of CPCs. What is more, before the use of surfactant in material preparation, in order to reach the best outcomes we should deal with its own properties.

**Acknowledgement**

This study was supported by the National Science Centre, Poland (project no. 2017/27/N/ST8/00913) and the Faculty of Materials Science and Ceramics AGH UST - University of Science and Technology, Kraków, Poland, Project No. 16.16.160.557 (2021). EC acknowledges financial support from the National Science Centre, Poland under doctoral scholarship no. 2019/32/T/ST5/00207.

EC has been partly supported by the EU Project POWR.03.00-IP.08-00-P13/18 - PROM NAWA

References


Development of a Personalized 3D Printed Bioactive and Biodegradable Implant for Guided Bone Regeneration

Sergi Rey-Vinolas, Soledad Perez-Amodio, Oscar Castano, Miguel Angel Mateos-Timoneda, Elisabeth Engel

1 Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Baldiri Reixac 10-12, 08028, Barcelona, ES; 2 CIBER Bioingeniería, Biomateriales y Nanotecnología (CIBER-BBN), Madrid, ES; 3 Department of Materials Science, EEBE, Technical University of Catalonia (UPC), d'Eduard Maristany 16, 08019, Barcelona, ES; 4 Department of Electronics and Biomedical Engineering, University of Barcelona (UB), Martí i Franquès 1, 08028, Barcelona, ES

Introduction
The interest in titanium meshes for guided bone regeneration (GBR) has increased over recent years due to their good stability, volume preservation, and predictable tissue regeneration [1]. However, they also present important drawbacks such as the need for a second surgery to remove the mesh after bone regeneration, and the mesh exposure prevalence [2]. In addition, the combined use with autologous bone graft for larger bone defects is still the gold standard [3]. This technique implies harvesting a healthy bone graft from other anatomic locations, thus can cause side effects such as morbidity, extra pain, and extra surgery to collect the graft. Consequently, this industrial project aims to develop a biodegradable and bioactive patient-specific implant for guided bone regeneration, capable to reproduce complex geometries based on additive manufacturing technologies. As it is based on a resorbable polymer (Polycaprolactone-PCL) and loaded with bioactive microparticles (MPs), the device is a temporal bone substitute able to stimulate angiogenic and osteogenic processes.

Experimental Methods
Different bioactive calcium phosphate-based MPs have been synthesized and dispersed into PCL obtaining thin films. Those films have been used as a raw material to be extruded by 3D Printing (3D Discovery, RegenHU). Apart from a condition with pure PCL, three more conditions with the synthesized MPs have been studied (PCLA, PCLB, and PCLC) in order to enhance the bioactivity of the implant. To assess the stability along the regeneration process, a degradation assay was performed under physiological conditions (HEPES 10mM pH 7.4 at 37ºC) for one year. Scaffolds were evaluated in vitro with human gingival fibroblasts (hGFib) to study the proper gingival attachment to the implant. As well, human mesenchymal stem cells (hMSCs) to evaluate cells’ viability by the quantification of their metabolic activity by Alamar Blue and cytotoxicity by LDH following ISO10993 standards. An angiogenesis protein-related array was performed to identify key proteins present in hMSCs supernatant culture media from seeded scaffolds. Polyamide bone defect models were also kindly provided by AVINENT Implant System.

Results and Discussion
Scaffolds with a parallel patterned design were fabricated by 3D-Printing with a highly interconnected macro-porosity to allow cell migration and blood vessel infiltration. For all conditions with MPs, a good homogeneous dispersion was evidenced according to μCT and FESEM images, facilitating ions release during their degradation process. The degradation assay carried out over one year showed that scaffolds maintained their structure and mechanical properties, with a maximum weight loss of 10% without collapsing.

One of the most frequent clinical complications is meshing exposure, so hGFibs were cultured on scaffolds showing a good biocompatible behavior according to the metabolic activity results, and full colonization of the scaffolds observed by fluorescent confocal images. Furthermore, no cytotoxic effects were detected in all conditions. Vascular endothelial growth factor (VEGF) was quantified among others proteins, showing its highest production levels after
3 days of culture. To support the *in vitro* results achieved, an *in vivo* subcutaneous model in mice is currently in progress.

Finally, complex geometries obtained from clinical cases were 3D printed and compared to polyamide defect models, obtaining a correct fit and, hence, validating patient personalization (fig 1).

**Conclusion**

We have developed a personalized bioactive and biodegradable 3D printed implant for guided bone regeneration, as a promising substitute of current techniques solving their main drawbacks such as a second surgery, extra pain, and morbidity. Although PCL loaded with different bioactive MPs was studied, further experiments are ongoing to select the most efficient condition for the application.

**Acknowledgement**

This project is being developed in collaboration and with funding from AVINENT Implant System. Authors would like to thank AGAUR for founding with “Doctorat Industrial” grant (2017DI076) and Dr. Elena Xuriguera from the University of Barcelona for her help in performing mechanical testing.

---

**fig 1**
Polyamide bone defect model (left) and developed bioactive and biodegradable implant (right).

---

**References**


Fabrication of injectable calcium phosphate cements containing PLGA microspheres as drug delivery system for bone regeneration

Carole Barou1,2,3, Habib Belaid1,2, Vincent Cavaillès2, Mikhael Bechelany1

1 Institut Européen des Membranes, Montpellier, FR; 2 Institut de Cancérologie de Montpellier, Montpellier, FR; 3 Biologics 4 Life, Pertuis, FR

Introduction
Bone is a living tissue which undergoes constant remodelling and whose performance could be altered by various diseases such as osteoporosis, fibrous dysplasia, or cancer metastases. In addition to be painful for patients, these diseases lead to pathological fractures, which require multidisciplinary approaches for their treatment. In many cases, a bone substitute material is used to fix the damaged tissue and to provide a mechanical support. The goal of this work is to propose a combined matrix having the potential to improve drug targeting to obtain a selective accumulation in the bone lesion with tunable characteristics. For this purpose, we focus on improvement of rheological properties of the injectable cement and the polymeric loading efficiency of a bisphosphonate.

Experimental Methods
The solid mineral phase was constituted by β- tricalcium phosphate (β-TCP) (50µm) and mono calcium phosphate monohydrate MCPM (150µm) powders at a 45:55 molar ratio, and 1 wt% dihydrogen pyrophosphate. The calcium phosphate cement (CPC) was obtained by addition of a citric acid solution (0.5M) with a powder-to-liquid ratio of 0.4mL per gram of solid content. A second type of cement is currently investigating, with magnesium and calcium phosphates as raw materials (MgCPC). The solid phase and the liquid phase were then respectively loaded into two connected syringes and a push-pull action for 30 seconds allowed to mix the cement. Different percentages of zirconia were added in the solid phase to establish the ideal content for the radio-opacity performance. Subsequently, acid poly-(lactic-co-glycolic) (PLGA) were designed by double-emulsion-solvent-evaporation method. A range of polymeric particles size (from 1µm to 200µm) and sodium alendronate content (0.5 to 5wt%) were investigated. Different formulations (CPC/PLGA5 to CPC/PLGA30) were tested by the addition of PLGA microspheres into the cement (5 to 30 wt%).

Results and Discussion
The initial CPC formulation containing 20 wt% of ZrO2 showed the best radio-opacity, with a compressive strength of 23 MPa and a Young’s modulus of 3.5 GPa. Then, CPC containing PLGA microspheres were characterized by scanning electron microscopy (SEM) to show the incorporation of microspheres. Physicochemical analyses by X-ray diffraction (XRD) and infrared spectroscopy (FTIR) confirmed the final cement phase conversion. The CPC/PLGA30 induced a global diminution of the initial setting time compared to CPC (from 12 to 7 minutes). Although the incorporation of PLGA microspheres increased the force necessary to inject the cement, the CPC/PLGA30 appeared fully injectable after 4 minutes. Additionally, the cohesiveness was improved by addition of the biopolymer. Due to the dissolution-precipitation reaction and the mixing preparation, the CPC has naturally a microporous structure. The addition of PLGA microspheres in the formulation increased drastically the porosity (from 4 to 45%), thus the degradation kinetics of the biomaterials was increased too. In vitro results showed a controlled release of sodium alendronate in the medium from the composite cement. The burst effect was limited compared to the formulation directly incorporating the drug into the cement, without PLGA particles. Finally, biocompatibility of formulations was validated via a viability test using MC3T-3 and hFOB 1.19 cells.
in presence of CPC and CPC/PLGA30. In vivo studies are ongoing in rat caudal vertebrae, to access the bone regeneration ability of the formulations.

**Conclusion**

The results validated this calcium phosphate cement as drug delivery system with PLGA particles as drug carriers. The improvement of the composite is ongoing to increase biologic efficiency of the matrix with magnesium ions, using MgCPC base. This appears as a very promising material for a local release of sodium alendronate to address osteoporosis or metastasis fracture sites, suitable with the clinician needs.
N02-05

Development of a siRNA-activated scaffold delivery system targeting proinflammatory signalling to promote regeneration of osteoarthritic cartilage

Tom Hodgkinson1, Domhnall Kelly1, Claudio Intini1, James E. Dixon2, Fergal J. O’Brien1,3

1 Royal College of Surgeons in Ireland, Tissue Engineering Research Group, Dept. Anatomy and Regenerative Medicine, Dublin, IE; 2 University of Nottingham, School of Pharmacy, Nottingham, GB; 3 Advanced Materials and Biomedical Engineering Research Centre (AMBER), Dublin, IE

Introduction

In osteoarthritis (OA), chondrocytes propagate and experience an altered physiochemical microenvironment, which hinders the success of biomaterial guided cell therapies and regenerative strategies. Functionally deficient pathological extracellular matrix (ECM) production and increased catabolism drive abnormal loading and further increase proinflammatory cytokine (e.g., interleukin-1B and tumour necrosis factor-α) production. Gene activated scaffolds targeting the transient attenuation of proinflammatory signalling have the potential to interrupt degenerative positive feedback loops while promoting anabolic processes to restore tissue functionality. In this study, we aimed to functionalise a chondrogenic freeze-dried collagen-hyaluronic acid (Col-Hya) scaffold (developed in the Tissue Engineering Research Group at the RCSI1) to deliver an immunomodulatory small interfering (si)RNA and increase scaffold effectiveness in inflammatory environments. For this, we investigated the targeted knockdown of connexin-43 (Cx43), a membrane channel protein that is significantly increased in OA cartilage (~40-fold) and has increasingly recognised roles regulating gene and protein activity pertinent to OA progression, including the activity of mechanosignalling molecule YAP (yes-associated protein) and proinflammatory transcription factor NFκB1,2,3,4. We developed and characterized a non-viral scaffold mediated Cx43 siRNA delivery system using a cell penetrating peptide with a glycosaminoglycan-binding domain (GET peptide5). Next, we used this siRNA delivery platform to investigate the effects of Cx43 knockdown on human articular chondrocyte (hAC) responses to OA-associated proinflammatory signalling (IL-1B and TNF-α) and the link between Cx43 and the YAP1-NFκB signalling axis in chondrocytes. Finally, we tested the ability of our siRNA-activated scaffolds to promote cartilage ECM production and healthy gene and protein expression in the presence of IL-1B and TNF-α in 3D culture models.

Experimental Methods

To optimise GET-Cx43 siRNA encapsulation and delivery, physiochemical characterisation (size, charge, morphology) and encapsulation efficiency assays were performed. Efficiency of siRNA knockdown in hACs was tested in 2D culture (quantitative reverse-transcription (qRT-)PCR, western blot). Optimised GET-siRNA formulations were then incorporated into Col-Hya scaffolds and controlled complex release profiled and Cx43 knockdown tested (qRT-PCR, western blot). Immunomodulatory effects of scaffold delivered siRNA were investigated through hAC culture with IL1b or TNFa and analysis of gene (qRT-PCR) and protein (western blot, immunohistochemistry, ELISA) expression and ECM production (histology, glycosaminoglycan (GAG) assay). The interactions of Cx43, YAP and NFκB were investigated through co-immunoprecipitation, small molecule inhibitors and promotors (YAP-Verteporfin, VP, 1 mM; Lysophosphatidic Acid, LPA, 20 mM) and immunofluorescent staining. The effect of scaffold-mediated Cx43 knockdown in responses to IL-1B and TNF-α in a 3D model were assessed by qRT-PCR, western blot, histology, GAG assay and ELISA over 28 days culture.

Results and Discussion

GET-siRNA complexes demonstrated favourable physiochemical and encapsulation properties. Complexes achieved successful Cx43 gene and protein knockdown in hACs in 2D culture (up to 90 % until 7 days culture).
Release profile analysis of GET-siRNA in Col-Hya scaffolds demonstrated successful complex incorporation, with Cx43 knockdown observed in scaffold invading hACs (>80% until 7 days) (Figure 1A). Cx43 knockdown reduced hAC responses to cytokine stimulation, increased healthy anabolic chondrocyte gene expression, GAG production and reduced catabolic responses (Figure 1B). In addition to reduced connexon-mediated cell-cell inflammatory signalling, co-immunoprecipitation experiments and immunofluorescent staining identified a protein-level interaction between Cx43 and YAP, a modulator of IL-1B and TNF-a (Figure 1C). In 3D models, IL-1B and TNF-a decreased healthy chondrogenic gene/protein expression and GAG production over 28 days chondrogenic culture. GET-Cx43 functionalisation decreased these catabolic cell responses and increased healthy ECM and GAG production over 28 days culture.

**Conclusion**

This study successfully functionalises a chondrogenic scaffold through incorporation of an immunomodulatory siRNA, allowing transient interruption of degenerative positive feedback loops while tissue functionality is restored. Using this scaffold-mediated non-viral siRNA delivery, we knocked down Cx43 expression in vitro, which decreased cellular responses to IL-1B/ TNF-a and promoted anabolic phenotypes. Further, we also describe a mechanistic link between Cx43 and IL-1N/ TNF-a signalling, identifying Cx43 as a potential therapeutic target in OA. The development of the siRNA-activated scaffold delivery system potentially offers a new paradigm in biomaterials in cartilage repair.

**Acknowledgement**

This study has received funding from the European Research Council under the European Community’s Horizon 2020 research and innovation programme under a Marie Sklodowska-Curie Individual Fellowship (MSCA-IF-EF-ST, Grant Number: 894837, ChondroCONNECT) and an ERC Advanced Grant (Number 788753, ReCaP).

---

**Figure 1**

A) QRT-PCR analysis of Cx43 gene expression in hACs following GET-Cx43 siRNA activated scaffold delivery (**p<0.01,***p<0.001, n=9) (B) GAG production in hACs after 28 days culture with and without 10 ng ml^-1 IL1b stimulation. IL1b stimulation decreased GAG production (*p<0.05, n=3) but Cx43 knockdown through GET-Cx43 siRNA activated scaffolds returned GAG production to positive control levels (*p<0.05, n=3) (C) Immunofluorescent staining of human articular chondrocytes (hACs) with and without Cx43 GET-siRNA knockdown showing increased nuclear YAP translocation with Cx43 knockdown.

**References**

IL-1 receptor antagonist enhances rhBMP-2-induced new bone formation during femoral fracture healing in rats

William A. Lackington\textsuperscript{1,2}, Dominic Gehweiler\textsuperscript{2}, Ivan Zderic\textsuperscript{2}, Dirk Nehr bass\textsuperscript{2}, Stephan Zeiter\textsuperscript{2}, Arlyng González-Vázquez\textsuperscript{3,4}, Fergal O’Brien\textsuperscript{3,4}, Martin Stoddart\textsuperscript{2}, Keith Thompson\textsuperscript{2}

\textsuperscript{1} Swiss Federal Laboratories for Materials Science and Technology (Empa), Biointerfaces Lab, St. Gallen, CH; \textsuperscript{2} AO Foundation, AO Research Institute, Davos, CH; \textsuperscript{3} Royal College of Surgeons in Ireland, Tissue Engineering Research Group, Dublin, IE; \textsuperscript{4} Royal College of Surgeons in Ireland, AMBER Centre, Dublin, IE

Introduction

Traditional methods to promote bone healing (e.g. autograft) and advanced therapeutics (e.g. recombinant human bone morphogenetic protein-2 [rhBMP-2]) have had a limited impact on easing the burden of a proportion (<20%) of patients with fractures who suffer from delayed healing or non-union. Increasingly, the response of the immune system to a fracture, and during the stages of bone healing, has been identified as a major regulator and, in some cases, as a predictor of healing outcome, shifting research focus towards the development of novel immunomodulatory therapies. Interleukin-1 receptor antagonist (IL-1ra), the natural antagonist of the potent pro-inflammatory cytokine IL-1b, has recently been shown to enhance bone healing in mouse calvaria. However, its therapeutic efficacy in long bone defects alone, or as an adjuvant in rhBMP-2 therapy, remains unknown. This study sought to determine the therapeutic efficacy of IL-1ra (2.5 μg) alone, or in combination with a low dose of rhBMP-2 (1 μg), in long bone defects when delivered using a collagen-hydroxyapatite scaffold (CHA) in a rat model.

Experimental Methods

Therapeutic efficacy was evaluated over 14 weeks using 2 mm segmental femoral defects in skeletally mature (20 – 24 week old) female F344 rats (n=8 per group), internally fixated using a 1.25 mm-thick polyetheretherketone RatFix plate (RISystem, Switzerland). Animals received either no treatment (empty defect), a CHA scaffold, CHA loaded with IL-1ra, or CHA loaded with a combination of IL-1ra + rhBMP-2. New bone formation within and around the defect site was monitored using \textit{in vivo} microCT and radiographs, immediately after surgery, and at 3, 4, 6, 8, 10, and 14 weeks post-op. Lateral and anteroposterior digitally reconstructed radiographs (DRRs) were generated from \textit{in vivo} microCT scans using Amira software to evaluate cortical bridging using a RUST-based scoring system, which is typically used in clinical practice to evaluate tibial fracture healing. The biomechanical resilience of newly formed bone was evaluated using non-destructive 4-point bending, while tissue morphology was evaluated by histopathological analysis, at 14 weeks.

Results and Discussion

Empty defects ultimately failed to bridge after 14 weeks (31±11% defect filled with new bone, 0/4 defects bridged). Treatment with CHA scaffolds led to a limited improvement in new bone formation (46±16%, 1/8 bridged) compared to empty defects, while IL-1ra (2.5 μg) loaded CHA scaffolds led to a similar outcome compared to empty defects (34±20%, 1/8 bridged) (Fig. 1). CHA scaffolds loaded with a combination of IL-1ra (2.5 μg) and rhBMP-2 (1 μg) led to superior bone healing (95±3%, 8/8 bridged) compared to CHA scaffolds loaded with rhBMP-2 (1 μg) (88±7%, 8/8 bridged), comparable to that of a higher 5 μg rhBMP-2 dose (93±4%, 8/8 bridged) (Fig. 1). The combination of IL-1ra (2.5 μg) and rhBMP-2 (1 μg) led to significantly accelerated bone formation at both week 4 and 6 compared to rhBMP-2 alone (1 μg) (Fig. 1). The combination of IL-1ra (2.5 μg) and rhBMP-2 (1 μg) also resulted in stiffer and more resilient new bone formation (16% failed the mechanical test) compared to CHA scaffolds loaded with rhBMP-2 (1 μg) (50% failed the mechanical test).

Conclusion

Page 160 of 2028
Taken together, this study is the first to determine that a single, locally administered, dose of IL-1ra can significantly enhance femoral fracture bone healing when used in combination with low dose rhBMP-2. This finding was observed in a clinically relevant internal plate fixation context, rather than the more widely used ExFix. This study suggests that the immunomodulatory effects of IL-1ra accelerates rhBMP-2-induced early bone formation, ultimately resulting in greater volume and stiffer new bone. This evidence supports the use of IL-1ra as an immunomodulatory strategy to enhance the efficacy of low dose rhBMP-2 therapy in future bone healing approaches.

Acknowledgement

WL, DG, IZ, DN, SZ, MS, and KT acknowledge funding from the AO Foundation. FO’B and AGV acknowledge funding from the European Research Council under the European Community’s Horizon 2020 Research and Innovation Programme under ERC grant agreement no. 788753 (ReCaP).

Figure 1. MicroCT imaging-based quantification of new bone formation after treatment with IL-1ra
Percentage of the defect site filled with new bone, excluding new bone formation outside the defect site. Data plotted are from individual animals with mean ± standard deviation indicated; * and ** denotes p<0.05 and p<0.01, two-way ANOVA.
Microwell-Based Engineering of the Bone Microenvironment through Ionic Calcium and Phosphate Signaling

Steven Vermeulen, Zeinab T. Birgani, Roman Truckenmüller, Pamela Habibovic

Maastricht University, MERLN Institute, Department of Instructive Biomaterials Engineering (IBE), Maastricht, NL

Introduction

Bone fractures or musculoskeletal disorders such as osteoporosis present a significant burden on individuals and the healthcare system [1]. A challenge in regenerative medicine is recapitulating the complex three-dimensional (3D) bone microenvironment in vitro to study these disorders or to develop regenerative therapies. This study applies a microwell-based platform to create aggregates of human mesenchymal stromal cells (hMSCs) and supplement media with inorganic compounds such as calcium (Ca$^{2+}$) and phosphate ions (Pi) to support differentiation. We found that combining a spheroid environment with inorganic signaling allowed rapid mineralization and induced a synergistic effect on osteogenic markers. This opens up possibilities for the generation of complex bone organoid models for regenerative medicine applications.

Experimental Methods

Polycarbonate arrays consisting of 500 µm diameter microwells were generated using film microthermoforming (Fig. 1A-B) [2]. The seeding of hMSCs inside these microwells allowed rapid aggregation into spheroids. 24h after cell seeding in micro-wells and standard polystyrene culture plates, cell culture medium was supplemented with either 4 mM Ca$^{2+}$ or 2 mM sodium phosphate dibasic dihydrate (Pi) with or without dexamethasone (DEX). RNA was extracted at 5, 10, and 20 days, after which osteogenic marker expression was assessed through quantitative PCR (qPCR). Quantification of mineralization was performed using OsteoImage staining.

Results and Discussion

To evaluate the effect of the 3D environment and Ca$^{2+}$ or Pi signaling on osteogenic marker expression, we harvested RNA and subsequently performed qPCR analysis. Targeting the osteogenic marker BMP2 [3], we found that already after 5 days an increase in expression levels occurred when cells were subjected to either Ca$^{2+}$ and Pi signaling on flat tissue culture plastic (Fig. 2A). Interestingly, the 3D spheroid microenvironment was sufficient to elevate BMP2 levels as compared to 2D culture, with Ca$^{2+}$ and Pi signaling not further enhancing expression. Similarly, Osteopontin (OPN) [4] was strongly elevated due to the spheroid environment (Fig. 2B). In contrast with BMP2, OPN demonstrated a further increase in expression levels when treated with the inorganic supplements. Of interest, DEX lowered both BMP2 and OPN expression levels when combined with Ca$^{2+}$ and Pi. Next to enhancing the expression of osteogenic markers, mineralization was induced as early as 10 days in hMSCs spheroids when treated with Ca$^{2+}$ or Pi (Fig. 2C). These findings support the notion that a spheroid environment, together with inorganic signaling, is an efficient way to enhance osteogenic differentiation.

Conclusion

Microwell-based spheroid formation of hMSCs in combination with either Ca$^{2+}$ or Pi supplements allows rapid mineralization and enhances the expression of osteogenic markers.

Acknowledgement

This work was financially supported by the European Union Interreg Vlaanderen-Nederland project “BIOMAT on microfluidic chip” (grant no. 0433). This research has been in part made possible by the Dutch Province of Limburg (program “Limburg INvesteert in haar Kenniseconomie/LINK”; grant nos. SAS-2014-00837 and SAS-2018-02477). PH gratefully acknowledges the Gravitation Program of the Netherlands Organisation for Scientific Research (NWO)
Figure 1: Microwells to generate MSC spheroids.
A) SEM image of a part of a 289 microwell array arranged in a honeycomb-like fashion. Scale bar represents 500 µm (up) and 100 µm (bottom). B) Profilometric image analysis of a fabricated microwell confirms the dimensions of 500 µm diameter and 300 µm depth.

Figure 2: Spheroid generation and inorganic supplements support an osteogenic phenotype in hMSCs.
A) Ca²⁺ and Pi improve BMP2 levels when MSCs are cultured on flat. In a spheroid environment, BMP2 levels are elevated without the need for additional stimulants. B) Synergistic effects on OPN levels are observed when the spheroid environment is combined with Ca²⁺ and Pi. DEX has a negative impact on BMP2 and OPN levels when combined with inorganic supplements. (* P<0.05; ** P<0.01; *** P<0.001). C) After 10 days, Ca²⁺ and Pi supplements strongly induce mineralization. Hydroxyapatite was visualized through OsteoImage staining. Scale bar represents 20 µm.

References
Hyaluronic acid based antibacterial injectable bionanocomposite containing green synthesized silver nanoparticles for bone tissue engineering

Assunta Borzacchiello1, Mario di Gennaro1,2, Pooyan Makvandi3, Ghereib W Ali4, Francesca Della Sala1, Wafa I. Abdel-Fattah4, Luigi Ambrosio1

1 National Research Council of Italy, Institute of Polymers, Composites and Biomaterials, Napoli, IT; 2 Università degli studi della Campania "Luigi Vanvitelli", Caserta, IT; 3 Istituto Italiano di Tecnologia, Pisa, IT; 4 National Research Centre, Inorganic Chemistry Division and Mineral Resources, Refractories and Ceramics Dept., Cairo, EG

Introduction
In bone tissue engineering, biomimetic scaffolds comprising calcium phosphate bioceramics, e.g. β-tricalcium phosphate (β-TCP), are aimed to serve as an artificial temporary extracellular matrix (ECM) in order to support cell adhesion and guide new bone tissue formation. The combination of bioceramics with organic materials, such as hydrogels containing hyaluronic acid (HA), naturally occurring polysaccharide that serves as a major component of extracellular matrix in mammalian connective tissues, is highly advantageous to overcome the brittleness of their intrinsic nature. Among the different types of hydrogels, thermosensitive hydrogels based on amphiphilic copolymers, such as Pluronics (polyethylene oxide-polypropylene oxide copolymers, PPO-PEO-PPO), thanks to their ability to undergo thermal gelation as well as low cytotoxicity, have attracted significant attention in biomedical applications and pharmaceutical industries [1,2]. However, infections during or post scaffold transplantation are still challenging which reduce the efficacy of bone healing. After the transplantation, infections may also be distributed to the scaffold from other sources of inflammation through bloodstream. Silver nanoparticles have shown a strong capability to inhibit or decline infections and have been also utilized for bone regeneration applications. Among different methods, biosynthesis of silver NPs, by using plant extracts, has received considerable attention due to the growing need to develop environmentally and non-toxic technologies [3]. On considering the properties of β-TCP, HA, Pluronic, and Ag NPs, our work was aimed to fabricate thermosensitive hydrogel biocomposites for bone tissue repair, which can be injected easily and possess antimicrobial properties to prevent infection.

Experimental Methods
Ag NPs were synthesized within corn silk extract (CSE) by microwave assisted method. The hydrogels were prepared by dissolving different amounts of HA, β-TCP, Pluronics F127 and F68 in CSE with and without AgNPs. The hydrogels composition was optimised by rheological analysis.

Results and Discussion
Fig. 1A shows the schematic illustration for synthesis and stabilization of Ag NPs by CSE. The absorption spectra of samples before and after microwave treatment are presented in Fig. 1B. Fig. 1C shows the results of DLS analysis of silver NPs and it can be seen that the prepared NPs have a narrow size distribution with a mean diameter of 49±2 nm. TEM image (Fig. 1D) shows well-dispersed silver nanoparticles which are spherical in shape with an average size of 13±1 nm. Rheological experiments demonstrated that the thermosensitive hydrogels have good mechanical properties with gelification temperature (Tgel) close to body temperature (Fig. 2A). The systems showed antibacterial activity toward gram-positive (Bacillus Subtilis, Staphylococcus Aureus) and gram-negative (Pseudomonas Aeruginosa, Escherichia Coli) bacteria (Fig. 2B). L929 cells exhibited a noncytotoxic and typical mouse fibroblast-like cellular morphology after 24 h of the incubation with the samples (Fig. 2C and D). Schematic representation of injection of the thermosensitive nanocomposite are presented in Fig. 2E. Thermosensitive hydrogels, which
possess good retention at the application site, have appealed a great deal in biomedical and clinical fields. At room temperatures, the systems are liquid and could be injectable whereas, at the body temperature, they became a gel. This fast sol-to-gel phase transition behavior is beneficial for cell entrapment to give a uniform distribution of cells within the gelled matrix. HA offers many advantages as a tissue scaffold which including biodegradability, biocompatibility, and bioresorbability which have been shown in many studies. Hyaluronic acid could activate cell surface receptors, influencing intracellular signaling cascades for cell growth, migration, proliferation, and differentiation [4]. One of the main reasons of scaffold failures is due to the implant-associated bacterial infections. Hence, using antibacterial scaffolds helps more success for bone tissue formation which are of clinical importance. In this study, we used spherical particles of Ag to impart antibacterial activity to the thermosensitive hydrogels to avoid bacterial contamination [5].

**Conclusion**

In conclusion, silver NPs were biosynthesized in an aqueous medium of corn silk extract without using toxic chemical reagents. The new thermosensitive HA-based nanocomposite hydrogels demonstrated good mechanical properties with \( T_{\text{gel}} \) close to the body temperature. The system revealed desired antibacterial activity against several gram-positive and gram-negative bacterial strains which can prevents bacterial infection. In addition, from the biological point of view, the nanocomposites revealed appropriate biocompatibility in comparison with the control samples.

**Acknowledgement**

The authors acknowledge the research project "ADVISE DRUGS AND ANTI-TUMORAL VACCINES FROM THE SEA" - POR CAMPANIA FESR 2014-2020 AND WITH THE AXIS 1 00.SS. 1.2.2 / 1.1 CUP B43D18000240007"
Figure 2

The sol-gel phase transition by rheological experiments. Elastic and viscous moduli as a function of the temperature at a frequency value of 0.01 Hz. (B) Antibacterial activity of different concentration of Ag NPs against Gram-positive (B. subtilis and S. aureus) and Gram-negative (P. aeruginosa and E. coli) bacteria after 24 h. Cell morphology (C) and cyrotoxicity (D) of samples after 24 h. (E) Schematic representation of injection of the thermosensitive nanocomposite containing β-tricalcium phosphate for bone tissue regeneration.

References

N02-09

Targeting macrophages to modulate inflammation in cartilage degeneration: using poly(lactic-co-glycolic acid)-based nanoparticles for ibuprofen delivery

Kaoutar Chattahy1,2, Catarina L. Pereira1,2, Flávia Sousa1,2, Cristiana Couto1,2,4, Meriem Lamghari1,2,4, Mário A. Barbosa1,2,4, Bruno Sarmento1,2,3, Raquel M. Gonçalves1,2,4, Susana G. Santos1,2

1 Universidade do Porto, i3S – Instituto de Inovação e Investigação em Saúde, Porto, PT; 2 Universidade do Porto, INEB – Instituto de Engenharia Biomédica, Porto, PT; 3 CESPU- Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Gandra, PT; 4 Universidade do Porto, ICBAS- Instituto Ciências Biomédicas Abel Salazar, Porto, PT

Introduction

Osteoarthritis (OA) is the most prevalent chronic joint disorder worldwide, leading to cartilage destruction, pain, loss of function, and disability(1). Controlling inflammation and pain are major challenges in OA therapy, as infiltrating macrophages contribute to the inflammatory joint microenvironment(2). The use of nanocarriers improves drug retention and targeting, providing effective local therapeutic dosages(3). Polylactic acid-glycolic acid (PLGA), has been widely used as a drug carrier. PLGA nanoparticles are degradable, biocompatible, and can be produced to deliver different kinds of drugs(4). Herein, we propose the development of PLGA-based nanoparticles (NPs) loaded with clinically used ibuprofen, as a strategy to control the inflammatory response, thus improving the microenvironment for cartilage repair. The hypothesis underlying this work is that macrophages can be effectively modulated by delivering ibuprofen encapsulated in PLGA NPs.

Experimental Methods

PLGA-based NPs were prepared by the nanoprecipitation method. The organic phase (PLGA + Ibuprofen in acetone) was added to the aqueous phase (Poloxamer 407 in water) under stirring and NPs were purified by subsequent washes. Formulations containing theoretical drug loadings of 5, 10 and 20% were produced to achieve the highest drug loading. Association efficiency and drug loading were quantified by a validated High-Performance Liquid Chromatography (HPLC) method. NPs were characterized for size, Pdl and charge by Dynamic Light Scattering (DLS). NP ibuprofen drug release profile was addressed in PBS (pH 7.4) at different time points (0, 4, 24, 48, 72 and 168h). Samples were collected and centrifuged (20 min at 9600g), and ibuprofen in the supernatant was quantified by HPLC. Human monocytes were obtained from buffy coats (donated by Serviço de Immunohemoterapia, CHUSJ-Centro Hospitalar Universitário de São João), differentiated to macrophages using M-CSF, and stimulated at day 10 towards pro-inflammatory (M1) phenotype with LPS+IFNγ. Ibuprofen-loaded PLGA NPs were added to M1 macrophages, to achieve 15 or 30 μg/ml of ibuprofen. Equivalent amounts of empty PLGA NPs and the same concentrations of free Ibuprofen were used as controls. NP internalization was characterized at day 12, by cell surface labelling (CD14, CD86, HLA-DR, CD163) and conventional or imaging flow cytometry. PGE2 and cytokines (IL-10, IL-1β and TNF-α) secretion were quantified in culture supernatants, using commercial ELISA kits.

Results and Discussion

Ibuprofen loaded PLGA NPs presented an average size by intensity of 206±2.9 nm, a Pdl of 0.06±0.02, and zeta potential was -1.77±0.25mV. Results showed that differences in association efficiency (AE) and effective drug loading were less pronounced in 20% formulation. Thus, the formulation of Ibuprofen loaded PLGA NPs with 20% of
theoretical drug loading and 75% of AE was used for further experiments. Drug release experiments demonstrated an initial burst release (30%) and extended drug release up to 40% after 7 days. PLGA NPs were readily internalized by up to 75% of cells, without significant impact on cell morphology. Low LDH release indicates that NPs are not cytotoxic towards macrophages after 72h of incubation. Upon macrophage M1 polarization, treatment with Ibuprofen loaded PLGA NPs, particularly the 30 μg/ml concentration, reduced cell surface levels of M1 markers CD86 and HLA-DR, when compared with untreated M1 stimulation, and free Ibuprofen treatment. Levels of secreted PGE2, in response to M1 stimulation, were reduced by treatment with Ibuprofen loaded PLGA NPs, using both concentrations, albeit not as significantly as for free Ibuprofen. Cytokine secretion profile for all samples is currently being analyzed to determine the impact of Ibuprofen loaded PLGA NPs on the modulation of macrophage secretory profile.

Conclusion
PLGA NPs are internalized by macrophages without cytotoxicity, and are suitable carriers for Ibuprofen, acting to modulate macrophage pro-inflammatory response, and the production of key mediators of cartilage-associated inflammation and degradation.

Acknowledgement
The authors thank Serviço de Immunohemoterapia, CHUSJ, for kindly donating buffy coats, and i3S Translational Cytometry and Bioimaging platforms. This study was supported by the European Union’s Horizon 2020 research and innovation program under grant agreement number 814558 project Restore.

References
1:00 p.m. – 2:00 p.m.

Track 01

**YSF GA | YSF General Assembly**

**Chairs**

**Karine Anselme**  
Institute of Materials Science of Mulhouse, FR

**David Barata**  
Instituto Medicina Molecular, Lisboa, PT

**Aldo R. Boccaccini**  
University of Erlangen-Nuremberg, DE

**Nicola Contessi Negrini**  
Imperial College London, London, UK

**Liliana Liverani**  
University of Erlangen-Nuremberg, Institute of Biomaterials, Erlangen, DE

**Arn Mignon**  
KU Leuven, Engineering Technology, Group T, Leuven, BE

**Liliana Moreira Teixeira**  
University of Twente, Developmental BioEngineering, Enschede, NL

**Elena Maria Varoni**  
University of Milano, IT
1:15 p.m. – 1:45 p.m.

Track02

Social Activity 1 | Yoga

Prof. Filipa Ribeiro will give 30min yoga class at lunch time.

Filipa Ribeiro

YOGA SHALA MATOSINHOS, Matosinhos, Portugal
2:00 p.m. – 3:30 p.m.

Track01

**TRS-S02 | Translation Research Symposia**

**Chairs**

**Fabrizio Barberis**  
Universita' degli Studi di Genova, Department of Civil Chemical and Environmental Engineering, Genova, IT

**Yves Bayon**  
Medtronic, Trévoux, FR

**Peter Dubruel**  
Department of Organic and Macromolecular chemistry (Chair); Polymer Chemistry and Biomaterials Group Leader; ESB Industry Liaison Delegate; Ghent University, BE

*Start-up investment rounds are fundamental to boost business toward the market. In the biomedical field start-ups move in a highly competitive international scenario, therefore financial support must be framed in an effective scaling-up strategy.*

*The speakers, based up on their first-hand experience on these topics, will discuss these items with ESB scientists and researchers.*
TRS-S02-01

BestHealth4U (Porto, Portugal)

Sónia Ferreira

BestHealth4U, Braga, PT

TRS-S02-02

Bill Barber consulting (USA)

Bill Barber

Bill Barber Consulting, Los Angeles, California, US

TRS-S02-03

Zcube srl (Italy)

Fabrizio Conicella

Zcube srl, Bresso, Italy, IT

TRS-S02-04

EIC Health (Belgium)

Andreas Lymberis

EIC Health, Brussels, BE

TRS-S02-05

EIT Health Spain (Spain/Portugal)

Irene Sánchez

EIT Health Spain, Barcelona, ES
2:00 p.m. – 3:30 p.m.

Track02

**K02 | Organ-on-a-Chip Systems in Precision Medicine**

**Chairs**

**Meriem Lamghari**
INEB - Instituto de Engenharia Biomédica, Porto, PT

**Roman Truckenmüller**
Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, NL

**Michael Joyce (YSF)**
Royal College of Surgeons in Ireland, Anatomy and Regenerative Medicine, Dublin, IE
K02-KL01

Application of Organ-on-a-Chip Systems in Precision Medicine

Peter Ertl

Technische Universität Wien, Technical Chemistry, Wien, AT

Personalized medicine is broadly defined as the use of information from a patient’s genotype to either initiate a preventative measure against the development of a disease or the ability to select the most appropriate therapy that is particularly suited to a patient. Despite the benefits of using DNA based personalized diagnostic approaches, in many cases the underlying genetic risk factors are too many to reliably predict cellular responses to drug treatments. To overcome this challenge, next generation personalized diagnostic tools also need to make use of information obtained from living cell assemblies that resemble the (patho)physiological microenvironment of individual patients to accurately predict disease onset, progression and remission. In course of the presentation current state-of-the-art of personalized diagnostics will be reviewed and three different organ-on-a-chip applications in precision medicine introduced including cancer drug screening, chronic inflammation research and neurodegenerative diseases.
K02-02

Stereolithography-based 3D-printing of transparent and biocompatible microfluidics for Organs-on-a-Chip applications

Anna Fritschen¹, Alena K. Bell²,³, Lukas Stühn², Robert W. Stark², Andreas Blaeser¹,⁴

¹ Technical University of Darmstadt, Department of Mechanical Engineering, BioMedical Printing Technology, Darmstadt, DE; ² Technical University of Darmstadt, Institute of Materials Science, Physics of Surfaces, Darmstadt, DE; ³ Technical University of Darmstadt, Collaborative Research Center (CRC) 1194 – Interaction between Transport and Wetting Processes, Project A07, Darmstadt, DE; ⁴ Technical University of Darmstadt, Centre for Synthetic Biology, Darmstadt, DE

Introduction

Soft-lithography methods are routinely applied in biomedical research, for instance for the fabrication of microfluidic devices, multichannel bioprinting print heads and Organs-on-a-Chip. While soft-lithography offers excellent resolution, it is a complex process and geometrically limited. Transparent 3D-printing could overcome these challenges by enabling complex 3D-shapes, round features and single step fabrication of closed channel systems. However, to compete 3D-printed microfluidics need to comply with multiple requirements, such as low surface roughness, good optical transparency, highly resolved features and high cytocompatibility. Stereolithography printing has proven to be a suitable method for this purpose, as it offers excellent resolution (down to 30–40 µm) and is generally suited to process transparent and biocompatible materials. However, sufficient transparency for high magnification and fluorescence microscopy, closed channel fabrication and long-term cytocompatibility currently remain a challenge.

Experimental Methods

In order to fabricate 3D-printed components that fulfil the above-mentioned criteria, we established a novel stereolithography strategy based on a modified Asiga Pico ²HD² printer. We investigated a commercial resin (Asiga PlasClear) and two own PEG-DA formulations. To reduce surface roughness and bulk defects, modifications of the build plate and resin vat were realized. The surface quality, optical transparency and chemical composition of the prints were characterized by confocal microscopy, AFM as well as UV/VIS and Raman spectroscopy. Finally, cytocompatibility of the applied materials was fluorescence microscopically assessed using primary endothelial cells and fibroblast cell lines.

Results and Discussion

The confocal microscopy and UV/VIS spectroscopy results revealed a significant advantage of the build plate and resin vat modification compared to a non-modified printer. All prints exhibited low surface roughness of less than 0.3 µm and superior optical transparency of over 90% in the visible light wavelength range (400 – 800 nm). However, during light microscopy, a periodic structure was visible in all printed parts indicating interference patterns caused by the voxel illumination during DLP. Additional AFM microscopy supported this assumption, as the dimensions of the surface features were of the size of the DLP pixels of 27 µm. Interestingly, the height of these periodic features were found to be dependent on the photoinitiator (BAPO) and -sensitizer (ITX) concentration and varied between 40 and 400 nm. Even more, the presence and concentration of photoinitiator and photosensitizer proved crucial for optimum print resolution and defined channel geometries. For this reason, different combinations and concentrations of the photoinitiator and photosensitizer were systematically investigated regarding minimum feature size and general printability. The commercial resin proved to be easy in use and offered a minimum feature size of 200 µm. Open channel structures could be achieved with a diameter of down to 350 µm. For the PEG-DA based resins, prints without photosensitizer exhibited low spatial resolution and low channel quality. The addition of low concentrations
of photosensitizers led to clearly defined prints with minimum feature sizes of 150 µm outperforming the commercial resin. Cytocompatibility tests with primary endothelial cells and established fibroblast cell lines and Raman spectroscopy revealed the need for post-fabrication treatments such as prolonged solvent extraction and UV-exposure to remove toxic photoinitiator and photosensitizer residues. Additionally, resin formulation and photoinitiator concentration were found to further impact post-fabrication cytocompatibility. Finally, high magnification phase contrast and fluorescence microscopy (up to 400 x) could be successfully demonstrated with various fluorescent dyes. Still, the commercial resin was shown not to be compatible with DAPI staining, as the cured parts absorbed most of the light with wavelength below 400 nm, which lies within the excitation wavelength band of DAPI. Finally, the printed microfluidic chips were used in Organ-on-a-Chip models containing cells directly cultured on the surface of parts as well as in a hydrogel matrix. Cell culture under perfusion was feasible for various days with cells showing the expected morphology under perfusion.

**Conclusion**

In summary, our work reveals that DLP printing is a versatile and reliable platform for the fabrication of transparent microfluidic devices that entail high feature resolution, optical clarity and cytocompatibility. Even more, we could prove that printer modification and resin optimization can be advisable to fully exploiting its potential. These qualities make DLP printing a promising technology for rapid prototyping of microfluidic components, customized cell culture dishes and Organ-on-a-Chip devices.

**Acknowledgement**

The authors thank the Deutsche Forschungsgesellschaft (Project ID 265191195 – SFB 1194, Project A07 and Sachbeihilfe DI 2176/2-1) for financial support.
**Introduction**

It is estimated that 10% of the worldwide population suffers from chronic kidney disease (CKD) with a rising tendency. [1] Patients with CKD have limited treatment options, with fibrosis being the pathological endpoint of CKD, where an increase of ECM deposition is normally observed. [2,3] Therefore, novel therapies that could halt or even reverse the progression of CKD are urgently needed. [1] Bioprinting is considered one of the most promising approaches to generate novel 3D *in vitro* models and organ-like constructs [4], which can offer viable alternatives to investigate underlying pathomechanisms and progression of kidney diseases. [5] In this work, we aim to establish a robust protocol for the isolation of primary kidney cells from a transgenic reporter mouse, showing keratin 8 conjugated with a yellow fluorescent protein (K8-YFP), and to test their suitability for bioprinting.

**Experimental Methods**

A method to isolate primary renal cells was established (*Figure 1a*). Primary murine tubular epithelial cells (pmTECs), endothelial and fibroblast (pmFibroblasts) cells were successfully isolated, but further optimization is required for the culture and expansion of primary endothelial cells. These cells were used in a new bioprinting platform laying the foundation for the development of a 3D renal tubulointerstitium model for *in vitro* studies. Polysaccharide biomaterial inks were characterized in terms of viscosity and printability. Endothelial cell line (HUVECs) and pmTECs were combined with polysaccharide biomaterial ink (alginate and pectin) and processed with a microfluidic 3D bioprinter. Cell viability and metabolic activity were evaluated for co-culture conditions. The production of core-shell bioprinted constructs was investigated by including HUVECs in the shell and pmTECs in the core.

**Results and Discussion**

As tubular epithelial cells are the major cell type in the kidney, we primarily isolated these cells. The established method allowed a successful isolation of pmTECs, fibroblast and endothelial cells, but further culturing was only successful for pmTECs and pmFibroblasts. pmTECs and pmFibroblasts were positive for cell-specific markers (*Figure 1b*). Immunofluorescence of keratin expression showed increase expression in fibrotic tissue (*Figure 1c*), as well as a different cell morphology in diseased tissue (*Figure 1d*). The processing parameters to bioprint polysaccharide based biomaterial inks were optimized. We tested the optimized bioprinting protocol with bioinks containing pmTECs and HUVECs, investigating cell survival and metabolic activity of the bioprinted constructs, leading to cell viability above 90% after one week in culture. Finally, bioinks were processed in a core-shell arrangement to mimic the tubulointerstitium, where the peritubular capillaries wrap the renal tubule, showing high accuracy in cell deposition (*Figure 2a*), dimension of the bioprinted constructs and capability to produce hollow filaments (*Figure 2b*).

**Conclusion**

Microfluidic bioprinting strategy was used to build a novel 3D kidney *in vitro* model presenting primary murine tubular epithelial cells bioprinted for the first time. This model will facilitate the investigation of the interstitial fibrosis, whose underlying mechanisms are currently not completely understood. This study lays the basis for an alternative 3D *in vitro* model for the investigation of mechanisms, potential therapies and the development of renal fibrosis.
Acknowledgement

We are grateful to the Dutch Kidney Foundation (Nierstichting Nederland, grant 18OI17 – Innovation Call 2018) and to the funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860715. We also acknowledge support from the Dutch Province of Limburg. This study was financially supported by the German Research Foundation (DFG: SFB/TRR57, SFB/TRR219, BO3755/3-1, BO3755/6-1 to PB and DJ100/1-1 to SD) and the German Ministry of Education and Research (BMBF: STOP-FSGS-01GM1901A) to PB and SD. Carmen. G. Tag (Division of Gastroenterology, RWTH Aachen, Germany) is acknowledge for FACS support.

Figure 1: a) Isolation procedure for pmTECs, endothelial cells and pmFibroblasts from K8-YFP mice. b) pmFibroblasts and pmTECs were positive for cell-specific markers (inlays as negative control, scale bar=25 μm). c) Immunohistochemistry of keratin (K) 8 in wildtype mice showed increased expression in fibrotic kidney compared to healthy contralateral kidney (scale bar=100 μm). d) pmTECs from healthy kidneys showed fine fibers of the keratin network and cobblestone formation, whereas pmTECs from fibrotic model showed a more spindle shaped morphology (scale bar=100 μm).
Core-shell bioprinting

Figure 2: Core-shell bioprinted filaments made with blue-stained HUVEC as part of the shell and green-stained pmTEC as part of the core. a) Individual samples where imaged on day 0 (after bioprinting), day 1, 2, 7, 14, 21 and 28. b) Confocal images of the core-shell filaments showing the formation of the hollow filament during the culture period, from day 0 to day 14 (scale bar=200 μm).

References
K02-04

3D Bioprinting of prevascularized full thickness constructs with an endothelialization of hollow channels

Bastian Böttcher, Karl-Heinz Feller
Ernst-Abbe-Hochschule Jena, IMPT Jena, Jena, DE

Introduction
The 3D printing of cell-laden hydrogels allows the creation of complex three-dimensional structures with ECM similar materials and a spatial arrangement of different cell lines. Consequential in vivo alike 3D cultures with different functional regions can be fabricated. However, a major drawback in current 3D models is the insufficient nutrient supply due to high distance between cells and surrounding medium. To reduce the subsequent concentration gradient, the integration of prevascular structures is helpful. Coaxial extrusion allows printing hollow structures with an on-site delivery of cells around the channel. Herein we investigated the effect of prevascularized structures in small full-thickness constructs made of a gelatin/alginate hydrogel by investigating if it is beneficial for cell survival and if HUVEC can form an endothelium when printed around the channel.

Experimental Methods
3D printing of the prevascularized constructs was achieved using an extrusion-based bioprinter by combining pneumatic cartridge dispensers and coaxial printing tools. The whole cell-laden gelatin/alginate construct was made in a three-step process. Firstly, a base layer was printed that served as a substrate for other gel components. Afterwards a coaxial printed strand was printed while the core consisted of a CaCl$_2$-laden gelatin that crosslinked the surrounding gelatin/alginate gel during printing. These coaxial printed struts were printed in a parallel manner. The space between these layers was filled in the third step with another cell-laden gel. For investigating the effect of prevascularization on cell viability, only HepG2 cells were used in all cell-laden gels, while HUVEC instead of HepG2 were printed in the shell of the coaxial printed strand for determination of a possible endothelialization. A day after printing, the cell-laden constructs were treated with sodium citrate and investigated at different time intervals for cell specific activity.

Results and Discussion
The treatment of the cell-laden constructs with sodium citrate lead to an improvement of cell survival for every cell line used. In general, the cell viability remained higher with greater concentration of sodium citrate used. That might be connected to the removal of bio-inert alginate causing an improved diffusion and a higher porosity. When prevascularized structures were integrated into full thickness constructs, an increase in cell viability compared to non-prevascularized constructs could be observed. However, with prolonged cultivation times this effect was extenuated. When HUVEC were delivered into the shell of the coaxial printed strand an endothelialization occurred, though it took several weeks until the inner channel wall was covered with HUVEC, as can be seen in the figure. Consequential coaxial extrusion allows the creation of hollow channels with an endothelium.

Conclusion
The integration of hollow strands into full thickness constructs using coaxial extrusion is an appropriate method for improving nutrient supply. When HUVEC were delivered around the hollow strand, an endothelia was formed that might be a basis for angiogenesis processes to further improve nutrient supply.

Acknowledgement
The authors would like to thank the BMBF for funding (funding number: 13FH134IN6).
Endothel cells grown in a hollow channel
Figure: Immunofluorescence staining of HUVEC in a prevascularized construct with a co-culture after four weeks. Picture A: lower region of the hollow strand; B: central area; C: upper region. Red staining: CD31; Blue staining: DAPI
Microfluidic-Driven Hydrogel Biofabrication for Engineering Functional Microtissue Architectures

Carlos F. Guimarães, Luca Gasperini, Alexandra P. Marques, Rui L. Reis

Introduction

The microfluidic manipulation of hydrogels can be a powerful tool for the biofabrication of cell-laden, soft fiber-like structures that can be used as building blocks to engineer live-like tissues by approaching their shape and intricate mechanics [1,2]. In the past decade, a wide range of microfluidic setups, chip designs, and printing nozzle configurations have been employed to fabricate structures with varied shapes and compartment organizations, requiring equivalently complex arrangements to be individually assembled for each structure. Simultaneously, most current microfluidic hydrogel fabrication strategies result in continuous structures with uniform architecture, which fail to approach the complex space-varying shape and mechanics that exist in several biological tissues [2]. We have demonstrated that the microfluidic manipulation of hydrogel flows and controlled laminar mixing can be employed for the sequential, high-throughput fabrication of 3D hydrogel cell-laden gradients. These can integrate broad ranges of mechanical properties and material characteristics, such as adhesiveness, biodegradation, and multiple crosslinking. We further explored how these gradients can be used as libraries for high-content screening cell responses to varying 3D environments (Figure 1), ranging from cell adhesion and proliferation to more complex triggering of adipogenic versus chondrogenic differentiation and mechanotransduction [3].

Experimental Methods

Now, we demonstrate a novel biofabrication technique with unprecedented versatility for the creation and tuning of micro-bioarchitectures within 3D hydrogel fibers. We show how one single 3D hydrodynamic flow-focusing chip can be employed to obtain a continuous flow of hydrogel precursors, which organize themselves based on their viscosity and inlet pressure, crosslinking into fibers with a plethora of multi-compartment shapes (Figure 2) [4]. We outline how the material's viscosity and microfluidic channel configuration can be adjusted in order to continuously spin fibers with over 20 different microarchitectures. We further present how the different materials and flows can be tuned to control compartment and fiber sizes down to full diameters below 50µm. To the best of our knowledge, this size represents the smallest multi-compartment hydrogel fibers so far fabricated, approaching the size magnitude of single cells and small vascular structures while simultaneously maintaining the designed architecture at downsized scales.

Results and Discussion

To prove the potential of our system for the biofabrication of complex, multi-compartment structures, we explored ribbon-like cancer/basement-membrane/stroma models for creating a novel cancer-on-a-fiber platform. The effect of 3D architecture and designed cancer microenvironment components (Stroma and Basement Membrane) significantly affected cancer cell's response to doxorubicin, enabling a more faithful in vitro recapitulation of therapeutic approaches. We also leveraged flow-focused core-shell architectures to continuously fabricate vascular-like structures and assemble them in 3D biological networks, a facile approach for large-length vascularization of engineered tissues that can be used to accelerate construct vascularization in multiple settings. We further combined hydrogels and hydrophobic molecule depots as all-in-one tissue engineering constructs, simultaneously integrating stem cells, biomaterials, and pro-differentiation factors. The gradual release of purely hydrophobic dexamethasone
transported in isolated droplets with tunable distribution was capable of triggering stem cell differentiation upon release, doing so to a higher extent than that of its soluble counterpart, which is commonly dissolved in media.

**Conclusion**

The innovative structures we report open several avenues in the hydrogel biofabrication fields, namely those focusing on creating evermore complex biological architectures and micro-modeling 3D environments of health and disease. Additionally, our discovery’s versatility via the use of one single chip might lead to the widespread application of 3D microfluidic flow-focusing as a new biofabrication technique on its own, with a myriad of possibilities for the fields of clinical and experimental tissue engineering.

**Acknowledgement**

The authors acknowledge Advanced ERC-2012-AdG-20120216-321266 project ComplexITE. CFG acknowledges support from FCT grant PD/BD/135253/2017.

---

**References**


K02-06

3D reactive inkjet printing of peptide based hydrogels for corneal tissue engineering

Georgia L. Duffy¹, He Liang¹, Don Wellings³, Kate Black², Rachel Williams¹

¹ University of Liverpool, Eye and Vision Science, Liverpool, GB; ² University of Liverpool, School of Engineering, Liverpool, GB; ³ Spheritech Ltd., Runcorn, GB

Introduction
The human cornea is the window to the eye, and acts to focus light onto the retina. It is composed of three main layers: a stratified epithelium, the stroma, and a monolayer of endothelial cells. Corneal opacities are the 4th leading cause of blindness worldwide, with the only current solution being a corneal transplant from a cadaveric donor. This treatment method has several limitations, such as the limited availability of tissue; which has prompted research into finding suitable alternatives.

3D reactive inkjet printing is an additive manufacturing method where more than one ink is printed together, reacting to form a solid structure. This is a method which can be utilised for tissue engineering applications due to its high resolution and capability of printing cells into the 3D construct. This work aimed to use reactive inkjet printing of natural polymers poly-ε-lysine (pεK) and gellan gum (GG), to form a porous hydrogel construct based on their electrostatic interaction. The hydrogel formed was analysed for its suitability in corneal tissue engineering.

Experimental Methods

Ink characterisation: 20 wt % pεK and 0.35 wt % GG were made up in DI water and their rheological properties characterised. Viscosity, density, contact angle and surface tension measurements were performed to establish the printability of the inks using the Ohnesorge (Oh) equation.

3D printing: Track optimisations were performed to establish the optimal speed and step spacing for the polymer inks. An ideal waveform was designed for each ink to achieve a stable droplet prior to printing. Using the parameters, hydrogel arrays were printed, and bitmaps were designed to print porous constructs.

Imaging of printed constructs: Hydrogel constructs were imaged using optical microscopy (Nikon Ti-E fluorescent microscope) and scanning electron microscopy (Hitachi S4800 SEM). Square arrays and porous constructs were imaged at 1, 3 and 5 passes of each ink to assess the hydrogel microstructure and quality of the print.

Cell culture on cast pεK/GG constructs: Human corneal epithelial cells (HCE-Ts) and human corneal endothelial cells (HCEC-12s) were seeded onto cast hydrogels made with 20 wt % pεK and 0.35 wt % and 0.75 wt % GG. They were also seeded onto TCP as a positive control. The cells were cultured for 7 days and then fixed and stained with DAPI and Phalloidin to analyse their morphology.

Results and Discussion

Ink characterisation: Both 20 wt % pεK and 0.35 wt % GG showed rheological properties suitable for inkjet printing. The Z numbers (Z= 1/Oh) were equal to 9 and 5 respectively, which places them both in the ‘printable range’ of 1-14 [1,2].

Surface structure: Optical imaging of printed arrays showed a unique pattern formed in the hydrogel surface, where the instantaneous reaction of the polymers causes the formation of craters across the surface, Figure 1. This patterning was seen in all printed constructs, from 1 pass per ink up to 5 passes per ink. SEM imaging was done at high magnification on 1 and 5 passes of ink and the crater-like structure can also be seen here.

Porous structure: Optical microscopy and SEM demonstrated that porous circular structures could be printed based on bitmaps.
Cell culture: HCE-Ts formed a monolayer across the surface of the cast hydrogels with both wt % of GG, demonstrating cyto-compatibility of the hydrogels with corneal epithelial cells, Figure 2. HCEC-12s formed a monolayer across the surface of the lower wt % GG hydrogel but fewer cells attached to the surface of the 0.75 wt % GG variant. This suggests the relatively higher amount of the pεK is important to support the cell attachment.

Conclusion
We have shown that it is possible to print porous hydrogel constructs based on the natural polymers poly-ε-lysine and gellan gum using reactive inkjet printing methods. These hydrogels display a unique surface patterning due to the instantaneous reaction between poly-ε-lysine and gellan gum. Preliminary cell culture data on cast hydrogels with the same chemistry demonstrated that both corneal epithelial and endothelial cell lines formed a monolayer across the hydrogel surface, demonstrating the materials cyto-compatibility. Future work includes further characterisation of the printed construct, including physical and mechanical characterisation and further cyto-compatibility testing with human corneal cells.

Figure 2
DAPI and Phalloidin staining of HCE-Ts seeded onto cast pεK/GG constructs and Tissue culture plastic (TCP) for 7 days. Images using a Nikon Ti-E fluorescent microscope. 20X magnification. Scale bars equal 100 µm. Blue stain shows nuclei of cells and green stain shows cytoplasm.

Figure 1
Optical micrographs of 75 mm² printed array on a glass substrate A) 1 pass B) 3 passes C) 5 passes of each ink. Magnification 4 X Scale bar = 500 µm. D) SEM micrographs of 75 mm² printed array on a glass substrate with 1 and 5 passes of each ink. Magnifications = 100 X and 250 X Scale bars = 500 µm and 200 µm.

References

Page 185 of 2028
K02-07

3D Bioprinting in Self-Assembled Nanoparticles to Fabricate Microphysiological Systems for Tissue/Organ-On-A Chip Applications

Syeda M. Bakht¹,², Manuel Gomez-Florit¹,², Tara Lamers¹,², Milan Sixt¹,², Bárbara B. Mendes¹,², Rui L. Reis¹,², Rui M. A. Domingues¹,², Manuela E. Gomes¹,²

¹ I3Bs – Research Institute of Biomaterials, Biodegradable and Biomimetics, Guimarães, PT; ² ICVS/3B’s – PT Government Associate Laboratory, Braga/Guimarães, PT

Introduction
Organ/Tissue-on-chip have seen an emerging interest in developing biomimetic systems that emulate relevant physiological micro-environment of cells to understand physiological or pathological mechanisms. However, the majority of these systems are fabricated with polymeric materials lacking the ability to imitate cell-cell and cell-extracellular matrix (ECM) crosstalk prevailing in living organisms. These conventional miniaturized microfluidic devices not only require a cumbersome manufacturing process but also have complicated loading processes for cells and biomaterials, none have yet proven to be sufficiently efficient, feasible, or economical. Here, we present a unique strategy to efficiently produce 3D microfluidic fibrillar devices as a green alternative to conventional polymeric materials. The proposed system combines 3D printing of sacrificial/cell-laden inks with the controlled self-assembly of colloidal suspensions of cellulose nanocrystals (CNC) to form non-degradable and fibrillar hydrogel via ionic crosslinking in biocompatible conditions. This system allows the fabrication of complex and perfusable multicellular constructs with high resolution and can support their long-term culture. The transparent CNC fibrillar hydrogel has the capacity to withhold perfusable complex channels and chambers for dynamic culture, which make them ideal for 3D free-form bioprinting to fabricate microphysiological systems for tissue/organ-on-chip applications.

Experimental Methods
CNC fluid gel properties were optimized for matrix-assisted 3D printing to define the printability window by combining different CNC and crosslinking agent (Ca²⁺) concentrations. Characterization of CNC fluid gel viscoelastic properties were performed by rheometry. BioX 3D bioprinter (Cellink) was used to print cell-laden/sacrificial inks. Post printing, CNC fibrillar self-assembly was induced with excess of Ca²⁺ to create stable constructs with embedded 3D printed freeform structures and perfusable channels. To demonstrate the versatility of CNC as support material for embedded 3D printing/bioprinting, various ink hydrogels based on thermal, ionic, and photo-crosslinking were tested. The biocompatibility and stability of our fibrillar matrix for long-term in vitro maturation was evaluated using different types of cellular bioinks, cultured under static and/or dynamic conditions. Cellulase enzyme was used for CNC digestion and release of the biofabricated tissue models.

Results and Discussion
The ionically crosslinked CNC showed ideal shear-thinning and self-healing properties which lead to 3D printing with high precision and resolution. We were able to prove the potential of the biomimetic platform by efficiently printing the most commonly used bioink hydrogels and perfusable complex structures with distinct designs embedded in the CNC fibrillar hydrogel matrix. The optical transparency of the CNC makes them an ideal candidate for post-printing analysis, which is crucial for microfluidic applications. The fibrillar matrix of CNC allowed for diffusion of nutrients and cross-talk between cells in different compartments, which has been a major limitation of microfluidic chips fabricated with conventional polymeric materials such as poly(dimethylsiloxane) (PDMS). CNC matrix was bioorthogonally digested in culture conditions to successfully release the engineered tissue. The preliminary data highlight potential of the ionically crosslinked CNC for tissue/organ-on-chip applications in a simple and integrated single process which will be further investigated in further studies for disease modeling and drug screening.
Conclusion
The results indicate that controlled self-assembly of CNC combined with 3D printing can be a promising and green alternative to conventional polymeric materials and microfabrication technologies. This strategy enables the fabrication of complex and perfusable 3D channels with micro-scale precision in a fibrillar matrix that allows crosstalk between cultured cells. This biomimetic platform will not only allow biofabrication of perfusable channels and chambers for dynamic 3D cell culture and for physiological studies but also the direct bioprinting of freeform cellularized constructs with relevant clinical size within its own support bioreactor for in vitro tissue maturation.

Acknowledgement
FCT for PD/BD/129403/2017, NORTE45_3Bs_RL1, PTDC/NAN-MAT/30595/2017, ERC for MagTendon (No. 772817) and H2020 for Achilles (No. 810850).
2:00 p.m. – 3:30 p.m.

Track03

**S04 | Smart (Nano)biomaterials and their Applications**

**Chairs**

*Gianni Ciofani*
Istituto Italiano di Tecnologia, Smart Bio-Interfaces, Pontedera, IT

*Jinlian Hu*
City University of Hong Kong, Biomedical Engineering, Hong Kong, HK

*Catarina P. Chaparro (YSF)*
NOVA School of Science and Technology (FCT-NOVA), CENIMAT/i3N (Centro de Investigação em Materiais), Almada, PT

*The symposium aims at introducing the fundamentals and new breakthroughs in the application of smart biomaterials in medicine. Recent advances of smart polymeric materials responsive to electricity, magnetic field, light, etc. will be approached. A particular attention will be moreover devoted to the exploitation of piezoelectric (nano)materials, for therapeutic and diagnostic purposes, and for tissue regeneration.*
Shape Memory Biomaterials and Their Applications

Jinlian Hu

City University of Hong Kong, Department of Biomedical Engineering, Hong Kong, CN

Shape memory polymers and their composites as biomaterials have received broad attention in various applications due to their unique advantages of stimuli responsive shape, displacement, strain and stress adaptiveness. With the advancement of knowledge, science and technology in materials design, fabrication and characterization, a wide variety of shape memory polymeric materials can be engineered for tissue regeneration, soft robotics, cellular manipulation, 4D printing, smart medical devices and minimally invasive implantation. The talk will first give a brief review on their recent progress in terms of cellular mechanisms, structural design and applications. Then the focus of the lecture will put on an innovative solvent-responsive coiling-expanding stent and their sister laminated structural materials.

Acknowledgement

The speaker gratefully acknowledge the financial support from the Hong Kong General Research Fund ("Development of ‘All-In-One Smart’ Polyurethanes Through Rediscovering Wool", RGC Project No. 152098/15E), National Natural Science Foundation of China (51373147 and 11802104), the Hong Kong Research Grants Council (RGC-GRF/5161/11E),

Figure 1. Two-Way Shape Memory Behavior with Multi-Turn Coil of a Laminated Film
(A) shape change of laminated film in alcohol and water at body temperature; (B) circulation curve of laminated film at body temperature; (C) central angle, diameter change, and recovery ratio of laminated film; length change and weight change of CEF (D) and UV resin (E) in different cycles stimulated by water and alcohol; expanding (F) and curling (G) process of laminated film in different temperature within 1 minute
Figure 2. Schematic of two-way shape memory laminated film stent

This figure describes the process of making the two-way shape memory laminated film and the final multi-turn coiled stent.

References
Piezoelectric materials for biomedical applications: From tissue engineering to cancer nanomedicine

Gianni Ciofani

Istituto Italiano di Tecnologia, Smart Bio-Interfaces, Pontedera, IT

Electrical stimulation of cells and tissues is an important approach of interaction with living matter, which has been traditionally exploited in the clinical practice for a wide range of pathological conditions; standard methods of stimulation are, however, often invasive, being based on electrodes and wired connections. The possibility to achieve an indirect electrical stimulation, by means of piezoelectric materials, is therefore of outstanding interest for all the biomedical research, and it emerged in the latest decade as a most promising tool in many bioapplications [1].

The generation of small electric charges upon the application of mechanical stimuli to piezoelectric nanomaterials is a unique phenomenon in the context of remote stimulation of cells and tissues. Electrical cues are known to foster specific biological responses, and piezoelectric nanomaterials own the ability to act as real “nanotransducers”, thus allowing obtaining “wireless” and remote electric stimulation thanks to non-invasive excitation through mechanical sources (usually ultrasound or vibrations).

Piezoelectric nanomaterials are usually ceramic or polymeric, with piezoelectric nanoceramics typically showing higher piezoelectric features than polymers. Concerning piezoelectric polymers, poly(vinylidene difluoride) (PVDF) and its copolymers show the best piezoelectric features, and have been widely investigated to promote cell stimulation: some works of our group, for example, reported on PVDF-based nanocomposites for stimulation of neuronal [2] and bone [3] cells.

Finally, it is worth to mention the potentialities of piezoelectric stimulation of cancer cells. It is in fact well-known as low-intensity electric stimulation represents an alternative treatment able to affect cancer cells without the use of any drugs/chemicals, and to significantly enhance the effects of chemotherapy by reducing multidrug resistance. Recently, our group provided the first evidences of the efficacy of this antitumor approach mediated by piezoelectric barium titanate nanoparticles and ultrasound, respectively on breast cancer [4] and on glioblastoma multiforme [5] cells. Efforts are now focused on replacing inorganic and non-biodegradable ceramic materials with piezoelectric polymers (such as the aforementioned PVDF) exploitable in the clinical practice.

References
S04-03

Graphene containing hydrogels as biocompatible materials towards self-powered electronic medical devices

Andreia T. Pereira1, Catia R.S. Rodrigues2, Ricardo Vidal1, João O. Ventura2, André M. Pereira2, Inês C. Gonçalves1

1 INEB/3S - Instituto de Engenharia Biomédica, Porto, PT; 2 IFIMUP – Instituto de Física de Materiais Avançados, Nanotecnologias e Fotónicas, Departamento de Física e Astronomia, Faculdade de Ciências, Universidade do Porto, 4169-007 Porto, Portugal, Porto, PT

Introduction
With the rise of the digital era, the Internet of Medical Things (IoMT), which establishes networks that exchange healthcare data between medical devices and people, through the use of biosensors, appears as the edge of the next breakthrough in the biomedical field. Moreover, electronic medical devices (EMD) such as pacemakers, left-ventricular assist devices (LVAD), and brain stimulators have been widely used to treat several diseases. To power supply the biosensors and these EMD, batteries are needed, but have several limitations such as the need for replacement, which frequently involves surgical procedures, their big size/weight, which limits devices miniaturization (specially for biosensors) and the risk of leakage of their content to surrounding tissue, which represents a hazard for patients [1]. Thus, to allow the "boom" of IoMT and improve the performance of electronic medical devices, there is a demand for an endless electrical energy source.

Triboelectric nanogenerators (TENG), reported for the first time in 2012, can convert mechanical energy into electricity by contact-separation or relative sliding between two materials with different triboelectric polarity, allowing scavenging mechanical energy from body movement, muscle contraction/relaxation, and cardiac/lung motions. However, most polymer-based TENG materials are not biocompatible, present limited performance when in the presence of body fluids and are still inefficient to supply EMD.

This work aims to give a step forward on this barrier by proposing as triboelectric nanogenerator an hemo/biocompatible FDA-approved hydrogel, poly(2-hydroxyethyl methacrylate) (pHEMA), which is characterized by its high water content (~60%). To tune the tribopolarity of pHEMA, we envisioned the use of graphene oxide aiming to reach materials with different tribopolarities.

Experimental Methods
GO was produced by modified Hummers’ method [2] and characterized regarding oxidation degree by X-Ray Photo Spectroscopy (XPS), surface topography by Scanning Electron Microscopy (SEM), and lateral size/charge by Zetasizer. pHEMA/GO composites were produced by in situ polymerization of 2-hydroxyethyl methacrylate and tetraethylene glycol dimethacrylate with different amounts of GO (0.6 – 27.5% v/v) [2]. The resulting composites were evaluated regarding their surface topography (SEM) and charge (Electro Kinetic Analyzer). The pHEMA/GO composites’ triboelectric polarity was measured in contact-separation mode towards PTFE (reference material with negative tribopolarity), being evaluated the generated voltage, current, and power density [4]. The storage capacity of produced energy in a capacitor of 1 µF was assessed [4]. Cytocompatibility of pHEMA/GO was evaluated accordingly to ISO 10993-5/12 by assessing the medium extracts’ cytotoxicity towards a human fibroblast cell line (HFF-1) during 24h.

Results and Discussion
The produced GO sheets contain 66% and 34% of carbon and oxygen atoms, respectively. GO has a lateral size of \( \sim 1.9 \mu m \), exhibits a wrinkled structure and presents a surface charge of \(-33.0 \pm 1.3 \text{ mV}\), characteristic of the graphene oxidized forms. When incorporated in pHEMA, GO increased the hydrogel's surface roughness, especially in the...
highest tested concentration (27.5% w/v). Besides this change in surface topography, pHEMA's surface charge increases within the amount of incorporated GO, ranging from -7.3 to 0.5 mV/cm².

Regarding the triboelectric outputs, in an open-circuit under contact-separation mode with PTFE the voltage varies in the range of 50-120 V and the maximum current reaches 1.5-3.0 µA with a maximum power of 0.15-1.38 W/m², even in a hydrated environment. Upon incorporation of 5% v/v GO, a plateau in the triboelectric outputs is achieved. All materials revealed the capacity to charge a capacitor of 1 µF, in 200 s, generating voltage between 0.3-1.5 V. Finally, the in vitro biocompatibility of the polymer composites was confirmed, as pHEMA/GO extracts are not cytotoxic towards human fibroblasts.

**Conclusion**

Incorporation of GO in pHEMA can tailor its tribopolarity, producing materials with different tribopolarity which could be used to assemble a TENG. The electricity generated using biomechanical movements between such materials is suitable to supply diversified implantable devices (~1 µW).

**Acknowledgement**

This work was financially supported by Fundação para a Ciência e a Tecnologia (FCT)/MEC and FEDER under Program PT2020 through the projects PTDC/CTM-COM/32431/2017 (SoftStrong), UIDB/04293/2020 (i3S), UID/NAN/50024/2019, PTDC/CTM-NAN/5414/2014, POCI-01-0145-FEDER-029454, and NORTE-01-0145-FEDER-022096 from NECL. The authors also acknowledge support from the International Consortium of Nanotechnologies (ICON) funded by Lloyd's Register Foundation, a charitable foundation that helps to protect life and property by supporting engineering-related education, public engagement, and the application of research. C. Rodrigues is thankful to FCT for the grant (SFRH/BD/147811/2019).

**References**


Towards Spatiotemporal Control of Biological Activity via Patterned Magnetic Hydrogels


1 University College Dublin, School of Chemistry, Dublin, IE; 2 Royal College of Surgeons in Ireland, Department of Chemistry, Dublin, IE; 3 University College Dublin, Conway Institute of Biomolecular and Biomedical Research, Dublin, IE; 4 University College Dublin, School of Physics, Dublin, IE

Introduction

Multifunctional nanocomposites which exhibit well-defined physical properties and encode spatio-temporally controlled responses (including changes of shape, microscopic morphology, mechanical strength and permeability) are emerging as components for advanced responsive systems. For instance in the case of biomedical applications magnetic nanocomposite materials have attracted significant attention due to their ability to respond to spatially and temporally varying magnetic fields changing their intrinsic structure, undergoing time-dependent deformations, or releasing cargo on demand.1-4

Experimental Methods

A combination of in-house built multi-head and commercial 3D printers was used to extrude selection of magnetic composite hydrogels. Magnetic nanoparticles were synthesised, stabilized and dispersed homogenously through the gels to optimise their hyperthermic responses. Oscillatory and rotational rheology measurements confirmed the viscoelastic properties providing ideal materials for 3D printing well-defined architectures with high fidelity for both magnetic and non-magnetic components of integrated multi-component builds. The nanocomposite inks showed complete shear- and temperature-recoverability/reversibility to their initial state, confirming that at particle concentrations that enable magnetic responses the necessary printability is not lost. Multiple complex structures were printed with high resolution (~150 mm) with independent magnetic and non-magnetic patterned components and these were shown to be reproducible and robust. Post-printing chemical crosslinking was used to retain long-term fidelity of the printed structures, whilst retaining magnetically responsive hyperthermic responses at low particle concentrations.

Results and Discussion

The combination of MNPs and established 3D printable polymeric hydrogel formulations can provide multifunctional and stimuli-responsive systems with spatial-, temporal- and dosage-controlled release properties. In our recent work towards bio-applications of magnetic hydrogels, we described spatiotemporal hyperthermic heating rules from spatially patterned magnetic hydrogels and demonstrated controllable release of dyes (Fig. 1).4,5 We also fabricated exemplar multi-component magnetically responsive structures and used high resolution in-situ IR thermography to confirm that the incorporated magnetic nanoparticles retain up to 100% of the original hyperthermic efficiency to provide spatial temperature gradients for cell stimulus and for stimulus-responsive timed delivery of biomolecules. Use of thermoresponsive control over hyperthermic release achieved by embedded selection of well-controlled microgels and nanogels made of poly(N-isopropylmethacrylamide) with chemically programmed volume temperature transitions at physiologically relevant range of 32 to 45°C will also be shown. The advantages of spatial patterning of thermally active components will be described in multiple biological scenarios, including timed monitoring of induced disease of kidney and cerebral organoids, chemotherapeutic cancer irradiation, as well as for controlled stem cell
differentiation. Applications of DC-magnetic fields to physical stimulation of patterned magnetic gels will also be presented.

Conclusion
The magnetic responsiveness of conventional magnetic materials and those magnetically manufactured; deformation, hyperthermia and combinations of these are described along with some of our recent developments including timed-automation of interventions into biological processes are briefly discussed.

Acknowledgement
The authors acknowledge support from Science Foundation Ireland (16/IA/4584 and 13/IA/1840) and from the Royal Society of Chemistry (M19-6613).

Figure 1
In recent works we demonstrated 3D printable responsive magnetic hydrogels composed of magnetic iron oxide nanoflowers and Pluronic polymers that encode spatiotemporally controlled temperature increase and dye release on alternating magnetic field irradiation. Inside Front Cover reproduced with permissions from [5].

References
Optimization of a shape memory-based platform for drug delivery

Nicoletta Inverardi1, Chiara Pasini1, Alice Melocchi2, Marco Uboldi2, Giulia Scalet3, Alessandra Maroni2, Lucia Zema2, Andrea Gazzaniga2, Francesco Briatico-Vangosa4, Ferdinando Auricchio3, Francesco Baldi1, Stefano Pandini1

1 University of Brescia, Department of Mechanical and Industrial Engineering, Brescia, IT; 2 University of Milano, Department of Pharmaceutical Sciences, Milano, IT; 3 University of Pavia, Department of Civil Engineering and Architecture, Pavia, IT; 4 Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering “Giulio Natta”, Milano, IT

Introduction
Shape memory polymers (SMPs) are materials capable of programmable shape changes, triggered by the exposure to an external stimulus [1]. Their smart response holds potential for the development of shape-shifting medical devices, e.g. self-expandable stents and dynamically active substrates for tissue engineering. Recently, the possibility to leverage the peculiarities of SMPs also for pharmaceutical applications was envisioned for site-specific delivery of active molecules inside the human body [2,3]. The shape memory effect of a pharmaceutical-grade polymer was proved to be useful for the prototyping of a gastroretentive drug delivery system (DDS), first by programming a temporary shape, which can be fitted inside commercially available capsules, and secondly, by exploiting the recovery of the permanent bulky shape (upon contact with simulated gastric fluid at 37°C) to achieve retention and controlled release of the conveyed drug [4]. The core aim of this work is to propose an experimental strategy, supported by a computational activity, to optimize the performance of this system in terms of the mechanical response and controlled release.

Experimental Methods
Formulations based on pharmaceutical-grade poly(vinyl alcohol) (PVA), glycerol and allopurinol, selected as drug tracer, were processed by fused deposition modeling (FDM) or by hot-melt extrusion. The extruded material was either cut in 50mm long rods or wrapped around specifically developed templates, while still hot, to obtain a permanent shape evaluated as gastroretentive (e.g. cylindrical or conical helix) [4]. Rods were spray coated with an ethanol-based 3:1 solution of Eudragit® RS:RL plasticized with 15% of triethyl citrate, and samples with different coating thickness were collected. Thermo-mechanical characteristics useful for the shape-memory effect were assessed by differential scanning calorimetry and dynamic mechanical analysis. Numerical analyses were performed in a finite element software (Abaqus) by using the generalized Maxwell model whose parameters were fitted from experimental thermo-mechanical data. Mechanical tests were carried out on dry and swollen specimens (i.e. upon immersion in water for 1, 4 and 6h) by using an electromechanical dynamometer under tensile conditions. The release performance was studied in a dissolution apparatus II (900 mL HCl 0.1 N, 37 ± 0.05 °C, 100 rpm), by assaying spectrophotometrically (λ = 251) fluid samples withdrawn at various time points (up to 24 h).

Results and Discussion
Helices and rod-shaped specimens based on a swellable/soluble hydrophilic SMP and containing a drug tracer were successfully obtained by FDM and hot-melt extrusion. Uncoated and coated rods were characterized for mechanical, shape memory and release properties. The coating was found to be fundamental in limiting the polymer swelling and consequently the drug release rate, which was reduced from 98% up to 8% after 6h. Similarly, after 6h of water immersion, the decrease in the mechanical properties was lower than that observed with uncoated ones. Interestingly, shape memory properties were found to be optimal regardless of the presence of the coating. Finally, the modeling activity provided useful guidelines for an efficient and time-saving design of shapes to be potentially...
used for self-expandable DDSs, by well predicting the shape changes occurring during isothermal recovery at body temperature, even in the case of samples with more complex geometries.

Conclusion
The results of this work confirm the possibility to employ PVA as base material for shape-changing DDSs towards 4D printing and propose a modeling approach and technological strategies to optimize their shape, mechanical properties and release performance. In fact, by employing thin coatings (less than 100μm thick) based on insoluble but permeable materials on samples, it was possible to fine-tune the release rate. Furthermore, coatings limit the water uptake and consequently the softening of the core polymer, thus modulating the reduction of mechanical properties over time, without significantly affecting the shape-memory kinetics.

References
2:00 p.m. – 3:30 p.m.

Track04

ESB-CSBM S | ESB-Chinese Society for Biomaterials (CSBM) Joint Symposium: Frontiers of Materiobiology

Chairs
Aldo R. Boccaccini
University of Erlangen-Nuremberg, DE
Jian Ji
Zhejiang University, CN
Agnese Lucchetti (YSF)
RWTH, Institute of Textile Technology at RWTH Aachen University, Aachen, DE
Decoding the materiobiology: multiple roles of biomaterials in participating life activities

Changsheng Liu

China, Frontiers Science Center for Materiobiology and Dynamic Chemistry, Engineering Research Center for Biomedical Materials of the Ministry of Education, East China University of Science and Technology, Shanghai, CN

The repair and reconstruction of defected tissue is a difficult problem that modern medicine tries to resolve. Biomaterials are one of the important elements for tissue regeneration. After implantation, biomaterials will affect the immune response, behaviors of relevant cells and repairing process. Although the biological effects of materials are gradually being discovered, the current studies are still mainly focused on the characterization of the apparent state of materials and the evaluation of the final repair effect. There is a lack of systematic and detailed understanding of how implanted materials participate in the \textit{in vivo} microenvironment \cite{1}. These blind spots directly restrict the design of regenerative materials. Therefore, it is necessary to further decode the correlation between material properties and biological functions, and clarify the rules and relevant mechanisms of how materials influence on the biological functions of cells, tissues / organs and the whole organism at different levels.

Acknowledgement

The work is supported by the National Natural Science Foundation of China for Innovative Research Groups (No. 51621002) and the National Natural Science Foundation of China (No. 31870953).

References

ESB-CSBM S-KL02

Nanoscale control by materials of mesenchymal stem cells for identification of bioactive metabolites.

Matthew J. Dalby

University of Glasgow, Centre for the Cellular Microenvironment, Glasgow, GB

Metabolites, or biological small molecules, are usually considered in identification of biomarkers. However, they can be used to drive cellular processes, such as stem cell differentiation. Use of complex media recipes to control stem cell differentiation add artefact to metabolomics experiments and so bioengineering approaches are attractive as they can drive different stem cell fates without changing what the cells are ‘fed’. We have developed metabolomics pipelines to identify bioactive metabolites that control mesenchymal stem cell (MSC) self-renewal and differentiation. We started this research avenue using peptide hydrogels with defined stiffnesses that could control MSC chondrogenesis and osteogenesis, identifying GP18:0 and cholesterol sulphate as bioactive metabolites¹. Next, using our nanovibrational bioreactor, the Nanokick², along with synthetic chemistry modification of hit metabolites, we focused on refining our putative osteospecific metabolite candidates to tune potency and specificity identifying fludrocortisone acetate. Finally, we have used nanotopography to control MSC self-renewal³ to identify respiration-link metabolites that drive the immunomodulatory phenotype of MSCs⁴; this is critical if we wish to grow large numbers of high quality MSCs for use as immunosuppressive therapies in transplant procedures.

Acknowledgement

EPSRC, BBSRC, MRC and Sir Bobby Charlton Foundation for funding. Carol-Anne Smith for technical support. All my lab, past and present, for their great contributions.

References

Self-setting granules for bone repair

Wei Zhu¹, Wei Li², Sheng Cui³, Xisheng Weng¹, Häkan Engqvist⁴, Wei Xia⁴

¹ University of Tsinghua, Department of Orthopedics in Peking Union Medical College Hospital, Beijing, CN; ² South China University of Technology, School of Biology and Biological Engineering, Guangzhou, CN; ³ Nanjing Tech University, College of Materials Science and Engineering, Nanjing, CN; ⁴ Uppsala University, Department of Materials Science and Engineering, Uppsala, SE

Introduction
Bioactive ceramics have been widely studied and used in clinic, mainly for orthopaedics and dental repair. [1-2] Except for the materials from nature and animals, fully synthetic bioactive ceramic materials have shown their good performance, i.e. good biocompatibility, bioactivity, and bone repair and regeneration. [2-3] Among them, bone cements and porous scaffolds are popular choices. [4-5] For bone cement, the advantage is the injectibility, which can be used for minimized operation. However, generally, their porosity is relatively low and the pores are small, which is good for cell migration and bone growth. For porous scaffold, it’s the opposite. They have proper porosity and pores, but not injectable and generally are big. It will be attracting if we can combine the advantages of bone cement and porous scaffold. It is what we are aiming for in this study.

Experimental Methods
α-Calcium sulfate hemihydrate (α-CSH), magnesium oxide (MgO) and fumed silica (SiO2) were used. All materials were used as received without purification. Different ratios between α-CSH and (MgO+SiO2), 0 – 50wt%, were tested. The different powder/liquid ratios were also tested. The mixing paste was placed in a Teflon mould with holes (diameter: 0.5mm and 1mm). The granules were taken out after setting, following by a heat-treatment. The as-obtained granules were injected into the different mould to form porous scaffolds under wet conditions. Both granules and scaffolds were analysed by phase composition (XRD), morphology (SEM), compressive strength, degradation, ion release and changes of pH values of soaking medium. Cell studies were done by the toxicity, RNA extraction and RT-PCR. The in vivo study was done by using a modified femur defect model of New Zealand white rabbits. New bone growth and collagen expression were analysed after implantation for aiming months.

Results and Discussion
Calcium sulfate hemihydrate and amorphous magnesium silicate granules have been prepared. The granules can form porous scaffolds with different shapes. The pore size can be controlled by the size of granules. The formation of the scaffolds was induced by the surface reaction between CSH and water. The content of magnesium silicate in the granules will affect the mechanical strength and degradation. The small granules could be injected through a syringe for bone cement. Cell studies showed good biocompatibility and in vitro osteogenesis. The animal study presented better new bone formation compared with autologous bone chips.

Conclusion
A self-setting ceramic granule has been firstly fabricated with a dual setting system by using a reversible setting reaction of calcium sulfate. The new granules can form varied geometries of porous scaffolds by a simple setting process. The in vitro and in vivo studies show it’s bioactive and has good osteogenesis.

Acknowledgement
This work was supported by the Swedish Foundation For International Cooperation in Research and Higher Education (STINT, CH2015-6394), National Natural Science Foundation of China (Grant No. 81871786) and Youth Research Fund of Beijing Union Medical College Hospital (Grant No. pumch 201911847).

References
Injectable biphasic calcium phosphate functionalized by phosphoserine-presenting dendrons with osteoinductive properties

Maria Grazia Raucci¹, Xiao Yang², Xingdong Zhang², Matteo Santin³, Luigi Ambrosio¹

¹ National Research Council, Institute of Polymers, Composites and Biomaterials (IPCB-CNR), Naples, IT; ² University of Sichuan, National Engineering Research Center for Biomaterials, Chengdu, CN; ³ University of Brighton, School of Pharmacy & Biomolecular Sciences, Brighton, GB

Introduction
Clinical application of injectable bone pastes has been increasing rapidly in recent decades because of their advantages including ease to adaptation into irregular-structured defects (e.g. absence of gaps between the host bone and the implanted filler) and injectability suitable to minimally invasive surgery. These advantages are widely advocated to reduce patient complications and health care costs. Moreover, injectable bone pastes have shown significant bone regeneration potential in many clinical conditions, such as the treatment of maxillofacial deformities and defects, certain indications of spinal fusion and augmentation of osteoporotic fractures. However, significant efforts have been expended towards exploring the optimal formulation of the bone pastes in recent years [1]. In this study, we successfully fabricated injectable bone pastes integrating biphasic calcium phosphate (BCP, HA:β-TCP=2:8) nanoparticles with phosphoserine(PS)-tethered generation 3 poly(ɛ-lysine) dendron (G3-K PS) as osteoinductive component. Furthermore, the incorporation of strontium (Sr) element into the BCP nanocrystals was also considered to minimize bone resorption.

Experimental Methods
The preparation of materials including BCP, SrBCP (with 15 mol% Ca⁺² replaced by Sr²⁺), BCPG3 (BCP in G3-K PS carrier) and SrBCPG3 (SrBCP in G3-K PS carrier) was adapted from previously optimized protocols on SrHA and HA/G3-K PS preparation [2]. G3-K PS semi-dendrimers were synthesized by a solid-phase peptide synthesis (Biotage Initiator) as previously reported [2]. Freeze-dried G3-K PS semi-dendrimers in ethanol solution (5 mg mL⁻¹) was added at 1% (w/w) to the above BCP or SrBCP slurry (Ca+Sr/P in the range of 1.53) under 200 rpm and 37 °C stirring until gelation occurred. The morphology and crystal size of the nanoparticles in the material paste were evaluated by transmission electron microscopy (TEM) analysis, meanwhile the phase composition was analyzed by X-ray diffraction (XRD). The handling properties of the pastes were analyzed on the basis of their injectability and moulding.

In vitro cell tests to assess the osteogenic potential of the synthesized biomaterials were performed using bone marrow stromal cells MSCs isolated from ovariectomized rats and the expression of specific gene markers (Runx2, ALP, Cxcl9, RANKL) at 1, 3 and 7 days of cell culture were analyzed by RT-PCR. Furthermore, to confirm the role of Cxcl9 and Sr ions in material induced osteogenesis, additional Cxcl9 protein (250 ng mL⁻¹) was introduced into the BCPG3 and SrBCP groups in culture medium, and Sr ions (3 mM) were introduced into the BCP and BCPG3 groups in cell culture medium. In vivo studies were performed using ovariectomized female Sprague Dawley rats and paste materials were gently injected into the drilled holes (3.0 mm in diameter and 4.0 mm in depth) by syringe. The rats were euthanized at week 12 post implantation and post-mortem analyses were performed. Several bone metabolic markers including P1NP, CTX-I and Cxcl9 were evaluated using ELISA assay. Moreover, Micro-CT was used to estimate trabecular density and architectural parameters within the defect, including bone mineral density (BMD), structural model index (SMI), trabecular number (Tb.N), trabecular thickness (Tb.Th) and trabecular separation (Tb.Sp). Immunohistochemistry analysis and scanning electron microscopy (SEM) observation were also performed.

Results and Discussion

Page 203 of 2028
In this study, an injectable bone paste material integrating BCP nanoparticles with G3-K PS carrier was successfully synthesized with or without the doping of Sr element into the BCP nanocrystals. Both in vitro and in vivo findings showed that the integration of G3-K PS would downregulate Cxcl9 gene and protein expressions to achieve an enhanced bone regeneration effect, with respect to a higher BMD and BV/TV (Fig.1A-D). Immunohistochemical staining of Cxcl9, Runx2 and RANKL proteins were conducted in the current study. The positive expression of Cxcl9 seemed to be less in BCPG3 and SrBCPG3 groups. Runx2 and RANKL positive expressions were more in BCPG3 group than others. The positive staining signals were further quantified in Fig. 1E. The result was in accordance with in vitro gene and protein expression and in vivo serum biomarker analysis. In this study, it is for the first time reported that the G3-K PS carrier could down-regulate Cxcl9 expression, as characterized by PCR, in vivo serum ELISA and immunohistochemical staining of implanted sample seem to consistently demonstrate. No additional benefit to osteoporotic bone regenerating ability of BCPG3 material was found with Sr incorporation.

Conclusion
The results indicated that the BCPG3 bone paste can become a high-performance bone filler in the treatment of osteoporotic bone defects.

Acknowledgement
The authors would like to thank the Progetto Premiale di AREA SCIENCE PARK “OPEN LAB - A System of Open Research Facilities” and *H2020-MSCA-RISE-2016*, SECOND.R.I., Grant Agreement No 734391 for financial support.

References
Biofabrication of Artificial Lymph Vessels

Sara S. Majidi\textsuperscript{1,2}, Yingchun Su\textsuperscript{3}, Mathias L. Jørgensen\textsuperscript{3}, Christoph Müller\textsuperscript{3}, Pourya Foroogh\textsuperscript{4}, Guangjun Nie\textsuperscript{5}, Menglin Chen\textsuperscript{1,3}

\textsuperscript{1} Aarhus University, Interdisciplinary Nanoscience Center, Aarhus, DK; \textsuperscript{2} Sino-Danish College, University of Chinese Academy of Sciences, Beijing, CN; \textsuperscript{3} Aarhus University, Department of Biological and Chemical Engineering, Aarhus, DK; \textsuperscript{4} Aarhus University, Department of Mechanical and Production Engineering, Aarhus, DK; \textsuperscript{5} National Center for Nanoscience and Technology, Beijing, CN

Introduction

The overall aim of this study was to fabricate beads-on-a-string structures in order to make a simple model of the lymphatic system. The developed model could potentially help understanding disease pathologies that can not be investigated in patients suffering from the disease. Furthermore, the fabricated structure could aid in the development of new immunotherapies.

In this study, an alginate hydrogel was proposed for the fabrication of the knotted fibers. As the water-abundant extracellular matrix secreted by the cells can be recognized as a hydrogel, these materials are becoming more and more attractive in the field of tissue engineering [1]. In order to fabricate the specific beads-on-a-string structure (knotted fibers), the Plateau-Rayleigh Instability phenomenon was exploited.

Experimental Methods

In order to fabricate the beads-on-a-string structures, the Plateau-Rayleigh instability theory was explored during co-axial spinning of alginate. First, it was investigated if variations in flow rate and concentrations of the alginate shell had an effect on the created beads-on-a-string structure. Second, the platform was tested in terms of cell encapsulation and culturing. Here, eGFP-expressing mesenchymal stem cells (eGFP-MSC), human peripheral blood mononuclear cells (PBMC), and Jurkat cells were encapsulated in alginate hydrogel environments with varying concentrations (2\textsuperscript{\textdegree}, 3\textsuperscript{\textdegree} and 4\textsuperscript{\textdegree}, w/v). Subsequently, the cytotoxicity was evaluated using live/dead staining and an LDH assay. To study long-term culture, cell-laden beads-on-a-string structures were fabricated and cultured for 13 days. The cells and material structure were evaluated using microscopy. Finally, PBMC activation with and without encapsulation in the alginate material was investigated to assess IL2 secretion from the material. PBMCs isolated from a healthy donor were purified. After purification, the PBMCs were pre-activated using an anti-CD3 antibody (OKT3) followed by encapsulation in the alginate material. The activation was evaluated by IL-2 specific ELISA of culture medium.

Results and Discussion

The results from the structure characterization, show that the diameter of the fabricated beads increases with both flowrate and alginate concentration. From the LDH and live/dead staining, the results show that the material is non-toxic. After long-term culture of the cell-laden beads-on-a-string, the encapsulated cells survived and adapted to the hydrogel environment by spreading out; thereby, suggesting that the system is applicable for cell culturing. The activation study showed that PBMCs successfully secreted IL2 from both non-encapsulated and encapsulated cells, which could be detected in the cell media. These findings show that the PBMCs maintain their functionality after encapsulation, indicating that the system is suitable for immunological studies.

Conclusion

In conclusion, this work brings us one step closer to successfully fabricating artificial lymph vessels. However, further work and optimization are needed to reach the end goal of creating in vitro lymphatic systems.

References
2:00 p.m. – 3:30 p.m.

Track05

N03 | Surface-Modified Biomaterials

Chairs
Jonathan Massera
Tampere University, Medicine and Health Technology, Tampere, FI
Hugo Oliveira
Inserm U1026 BioTis: Bioingénierie Tissulaire, Université Bordeaux, Bordeaux, FR
Ezgi Bakirci (YSF)
University of Wuerzburg, Wuerzburg, DE
Alternating Current Electrophoretic Deposition of Anti-infective DNase I Coatings on Polydopamine Activated Titanium Surfaces

Marie Van der Gucht, Rob Lavigne, Annabel Braem, Merve K. Aktan

1 KU Leuven, Department of Materials Engineering, Biomaterials and Tissue Engineering Research Group, Leuven, BE; 2 KU Leuven, Department of Biosystems, Laboratory of Gene Technology, Leuven, BE

Introduction
The difficult-to-treat nature of biofilms encourages researchers to develop strategies that allow preventing the formation of implant-associated biofilms rather than eradicate established biofilms. A promising approach is to inhibit biofilm growth indirectly by the degradation of extracellular DNA (eDNA) present in the matrix using an enzyme, such as deoxyribonuclease (DNase I). Here, we investigate the immobilization of DNase I on titanium (Ti) implant materials for a prolonged protection against biofilm formation at the implant surface. A schematic representation of the coating process is given in Fig. 1. In a first step, Ti surfaces were functionalized using dopamine (DA) chemistry which can enable permanent attachment of the enzyme. Next, PDA substrates are coupled as electrodes during AC-EPD for fast grafting in comparison to a classical diffusion methodology (i.e. simple dipping). After morphological and chemical characterization, the activity, release kinetics, and stability of both types of DNase I coatings were monitored in real-time. Furthermore, the activity of the coatings against Staphylococcus epidermidis (S. epidermidis) biofilm formation was evaluated.

Experimental Methods
Polished Ti substrates were functionalized by immersion in a DA solution (in Tris buffer). The resulting PDA-Ti substrates served as an electrode during AC-EPD or dip coating in a 0.0125 mg/ml DNase I aqueous suspension, a schematic representation of the EPD and dipping setup is shown in Figure 1. An unbalanced triangular AC-EPD signal was adjusted in order to couple the working electrode as an anode during the high-amplitude half cycle. After 10 min of AC-EPD resp. dip coating, samples were rinsed with Milli-Q to remove untethered DNase I followed by drying at room temperature. The coating’s surface morphology was investigated by means of atomic force microscopy (AFM). The thickness of coatings was evaluated using spectroscopic ellipsometry. Wettability and chemical composition of DNase I coatings were performed by contact angle measurements and by Fourier transform infrared spectroscopy (FTIR), respectively. Subsequently, the enzymatic activity of immobilized DNase I on PDA discs was evaluated using the quantitative DNase assay (qDNase assay, Fig. 1), as described previously. Furthermore, the release rate of DNase I molecules from the surface was monitored using qDNase assay. Moreover, the same assessment procedure was used to analyze AC-EPD and dip coatings shelf life (DNase I activity vs. time) at 4°C. Biofilm experiments were performed by adding a S. epidermidis suspension to each substrate followed by incubation at 37°C for 20 h. After gently washing, biofilms were fixed and examined qualitatively and quantitatively using SEM and confocal laser scanning microscopy (CLSM), respectively.

Results and Discussion
According to AFM, the roughness of polished Ti substrates, which initially appeared fairly smooth, increased with further treatment. The highest surface roughness was observed for AC-EPD DNase I in comparison to PDA-Ti and dipped DNase I coated surfaces. Whereas the wettability of pristine Ti increased after PDA activation, incorporation of DNase I resulted in reduced hydrophilicity. Coating thickness was approximately 5-fold higher for AC-EPD DNase I coatings in comparison to dipped DNase I coatings. This was also confirmed by FTIR analysis as the most prominent IR absorption amide I band was observed on AC-EPD coatings. Furthermore, the enzymatic activity of AC-EPD coatings was 3 times higher as compared to dipped coatings. The coating half-life for AC-EPD and dip DNase I coatings was evaluated.
coatings was estimated at 3 days of storage at 4°C, yet a significant activity could still be observed even after 7 days. Release tests, however, revealed that the majority of the AC-EPD coated enzymes were not covalently attached, but rather released from the surface in approximately 24 h. There was no activity observed on dip coating after 24h of release study. Finally, *S. epidermidis* biofilm formation was reduced more on AC-EPD DNase I coatings, as confirmed by SEM (Figure 2) and biofilm thickness observations conducted using CLSM.

**Conclusion**
A proof-of-concept is given for the use of AC-EPD as a fast grafting tool for the immobilization of DNase I on PDA-Ti implant surfaces. When compared to conventional dipping strategies, AC-EPD significantly improved the DNase I deposition yield, while the coating’s enzymatic activity was preserved for at least 7 days. Moreover, AC-EPD DNase I coatings effectively lowered biofilm formation on the Ti surface. Overall, AC-EPD DNase I coatings may hold a great potential to reduce biofilm formation on implant surfaces thereby increasing the success rates of such coated implants.

**Acknowledgement**
This work was supported by KU Leuven Internal Funds [C32/18/010; STG/17/024].

**References**
Development of surface treatment with artificial spider silk polymer nanofilm to improve cell adhesion

Masashi Fujiwara¹, Yutao Zhu³, Tomoyuki Koga⁴, Koji Yamamoto², Yusuke Morita²

¹ Doshisha University, Graduate School of Life and Medical Sciences, Kyoto, JP; ² Doshisha University, Department of Biomedical Engineering, Kyoto, JP; ³ Doshisha University, Graduate School of Science and Engineering, Kyoto, JP; ⁴ Doshisha University, Department of Molecular Chemistry and Biochemistry, Kyoto, JP

Introduction

Since implants such as artificial blood vessels applied for soft tissue are greatly deformed in the living body, flexibility and elasticity are required for the coating materials used for the implants to improve cell adhesion. It is also important not to impair the original surface microstructure of the implants. Spider silk has excellent biocompatibility, and we focused on nanofilm made from spider silk. The nanofilm has high flexibility and excellent self-adsorption property, and is expected to be applied to implants with various surface micro-structures. The purpose of this study was to develop the novel surface treatment method to enhance cell adhesion using an artificial spider silk polymer nanofilm.

Experimental Methods

Preparation of [(F8-PPG)] nanofilm: The peptide-multiblock polymer (F8-PPG) was prepared as an artificial spider silk polymer by the polyaddition of amine-terminated oligophenylalanine with poly(propylene glycol)(PPG) diisocyanate. 0.5, 1.0 and 2.0 wt% [(F8)-(PPG)] polymer solution were prepared by dissolving fluorescently labeled [(F8)-(PPG)] polymer in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP). A silicon wafer covered with calcium alginate layer was prepared using a spin coater, and then the [(F8)-(PPG)] nanofilms were produced on the silicon wafer from the [(F8)-(PPG)] polymer solution using a spin coater. The [(F8)-(PPG)] nanofilm formed on the calcium alginate layer was removed by immersing it in sodium citrate buffer. The glass slides (20×20×1 mm) were covered with the [(F8)-(PPG)] nanofilm for different polymer concentration. The film thickness of the each [(F8)-(PPG)] nanofilm was measured with a stylus profiler.

Evaluation of self-adsorption of [(F8)-(PPG)] nanofilm: The PDMS plates (Figure 1 (a)) having a micropattern with a height of 20 μm and an interval of 100 μm were prepared as a substrate to evaluate self-adsorptivity of [(F8)-(PPG)] nanofilm to a microstructure. The [(F8)-(PPG)] nanofilms for each solution concentration were put on the PDMS plates. The fluorescence images of the coated surface of PDMS plates were acquired with a fluorescence microscope. Horizontal brightness distribution at the center of the fluorescence images were quantitatively evaluated by an image analysis software.

Evaluation of extension of [(F8)-(PPG)] nanofilm: The PDMS sheets (10×30×1 mm) (Figure 1. (c)) were prepared to evaluate extensibility of the [(F8)-(PPG)] nanofilm with substrate elongation. Central area (10×10 mm) of the PDMS sheets were coated with the [(F8)-(PPG)] nanofilm for each solution concentration. Tensile deformation was applied to each PDMS sheet covered with the [(F8)-(PPG)] nanofilms up to strain of 20% by the tensile device (Figure 1. (d)). The [(F8)-(PPG)] nanofilm on the PDMS sheets were observed with a fluorescence microscope at strains of 5, 10 and 20% to evaluate occurrence of cracks.

Results and Discussion

The film thicknesses of the [(F8)-PPG] nanofilms for solution concentrations of 0.5, 1.0 and 2.0 wt% were 198±48 nm, 243±59 nm and 346±39 nm, respectively. Figure 2 (a)~(c) shows a fluorescence image of a micropattern coated with nanofilm, and Fig.1 (d) shows horizontal brightness distribution at the center of fluorescence images. The [(F8)-(PPG)] nanofilms for all solution concentrations absorbed uniformly on the micropattern. When a strain of 10% was applied to a PDMS sheet coated with the [(F8)-(PPG)] nanofilm, cracks were observed on the [(F8)-(PPG)] nanofilms.
for solution concentrations of 0.5 and 1.0 wt%, but no cracks were observed the nanofilm for solution concentrations of 2.0 wt% up to strain of 20%. These results showed that the [F8]-PPG nanofilm with a solution concentration of 2.0 wt% showed uniform self-adsorption and good extensibility as the coating material. It was expected that [F8]-PPG nanofilm with a solution concentration of 2.0 wt% can be effective surface treatment to enhance cell adhesion without impairing the surface morphology.

Conclusion
We have developed a surface treatment with nanofilms of artificial spider silk polymers that which has excellent self-adsorption and extensibility.

Figure 1
Images of substrates and device (a) PDMS plate (b) SEM image (×200, Bar: 100 μm), (c) PDMS sheet (d) tensile device.

Figure 2
Fluorescent images of a micropattern coated with nanofilm (×100, Bar: 200 μm), (a) 0.5 wt% (b) 1.0 wt% (c) 2.0 wt% (d) brightness distribution.
N03-03

Dual Keratinocyte-attachment and Anti-inflammatory Coatings for Permucosal Sealing of Dental Implants

Sunil K. Boda, Conrado Aparicio

University of Minnesota, Minneapolis, Minneapolis, US

Introduction

Unlike junctional epithelium attachment to teeth, permucosal sealing around dental implants is hindered by inflammation and epidermal down growth [1]. Previous research from our group showed that a combination of keratinocyte-specific and antimicrobial peptide coatings on titanium can promote soft tissue attachment and hinder bacterial colonization, respectively [2]. In the current work, we employ a dual coating on titanium made of a keratinocyte-specific peptide and a fatty acid with anti-inflammatory potential to promote oral epithelial tissue attachment around titanium implants. Taking cue from a previous study, we chose conjugated linoleic acid (CLA) to reduce inflammation triggered epidermal down growth [3]. For soft tissue attachment via the formation of hemidesmosomal structures by oral keratinocytes on dental implants, we tested two peptides; R3G3LamLG3 derived from Laminin 332, the major extracellular matrix component of the basement membrane in skin tissue and Netrin-1 capable of epithelial cell adhesion through α6β4 integrins [4].

In vitro hemidesmosome formation on modified titanium surfaces and modulation of inflammation was assessed using human oral keratinocytes and murine macrophages, respectively.

Experimental Methods

Finely polished titanium (pTi - grade II) discs of 6 mm diameter were either untreated or oxygen plasma (5% O₂, 95% Ar, medium intensity, 5 min) treated. Subsequently, the discs were coated with 1 mM Linoleic acid (LA) followed by coating with cationic peptides - R3G3LamLG3 and Netrin-1 at 1 mg/mL. The different coatings on pTi were characterized by x-ray photoelectron spectroscopy and water contact angle measurements. The proliferation and hemidesmosome formation of human oral keratinocytes (HOKs) were used to assess soft tissue attachment to modified titanium surfaces. Two hemidesmosomal marker proteins, Col XVII (BP180) and Integrin β-4 were evaluated by immunofluorescence microscopy and their expression was quantified by measuring the corrected total cell fluorescence (CTCF) using Image J software. For a favorable immunomodulation, conjugated Linoleic acid (CLA) coating was deployed to induce macrophage polarization from pro-inflammatory M1 to anti-inflammatory M2 phenotype in lipopolysaccharide (LPS) stimulated RAW 264.7 murine macrophages.

Results and Discussion

Consistent with a previous report on tuning the surface reactivity of anatase (TiO₂) on Ti foil to carboxylic acids [5], surface activation of titanium with oxygen plasma promoted the adsorption of LA as divulged by high resolution C1s XPS. Furthermore, XPS survey scan spectra revealed a significantly higher (1.3-1.5 times) recruitment of cationic peptides on plasma pre-treated LA coated surfaces in comparison to corresponding untreated LA coated ones. The proliferation of HOKs was greater on peptide coated pTi in comparison to uncoated or LA coated ones, irrespective of the plasma pre-treatment. This is consistent with earlier findings from own our group [2] and previous reports [5]. Further, both R3G3LamLG3 and Net-1 promoted hemidesmosome formation analyzed in terms of Integrin β-4 and Col XVII expression (CTCF) (Figure 1). Particularly, Integrin β-4 expression in HOKs was commensurate with the relative amounts (N 1s atom %) of peptide detected by XPS (Figure 2). With respect to inflammation, CLA adsorbed on pTi could reduce the induced nitric oxide synthase (iNOS) production; a pro-inflammatory cytokine generated in LPS stimulated murine macrophages, while promoting anti-inflammatory CD-206 expression.

Conclusion

Page 212 of 2028
Oxygen plasma treatment can be a facile approach for tethering small molecules/peptides on polished titanium without modifying surface topography. A dual keratinocyte-specific peptide and anti-inflammatory biomolecule coating on titanium may help promote permucosal sealing around dental implants. Such coatings may also help mitigate titanium allergy or hypersensitivity in patients.

**Acknowledgement**
This work was partially funded by Mozo Grau Dental Implants, S.L. (Valldolid, Spain)

---

**Figure 1**
Representative fluorescence microscopy images of HOK expressing Integrin β-4 hemidesmosomal marker protein on linoleic acid (LA) and/or R3G3LaMLG3 coated pTi without and with plasma pre-treatment. Red = Integrin β-4, Blue = DAPI
Figure 2
Hemidesmosomal markers - Integrin β-4 (A, B) and Col XVII (C, D) expression measured by corrected total cell fluorescence (CTCF) after 3 days of HOK culture on pTi with (A, C) ± Plasma treatment ± linoleic acid (LA) ± R3G3LamLG3 and (B, D) ± Plasma treatment ± LA ± Net-1 coatings. In the boxplots, the middle line depicts the median, boxes demark the 25th to 75th percentile and each point indicates one replicate, n = 36 per group.* indicates statistically significant difference at p < 0.05 between compared groups.

References
N03-04

Nanogrooved microdiscs and liquefied-core capsules as platforms for the development of vascularised bone tissue constructs

Isabel M. Bjørge1, Bárbara M. de Sousa2, Sónia G. Patrício1, A. Sofia Silva1, Liebert P. Nogueira3, Lúcia F. Santos1, Sandra I. Vieira2, Håvard J. Haugen3, Clara R. Correia1, João F. Mano1

1 University of Aveiro, CICECO, Department of Chemistry, Aveiro, PT; 2 University of Aveiro, Department of Medical Sciences, Institute of Biomedicine (iBiMED), Aveiro, PT; 3 University of Oslo, Oral Research Laboratory, Institute of Clinical Dentistry, Oslo, NO

Introduction

Cell behaviour and stromal cell differentiation into distinct lineages have been shown to be actively influenced by surface topography. Whereas this has been verified in a 2D context, the role of topography in 3D, which better mimics the natural cell environment, needs to be explored. To this end, we developed nanogrooved microdiscs (topodiscs) as substrates for a bottom-up cell-mediated 3D-construct fabrication and combined them with the technology to produce compartmentalised liquefied-core capsules. The liquid core is established upon mild liquefaction, enabling previously formed 3D aggregates of topodiscs and ASCs to serve as living support for freely dispersed HUVECs, via sequential seeding (figure: Project overview). The unique liquid core can be further exploited by employing dynamic culturing within standard spinner flasks, where each individual capsule is transformed into a microbioreactor. While topodiscs are expected to provide topographical cues to direct osteogenic differentiation of previously adhered ASCs, the resultant 3D aggregates will provide living domains for junctional intercellular communication with HUVECs. Our hypothesis is that by combining topographical cues with cell signalling pathways (e.g. connexin 43, a predominant gap junction protein between endothelial and osteogenic cells) and dynamic culturing, bone-like microtissues can be developed by a truly tissue engineering (TE) strategy and without requiring cell culture supplements. Such microtissues would present a close-to-native bone ultrastructure, thus presenting a mineralised and vascularised extracellular matrix.

Experimental Methods

Topodiscs were produced via nanoimprinting of spherical polycaprolactone microparticles between water-soluble polyvinyl alcohol counter-moulds of nanogrooved CD templates. This was performed at an optimised time, temperature, and pressure [1]. Spherical microparticles and smooth surface microdiscs were used as controls, replacing topodiscs in each experimental condition. ASCs-topodisc microaggregates with a controlled size were produced overnight using Aggrewell400 well plates. ASC-topodisc microaggregates and ECs were dispersed within the alginate core of a multilayered membrane encapsulation system, where straightforward core liquefaction enabled a sequential seeding. Each individual macrocapsule was enveloped by a 12-layered polyelectrolyte membrane composed of poly-L-lysine, alginate, and chitosan to both compartmentalise and maintain the liquefied core environment [2]. Encapsulated ASC-topodisc microaggregates were characterised and imaged upon fluorescent live/dead and F-actin/nuclei staining. ASCs and ECs were labelled with lipophilic fluorescent dyes to assess relative cell positioning and alignment. Furthermore, the osteogenic potential of the topodisc-capsule system composed of ASCs alone or ASCs co-cultured with ECs in both static and dynamic conditions was assessed up to 21 days. Construct pre-vascularisation was concurrently evaluated for co-cultures conditions.

Results and Discussion

Topodisc production via nanoimprinting led to a homogeneously nanogrooved surface topography. After 24h in culture, uniform ASC-topodisc microaggregates were formed, which were subsequently encapsulated within liquefied-core capsules, with or without ECs. Merging of several microaggregates into defined 3D constructs within
the liquefied-core capsules was observed for all conditions (figure: Highlighted results). Upon 21 days in culture, osteogenic differentiation was confirmed by the presence of osteopontin and hydroxyapatite for all conditions except for the ASC monoculture with spherical microparticles and smooth surface microdiscs, highlighting the importance of surface nanogrooves. The presence of angiogenesis marker von Willebrand factor and vascular markers laminin and podocalyxin in co-culture systems were indicative of construct pre-vascularisation.

**Conclusion**

Pre-vascularised, mineralised bone-like constructs were effectively achieved within compartmentalised liquefied-core capsules. The nanogrooved surface of topodiscs proved, in part, to be an impacting factor to induce osteogenic differentiation of ASCs. ASC-topodisc aggregates were demonstrated to be optimal supports for EC adhesion. As a next step, proteomic analysis of matrix vesicle content for dynamic co-culture conditions featuring either topodiscs or spherical microparticles will be performed to assess the up- and down-regulation of specific proteins related to osteogenesis, angiogenesis, and mechanotransduction pathways. We envision to propose the developed technology as a patient-specific bone TE strategy for minimally invasive procedures.

**Acknowledgement**

The authors acknowledge grants from FCT (SFRH/BD/129224/2017 and PTDC/BTM-MAT/31064/2017) and European Research Council (ERC-2014-ADG-669858-ATLAS). This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement.

References


High resolution replica molding of naturally inspired surfaces to form polymeric nanostructured biomaterials

Susan Kelleher¹, James MCormack², Lucia Podhorska², Shauna Flynn¹

¹ Dublin City University, Dublin, IE; ² University College Dublin, Dublin, IE

Introduction
Many nanostructured surfaces have shown to display bactericidal properties as a result of surface topography alone. Moreover, the size and distribution of the surface features (on the nano- and micro scale) is known to influence the efficacy of the surface at inhibiting bacterial cell growth. The surface studies to date largely comprise of either natural surfaces e.g. the wings of a number of different species of insects, or of inorganic, hard materials e.g. structured silicon. However, reproducible and controlled production of nanoscale features on the surface of soft, polymeric biomaterials remains a challenge. Herein we present a series of replica molding experiments using a range of templates to produce PEG gels with upright nanopillars on the surface.

Experimental Methods
Our work uses two key templates for replica molding: 1) silicon nanoneedle arrays and 2) natural cicada wings. The silicon nanoneedles were fabricated using block copolymer micelle lithography. PS-b-P2VP was used to fabricate a monolayer of hexagonally arranged spherical micelles on silicon substrates. By controlling the loading of FeCl₃ into these micelles, monolayers of metal nanodots hard masks were deposited onto the silicon surface. These nanodots were then used as hard masks to produced plasma-etched silicon arrays in the nanoscale. Cicada wings were cleaned with acetone, and dried under a stream of nitrogen prior to replication. Replica molding of the templates was carried out using acrylate-terminated polypropylene glycol (PPG) and polyethylene glycol (PEG). Depending on the template, either a UV light/ UV-activated photoinitiator or white light/white light photoinitiator mix were used to cure the materials. A number of different molding method were used for the different templates, including drop casting and capillary-assisted molding. To study the surface nanostructures of the different molds, AFM, SEM, and FESEM were used.

Results and Discussion
Replica molding of the surface of cicada wings and nanostructured silicon in polymeric materials gave excellent results. Highly fluid short-chain prepolymers allowed for full coverage of nanoscale features, even with high aspect ratios. Our 2-step process produced a replica (primary mold) of both the silicon and cicada wing templates. This primary mold was then used to produce a replica (secondary mold). Our wide range of templates shows the diversity of our molding techniques. Using block copolymer patterning techniques, we have produced silicon templates which possess nanoneedles of controlled diameter (30-55 nm), pitch (95-330 nm) and height (45-100 nm). The subsequent replica molding process allowed us to produce these features in PEG with excellent retention of the original structure dimensions. Additionally, we have demonstrated direct molding from the wings of cicadas, which possess sub micron scale pillars (dimension range for different wings = diameter 164-1307 nm and height 182-1003 nm). We ultimately produced polymer replicas of the wings on this scale, which also have excellent retention of the template.

Conclusion
Replica molding is a reliable process for replicating topographical features down to the nanoscale. Polymeric nanoneedle arrays can be produced from silicon templates by combining block copolymer micelle lithography and replica molding techniques. Additionally, direct replica molding of cicada wings is possible using a very
straightforward 2-step process. Our process of replica molding for both natural wings and nanostructured silicon gives excellent replicas of the surface.

Acknowledgement

This material is based upon works supported by the Science Foundation of Ireland under Grant No. 15/SIRG/3429(T).
'Degrading away my University' - Design and Controlled Degradation of Polycaprolactone-containing Microstructures

Mark T. O'Loughlin\textsuperscript{1,3}, Niamh Geoghegan\textsuperscript{1,3}, Colm Delaney\textsuperscript{2}, Susan Kelleher\textsuperscript{1,3}

\textsuperscript{1} University College Dublin, School of Chemistry, Dublin, IE; \textsuperscript{2} Trinity College Dublin, School of Chemistry, Dublin, IE; \textsuperscript{3} Dublin City University, Chemical Sciences, Dublin, IE

Introduction

Direct laser writing through two-photon polymerisation (2PP) can be employed to fabricate solid polymeric masters quickly and accurately from liquid polymer precursors on the nano- to microscale\textsuperscript{[1]}. Generation of reproducible positive moulds from these masters can then be achieved via the more traditional soft lithography technique of replica moulding\textsuperscript{[2]}. A wide variety of biocompatible polymers can be repeatedly moulded from the master negative, such as polyethylene glycol diacrylate (PEG-DA), polypropylene glycol diacrylate (PPG-DA) and polycaprolactone dimethacrylate (PCL-DMA).

Herein, this work specifically focusses on a novel blended polymeric system consisting of varying concentrations of PCL-DMA crosslinked with 1,6-hexanediol diacrylate (HDDA). Much of the focus of this work is on PCL, mainly due to two favourable properties - biodegradability and biocompatibility. The biodegradability characteristic associated with PCL derives from the ester bond found in each monomeric unit which has the capability to degrade in aqueous systems through hydrolysis\textsuperscript{[3]}. By optimising design, moulding and polymerisation protocols, and careful selection of the liquid polymer mixture, a wide variety of controllably degradable microstructures can be reproducibly achieved. In this work, the abbreviation of my university of study, 'UCD', has been incorporated into the polymeric microstructures by smart design of the laser printed master (Fig. 1). As such, this 'UCD' will be degraded in a controlled manner over time upon exposure to an alkaline environment. This degradation can then be monitored via imaging techniques and by mass loss calculations (Fig. 2).

Experimental Methods

Two-Photon Polymerisation of Pore Arrays: A commercial Direct Laser Writing apparatus (Photonic Professional, Nanoscribe Gmbh) was employed for pore fabrication. A single drop of the monomeric photoresist, IP-DIP (Nanoscribe) was deposited onto the centre of one side of the substrate before the holder was placed into the system prior to printing the 2PP array. The stereolithography structure file (stl) of the desired array of pores was loaded onto the software. For pore array fabrication, the laser power and scan speed were set to 15 mW and 6000 μm s\textsuperscript{-1}, respectively.

Replica Moulding: Replica moulding from 2PP master of pore arrays was carried out using PCL-DMA:HDDA blended solutions of varying concentrations. The monomeric cocktail was pipetted beneath a sealed cover glass and between two PDMS spacers until the space was filled. The solution was then cured under white light before being removed and inverted from the 2PP master.

Degradation Study: The degradation of the microstructures was performed in 5 M NaOH and was analysed by SEM. The study was monitored every 5 days for 25 days to try show sufficient degradation of the structures. The 3 polymeric cocktails studied in the process were neat HDDA, 1:9 PCL-DMA:HDDA and 1:4 PCL-DMA:HDDA. The degradation as mass loss was also investigated. Each sample (except 0 days) was immersed into distinct vials of 5 M NaOH (10 mL). After 5 days each sample was removed and rinsed with deionised water. The samples were then dried and weighed. This process of immersing and drying was repeated for 10, 15, 20 and 25 day timepoints. The mass loss percentage was calculated to give a more indicative value of the degradation as a percentage.

Results and Discussion

Page 219 of 2028
Fig. 1 shows the successful replica moulding from the 2PP pore array using 1:9 PCL-DMA:HDDA. Moulding using neat HDDA and 1:4 PCL-DMA:HDDA was also a reproducible success. The results of this work show that the microstructures depicting 'UCD' degrade in a controlled manner depending on the concentration of PCL-DMA used in the polymer cocktail. The greatest structural degradation is seen in the 1:4 (20 % PCL-DMA) samples, the next greatest in the 1:9 (10 % PCL-DMA) and the least in the neat HDDA (0 % PCL-DMA). This is evidence that the degradation that is occurring is due to the hydrolysis of the ester moieties within the polymer backbone of PCL-DMA. This hypothesis is supported by the data in Fig. 2 where the greatest decrease in mass is again seen in the 1:4 sample and the least in the neat HDDA sample. A study is currently underway to show the size-dependent release of fluorescent nanoparticles from the samples as the degradation of the microstructures progresses. Data for this will be obtained prior to the commencement of the conference.

**Conclusion**

This work shows that degradable materials can be accessed and used to fabricate degradable microstructures through the design of micro-scale printing technology in combination with the more conventional technique of replica moulding. This study demonstrates that SEM, mass loss analyses and release studies can be used to monitor the degradation by hydrolysis of the polymeric microstructures incorporated in this work.

**Acknowledgement**

M.O.L. and S.K. acknowledge the Irish Research Council (Grant number GOIPG/2017/1037). N.G. and S.K. also acknowledge the Science Foundation Ireland (SFI) and European Regional Development Fund (Grant Number 13/RC/2073 and 17/RC-PhD/ 3480) through CÚRAM, the SFI Research Centre for Medical Devices. C.D. acknowledges support from the Irish Research Council through the Government of Ireland Postdoctoral Fellowship Scheme (Grant Number GOIPD/2020/484).
Fig. 2. Mass loss (%) of neat HDDA (black), 1:9 PCL-DMA:HDDA (red) and 1:4 PCL-DMA:HDDA (blue) over 25 days in 5 M NaOH.

References
Protein-Polyelectrolyte complexes as versatile building blocks for the surface immobilization of proteins in layer-by-layer assemblies

Cédric Vranckx¹, Olivier Cornu², Christine Dupont-Gillain¹

¹ Université catholique de Louvain, Institute of Condensed Matter and Nanosciences (IMCN), Louvain-la-Neuve, BE; ² Université catholique de Louvain, Institut de Recherche Expérimentale et Clinique (IREC), Louvain-la-Neuve, BE

Introduction
After three decades of innovation, layer-by-layer (LbL) assembly is now amongst the most used methods to create nanostructured coatings and materials. With the growing interest in surface biofunctionalization in numerous applications such as biomaterial design, biocatalysis, biosensing, drug delivery or tissue engineering, LbL has been used to immobilize biomacromolecules such as DNA, RNA, peptides and proteins. Actually, controlling protein immobilization at interfaces through electrostatic LbL assembly is largely recognized as challenging due to their polyampholyte nature, conformation-dependent activity, shape and anisotropic charge distribution. vander Straeten et al. demonstrated that it is possible to standardize lysozyme (Lyz) electrical properties through complexation with poly(styrenesulfonate) (PSS) as polyanion, and to incorporate such protein-polyelectrolyte complexes (PPCs) in multilayers by the LbL method using poly(allylamine hydrochloride) (PAH) as polycation. [1,2] However, while PPCs have shown great potential to facilitate protein LbL assembly, this has only been proven for one system and the versatility of the method has only been suggested.

The objective of this work is, firstly, to investigate whether PPCs can be used to immobilize a wide range of proteins, in combination with various polyelectrolytes (PEs) and, thereby, demonstrate the versatility of this method. Secondly, it aims at quantifying the amount of protein in the multilayer.

Experimental Methods
LL-37, Lyz, insulin (Ins) and glucose-oxidase (Gox) were chosen because they are involved in various biomedical and biotechnological applications, and their surface immobilization is topical. These proteins were first complexed with oppositely charged PE, i.e., PSS, alginate (Alg) and heparin (Hep). The resulting PPCs were then assembled with another PE, i.e., chitosan (Chi) and PAH to form [PE-PPCs] multilayers. First, PPCs formation was investigated by optical density measurements. Then, PPCs were integrated into multilayers. As a matter of comparison, the LbL assembly of bare protein with PE, i.e., classical LbL assembly was carried out. In the latter case, the PE used to complex the protein was alternately adsorbed with the bare protein molecule to form a multilayer. The multilayer growth was monitored using quartz-crystal microbalance with dissipation (QCM-D) (Figure 1). Finally, in the case of LL-37, bicinchoninic acid assays (BCA) allowed the quantification of LL-37 in the Chi‒PPCsLL-37‒Hep multilayer.

Results and Discussion
By combining the different proteins and PEs, 32 different self-assemblies were built. In order to better capture the mass difference, i.e., the improved protein adsorption when PPCs are used, the mass increase compared to classical LbL assembly was computed for each protein-PE combination. Results are presented in Figure 2 and show the mass increase in percent for each of the 16 protein-PE couples tested. For instance, the mass increase is 198 % for the Chi–PPCsLL-37–PSS system and up to 509 % for the PAH–PPCsGox–Hep. These results clearly demonstrate that a few hundred percent more protein can be immobilized using PPCs as the building blocks of the LbL assembly. Moreover, the quantification of LL-37 into the Chi–PPCsLL-37–Hep multilayer showed that the adsorbed quantity is linearly proportional to the number of adsorbed layers. For instance, after 25 bilayers, 5.2 µg*cm⁻² of LL-37 were adsorbed. These results are the first to demonstrate the versatility of PPCs for immobilizing proteins on surfaces.
The second parameter that can be extracted from the multilayer growth data is the magnitude of desorption upon PE adsorption. vander Straeten et al. have recently demonstrated that these PAH–PPCs$_{\text{Lys-PSS}}$ multilayers are highly dynamic self-assemblies, leading to the potential release of proteins.[3] However, this has only been demonstrated in one specific case. Our in-depth study determined that a desorption is almost systematically observed upon PAH adsorption and not with Chi. This is a very interesting result because it shows that depending on the PE used, it is possible to control the multilayer reorganization and thus the release of proteins, which has a strong interest in drug release studies.

**Conclusion**

Therefore, with the goal to immobilize proteins at surfaces, PPCs allow a better multilayer growth, ranging from two to four times more, compared to the use of bare proteins.

Very importantly, our approach establishes a way to circumvent the major issues related to LbL assembly of proteins, offering great versatility, in terms of protein and polyelectrolyte nature. It will therefore be beneficial to the many scientific communities willing to modify interfaces with proteins and other challenging biomacromolecules in an efficient manner.
Figure 2
Heat map representing the mass increase when PPCs are used compared to bare proteins. Results with PPCs are expressed in percent of the total mass adsorbed after the adsorption of a two bilayers cushion and five bilayers of PE and bare proteins.

References
Development of the Evaluation Method for the Material Properties of the Hydration Layer Formed on the Material Surface

Kyosuke Hatayama\textsuperscript{1}, Yusuke Morita\textsuperscript{2}, Koji Yamamoto\textsuperscript{2}

\textsuperscript{1} Doshisha University, Graduate School of Life and Medical Sciences, Kyoto, JP; \textsuperscript{2} Doshisha University, Department of Biomedical Engineering, Kyoto, JP

Introduction
The surface of articular cartilage shows superior lubricating properties owing to the expression of many molecules which possess a hydration function, such as lubricin, hyaluronic acid and highly sulphated proteoglycan. This molecular hydration ability has been applied to the surface modification to reduce the friction between implant device and living body, for instance, an artificial hip joint grafted with MPC polymer\textsuperscript{11}. Although the mechanical properties of these material surfaces are principally evaluated by using atomic force microscopy (AFM), it would be difficult to detect a mechanical characteristic attributed to the fluid-like flow generated in the hydrated layer, such as the Squeeze effect that can affect a decrease of friction in fluid lubrication. Since such effect depends on the size of the contact area and the approach speed of the indenter, we have developed a device that has a larger spherical probe (diameter: 1 mm) than AFM and measured the compressive resistance force generated by the hydrated layer. By applying a viscoelastic calculation model considering changes of the contact area, we estimated the material properties of the hydrated layer originated from its solid and liquid characteristics.

Experimental Methods
Measurement of the compressive resistance force: A slide glass grafted with polyethylene glycol (PEG) was used as a material having a hydration function, and all experiments were performed in PBS. Figure 1 shows a schematic drawing of the spherical stainless probe (diameter: 1 mm) of the indenter and the hydrated layer, and a magnified photo of the major components of the measurement part of the device. The compressive resistance force generated when the tip of the indenter approached to the hydrated layer was detected by measuring the displacement of the leaf spring. The approaching speed of the indenter was controlled by using a piezo-driven fine movement stage. All experiments were conducted under the conditions of approach speeds at 1.8 mm/s and 0.18 mm/s.

Identification of mechanical properties: We assumed that the hydrated layer consisted of PBS and PEG could be described by using multiple Voigt elements, and calculated the compressive resistance force using a calculation model considering changes of the contact area under the approach of the indenter. The viscoelastic property of the hydrated layer was evaluated by identifying the spring stiffness $k$ and the viscous property $c$, which represent material property values independent of the contact area.

Results and Discussion
Figure 2 shows the results of the compressive resistance force as a function of the displacement of the hydrated layer and the numerical calculation values simulated under the conditions of 1.8 mm/s and 0.18 mm/s. Viscous properties due to the difference of approaching speed could be detected in the same deformation of the hydrated layer and those depended on the contact area. From the calculation results considering changes of the contact area, the spring stiffness $k$ and the viscous property $c$ of the hydrated layer were successfully identified as $56.576 \times 10^{-9}$ N/m and $0.4949 \times 10^{-9}$ N·s/m$^2$.

Conclusion
We developed the device that can measure the compressive resistance force generated in the hydrated layer formed on the material surface and constructed the viscoelastic model to numerically calculate the force. It was found that by considering changes of the contact area, the material properties of the hydrated layer could be estimated.
Figure 1: Schematic diagram of measurement of compressive resistance force by hydration layer.
A schematic drawing of the spherical stainless probe (diameter: 1mm) of the indenter and the hydrated layer, and a magnified photo of the major components of the measurement part of the device.

Figure 2: Results of experiments and numerical calculations.
The results of the compressive resistance force as a function of the displacement of the hydrated layer and the numerical calculation values simulated under the conditions of 1.8 mm/s and 0.18 mm/s.

References
Corneal endothelial tissue engineering using multi-layered polymer sheets

Jasper Delaey¹, Jasper Van Hoorick¹, Bert Van Den Bogerd², Carina Koppen², Peter Dubreuil¹, Sandra Van Vlierberghe¹

¹ Ghent University, Polymer Chemistry & Biomaterials group, Centre of Macromolecular Chemistry (CMAc), Department of Organic and Macromolecular Chemistry, Ghent, BE; ² University of Antwerp, Department of Ophthalmology, Visual Optics and Visual Rehabilitation, Faculty of Medicine and Health Sciences, Antwerp, BE

Introduction
Corneal endothelial damage and diseases are two of the major contributors to blindness or severe visual impairment worldwide [1]. The corneal endothelium is the innermost cell layer of the cornea and consists of a cellular monolayer that maintains the stroma in a dehydrated state through a “pump-and-leak” mechanism. A critical loss of cells due to damage, disease or aging leads to corneal edema which in turn results in opacification of the cornea [2]. Currently, the only treatment consists of a corneal transplantation from healthy cadaveric donor tissue. Unfortunately, only 1 donor is available for every 70 cases [3]. To tackle this shortage, the present work focusses on the development of transparent (>90%), thin (≤5µm), multilayered sheets constituting a poly(D,L-lactide) (PDLLA) layer to provide structural rigidity and a crosslinkable gelatin-based hydrogel as an extracellular matrix (ECM) mimic. These sheets provide a substrate for corneal endothelial cells (CECs) to enable subsequent ocular implantation thereby restoring the damaged endothelium and the patient's vision.[4] Additionally, we aim to scale up the production of these membranes to make them easily tunable in thickness, as well as increasing and facilitating their large scale-production possibilities by using doctor blading.

Experimental Methods
Multi-layered sheets (ø=12mm) were developed through successive spincoating steps. A sacrificial gelatin layer (H₂O, 10w/v%) was spincoated on a glass plate followed by a PDLLA layer (Corbion, PLURASORB 20, THF, 4w/w%). Next, a layer of gelatin-methacrylamide-amino-ethylmethacrylate (Gel-MA-AEMA) [5], was spincoated (H₂O, 10w/v%) as final layer, after applying an argon plasma treatment (0.8mbar, 30s) to the PDLLA. Finally, crosslinkable gelatin was crosslinked using UV-A (6 mW/cm², 30 min) irradiation. Isolation of the sheets occurred by dissolving the sacrificial layer (H₂O, 40°C). Sheets were characterized for their transparency (UV-VIS/NIR, 390-700 nm), thickness (white light interferometry) and glucose permeability (side-by-side diffusion setup,) as well as for their surface composition (XPS) and compatibility with CECs using B4G12 cells. Additionally, PDLLA and gelatin were doctor bladed and the relationship between processing parameters and film thickness was investigated.

Results and Discussion
Multi-layered sheets were successfully produced. The sheet thicknesses ranged between 0.8 and 1.5µm, thinner than the natural Descemet's membrane (10-20µm). All produced sheets showed a transparency of >98% (wet state, 380-700nm). The sheets were sufficiently permeable (>2.36*10⁻³ cm/s) towards glucose, both in the presence and absence of the gelatin-derived coating. Upon seeding the sheets with B4G12 CECs, the cells developed their characteristic shape. Immunocytochemical staining confirmed the presence of Na⁺/K⁺ ATPase pumps and tight junctions (ZO-1), indicating good cellular proliferation. Doctor-blading proved to be an effective method to scale-up membrane production as well as to easily tune membrane thickness (2-50 µm).

Conclusion
Transparent (>90%), thin (<10 µm), multi-layered sheets were successfully produced. These sheets were sufficiently permeable for glucose. Additionally, the sheets were able to support the proliferation of B4G12 CECs. Additionally,
for future large-scale production, doctor blading can be an effective method, allowing for less inter-sample variability by producing large membrane sheets at one time. On top of this, the technique allows for facile tuning of membrane thickness, opening up production of membranes for other ocular applications.

Acknowledgement
J.D. holds an SB PhD grant from the research foundation Flanders (FWO) with project number 1SA2119N.

Production of multi-layered membranes
Multi-layered membranes are obtained through successive spincoating steps. In a first step, a sacrificial gelatin layer is spincoated. Next, a PDLLA-based layer is coated to provide structural integrity. Before coating the final layer, a plasma treatment is performed to ensure covalent attachment of the final layer. Last, the final, gelatin based, layer is coated. The membrane is the subjected to UV-A irradiation to crosslink the crosslinkable gelatin derivative. Finally, the membrane is isolated by dissolving the sacrificial layer in warm water.

References
2:00 p.m. – 3:30 p.m.

Track06

N04 | Biomaterials for Orthopedic Applications II

Chairs
Michael Doser
Deutsche Institute für Textil- u. Faserforschung, Biomedical Engineering Dept., Denkendorf, DE
Pierre Weiss
University of Nantes, FR
Phuong Anh Dang (YSF)
ESPCI, Paris, FR
Plasma treated alginate-based hydrogels for delivery of RONS in osteosarcoma cancer therapy

Albert Espona-Noguera$^{1,2,3}$, Maria Pau Ginebra$^{1,2,3}$, Cristina Canal$^{1,2,3}$

$^1$ Universitat Politècnica de Catalunya, Biomaterials, Biomechanics & Tissue Engineering group, Dept. of Materials Science and Engineering, Barcelona, ES; $^2$ Universitat Politècnica de Catalunya, Barcelona Research Center in Multiscale Science and Engineering, Barcelona, ES; $^3$ Universitat Politècnica de Catalunya, Research Centre for Biomedical Engineering (CREB), Barcelona, ES

Introduction
Cold atmospheric plasma (CAP) is a source of complex chemically reactive components, which can exert many biological effects on cells. Interestingly, this novel technology has been shown to selectively kill cancer cells without damaging the surrounding tissues. Thereby, it would enable an alternative anticancer therapy, while avoiding undesired side effects found in radio- and chemotherapy. Among all components present in CAP, it has been shown that the oxygen and nitrogen reactive species (RONS) have a major contribution to this selective cytotoxic effect [1]. Currently, the way to apply a plasma treatment on a patient is still challenging because most of the direct CAP treatments of the tumor area would involve surgery. Interestingly, many studies demonstrated that RONS can be transferred to liquids, which can exert very similar anticancer effects compared to direct CAP treatment [2]. However, the delivery of RONS through non-viscous liquids is fast and poorly controlled, thus reducing the efficacy of the treatment [3]. To ameliorate the delivery of RONS, it has been shown that CAP-treated biopolymer solutions with the ability to form hydrogels can enhance the generation, storage, and release of RONS, resulting in an ideal approach for indirect CAP treatment of tumors [4]. Based on these findings, this work aims to explore the capacity of alginate-based formulations for generating and releasing RONS as a potential therapeutic approach in osteosarcoma cancer.

Experimental Methods
Sodium alginate (Panreac), was dissolved in distilled water at a concentration of 0.25%. The salt Na$_2$HPO$_4$ (Fluka) was added at different concentrations (100, 125, 150, 175, and 200 mM) to delay the gelation process, thereby obtaining an injectable formulation that solidifies in situ. The formulations were studied by oscillatory measurements in a rheometer (TA instruments) to determine the gelation time. To produce the plasma-treated hydrogels (PTH), 1 mL of 0.25% alginate was placed under the kINPen® plasma jet at 1sL/min gas flow and a distance of 10 mm. Plasma treatment times between 15s-5min were investigated. Afterward, 1mL of plasma-treated alginate was mixed with 50µL of 2.44M CaSO$_4$ (Merck) and 50µL of 125mM Na$_2$HPO$_4$ to trigger the crosslinking reaction. Finally, 200µL of PTH were poured onto MG-63 human osteosarcoma cells (ATCC). Then, the metabolic activity (Prestoblue™, ThermoFisher Scientific) and the cell viability (LIVE/DEAD™, ThermoFisher Scientific) were evaluated after 24 and 72h.

Results and Discussion
Regarding the formulation, results indicated that increasing Na$_2$HPO$_4$ concentration increases gelation time from 3 to 25 min. 125mM Na$_2$HPO$_4$ was selected for subsequent experiments since it showed a gelation time of 10min, which allowed easy handling of the hydrogel while providing feasible gelation time to work with cells. On the other hand, results demonstrated that plasma-treated alginate hydrogels can exert cytotoxic effects on MG-63 cells in a RONS-concentration dependent manner. A significant reduction was observed in the metabolic activity by the exposition of the cells to plasma-treated hydrogels as a function of plasma treatment time (Figure 1A). Short plasma treatment times did not affect cells, but from 1 min treatment on, a progressive cytotoxic effect was observed up to 5 min of plasma treatment. These results correlated with the live/dead staining (Figure 1B), where the number of viable...
cells (in green) decreased when increasing the plasma treatment time, showing the lowest number of viable cells in the 5 min plasma treatment condition.

**Conclusion**

The results obtained in the present work indicate that plasma-treated alginate hydrogels can be easily manipulated and injected in the desired location and, more importantly, that such hydrogels have a cytotoxic effect on MG-63 human osteosarcoma cells in a RONS-concentration dependent manner. Therefore, these results open the door for the use of alginate-based formulations as injectable RONS delivery systems in CAP-associated therapies for osteosarcoma treatment.

**Acknowledgement**

This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (Grant agreement Nº 714793). Authors acknowledge MINECO for PID2019-103892RB I00/AEI/10.13039/501100011033. Authors belong to the SGR2017 1165.

**References**


Freeze-casted bioglass-PDLLA nanocomposite scaffolds for mandibular osteoradionecrosis treatment: from building units design to preliminary in vivo study

Prescillia Lagarrigue1,2, Vincent Darcos2, David Grossin1, Audrey Bethry2, Patricia Licznar3,4, Agnès Masnou3, Béatrice Herbault-Barres5, Agnès Dupret-Bories5,1, Christèle Combes1, Jérémy Soulié1

1 Université de Toulouse, CNRS, Toulouse INP - ENSIACET, CIRIMAT, Toulouse, FR; 2 Université de Montpellier, CNRS, ENSCM, IBMM, Montpellier, FR; 3 Université de Montpellier, HydroSciences, Montpellier, FR; 4 CHRU Montpellier, Hôpital St Eloi, Montpellier, FR; 5 Institut Universitaire du Cancer de Toulouse Oncopole, ICR, Toulouse, FR

Introduction

Mandibular osteoradionecrosis (ORN) appears in 10% of patients after radiation therapy for ENT cancer treatment. For bone defects larger than 2 cm, the only existing treatment is major surgery with strong handicap for the patient. To the best of our knowledge, no synthetic bone substitute material is actually available to promote bone regeneration for ORN. In this context, the recent bone tissue engineering advances lead to the development of 3D porous scaffolds allowing cells colonization and growth [1]. The ideal scaffold suitable for ORN should be i) highly porous (volume, size), ii) bioreabsorbable with a controllable degradation rate, iii) antibacterial in order to limit infections and iv) bioactive to stimulate new bone formation and angiogenesis. Consequently, the aim of this work is to develop new macroporous organic/inorganic nanocomposite scaffolds respecting those criteria. Their preparation combines an innovative “bricks and mortar” strategy with the freeze casting process [2]. Indeed, these composites are based on biodegradable customized polymer (poly(D,L-lactide) (PDLLA), the “mortar”) grafted on Cu-doped silicate-based bioactive glass nanoparticles (BGN) (“bricks”) to improve both mechanical properties and tissue regeneration.

Experimental Methods

Binary SiO2-CaO BGN with and without Cu-doping were synthesized using Stöber-derived methods. Hybrid nanoparticles were obtained using a “grafting onto” strategy, leading to PDLLA-covalently functionalized BGN. Suspensions of those hybrid nanoparticles was then freeze casted to obtain a macroporous scaffold with a controlled continuous and anisotropic porosity. Physico-chemical (composition, structure), mechanical, in vitro (cytocompatibility and antibacterial tests on S. aureus and E. coli), and in vivo (biocompatibility and osteoconductivity after implantation in rat calvaria) properties were characterized.

Results and Discussion

Characterization of BGN (TEM, SSNMR, ICP-AES) highlighted the influence of synthesis condition (pH, order of reagent addition) on their final shape, diameter, composition and particularly Ca and Cu spatial location and insertion in the glass network. Thereafter well-defined PDLLA chains were successfully grafted onto BGN surface and the resulting hybrid particles fully described (SEM, DLS/zeta-potential, TGA and FTIR), in particular quantifying the amount of grafted polymer [3]. We demonstrated that the freeze casting process parameters such as temperature gradient) and/or suspension composition such as fillers rate strongly affect the final scaffold porosity and thus mechanical properties (SEM, X-ray μ-tomography, compression tests). In vitro biological study showed that SiO2-CaO BGN-PDLLA composite scaffolds were non cytotoxic for primary human fibroblasts culture. Unexpected results from both cytotoxicity and bacterial tests on Cu-doped materials demonstrated the prominence step of copper incorporation within bioglass network. Finally, in vivo implantations and the resulting cell colonization of pores emphasized the potential of these biomaterials.

Conclusion
All together these results validated for the first time that the “bricks and mortar” approach combined with the freeze-casting process is relevant to obtain scaffolds for bone substitution and that such macroporous nanocomposites are promising candidates for the treatment of ORN.

Acknowledgement

The authors thank Institut Carnot Chimie Balard Cirimat (ANR program n°16 CARN 0008-01), “Fondation des Gueules Cassées” (n° 38-2019) and ANR CongOs (ANR-20-CE19-0019) for supporting this research work.

References


Building on established technology is no guarantee for success: challenges with the in vivo evaluation of an osteochondral implant based on a proven bone component

Florence Abinzano¹, Pawena Diloksumpan², Mylène de Ruijter¹, Anneloes Mensinga¹,², Saskia Plomp², Ilyas Khan³, Harold Brommer², Ineke Smit², Miguel Dias Castilho¹, Rene van Weeren², Jos Malda¹,², Riccardo Levato¹,²

¹ University Medical Centre Utrecht, Department of Orthopaedics, Utrecht, NL; ² Utrecht University, Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht, NL; ³ Swansea University, Center for Nanohealth, Institute of Life Science, College of Medicine, Swansea, GB

Introduction
Articular cartilage defects remain a major challenge, as they will not heal naturally and often lead to the development of osteoarthritis. There are still no long-term regenerative options, with currently available treatments leading to fibrous repair tissue of insufficient quality. Implant-based approaches have shown promise, yet the fixation of the constructs is problematic.¹ Preculturing the construct prior to implantation, generating cartilage-like matrix production, might lead to improved repair tissue. The aim of this study was to evaluate the long term performance of a precultured, integrated osteochondral implant in an orthotopic equine model, based on a previously tested bone component.

Experimental Methods
The construct consisted of a 3D printed low-temperature setting bioceramic bone anchor which was firmly integrated to a cartilage-mimetic compartment. This cartilage zone consisted of a melt-electrowritten reinforcing polycaprolactone (PCL) mesh,² with articular cartilage-resident progenitor cells (ACPCs) seeded on top of it (Figure 1). As a control, implants without cells were used. The bone anchor had been previously shown to induce osteoregeneration when implanted in vivo into the hip bone (tuber coxae) of warmblood horses.³ To maximize cartilage matrix production before implantation, the constructs were precultured for 28 days supplementing the media with bone morphogenetic protein-9 (BMP-9).⁴ A cell-laden construct was press-fit implanted into surgically created defect site in the stifle joint of Shetland ponies, while a cell-free implant in the contralateral joint (n=8). Healing was monitored for 6 months non-invasively through radiographic examinations and quantitative gait analysis, after which the animals were humanely euthanized. After termination, implants were harvested and micro-computer tomography scans, mechanical analysis, biochemical analysis and histological examination were performed to assess functional outcomes.

Results and Discussion
In vitro maturation of the constructs prior to implantation resulted in abundant extracellular matrix production, rich in glycosaminoglycans (GAGs) and type II collagen (Figure 2.1), and low in type I collagen. During press-fit implantation, minor imperfections of the contours of the printed bone compartment lead to fragmentation of the edges of the brittle bioceramic scaffold. Gait analysis and radiographic examinations showed no clinical signs of lameness and no differences between cell-laden and cell-free constructs in terms of gait profile parameters (stride duration, head, pelvis and joint range of motion). Six months after implantation, fibrous tissue was found in all the defects, with no mechanical differences between the cell-laden and cell-free controls. Histological and biochemical analyses showed a remodelled matrix resembling fibrocartilage, with minimal amounts of GAGs and type II collagen present in the chondral region for both cell-laden and cell-free implants (Figure 2.2). The PCL meshes within the chondral compartment were still traceable in histological images. Quantitative micro-computed tomography showed collapse
of the bone anchor, low volume of mineralized neo-bone formation and minimal integration to the surrounding tissue, with no differences between cell-laden and cell-free implants (Figure 2.3).

Conclusion

It was concluded that the failure of the bone component of the integrated implant, resulting in the loss of mechanical support and improper fixation, strongly affected the overall outcome, hindering the evaluation of BMP-9 stimulated articular cartilage progenitor cells for \textit{in vivo} cartilage repair. Minor differences in the implant and the change in testing location, from the non-loading tuber coxae to the loading stifle joint, lead to completely different results. This outcome stresses the need of carrying out \textit{in vivo} pilot studies under exactly the same conditions before moving into a larger \textit{in vivo} study.

Acknowledgement

The authors would like to thank Dr. van Loon, Dr. de Grauw, Dr. Veraa and Paulina Nunez Bernal for their assistance with this experiment. The authors also wish to acknowledge the funding support from the AO Foundation ARI collaborative research program Osteochondral Defect, the Royal Thai government scholarship, the Dutch Arthritis Foundation (LLP-12 and LLP-22), the European Research Council (3D-JOINT) and the UK Regenerative Medicine Platform.

Figure 1

Schematic representation of the fabrication process of the tissue engineered osteochondral constructs: 3D printing of the combined osteochondral graft (A), and its seeding with ACPCs and subsequent \textit{in vitro} preculture with BMP-9 supplementation (B).
Figure 2

Figure 2.1 - Histological staining of the constructs before implantation (Scale bar = 50mm). Figure 2.2 - Representative histological images after 6 months of in vivo implantation. Safranin-O/fast green (A, E); Collagen type II (B, F); Picrosirius-red (C, G); collagen type I (D, H) of cell-laden (A-D) and cell-free structures (E-H) (Scale bar = 1mm). Figure 2.3 - Representative µ-CT images from the middle of the sagittal plane of the osteochondral constructs.

References


Development of biomimetic composites of PVA with Kevlar nanofibers, with superior tribomechanical properties

Andreia S. Oliveira¹,²,³, Rogério Colaço², Ana P. Serro¹,³, Nicholas A. Kotov⁴,⁵,⁶

¹ Instituto Superior Técnico, Universidade de Lisboa, Centro de Química Estrutural, Lisbon, PT; ² Instituto Superior Técnico, Universidade de Lisboa, Instituto de Engenharia Mecânica, Lisbon, PT; ³ Instituto Universitário Egas Moniz, Centro de Investigação Interdisciplinar Egas Moniz, Caparica, PT; ⁴ University of Michigan, Department of Chemical Engineering, Michigan, US; ⁵ University of Michigan, Biointerfaces Institute, Michigan, US; ⁶ University of Michigan, Department of Materials Science and Engineering, Michigan, US

Introduction
Aramid (poly(p-phenylene terephthalamide)) fibers, commercially known as Kevlar, are a class of organic materials with outstanding properties, such as mechanical robustness and thermal stability [1]. These fibers have been widely used to reinforce other polymers, improving their stiffness and strength. Recently, it has been shown that a nanoscale version of Kevlar - aramid nanofibers (ANFs) synthesized from their macroscale fabrics and yarns - can effectively reinforce polyvinyl alcohol (PVA) for the replacement of cartilage tissues, due to their proven biocompatibility and exceptional mechanical properties [2]. In this work, we prepare PVA+ANF composites under different conditions, to identify which production method can generate materials with superior mechanical and tribological performance, without compromising their water-retention ability, relevant in the mimicking of the natural tissues.

Experimental Methods
PVA (Mw146000–186000 Da) was dissolved in dimethyl sulfoxide (DMSO) (15% m/v), water (15% m/v), or trifluoroacetic acid (TFA) (7.5% m/v). Bulk Kevlar K29 (TEX 69) was used to obtain ANF solutions (2% m/v), which were also prepared in three different solvents: DMSO in the presence of potassium hydroxide [3]; water, pre-submitting the ANFs to a hydrothermal treatment under acidic conditions [4]; and a mixture of TFA and methanesulfonic acid (MSA) in a volumetric ratio of 4:1. Three different hydrogels (H-D, H-W and H-T) resulted from the combination of PVA:ANF solutions in a ratio of 10:1 (w/w), prepared in common solvents. The mixtures were poured into flat molds, cooled to room temperature, and dried at 40 ºC until reach a constant weight. Samples H-D and H-T were submerged in water for 3 days to solvent-exchange, before being put to dry. Then, the materials were washed in water and stored hydrated.

The nanofibers resultant from the ANF solutions were analyzed by scanning electron microscopy (SEM, JEOL JSM-7001F). The tert-butyl alcohol freeze-drying method [5] was applied to ANF samples prior to imaging. Hydrogels were characterized concerning their equilibrium water content, by comparing the hydrated weight of the materials with their dry weight. The mechanical performance was assessed through uniaxial tensile (0.5 mm/s) and unconfined compression (0.1 mm/s) tests, carried out in a texturometer (TA.XT Express Texture Analyzer, Stable Micro Systems). The friction coefficient of samples against 316L stainless steel (Ø6 mm balls), was measured in a pin-on-disk tribometer (TRB³, Anton Paar), in reciprocal linear mode, with loads of 5 and 20 N, using phosphate-buffered saline solution as lubricant.

Results and Discussion
SEM micrographs of ANF samples prepared in DMSO (ANFD), TFA/MSA (ANFTM), or hydrolysed with acid (ANFH) are displayed in Figure1. The images show the microscale morphology of ANFs, in which the nanofibers form a highly interconnected network.

The values of equilibrium water content (EWC) of PVA-ANF composites did not vary between H-D and H-W (≈61-63%) but were increased for H-T (≈68%).
The composites prepared in DMSO showed a maximum tensile strain of ≈258%, and ultimate tensile strength and elastic modulus of ≈4 MPa. In response to compression, these materials were the most rigid, presenting a modulus of ≈3 MPa. With regard to H-W samples, these hydrogels demonstrated an excellent improvement in tensile tests, revealing an elongation-to-break of ≈409% and ultimate strength of ≈9 MPa. This improvement may be explained by the increase in hydrogen bonding between PVA and ANF, caused by the acidic hydrothermal treatment, which led to the breakdown of amino bonds in the ANF chains, thus providing more available sites (–NH₂ and –COOH) to react with other functional groups [4]. H-T samples had the lowest mechanical strength and modulus, both in tensile and compression. In these composites, the increase in the water content and the reduction of the mechanical performance, suggest a decrease in hydrogen bonding between the forming polymers, resulting in a more porous and weakened structure.

Concerning tribological behaviour, the coefficient of friction (CoF) values of all composites were low and similar (≈0.07) when 5 N of force was applied. For 20 N, the CoF was 1.2x and 1.6x higher for H-D and H-T respectively, in comparison to H-W (≈0.09).

**Conclusion**

Our results indicated that the properties of PVA+ANF composites are dependent on the preparation method. ANF materials made from corresponding hydrogels, prepared in water (H-W) had an improved mechanical and tribological performance, without compromising their water-retention ability. The unique combination of properties should be attributed to the establishment of dense networks of hydrogen bonding interactions between the PVA and nanofibers.

**Acknowledgement**

The authors gratefully acknowledge to Fundação para a Ciência e a Tecnologia for the financial support (grant numbers: PD/BD/128140/2016 [A.S. Oliveira, MIT - Portugal program], PTDC/CTM-CTM/29593/2017 [CartHeal], UIDB/00100/2020 [CQE], UIDB/50022/2020 [IDMEC/LAETA], and UIDB/04585/2020 [CiiEM]).

**References**


Bioactive membranes for the treatment of osteoporosis-related fractures

Lúcia F. Santos, Ana S. Silva, Sara Nadine, Clara R. Correia, João F. Mano

University of Aveiro, CICECO - Aveiro Institute of Materials, Aveiro, PT

Introduction

Fragility fractures are a main consequence of osteoporosis, a major public health problematic whose treatment remains a challenge in the orthopaedic field. Recent evidence have demonstrated that an impaired periosteal activity is responsible for recurrent fractures. Thus, bone tissue engineering strategies focusing on periosteum repair may represent a promising approach to treat the fragility fractures occurring in osteoporotic people. We suggest the development of a natural-based regenerative membrane able to mechanically fix biological active capsules for bone regeneration. For that, we propose a micropatterned laminarin hydrogel composed of spaced micropillars that can act as on-site fixing agent for the biological active capsules, with suitable mechanical properties and adequate resorbable times for the regeneration of the injury. We expected that such device could be implanted by wrapping the membrane around the defect and guide bone regeneration.

Experimental Methods

Liquified capsules were produced by well-established procedures in the group,[1,2] comprising i) polycaprolactone microparticles for cell adhesion and ii) a rich cell niche of human adipose stem cells (hASCs) and human umbilical vein endothelial cells (HUVECs). Cells and microparticles were resuspended in a low viscosity sodium alginate solution and then, alginate microgels (capsules) were produced by electrohydrodynamic spraying using calcium chloride solution as a crosslinking bath. Lastly, layer-by-layer was performed using 3 different polyelectrolytes (chitosan, alginate and poly-L-lysine) and the process was repeated until a 10-layered membrane was created. Methacryloyl laminarin (MeLam) hydrogels composed by spaced micropillars were obtained by bringing a solution of MeLam in contact with an optimized PDMS master,[3] previously treated with plasma. The mold was exposed to UV irradiation during 10 min at 1.2 W/cm². Capsules with optimized sizes were entrapped within the micropillars of the MeLam hydrogels. To avoid the rupture of the capsules, the liquefied core was only obtained after capsules’ entrapment within the laminarin-based membrane, by chelation with ethylenediaminetetraacetic acid solution (EDTA). Since the periosteum is highly rich in fibroblasts, the bioactive membrane as placed in contact with a 2D bed of fibroblasts, to in vitro mimic the natural environment of the periosteum. The characterization of the capsules and membranes, the in vitro osteo- and angiogenic potential was also accessed for the individual components (membranes and capsules) and after their conjugation (membrane + capsules).

Results and Discussion

A MeLam micropatterned hydrogel with spaced micropillars was fabricated by soft lithography with pillars’ height of 300µm and a micropillars’ space of 160µm. As previously reported by the group, the maximum entrapment efficiency was obtained by matching the diameter of the micro-objects and the micropillars spacing.[3] In this sense, liquified capsules (containing cells and microparticles) were obtained with approximately 160 μm of diameter and were successfully entrapped within the laminarin membrane. After confirming the cell viability and proliferation for the individual components (membranes and capsules), the bioactive membrane (laminarin micropatterned hydrogel + capsules) was placed in contact with a 2D bed of fibroblasts during 21 days of culture to mimic the natural fibroblast environment of the periosteum. The results showed that the capsules were released from the membranes in the first days of culture and also incorporated the fibroblast cell layer. The osteogenesis of stem cells in our system was also demonstrated after 21 days of culture with the presence of osteogenic markers like osteopontin.
Conclusion
A bioinspired and natural-based membrane was microfabricated to accommodate bioactive capsules for periosteum regeneration purposes. The bioactive membrane is expected to induce regional bone formation and an overall stimulation of bone regeneration.

Acknowledgement
We acknowledge the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. This work was also supported by the project ATLAS (ref.ERC-2014-ADG-669858) and through the doctoral grant SFRH/BD/141523/2018 (Lúcia F. Santos).

Fabrication of the bioactive membranes.
A. Schematic representation of the development of a natural-based regenerative membrane able to fix a large amount of bioactive capsules for bone regeneration; B. Brightfield images of the produced MeLam micropatterned hydrogels composed by spaced micropillars; C. Fluorescence images of the bioactive capsules produced by electrospray technique, containing hASCs (in purple) and HUVECs (in orange).

in vitro assays of bioactive membranes
A. Entrapment of the bioactive capsules containing hASCs (in purple) and HUVECs (in orange) in the micropatterned membrane; B. Assessment of the cell viability of the bioactive capsules during 21 days of culture, where live cells are depicted in green. The formation of the cell aggregates along the time was also accessed by the staining of the F-actin filaments (Phalloidin, in red). The osteogenesis ability of the proposed system was also evaluated by the presence of the osteopontin (in red). All the nuclei are staining in blue (DAPI).

References
Assessment of the osteogenic and osteoclastogenic potential of pure and strontium-substituted nanohydroxyapatite

Georgia-Ioanna Kontogianni1,2, Catarina Coelho3, António Azevedo3, Paulo Quadros3, Giorgia Montalbano4, Sonia Fiorilli4, Chiara Vitale Brovarone4, Maria Chatzinikolaidou1,2

1 University of Crete, Department of Materials Science and Technology, Heraklion, GR; 2 Foundation for Research and Technology Hellas (F.O.R.T.H)-IESL, Heraklion, GR; 3 FLUIDINOVA SA, Moreira da Maia, PT; 4 Politecnico di Torino, Department of Applied Science and Technology, Turin, IT

Introduction
The role of a biomaterial in osteogenesis is to act as a matrix for cell proliferation, osteogenic differentiation, and extracellular matrix formation, therefore facilitating the process of bone regeneration. The repair of critical-sized bone defects using tissue engineering approaches has been previously shown to be effective. Nano-hydroxyapatite (nano-HA) is the major mineral component of bone and part of the calcium phosphate family. Consequently, it is highly biocompatible, osteoconductive and forms strong bonds with native bone, making it an excellent candidate for use in bone tissue engineering [1].

Experimental Methods
Pure nano-HA in a powder and paste form, as well as substituted nano-HA with 50% and 100% Sr, were produced by wet chemical precipitation in the NETmix® reactor (FLUIDINOVA S.A.) using calcium, strontium and phosphorous salts as starting materials. Each material was suspended to a final concentration of 0.25% v/v against both pre-osteoblastic MC3T3-E1 cells and Peripheral Blood Mononuclear Cells (PBMCs). The latter have the ability to differentiate into fully formed osteoclasts with the use of macrophage stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B ligand (RANKL). Cytotoxicity of the materials was investigated in pre-osteoblasts using the PrestoBlue™ assay. Alkaline phosphatase (ALP) indicating osteoblast differentiation was determined using an enzymatic activity assay. Total soluble collagen secretion in the culture medium and calcium biomineralization were assessed in order to evaluate the formation of extracellular matrix. Gene expression of the osteogenesis-related markers ALP, Coll1-a1, osteonectin, osteopontin and osteocalcin was investigated by means of quantitative real time polymerase chain reaction (qPCR). Moreover, qPCR was performed to evaluate the osteoclastogenesis-related markers DC-STAMP, NFATC1, OSCAR and TRAP. Tissue culture treated polystyrene (TCPS) was used as control surface.

Results and Discussion
All nano-HA materials tested are not cytotoxic, show high cell viability and present a higher ALP activity on days 3 and 7 compared to the control, with the 50% Sr-containing nano-HA showing a significant increase. Calcium mineralization was higher on all materials compared to the control after 7 days in culture. Collagen secretion was high in all nano-HA materials, with the powder showing significantly higher values after 14 days. Gene expression levels of osteocalcin with the nano-HA materials were higher compared to the control after 14 days of incubation. As osteocalcin is produced by osteoblasts as a late marker of osteogenesis, we conclude that all nano-HA materials induce osteoblastic formation after 14 days of culture. Finally, the expression of the osteoclastogenesis related markers that did not show any significant differences compared to the TCPS control. Therefore, these results demonstrate that nano-HAs are promising materials for bone tissue formation.

Conclusion
The different compositions of nano-HA materials that were investigated displayed an excellent biocompatibility, significant cell adhesion and a high capacity of pre-osteoblastic cells for proliferation and osteogenic differentiation.
These data suggest that all types of the nano-HA materials have high osteogenic and low osteoclastogenic potential, rendering them promising for therapeutic approaches against osteoporotic pathological conditions.

Acknowledgement

This project has received funding from the European Union’s Horizon 2020 research and innovation program under grant agreement No. 814410.

References

Functional *in vitro* engineered osteochondral tissue for modelling osteoarthritis disease

**Annachiara Scalzone**, Giorgia Cerqueni, Michele Pistillo, Xiao Nong Wang, Kenny Dalgarno, Ana Marina Ferreira-Duarte, Monica Mattioli-Belmonte, Piergiorgio Gentile

1 Newcastle University, School of Engineering, Newcastle upon Tyne, GB; 2 Università Politecnica delle Marche, Department of Clinical and Molecular Sciences, Ancona, IT; 3 Newcastle University, Translational and Clinical Research Institute, Newcastle upon Tyne, GB

**Introduction**

Osteoarthritis (OA) is a degenerative pathology with a global prevalence of 22.9% in individuals aged 40 [1], which leads to significant impaired of the quality of life and disabilities. The main features of OA are the complex interplay of inflammatory processes associated with the synovial membrane, articular cartilage (AC) degradation and remodelling of the subchondral bone (SB) [2]. Furthermore, during the OA pathogenesis, there is an increase in the catabolic protein’s expression by synoviocytes, chondrocytes and mononuclear cells, followed by action of stimuli such as cytokines or chemokine[3]. Although, several studies have been carried on deepening the knowledge on the onset of OA and its treatment, there are still uncertainties about a long-term surgical treatment for OA joints [4]. Our work aimed to develop an engineered in vitro model of healthy and osteoarthritic osteochondral human tissues (composed by subchondral bone and deep layer of AC) in order to increase our understanding on physiology, biology, and progression of OA diseases. The findings of this work could advance the understanding of the fundamental mechanisms underlying OA as a key tool for testing new therapeutic options that could be employed in the medical management of the disease.

**Experimental Methods**

For the SB layer, a polylactic acid (PLA) porous trabecular-like construct was 3D printed via Fused Deposition Modelling (Rokit INVIVO Bioprinter) and functionalized with gelatin (Gel) and hydroxyapatite (nHA) through immobilisation via poly-dopamine (DP) coating. For the AC deep zone, a photocurable Gellan Gum (GG; 3%w/v) multi-channels hydrogel structure was obtained via soft-lithography, adherent to the PLA scaffold. Chondrocytes (differentiated from Y201 bone marrow stromal mesenchymal stem cells) were encapsulated in each channel of GG layer within a chondroitin sulphate-based hydrogel [5], while Y2O1 cells were seeded on PLA scaffold (Figure 1). GG physical properties (water uptake capability, porosity via Scanning Electron Microscopy (SEM) and mechanical properties via compression and stress relaxation analyses) were evaluated. OA milieu was induced by IL-6, IL-1β and TNF-α addition to the medium. Cell viability was assessed for both phases of the construct via Live/Dead, metabolic activity (MTT for PLA and MTS for GG) and Immunostaining of nuclei and cytoskeleton (DAPI and phalloidin). The chondrogenic potential of cells and the effect of cytokines in the OA samples were evaluated through gene expression analysis, mucopolysaccharides quantification (Alcian blue) and calcium deposition (Alizarin Red).

**Results and Discussion**

GG hydrogel showed high water uptake capability (initial rapid uptake of 1433±57% at 3 h then achieving plateau), high porosity (pore range size of 100-150μm 30%) and suitable mechanical properties for AC application (48±5kPa as compressive Young's modulus). All the materials showed cytocompatibility in terms of cells viability and metabolic activity up to 7 days (Figure 2A). The effects of cytokines inducing OA decreased the mechanical properties (Figure 2B shows the stress-relaxation behaviour at day 14). The expression of chondrogenic markers (COL2A1, ACAN, SOX9) and the mucopolysaccharide quantification (0.36±0.01µg in the Healthy model compared to 0.22±0.02µg in the OA samples at day 21) (Figure 2D). OA model showed also increased OA-related molecule expression (COLL1,
MMP9-13, ADAMTS5) and Alizarin Red values (680±20µM for the OA-model compared to 550±10µM of the Healthy model) (Figure 2C).

Conclusion
We developed a high reproducible 3D in vitro OA model characterized by quickly manufacturing, easy manipulation and large availability. Our analyses demonstrated that the selected “cytokines cocktail” medium enrichment was a feasible process to reproduce the OA milieu in vitro. This construct could be applied to test new therapeutics and to study on cells crosstalk interactions.

Acknowledgement
Annachiara Scalzone acknowledges support from EPSRC for her Ph.D. studentship (EPSRC Grant EP/R51309X/1); Giorgia Cerqueni acknowledges support from Università Politecnica delle Marche for her Ph.D. studentship.

References
N04-08

Osteoarthritis pathogenesis: a cytokine-induced *in vitro* spheroid-based study model

Annachiara Scalzone¹, Giorgia Cerqueni², Xiao N. Wang¹, Monica Mattioli-Belmonte², Kenny Dalgarno¹, Marina Ferreira-Duarte¹, Piergiorgio Gentile¹

¹ Newcastle University, School of Engineering, Newcastle Upon Tyne, GB; ² Università Politecnica delle Marche, Department of Clinical and Molecular Sciences – DISCLIMO, Ancona, IT

**Introduction**

The progressive degeneration of Articular Cartilage (AC) due to Osteoarthritis (OA) is the most common and chronic form of joint disease in the Western World, affecting 10–12% of the global population for a total of 303 million people¹. To date, no specific therapy for a long-term surgical treatment for OA joints has been successful, mainly due to the lack of knowledge about the disease pathogenesis². Indeed, *in vitro* models of AC human tissues to study the OA progression are suitable candidates in increasing our understanding on AC physiology and biology and this could help in advancing the research into the causes of the OA disease at early stage as a key tool to investigate new therapeutic options in reliable and predictive models. In this work we developed an *in vitro* spheroid model of AC in Healthy and OA condition, with the aim of studying the disease evolution at cellular level. Current evidence suggests that pro-inflammatory cytokines are responsible for the catabolic process occurring in the pathological tissues. Herein, we designed and analysed a cytokine-induced OA spheroid model, obtained combining interleukin (IL)-1β, tumor necrosis factor-α (TNF-α) and IL-6.

**Experimental Methods**

For the formation of multicellular spheroids, human articular chondrocytes (HC) and immortalised mesenchymal cells differentiated in chondrocytes (Y201-Cs) were cultured in round-bottom 96-well plate. Three culture condition were set: Healthy (DMEM/F12), Low concentration Osteoarthritic (LC-OA) (DMEM/F12 loaded with: IL-1ß:1ng/mL, TNF-α:1ng/mL, IL-6:10ng/mL) and High Concentration Osteoarthritic (HC-OA) (DMEM/F12 loaded with: IL-1ß:5ng/mL, TNF-α:5ng/mL, IL-6:50ng/mL). Spheroids growth kinetics and metabolic activity was evaluated over 10 days of culture, in both healthy and OA conditions. Then, their metabolic activity was evaluated via MTS assay. After 10 days of culture, the spheroids were assembled and cultured for 14 days on a gelatin-coated poly(lactic-co-glycolic acid) electrospun membranes (10 spheroids/cm²), following a protocol in line with the clinically approved Chondrosphere® (CO.DON AG) technique³. Gene expression (SOX9, ACAN, COL2A1,MMP-13 and ADAMTS-5), Histology (H&E, PicroSirius Red and Alcian Blue) and Immunohistochemistry (IHC) (Collagen type II and Aggrecan) analyses were performed to evaluate the quality of the tissue produced in the set conditions.

**Results and Discussion**

Both cell type showed a decrease of spheroid diameter (~35%) over culture in healthy condition, while the dimension was stable for the spheroids under OA state. decreased over culture, while it increased for Y201-Cs in all the three condition. Instead, for both cells, at day 10 HC-OA sample showed an increased metabolic activity, possibly due to the biosynthesis of inflammatory and degradative proteins involved in the OA pathogenesis⁴. Gene expression, histology and immunohistochemistry demonstrated the successful role of the cytokine in modulating the expression of matrix metalloproteinases and cartilage ECM proteins (Figure 1). Both HCs and Y201-Cs showed a decreased expression of SOX9, ACAN and COL2A1 at day 21 compared to the Healthy model and an overexpression of pathological marker MMP-13 and ADAMTS-5. To confirm this, we demonstrated that both cells population in Healthy state, produced higher amount of collagen and glycosaminoglycans by Picrosirius red and Alcian Blue stainings and specifically Collagen Type II and Aggrecan, by IHC compared to OA conditions. Finally, H&E staining demonstrated...
that the obtained healthy cartilage-like tissue was more intact compared to the pathological one, that appeared more fragmented especially for the HC cells.

**Conclusion**

In this work we optimised an approach to obtain an *in vitro* model of articular cartilage tissue in healthy and pathological state. Also we demonstrated that a low concentration of cytokines is sufficient to obtain lower expression of anabolic genes and higher expression of catabolic genes by cells, together with a lower production of collagen type II and aggrecan. Finally, we showed how it is possible to get a reliable *in vitro* model even with a bankable cell line (Y201-C), with the related advantages in terms of low-cost, high reproducibility, high number of cells available, fast time for producing it compared to primary chondrocytes.

**Acknowledgement**

A.S. acknowledges support from EPSRC for her Ph.D. studentship (EPSRC Grant EP/R51309X/1). X.W. and K.D. acknowledge support from Versus Arthritis (Award 21156).

![Figure 1](image)

**Figure 1**

IHC (DAPI/Coll II, DAPI/ACAN) at day 1 and day 21 for Y201-C and HC and Gene expression for SOX9 and MMP13 for Y201-C and HC.

**References**


Development of osteogenic microenvironments using cationic liposomes embedded into polyelectrolyte multilayer system

Yazmin A. Brito Barrera1, Matthias Menzel2, Christian Schmelzer2, Christian Wölk3, Thomas Groth1,4

1 Martin Luther University Halle Wittenberg, Department Biomedical Materials/Institute of Pharmacy, Halle (Saale), DE; 2 Fraunhofer, Department of Biological and Macromolecular Materials/Institute for Microstructure of Materials and Systems (IMWS), Halle (Saale), DE; 3 Leipzig University, Pharmaceutical Technology/Institute of Pharmacy/Faculty of Medicine, Leipzig, DE; 4 Martin Luther University Halle Wittenberg, Interdisciplinary Center of Materials Science, Halle (Saale), DE

Introduction
Cationic liposomes of OO4 lipid and dioleoylphosphatidylethanolamine (DOPE) contain a higher amount of amino groups than classical lipids that can be used for lipofection. Here, a polyelectrolyte multilayer system (PEM) made of chondroitin sulphate (CS) and collagen type I (Col I) was prepared by Layer-by-Layer technique (LbL), which is a method to fabricate surface coatings by alternating adsorption of polyanions and polycations. Due to their intrinsic charge, the liposomes can be used as a polycation and can be embedded in the terminal layers of PEM for the controlled release of the cargo (lipophilic or hydrophilic models) in the liposomes into C2C12 cells (myoblast). As well, the use of Col I and CS should mimic the extracellular matrix of bone and the addition of a functional cargo like Dexamethasone can induce osteogenic differentiation for future applications such as bone replacement therapies.

Experimental Methods
Characterization of the liposomes was evaluated regarding their size distribution using dynamic light scattering (DLS) and morphology by micrographs of freeze-fracture TEM and Cryo TEM. Physicochemical studies of PEM were done to characterize layer growth (surface plasmon resonance), thickness (ellipsometry), topography (atomic force microscopy). The substrates were coated with PEM consisting of CS, liposomes, Col I as terminal layers. Cell adhesion and liposome uptake studies by C2C12 cells were carried out with immunofluorescence staining and flow cytometry analysis. The osteogenic differentiation was evaluated using histochemical staining by Alizarin Red and the detection of alkaline phosphate activity (ALP).

Results and Discussion
The DLS measurements show two sizes of populations between 70-100nm and 300-700nm. In the characterization of PEM, SPR results show a linear growth behavior with an increase in the angle shift. Liposome adsorption measured lead to a significant increase in thickness. AFM studies indicated a change on the surface before and after deposition of the liposomes on PEM. The cell adhesion studies show that there were significantly more cells adhering on PEM with liposomes as a terminal layer and with the addition of one bilayer as a terminal. The concept to add two layers more after the liposomes were not only to protect liposomes from degradation but also to improve cell adhesion. The quantification of liposome uptake indicated that 50.8% of the cells internalized liposomes with Texas Red dextran were positive and 90.5% of cells were Rhodamine-DOPE-positive. Consequently, efficient uptake of liposomes can be assumed for both conditions. As a proof of concept, dexamethasone (Dex) was incorporated in the lipid bilayer of liposomes to induce differentiation of C2C12 cells into osteoblasts. Quantitative measurement of ALP activity shows an increase in samples where Dex-loaded liposomes were embedded in comparison to the positive and negative control. Histochemical staining by alizarin red s indicates the formation hydroxyapatite.

Conclusion
This work has shown that deposition of cationic liposomes composed of the co-lipid DOPE and OO4 lipid with cargo like dexamethasone in a PEM made from CS and Col I can be used to induce osteogenic differentiation of cells \textit{in situ}.

\textbf{Acknowledgement}

This work was supported by CONACYT-México and the German Academic Exchange Service (DAAD). The German Research Society (DFG) through project number 396823779. The High-Performance Center Chemical and Biosystems Technology Halle/Leipzig and supported by the European Regional Development Fund (ERDF).
3:45 p.m. – 5:15 p.m.

Track01

**TRS-S03 | Translation Research Symposia**

**Chairs**

*Fabrizio Barberis*
Universita' degli Studi di Genova, Department of Civil Chemical and Environmental Engineering, Genova, IT

*João Cortez*
Catholic University of Portugal, Porto, PT

*Peter Dubruel*
Department of Organic and Macromolecular chemistry (Chair); Polymer Chemistry and Biomaterials Group Leader; ESB Industry Liaison Delegate; Ghent University, BE

*Hugo Prazeres*
University of Porto, Institute of Biomedical Engineering (INEB) and i3S- Instituto de Investigação e Inovação em Saúde, Porto, PT

*It is only sometimes that innovation takes a well-orchestrated route: a scientific breakthrough, a product derived from such knowledge, an academic-owned patent licenced to a start-up or a big company, regulatory approvals, market sales and shares climbing. Such orderly, stage-by-stage progression to success has been embodied in various widely circulated “bibles” of innovation models. However, this innovation cycle “fairy tale” is not entirely the rule, as most often an entrepreneur’s success story came from devising unexpected solutions to battle the same old problems and from “crazy” ideas to overcome false failures and to strive against all odds.*

*This session aims to tutor and inspire future promoters with entrepreneurship “war stories”, told in person by the chief executives of biomaterials-related companies, about the wildcards that were key to their success story.*
TRS-S03-01
WISE Srl
Luca Ravagnan
WISE Srl, Cologno Monzese, IT

TRS-S03-02
Lattice Medical (France)
Julien Payen
Lattice Medical, Loos, FR

TRS-S03-03
UpNano GmbH
Aleks Ovsianikov
UpNano GmbH, Wien, AT

TRS-S03-04
HydrUSTent SA
Alexandre Barros
HydrUSTent SA, Barco - Guimarães, PT

TRS-S03-05
Healper (Portugal)
Daniel Alves
Healper, Lisboa, PT
3:45 p.m. – 5:15 p.m.

Track02

K03 | Futuring Biomaterials

Chairs
Cristina C. Barrias
i3S-Institute for Investigation and Innovation in Health, Porto, PT
José Carlos Rodríguez-Cabello
University of Valladolid, ES
Jagoda Litowczenko-Cybulksa (YSF)
Adam Mickiewicz University in Poznan, NanoBioMedical Centre, Poznań, PL
Biofabrication in commercial setting

Gabor Forgacs

University of Missouri-Columbia, Missouri, US

Biofabrication is typically defined as the generation of biologically functional products composed, among others, of living cells. In this talk I will present examples of commercial products created by methods that fall within the above definition of biofabrication but at the same time point beyond it. Specifically I will discuss how biofabrication methods can be applied to making leatherlike materials and cultured meat even though the final products obviously do not have biological functionality, as they contain no living material when used or consumed. I will also point out how biofabrication can help to mitigate critically important sustainability issues associated with industrial leather and meat production.
3D Bioprinted Interphase-based bone in vitro models

Alvaro Sanchez-Rubio, Vineetha Jayawarna, Stylianos Sarrigiannidis, Matthew Dalby, Manuel Salmeron-Sanchez

University of Glasgow, Centre for the Cellular Microenvironment, Glasgow, GB

Introduction
In vitro models, which allow to study tissues and organs outside their biological context, are key tools in not only fundamental and applied research but also the pharma industry. However due to the complexity of native tissues, which display heterogeneity in topographical, physicochemical and biological features, creating these models remains a challenge. Until now, being able to mimic that heterogeneity was not possible. However, 3D bioprinting due to its free-form and multimaterial fabrication capabilities has enabled producing constructs that include specially tailored regions that can more appropriately mimic each of the areas present in native tissue, including different chemical cues, physical properties and cell types. In this work, by using 3D Bioprinting we overcome the tissue complexity challenge and engineer interphase-based in vitro models that consider the physicochemical and biological heterogeneity of native bone to achieve the complexity inherent to the vascular bone unit, where vascular and mineral component can be found together and in close interaction. Especially relevant considering that alterations to this interaction leads to several bone pathologies, like osteoarthritis or osteoporosis. Therefore, it is crucial to reproducibly fabricate models that allow to study this system and the interactions present within, so to gain a better understanding of the biological processes, and to more efficiently discover and develop drugs that would act upon it.

Experimental Methods
Different hydrogel systems (Alg/Gel and GelMA) were prepared by dissolving the given polymers at different ratios. Photoinitiators were added to UV-Crosslinked systems. The printed Alg/Gel and BiogelX™-bioink-RGD structures were exposed to different concentrations of CaCl2 solutions to crosslink after printing, while GelMA structures were exposed to UV light for different times. Different cell types (hMSCs and HUVEC) were laden to the hydrogels and printed, in mono- and co-culture conditions. Mechanical properties of the hydrogels were studied using Rheology and Nanoindentation. NeutrAvidin (NA) was used to assess chemical separation of compartments. Further modification of the hydrogels was done by using peptide-coupled alginate (Alg-RGD) or adding Fibronectin (FN) and custom peptide fragments (CPF), which include the gelatine binding domain of FN together with the Growth Factor (GF) binding domain. BMP-2 and VEGF were also added to the system. Release of these proteins was studied using BCA and ELISA. Viability was studied using commercially available Live/Dead kits.

Results and Discussion
Reproducible 7mm diameter, interphase-based constructs were obtained using extrusion-based bioprinting. Stiffness measured varied with polymer concentration and crosslinking times, ranging from below 10 KPa to over 40KPa for soft and rigid gels, and showed increasing stiffness values when either polymer concentration or crosslinking times were used. Constructs with different stiffnesses in each of the compartments were achieved. NA imaging showed clearly separated and adjacent compartments within the same construct and sustained released of the protein into the media. Similarly, we proved presence and clear separation of BMP-2 and VEGF in either compartment of the bone-chip. Immunostaining indicated the ability of FN and CPF to bind to Gelatine, in both 2D and 3D. In addition, when either FN or CPF were used, growth factors were released slower into the media, when compared with non-functionalized bioinks. Viability up to 21 days for MSCs remained over 80% while HUVEC, stayed around 70% for the first 3 days and then decreased, although the morphology of both cell types remained rounded.

Conclusion
Constructs with different compartments were obtained, which specifically mimic each of the regions in the vascular bone unit, having different physicochemical and biological properties. This ability to include different stiffnesses and GF in each compartment of the model overcomes the simplicity found in previous models and shows a higher resemblance to native bone architectures. The throughput of the system indicates the potential of 3D Bioprinting for the generation of reproducible organotypic in vitro models. The functionalization of the bioink by both FN and CPF showed promising results in producing Gel-based bioinks with enhanced GF-binding properties. Bioinks exhibited low toxicity, although further studies need to be done to assess osteogenic and vasculogenic differentiation within the bone-chips.

Acknowledgement
Medical Research Scotland and Biogelx Ltd. for funding this project.
Modeling of Pancreatic Tumors using 3D Bioprinting

Cristina Banda Sánchez, Nieves Cubo Mateo, Jesús Frutos Díaz-Alejo, Julie Earl, Icíar González Gómez, Luis M. Rodríguez Lorenzo

Introduction

Cancer modeling has been of special interest since it paves the way for easing and modulating preclinical research model’s conditions. Relevant advantages are the higher similarity to in vivo systems compared to 2D culture, and preclinical allogenic equivalence compared to animal models. Since pancreatic cancer holds still a low survival rate, strongly dependent on the moment of diagnosis, new therapies urge to be tested, thus advances in 3D modeling of equivalent tumor environments are required.

The objective of this project is the 3D bioprinting of pancreatic tumor models based on PANC-1 laden human plasma, sodium alginate (SA), methylcellulose (MC) bioinks.

Experimental Methods

A. Bioink’s Preparation

The proposed ink is composed of 3% SA and 9% MC in human plasma. Every procedure is accomplished under sterility conditions inside a laminar flow cabinet. Fresh frozen human plasma (obtained in compliance with the standards of the American Association of Blood Banks) is thawed at 37ºC and mixed first with SA under stirring, and then MC with a spatula. Thereafter, PANC-1 cells, cultured in 10% FBS 1% L-Glu 1%Pen/Strep RPMI medium at 37ºC and 5% CO₂, are trypsinized and counted to adjust the desired cell concentration in PBS. After mixing the cells with the ink using a spatula, a final concentration of 5·10⁵ cells in each of the printed constructs is obtained.

B. Optimization & Characterization of the bioink

Ink’s mechanical properties are rheologically characterized on rheometer ARG2 (TA instruments) with a sandblasted parallel plate D = 25 mm geometry at 37ºC before, during, and after gelation event upon addition of 1.5% CaCl₂ crosslinker. Oscillatory frequency sweeps are performed to evaluate the viscosity behavior at the shear thinning region; shear thinning coefficients are obtained as in [4]. Thixotropy and degree of recovery after structural disruption are computed for characterizing printability. Gelation kinetics are measured in a time sweep for 30 min, where crosslinker 1.5% CaCl₂ is added 1 min after start recording.

C. 3D Bioprinting

Cell-laden bioink solution is loaded into a 5 mL cartridge, then stored at RT for stabilization of bioink’s rheological properties. Pneumatic extrusion Inkredible 3D printer (Cellink) is used for dispensing the hydrogel ink through a conical nozzle (D = 410 μm). Cylindrical constructs of D = 15 mm are printed in 6P-culture well plates. Printed hydrogels are crosslinked for 20 min with 1.5% CaCl₂; it is then removed, replaced with fresh medium, and incubated at 37ºC 5% CO₂.

D. Viability assay on cell-laden constructs

Cellular survival and proliferation are assessed with a Live/Dead viability kit (ThermoFisher Scientific #L3224) according to the manufacturer’s protocol. Afterward, constructs are imaged in a fluorescence microscope (PAULA, Leica Microsystems) for green (live) and red (dead) spectra. Viability is screened at 1, 3, 7, and 11 days post-printing.

Results and Discussion
A. Optimization & Characterization of the bioink
The end of LVR is determined by the critical yield point, $\gamma_c = 154 \, \mu\text{Nm}$ in non-crosslinked plasma-based ink; frequency sweep experiments are performed below that value. The ink has a rate index $n = 0.28$, showing highly shear thinning behavior. It yields a gel-like consistency prior to crosslinking at rest, along with a fast recovery rate of 89% in the first 60 s after disruptive shear stress application, simulating the printing process.

B. 3D Bioprinting
At the time of printing, the bioink shows great printability in terms of layer stacking, shape consistency, CAD fidelity, no ink clumping at the tip of the nozzle, and homogeneous qualitative ink composition. The average required printing pressure, constant during the whole process, is 136 KPa. Past 20 min from crosslinker addition, the constructs have swelled slightly doubling the filament diameter, yet not clogging the printed grids destined for nutrient and oxygen exchange.

C. Viability assay on cell-laden constructs
Embedded PANC-1 cells survive and proliferate within the printed constructs after at least 7 days of culture renovating fresh medium periodically, as illustrated in Figure 1. Viability at day 1 suggests that the printing process was barely detrimental for cells. A week after, cells remain alive and proliferate; the red fluorescent channel is merged with the green one. Almost no red light was detected, meaning average low dead cell’s quantity, thus biocompatibility of the ink.

Conclusion
The presented bioink candidate, made of PANC-1 cell line, sodium alginate, methylcellulose, and human plasma has shown valuable characteristics: great printability, biocompatibility, structural integrity, and consistent mechanical properties. Simplified pancreatic tumor models were printed and cultured for 11 days, and cells were shown to be proliferating within the constructs for at least a week. Further experiments with different culture media conditions, printed patterns, and pancreatic cancer cell lines are ongoing.

Acknowledgement
This research was funded by AEI/FEDER UE (DPI2017-90147-R) Spain. Special thanks to Cellink for providing Inkredible printer, and Hospital Ciudad de Coria for the plasma supply.

Figure 1: Viability of cell-laden bioprinted constructs
Viability and proliferation of printed PANC-1 cell-laden plasma-based inks using LIVE/DEAD cell kit (L3224, Invitrogen). Live (green) and dead (red) cells within constructs were screened (from left to right) at day 1, day 3, and day 7 post-printing.

References
Hierarchical biomaterials via photopatterning-assisted additive manufacturing

Dalia Dranseikiene, Elia A. Guzzi, Raffaele Bischof, Dhananjay V. Deshmukh, Mark W. Tibbitt

ETH Zürich, D-MAVT / Macromolecular Engineering Lab, Zürich, CH

Introduction

Additive manufacturing (AM) or biofabrication of tissue mimics often requires control of bioink properties at different scales, which is not accessible by a single approach. AM approaches can be grouped into two broad subcategories: 1) extrusion-based and 2) stimulus-based (e.g. light). Extrusion-based AM allows the use of multiple bioinks in a single print, but the resolution is limited by the nozzle size. Photo-triggered AM offers higher spatial resolution, but usually only single material is utilized. To overcome these limitations, we developed an approach to fabricate hierarchical biomaterials via combined extrusion–photopatterning AM (Fig. 1).

Experimental Methods

The base formulation of universal nanocarrier ink (UNI) consisted of 1 wt% hydroxypropylmethylcellulose (HPMC) and 15 wt% PEG-b-PLA nanoparticles. Gelatin methacryloyl (GelMA, DoF = 70%) was combined with UNI formulation at either 5 or 10 wt%.

Shear rheometry (MCR 502; Anton-Paar; Switzerland) was used to characterize the viscoelastic properties of different biomaterial inks and to investigate the cross-linking kinetics for the control of mechanical properties via photopatterning.

To prepare the hierarchical structures a pneumatic-driven 3D printer (BioX; Cellink, Sweden) and a digital micromirror device DLP® E4750LC (EKB Technologies Ltd; Israel) with 405 nm LED light source were used.

Uniaxial tensile tests (MTS Systems; USA) were performed on the patterned structures to evaluate if the composite structures comply with the rule of mixtures.

Finally, to ensure cytocompatibility of the process human mesenchymal stem cells (hMSCs) were encapsulated in UNI–GelMA and 3D scaffolds were fabricated with a combined extrusion-photopatterning AM technique.

Results and Discussion

The combination of UNI platform with GelMA ensured shear-thinning and self-healing properties required for extrusion-based AM, which were not present in pure GelMA formulations at 37°C. All UNI–GelMA inks had similar shear-thinning behavior and were able to reform after high strain intervals (γ = 100 %, ω = 10 rad s⁻¹).

In order to replicate the process of photopatterning, in which specific hydrogel regions were photopolymerized for longer duration, the cross-linking kinetics of both UNI–GelMA-5 and UNI–GelMA-10 were investigated upon exposure to visible light (λ = 405 nm, I = 10 mW cm⁻²) for different time intervals. A step-wise photopolymerization approach was also tested, in which the sample was initially only partially cross-linked for a short period, equilibrated, and then further exposed to light to reach complete cross-linking of UNI–GelMA. This allowed us to tune the mechanical properties by altering the duration of photopolymerization.

Due to the large difference in storage modulus obtained with UNI–GelMA-10 after two intervals of photopolymerization (ΔG’ > 10 kPa), this formulation was further used for tensile test measurements. Bulk samples cross-linked for short (t = 10 s) and long (t = 60 s) durations had one order of magnitude difference in the final elastic modulus (E₁ = 10 s = 6.4 ± 2.9 kPa and E₁ = 60 s = 28.9 ± 4.4 kPa). Composite structures were fabricated in a step-wise approach: initially the matrix was homogeneously partially cross-linked, and in a second step the reinforcement structures were patterned on the matrix. Different volume fractions of reinforcement were photopatterned in the scaffolds (Fig. 2a). To determine the efficiency of reinforcement, the experimental mechanical data for the 16% and
33% fiber reinforced samples was compared with standard composite theory. The measured elastic moduli of hydrogel scaffolds were comparable to the theoretical rule of mixtures. Biocompatibility of the combined AM process was verified by processing UNI-GelMA-5 bioink with hMSCs via both extrusion and photopatterning. There was no significant difference in cell viability found between the groups of samples that were either only photocrosslinked for different durations or printed by extrusion and then crosslinked. Moreover, cell morphology changes over time in all tested conditions showed that the combined AM process did not impair cell spreading (Fig. 2b).

**Conclusion**

In this work, we showed the possibility to combine structural control from both photo-triggered and extrusion-based AM. In the future, this hybrid approach could be exploited to fabricate more complex constructs for tissue engineering with hierarchical material organization, from micro- to macro-scale.

**Acknowledgement**

This work was supported by start-up funds from ETH Zürich and the SNSF (200021_184697).

---

**Fig. 1.**
Schematic representation of photopatterning-assisted additive manufacturing technique.

**Fig. 2.**
(A) UNI-GelMA-10 scaffolds with 16 and 33% degree of reinforcement (scale – 500 µm). (B) Cell spreading in 16% reinforced UNI-GelMA-5 scaffolds after 0 and 5 days of incubation (scale – 50 µm).

**References**

Sacrificial thermoresponsive Poly(2-cyclopropyl-oxazoline) as a beneficial material for freeform 3D printing and channel network generation in hydrogels

Vincent T. Mair, Ilona Paulus, Jürgen Groll, Matthias Ryma

Julius-Maximilians-Universität Würzburg, Department for Functional Materials in Medicine and Dentistry, Würzburg, DE

Introduction

Most 3D-printing technologies rely on the vertical stacking of layers, whereas each layer provides the structural integrity for the upcoming one. An especially interesting technique, which overcomes these layer limitations, is freeform printing. In the context of biofabrication, this method has been applied for the creation of sacrificial 3D-structures based on carbohydrates for perfusable channel generation in tissue engineered constructs. However, carbohydrate scaffolds do not intrinsically provide a suitable tool for channel creation in extracellular matrix (ECM) mimicking hydrogels. The main reasons are the fast dissolution in aqueous solutions, which leads to the collapse of channels before hydrogels can crosslink, and the osmotic pressure on nearby cells during dissolution of the sugar. To overcome the disadvantages of carbohydrates, scaffolds needed to be dip or spray coated to increase stability during hydrogel gelation and hinder dissolution of the sugar in the hydrogels. However, these methods are cost-, time- and labor-intensive and coating becomes less accurate with increasingly complex scaffolds.

In this study, we established freeform printing of the thermoresponsive polymer poly(2-cyclopropyl-oxazoline) for the creation of freestanding sacrificial scaffolds, which overcomes the disadvantages of carbohydrates by providing stability during hydrogel creation and cytocompatibility during dissolution without requiring post-processing.

Experimental Methods

With PtycloPrOx, we were able to create single freestanding filaments. We observed that an increase in temperature during printing leads to the generation of fibers with increased diameters, based on the lower viscosity of the polymer during printing. An increasing feedrate also led to an increased fiber diameter and vice-versa, since faster movement leads to the deposition of less material in the same distance. With this, fibers can be created ranging from diameters below 100 µm up to 450 µm. Furthermore, we printed single freestanding fibers with various angles from 0° to 90°, proving the applicability of the polymer for more complex structures.

Results and Discussion

Another important factor for the creation of complex scaffolds via FFP is the introduction of fiber fusion. As a first proof-of-principle, we printed 2 straight fibers, which were connected by a bridging free-hanging filament (Figure 1 A). With this, we were able to stack several units in Z-axis by filament fusion (Figure 1 B, C). To prove the suitability in 3 dimensions, we further created stacked cube structures with several fiber thicknesses by varying the temperature (Figure 1 D, E) and generated spiral structures (Figure 1 F).

In conclusion, the basic requirements for FFP, namely control of fiber thickness, stacking and filament fusion were established. To prove the advantageous channel creation with scaffolds based on PtycloPrOx without the need for post-modification, we exemplary embedded the spiral structure in Agarose. After crosslinking and temperature decrease below the LCST of PtycloPrOx, we were able to flush out the polymer and create empty channel structures (Figure 2 A, B). Perfusion of these channels was shown by the addition of a dye (Coomassie blue) (Figure 2 C), proving the suitability of the polymer for channel creation.

Conclusion

Page 259 of 2028
In summary, we were able to adapt the thermoresponsive polymer PcycloPrOx to FFP. It was shown that freestanding interconnected scaffold structures with fiber fusion and stacking can be created. This method enables cytocompatible channel creation, without leaving non-dissolvable remnants behind. In conclusion, the addition of thermoresponsive poly(2-oxazoline)s to the FFP-library opens up great opportunities for the usability of one-step channel network creation in tissue engineering constructs.

Acknowledgement

We would like to thank the European Research Council (PoC 899609 Design2Flow) and the Deutsche Forschungsgemeinschaft (SFB Trr 225 Project B02).

Figure 1

Figure 1: Establishment of fiber fusion and complex structures via FFP of PcycloPrOx. A: Free hanging fiber between two straight fibers. B: Enlarged view of fused filaments. C: Stacked fibers. D: Stacked cube structure. E: Stacked cube structures with varying fiber diameter. Printing temperature from left to right: 200 °C, 210 °C, 215 °C, 225 °C. F: Spiral structure. Scale bar A, C, D, E, F: 2 mm; B: 100 µm.

Figure 2

Figure 2: A: Embedment of a PcycloPrOx spiral scaffold in Agarose. B: Channel generation after dissolution of PcycloPrOx by temperature decrease. C: Perfusion of the generated channels with Coomassie blue. Scale bar 2 mm.

References


Bioprinting of spatially defined co-cultures of mammalian cells and photosynthetically active microalgae as alternative concept for post-implantation oxygen supply

**Sophie Dani**¹, Johannes Windisch¹, Felix Krujatz², Michael Gelinsky¹, Anja Lode¹

¹ Technical University Dresden, Centre for Translational Bone, Joint and Soft Tissue Research, Dresden, DE; ² Technical University Dresden, Institute of Natural Materials Technology, Dresden, DE

**Introduction**

One strategy in the treatment of Diabetes Type 1 is the transplantation of allogenic pancreatic Islets of Langerhans to restore the endogenous insulin production. To avoid the administration of immunosuppressants and prolong the life span of the transplant, the islets can be protected from the recipient's immune system by encapsulation in alginate hydrogels. However, this measure impedes the islet's oxygen supply, reducing their functionality and survival rates.

The co-cultivation with photosynthetically active microalgae is an alternative concept for blood-independent oxygen supply. In our lab, both islets and microalgae were successfully plotted within a hydrogel blend of alginate and methylcellulose (algMC) previously [1, 2]. However, mammalian cells and microalgae have fundamentally different environmental and nutritional demands, yet the needs of both cell types needed to be met in a co-culture. Challenges are the identification of a suitable microalgae partner, the establishment of process conditions which support the survival and function of both cell types and the development of a co-culture medium that ensures a successful long-term cultivation.

**Experimental Methods**

As a model cell type for Islets of Langerhans, INS-1, an immortalised cell line of murine beta-cells was used for all experiments. Microalgae strands investigated were *Chlorella sorokiniana*, *Scenedesmus sp.*, *Coelastrella striolata* and *Coelastrella oocystiformis*. The mammalian cell culture medium is composed of Roswell Park Memorial Institute (RPMI) 1640 Medium (Gibco), 10 % heat inactivated fetal calf serum (Corning), 1% ampicillin (Roth), 5% HEPES pH 7.4 (Roth), 1% L-Glutamine (Biochrom GmbH) and 1% mercaptoethanol sodium pyruvate (Gibco). The microalgae medium “Tris minimal”, consisting of tris buffer, a salt solution and trace elements, was prepared as described previously [3], however ammonium chloride was substituted for 0.75 g/L sodium citrate. The bioink used for plotting consisted of 3 % alginate (Sigma) and 9 % methylcellulose (Sigma) dissolved in phosphate buffer saline (INS-1) or water (microalgae). Strands were deposited by extrusion-based multi-channel plotting using a 3.1 Bioscaffolder (GeSiM mbH). After plotting, the constructs were cross-linked in 70 mM strontium chloride solution. Both cell types were cultivated in 37 °C and with 5 % CO₂. If illuminated, a homogenous light source of 150 µmol/m²s (PAR) intensity was used. Different mixtures of the mammalian and the microalgae media were tested regarding their effect on viability and function of both cell types.

**Results and Discussion**

Using 3D bioplotting, both cell types suspended in a bioink consisting of algMC could be placed in close proximity, to ensure efficient oxygen transfer, but without direct cell-cell contact. Two parameters of co-cultivation were set already in the beginning of this work: a cultivation temperature of 37 °C and the necessity of a light source to enable photosynthesis.

The influence of light on INS-1 was not previously described in the literature. Cultivation of INS-1 under white and red illumination of the same intensity revealed that under white light, cell viability and insulin secretion were drastically reduced, while red light had no influence on either cell survival or functionality of INS-1.

In order to find a microalgae strand that can grow under these conditions, four heat-resistant strands were investigated regarding their photosynthetic activity and their survival and growth rates after plotting. *Scenedesmus*
sp. showed a high photosynthetic yield in red light that was not reduced in the presence of glucose - a mandatory component of mammalian cell culture medium –, indicating a stable autotrophic portion in its metabolism. Plotting and immobilization had no negative impact on viability and growth rate as well. Since the INS-1 as heterotrophic cells have very specific needs in order to grow and exhibit a natural insulin response, the co-culture medium suitable for both cell types is close to the standard medium composition for INS-1. It was shown that ¼ of RPMI could be substituted with microalgae medium without loss of function of the INS-1 when the additives, as described above, are kept in the original concentration. On the other side, replacement of ¼ of RPMI by TP is sufficient to ensure the viability of the microalgae, if the trace element supplement was kept in its original concentration and the in cell culture normally used antibiotic streptomycin was replaced by 1% ampicillin. With this co-culture medium, both cell types show unimpeded functionality and growth.

**Conclusion**

In this work, spatially defined co-cultures of *Scenedesmus* sp. and INS-1 were established and all necessary parameters for a successful co-culture were developed. Viability, proliferation, and function of both cell types in these conditions were confirmed which now paves the way for novel strategies of oxygen supply.

**Acknowledgement**

The work is funded by the German Research Society (DFG).

**References**


Combining shape and mechanical function in a fully resorbable 3D-printed bone implant for hip dysplasia treatment

Nasim Golafshan¹, Koen Willemsen¹, Firuz Babu², Elke Vorndran³, Alireza Dolatshaho-Pirouz², Harrie Weinans¹, Bart C H van Der Wal¹, Jos Malda¹, Miguel Castilho¹

¹ Department of Orthopaedics, University Medical Center Utrecht, GA Utrecht, The Netherlands, Utrecht, NL; ² Technical University of Denmark, Department of Health Technology, Center for Intestinal Absorption and Transport of Biopharmaceuticals, 2800 Kgs, Lyngby, Denmark, Lyngby, DK; ³ Department for Functional Materials in Medicine and Dentistry, University of Wurzburg, Germany, Wurzburg, DE

Introduction

Osteoarthritis (OA) is a painful and debilitating condition that affects over 40 million people just in Europe (1). Together with the knee, the hip is one of the joints most frequently involved in OA. One of the two main causes for hip OA is hip dysplasia (HD) (2). HD is characterized by the instability of the hip joint and is caused by incomplete coverage of the femoral hip by the acetabulum. The most common surgical treatment to correct dysplasia involves the re-alignment of the hip socket (osteotomy) or the insertion of a bone graft (shelf arthroplasty) to enlarge the acetabulum (3). The success rate of this procedure is relatively low (40 to 60%) due to the challenges in shaping and positioning of the bone graft to the defect size with associated accelerated graft resorption or impingement of the femoral head (4). In an attempt to overcome these limitations, implants are developed that can ensure a perfect fit to the defect size, facilitating optimal integration and durable restoration of the hip socket.

Experimental Methods

In this study, we have developed a mechanical competent and fully resorbable magnesium phosphate (MgP) - based implant to enlarge the acetabulum. The appropriate design was obtained from CT-scans of eight dog patients and fabricated using a room-temperature extrusion-based process (5). The implants were printed with two various core fiber spacing of 1 mm and 0.7 mm (abbreviated as IFS-1 and IFS-0.7, respectively). The mechanical compatibility of the implant as well as stability and optimal femoral coverage in the load-bearing quadrant of dog model is being investigated using a custom-built mechanical set-up simulating canine hip joint forces (Fig. 1).

Results and Discussion

In order to evaluated the mechanical performance of the base material and respective implants internal architecture three-point bending tests were carried out. Under three-point bending, IFS-1 and IFS-0.7 implants presented similar load-displacement curves (Fig. 2A-C). Curves started with steep slope in the elastic region, following by an inflexion point after the yield until maximum force was reached. Moreover, our results demonstrate that the 3D-printed implant design adapted well to the acetabulum of canine dysplastic hips, while the flexible MgP material composition supported fixation with metallic screws. Implants were able to withstand a maximum force of 200N without failure and a significant plastic deformation, which confirmed a proper fixation of the implant to the defect side (Fig. 2D-E). For the ex vivo biomechanical test, after 15 days in enzymatic media, implants exhibited not significant deterioration of the external architecture was observed to maintained but with the presence of slight material gaps and alternation of smoothness (Fig. 2F-H). Although a significant decrease in mechanical properties was observed after 15 days degradation under this extreme punctual compressive load, it was notable to observe that implant with a loss in weight fraction of material equivalent to 48% did not lose completely the mechanical integrity.

Conclusion
The patient-specific design will provide a perfect fit resulting in optimal integration and durable restoration of the hip socket. The implants will provide a stable, long-term and regenerative solution that will fully integrate with the host bone tissue.

Representation of workflow and fabrication of 3D printing implants.
A) Schematic illustration of the hip dysplasia in a canine model. B) The location of the personalized implant based on the described CAD design. C) Here, the 3D printing process of the personalized implant via bioactive materials for the implant and Pluronic as a supporting material are shown. D, E, F) The evaluation of flexural, in-vitro degradation and ex-vivo mechanical test of the implants.

Mechanical properties of the implants.
A) A-C) Representative load-displacement curves for a three-point flexure (3PF) test. D) Representative micro-CT images of the unloaded (left) and loaded (right) implants at maximum load of 200N (scale bar = 5mm). E) Representative load-extension curves at 100N and 200N maximum load. F) The snapshots of the 3D-printed hip implants immersed in enzymatic medium over 15 days. G) Load-displacement curves of implants. H) The snapshots of the as-printed implants before the test and after the failure.

References
3:45 p.m. – 5:15 p.m.

Track03

S05 | Advanced Technologies and Cellular Approaches for the Development of Precise 3D Tendon and other Musculoskeletal Tissue Substitutes and Models to Understand Regeneration Mechanisms

Chairs
Manuel Delgado Caceres
University Regensburg Medical Centre, Experimental Trauma Surgery, Department of Trauma Surgery, Regensburg, DE
Gianluca Ciardelli
Politecnico di Torino, Turin, Italy
Christina Mouchtaridi (YSF)
University of West Attica, Department of Biomedical Sciences / School of Health and Care Sciences, Athens, GR

Novel approaches in 3D and 4D printing, microfluidics and other emerging techniques for developing precise 3D tendon and other musculoskeletal tissue substitutes and 3D models to understand regeneration mechanisms. Cellular approaches, including those focusing on strategies for modulating inflammatory processes to trigger regenerative responses of tissue engineering therapies.

The keynote will focus on innovative technologies such as magnetic assisted 3D printing and microfluidics that, combined with adequate cellular approaches, can be used to either develop biomimetic/functional constructs for tissue regeneration or more realistic 3D tissue models to study tissue pathologies/healing mechanisms.
S05-KL01

Magnetic Assisted 3D bioprinting and mechanoactuation technologies for achieving advanced biofuntionality of 3D constructs and precise models to engineer the regeneration of tendons

Manuela Gomes1,2

1 3B’s Research Group-University of Minho, I3Bs Research Institute, Guimaraes, PT; 2 ICVS/3B’s, PT Government Associate Laboratory, Braga/Guimaraes, PT

The poor healing ability of tendons as well as the limitations of currently used therapies have motivated tissue engineering (TE) strategies to develop living tendon substitutes. However, the limited knowledge on tendon development and healing processes has hindered the design of TE procedures that more closely recapitulate tendon morphogenesis.

Our lab has been exploring the development of unique cell-laden 3D magnetically responsive systems that recapitulate key features of the native tissue and that can be further remotely modulated both in vitro and in vivo by the application of external magnetic stimuli. We are exploring conventional and innovative tools such as multimaterial 3D bioprinting to design magnetic responsive systems mimicking specific aspects of tendon tissue architecture, composition and biomechanical properties, which, combined with adequate stem cells, shall render appropriate behavioural instructions to stimulate the regeneration of tendon tissue. Simultaneously, the 3D cell-laden magnetic system shall enable sophisticated 3D tissue models to unravel mechanisms behind tendon homoeostasis and repair that will support the base knowledge to establish rational design criteria for the biofabrication of living tendon substitutes offering the prospect of tendon regeneration as opposed to simple tissue repair.

Acknowledgement

Authors thank Hospital da Prelada for tissue samples; FCT for project MagTT PTDC/CTM-CTM/29930/2017 and HORIZON 2020 for ERC CoG MagTendon (772817) and Twinning Project Achilles (810850)
S05-02

Developing a tubular, polymer-based electrospun construct with controlled anti-adhesion and -inflammation compounds for deep flexor tendon repair

Nele Pien2,3, Ian Peeters4, Liesbet Deconinck2, Lana Van Damme2, Lieven De Wilde4, Ann Martens5, Sandra Van vlierberge2, Peter Dubruel2, Arn Mignon1,2

1 KU Leuven, Biomaterials and Tissue Engineering, Surface and Interface Engineered Materials, Department of Materials Engineering, Leuven, BE; 2 UGent, Polymer Chemistry & Biomaterials Research Group, Centre of Macromolecular Chemistry, Gent, BE; 3 Laval University, Laboratory for Biomaterials and Bioengineering, Department of Min-Met-Materials Engineering & Regenerative Medicine, CHU de Quebec Research Center, Quebec, CA; 4 UGent, Faculty of Medicine and Health Sciences, Department of Human Structure and Repair, Ghent University Hospital, Gent, BE; 5 UGent, Faculty of Veterinary Medicine, Department of Physical Medicine and Orthopaedic Surgery, Gent, BE

Introduction

Tendons are indispensable in the human body. Connecting muscle to bone, they provide strength, withstand tension and release stored energy1. Hand tendon traumas comprise around 10% of all emergency department visits and up to 20% of all injuries treated2. Current treatment methods have several shortcomings, including an insufficient mechanical strength, possible adhesion reactions with surrounding tissue and inflammation issues. The current research aims to create a structure to counter both mechanical and biological problems. A reinforced, multi-layered tubular polymeric construct is electrospun. The construct is composed of three layers: an inner electrospun layer containing an anti-inflammatory component (Naproxen), a middle layer with a braided monofilament as a reinforcement layer and an outer electrospun layer containing an anti-adhesion component (hyaluronic acid)3 (Fig 1).

Experimental Methods

A novel acrylate endcapped urethane-based poly(ε-caprolactone) precursor (AUP) was developed and characterized measuring the acrylate content and the molar mass using 1H-NMR spectroscopy. This AUP polymer was benchmarked against commercially available poly(ε-caprolactone) (PCL). After characterization, the materials were electrospun into the construct layers, also including the active compounds (Naproxen and HA). The final constructs were mechanically evaluated using suture retention and ex vivo tensile tests on sheep tendons. An in vitro study was also performed using an MTS assay and Ca-AM/PI staining. Finally, the materials were tested on their degradability (Fig 2).

Results and Discussion

NMR allowed to determine the MM of the PCL diol (1931 g∙mol⁻¹), the MM of the AUP (3336 g∙mol⁻¹) and the acrylate content (0.554 mmol∙g⁻¹). During the electrospinning, a comparison was made between pure PCL and AUP/PCL blends. Both led to homogeneous fibers. However, PCL clearly led to thicker fibers (8.77 ± 1.88 µm) than the blend (4.30 ± 0.41 µm). The monolayered AUP/PCL reached a significantly higher (p < 0.05) average suture retention strength (0.79 ± 0.17 MPa) than the monolayered PCL (0.50 ± 0.09 MPa). This could be linked to the stronger, crosslinked network of AUP. Tensile tests indicated ultimate strengths of 0.81 ± 0.04 MPa and 1.17 ± 0.30 MPa respectively. Considering the strength required for the final application (4 MPa needed4), the constructs are not strong enough. In a subsequent step, the reinforced triple-layer electrospun construct was then processed including the active compounds (hyaluronic acid and Naproxen) and the intermediate tubular braid. In vitro assays using human fibroblasts showed that incorporation of the active components was successful and not-cytotoxic. It was hypothesized that the AUP/PCL HA is preferred over PCL HA as the high initial leaching of HA in the PCL HA could potentially be...
detrimental for the cells. This prolonged release could be attributed to the more densely covalently crosslinked network of the AUP/PCL. Ex vivo tensile tests on sheep tendons with the monolayered and multilayered PCL and AUP/PCL repair constructs resulted in ultimate stresses of 0.39 ± 0.10 MPa and 0.27 ± 0.11 MPa for the monolayered PCL and AUP/PCL respectively and 8.56 ± 1.92 MPa and 8.36 ± 0.57 MPa for the multi-layered PCL and AUP/PCL respectively. The period in which the constructs need to remain mechanically stable is 8 weeks. After that, the lesioned tendon passes to an advanced healing. Degradation tests have shown that no statistically significant decrease (p < 0.05) in weight was observed over this period, which proves their stability. Both multilayered structures fulfilled the required strength for tendon repair. Interestingly, the AUP/PCL electrospun multilayered structures showed smoother edges than the PCL constructs, making manipulations with tendons easier.

**Conclusion**
The aim of this study was to develop a better solution to repair deep flexor tendons compared to the current state-of-the-art. The constructs were proven to be stable for the required 8 weeks, after which the lesioned tendon moves to an advanced healing. Anti-adhesion (HA) and anti-inflammatory (Naproxen) compounds were successfully introduced in the electrospun constructs. In vitro assays showed that incorporation of these bio-active components was successful and not-cytotoxic for human fibroblasts. These tests also indicated that HA induced a burst release in the PCL constructs, while a prolonged release in the stronger and denser network of AUP/PCL. The mono-layered constructs were insufficiently strong (< 4 MPa) for the intended application. Introduction of an intermediate braided monofilament layer between the electrospun layers led to strengths of 8.56 ± 1.92 MPa as shown by ex vivo sheep tendon tensile tests. It can thus be concluded that the multilayered AUP/PCL electrospun structures represent a very promising mechanical and biological solution to repair deep flexor tendons. A follow-up in vivo study in rabbits is ongoing.

**Acknowledgement**
The authors would like to acknowledge Prof. J. Martins and Mr. Tim Courtin of the NMR department for helping with the NMR measurements and Cousin Biotech for providing the monofilament. The work of N. Pien was supported by a Vanier Canada Graduate Scholarship. P. Dubruel, S. Van Vlierberghe and A. Mignon would like to acknowledge the financial support of the Research Foundation Flanders (FWO) under the form of various research grants. I. Peeters would like to acknowledge the financial support of the UGent Bijzonder Onderzoeksfonds (BOF: Grant no: 01D28316).
Research concept

Within the project, the precursors were first synthesized. They then were combined with anti-adhesive and anti-inflammatory compounds and processed through electrospinning. The final construct exists out of 3 layers, whereby each active compound is in one of the outer layers with a braided monofilament in between those layers. Finally, the constructs were mechanically (ex vivo) and biologically (in vitro) characterized. Currently an in vivo study is ongoing on rabbits.

References

S05-03

A wear model to predict in-vivo damage of ACL grafts

Deyo Maeztu Redin¹², Julien Caroux¹², Pierre-Yves Rohan³, Hélène Pillet³, Alexia Cermolacce⁴, Julien Trnka⁴, Mathieu Manassero⁴, Véronique Viateau⁴, Laurent Corté¹²

¹ École Nationale Supérieure des Mines de Paris, Centre des Matériaux, Évry, FR; ² ESPCI, Molecular, Macromolecular Chemistry and Materials, Paris, FR; ³ Arts et Métiers, Institut de Biomécanique Humaine Georges-Charpak, Paris, FR; ⁴ École Nationale Vétérinaire d’Alfort, Laboratoire de Biologie, Bioingénierie et Bioimagerie Ostéo-Articulaire, Maisons-Alfort, FR

Introduction

Anterior cruciate ligament (ACL) reconstruction is a common surgical procedure with overall good outcomes (75%-97%) [1]. Nonetheless, the etiology of ACL reconstruction failure is often unclear. In the frame of surgery planning and graft design, it is of interest to understand and model the mechanisms underlying graft damage. Impingement — interference of the implant with other structures like cartilage and bone — is considered a major reason of failure [2]. Its occurrence is highly sensitive to anatomical variability and graft positioning during surgery. These uncertainties raise a special concern for preclinical trials, since the anatomy of sheep, standard animal model used for ACL grafts, is particularly prone to ACL impingement. In this study, we hypothesise that impingement alone is not sufficient to predict the damage of grafts in-vivo: knee kinematics and the effect of wear due to friction of the graft against the joint surfaces need to be considered. To address this question, we used a sheep model of arthroscopic ACL reconstruction to assess the location and severity of wear occurring on the graft during flexion-extension of the knee. We confronted this damage predictor to in-vivo indicators of damage.

Experimental Methods

Seven sheep (20-41 months, healthy, nulliparous, pre-alpine breed) underwent ACL reconstruction using tendon autografts. The limbs were explanted at three months after ligamentoplasty. For each specimen, biplanar radiographs were acquired using a low-dose X-ray system (EOS, EOS Imaging, Paris, France) and 3D digital models of the femur and the tibia were obtained through a reconstruction algorithm described in a previous study [3]. The cadaveric sheep knee specimens were then tested in vitro using a motorised device to record knee kinematics in flexion-extension. Graft damage was characterised by necropsy observations and ultimate tensile strength (UTS) measurements [4]. The 3D images and kinematic data were used to construct a digital model of each knee (Figure 1). Correlations (Pearson, r) between UTS and model variables (predictors) were studied.

Results and Discussion

From the reconstructed position of femur and tibia of each specimen, we computed the maximum values of strain, torsion angle and impingement volume underwent by the graft during knee flexion-extension. When compared to UTS, none of these variables explain the resistance of the grafts in vivo (r=0.175, r=-0.148 and r=-0.706 respectively) Using kinematic data, a wear model based on Archard’s equation⁵ was thus proposed to assess local wear produced in a knee flexion cycle. The maximum wear attained on the graft (wear index) showed a strong negative correlation with UTS (r=-0.898) and explained the differences among specimens (Figure 2 a). Local wear was compared to necropsy observations, where damage zones were consistent with the predictions (Figure 2 b and c).

Conclusion

These results show that wear is a good predictor of ACL graft damage in-vivo. The simplicity of the proposed wear criterion permits its direct use in surgery planning and implant design to minimise the risk of implant failure. Its application to sheep anatomy provides a straightforward way to increase efficiency and predictive power of preclinical testing, and to reduce the costs, time and number of animals.
Figure 1

Reconstructed 3D images of femur and tibia showing knee flexion-extension kinematics of a limb; $\theta$ knee flexion angle. Data obtained three months after ligamentoplasty.

Figure 2

Figure 2: a) Scatterplot of UTS and wear index. Both variables are negatively correlated; dashed line gives best linear fit. b) Necropsy observation of an operated knee at 3 months after ACL reconstruction. Black arrow indicates damage on the tendon autograft. Crosses show the location of the femoral and tibial insertion points. c) Corresponding prediction of local wear on the graft for the same knee. Black arrow indicates the location of the maximum wear index on the reconstructed ACL.

References

**In vitro** modelling of tendon physiology and pathology with 3D microengineered living fibers

**Rui M.A. Domingues**\(^1,2\), Isabel Calejo\(^1,2\), Claudia J. Labrador-Rached\(^1,2\), Manuel Gomez-Florit\(^1,2\), Denitsa Docheva\(^3\), Rui L. Reis\(^1,2\), Manuela E. Gomes\(^1,2\)

\(^1\) University of Minho, 3Bs Research Group, Guimarães, PT; \(^2\) ICVS/3B’s - PT Government Associate Laboratory, Braga/Guimarães, PT; \(^3\) University Hospital Regensburg, Department of Trauma Surgery, Regensburg, DE

**Introduction**

The hypovascular and hypocellular nature of tendon tissues severely limits their healing capacity and contribute for loss of functionality and propensity to injury. Although tendon healing follows the common tissue repairing stages of inflammation, proliferation and remodeling, this process is governed by complex biological factors and cellular crosstalk mechanisms that are still poorly understood. A major obstacle to the scientific and clinical advancement of tendon therapies is the lack of reliable and valid models allowing to decipher fundamental aspects of tendon physiology and pathology, as currently available 2D cell cultures and small animal models have shown limited biological relevance. Microengineered 3D models enabling the mimicry of native tissue’s structure and function through the precise deposition and assembly of materials and cells might provide a solution for this challenge. Here we hypothesize that by combining platelet lysate (PL) hydrogel coatings with tendon inspired fiber assemblies with defined 3D architecture, we would be able to recapitulate the biophysical and biological cues of both healthy and diseased tendon microenvironments at the required throughput to produce microphysiological systems for **in vitro** modeling.

**Experimental Methods**

3D microengineered tendon units recreating both physio- and pathological tendon microenvironments were fabricated. Electrospinning was first used for the production of continuous anisotropic fibers [1] that were afterward twisted into more complex yarns to reconstruct the hierarchical 3D structure of healthy tendon fascicles and used as core elements for the production of composite living fibers (CLFs) [2] recreating the structural anisotropy existing in healthy tendon microenvironments (CLFs-Healthy). In contrast, fibrous cores with isotropic topographies were produced to recreate diseased-like extracellular matrix (ECM) organization and induce the expression of degenerative-related markers, as commonly observed in tendinopathies (CLFs-diseased). Human tendon-derived cells (hTDCs) were selected as a native tissue cell source widely used in **in vitro** tendon modeling. To fabricate the CLFs, hTDCs were encapsulated within PL hydrogel coating shells. These PL shells were intended to replicate the interstitial tendon ECM and providing a source of growth factors, structural proteins, and other signaling biomolecules that promote fast construct cellularization and effective matrix deposition, while guiding the hTDCs phenotype commitment depending on the core’s topography. This concept was combined with 3D printed support frames to produce multiplexed assemblies allowing the rapid generation of large numbers of highly reproducible sample replicates fitting multi-well plate formats.

**Results and Discussion**

We harnessed on the synergy between the biological signaling of PL components with the controlled 3D architectures of electrospun microfibers to produce CLFs emulating healthy or diseased tendon tissue states (Fig.1). In CLF-Healthy, encapsulated hTDCs presented high cytoskeleton polarization and alignment, expressed tendon-related markers (scleraxis, tenomodulin, and Mohawk) and deposit a dense tenogenic ECM. In contrast, cell crowding without marked preferential orientation and high ECM deposition were observed in CLF-diseased. Moreover, the observed phenotypic drift characterized by the increased expression of non-tendon related markers and smooth
alpha muscle actin, a myofibroblastic marker, was also reflected on an increased collagen type III / collagen type I ratio. This diseased-like profile was further evident on the observed imbalance between matrix remodeling and degradation effectors, as characteristic of tendinopathy.

**Conclusion**

In summary, taking advantage of biophysical and biological cues for the induction of regenerative and pro-fibrotic phenotypes in human tendon-derived cells, we fabricate microengineered 3D *in vitro* models of tendon healthy and diseased states. Overall, the developed microphysiological systems hold great promise as tools to study tendon physiology and pathology, offering a reliable high-throughput alternative to existing options in the search for new tendon therapies.

**Acknowledgement**

Authors acknowledge Transplantação-IPST (Portugal) for providing platelet concentrates and Hospital da Prelada (Porto, Portugal) for providing tendon tissue samples. The authors acknowledge ERDF for project NORTE-01-0145-FEDER-000021; EU’s HORIZON 2020 for ERC 772817 and Twinning 810850-Achilles; FCT for the PhD grant of IC (PD/BD/128088/2016) and CL (PD/BD/150515/2019); contract of MGF (CEECIND/01375/2017); and SmarTendon project (PTDC/NAN-MAT/30595/2017).

![Fig.1](image)

*Fig.1* Multiplex micro-engineered 3D in vitro tendon units replicating physiological and pathophysiological tendon tissue profiles.

**References**


Piezoelectric bioink for skeletal muscle tissue engineering.

Claudia Paci1,2, Federica Iberite1,2, Lorenzo Vannozzi1,2, Lorenzo Arrico1,2, Enrico Catalano1,2, Leonardo Ricotti1,2

1 Scuola Superiore Sant'Anna, The BioRobotics Institute, Pisa, IT; 2 Scuola Superiore Sant'Anna, Department of Excellence in Robotics & AI, Pisa, IT

Introduction

3D bioprinting has the potential for becoming a future breakthrough method in the implementation of skeletal muscle tissue engineering. In this domain, the physico-chemical properties of bioinks constitute a crucial aspect that can drive an appropriate cell differentiation [1]. Material chemical cues and mechanical properties have been widely investigated in the last decade. Less explored, yet intriguing, can be the incorporation of exogenous factors (e.g., electrical, magnetic or mechanical stimulation) to further boost cell differentiative processes.

Piezoelectric nanomaterials have been used as nanoscale transducers able to convert mechanically-induced deformation into an electrical cue when invested by an ultrasound wave (acting as a wireless source of mechanical energy). This paradigm has shown beneficial effects on different cell types, in particular accelerating the differentiation of neural and muscle precursors [2,3]. However, no research groups have explored the inclusion of piezoelectric nanoparticles in a bioink used for 3D bioprinting of skeletal muscle cells so far. This work shows preliminary results on the properties of a piezoelectric bioink based on Pluronic/alginate doped with barium titanate nanoparticles and the biological response of C2C12 cells encapsulated in it.

Experimental Methods

BaTiO3 nanoparticles (BTNPs, diameter: ~ 80 nm) were sterilized by autoclave, then added to a filtered solution of propylen glycol alginate (PGA) in deionized H2O at a ratio 1:1 BTNPs/PGA. PGA was used to achieve a stable dispersion of the nanoparticles. A Pluronic/alginate hydrogel (20% w/w Pluronic F127, 2% w/w alginate) was prepared as described in [4]. Four bioink types were prepared: one based on bare Pluronic/alginate without nanoparticles and three ones doped with BTNPs at concentrations of 100, 250 and 500 μg/mL, respectively.

The four bioinks were loaded with C2C12 myoblast cells at a density of 2x10^6 cells/mL. The bioinks were then printed with a 3D Bioplotter (Envisiontec GmbH), depositing one layer (7x7 mm^2) of parallel fibers (nozzle diameter: ~ 250 μm) at 37°C with a pressure of 0.4 bar and a relative speed of 10 mm/s. The constructs were then crosslinked with 1 mL of a 25 mM CaCl2 solution for 10 min.

SEM imaging and EDX were carried out with a Helios NanoLab 600i Dual Beam system provided with a Bruker Quantax EDX Detector.

Rheometric analyses were carried out through an Antoon Paar MCR 302 Rheometer using a plate-and-plate geometry at 37°C.

For viability tests, the four constructs were maintained for 72 h in DMEM supplemented with 10% FBS and 1% Pen-Strep (basal medium). On day 1 and day 3, cell viability was evaluated using LIVE/DEAD™ and PrestoBlue™ assays (Invitrogen).

For differentiation experiments, two constructs (non-doped and doped with 250 μg/mL BTNPs) were cultured in basal medium for 5 days and then cultured for 7 days in two different differentiation media. Medium 1 consisted of DMEM supplemented with 1% ITS, 1% FBS and 1% Pen-Strep. Medium 2 consisted of DMEM supplemented with 10% HS, 1% Pen-Strep and 50 ng/mL IGF-1. After 7 days, the expression levels of GAPDH, MYOD1, MYOG, ACTA1, MYH2, MYH4, CSRP3 and ACTN2 were assessed through real-time qRT-PCR.
All experiments were performed at least in triplicates for each condition.

**Results and Discussion**

Preliminary printing efforts (Figure 1a) allowed selecting 0.4 bar as the optimal pressure value, which was used for the following experiments.

SEM images and EDX results (Figure 1b) showed that BNTPs were uniformly dispersed in the printed hydrogels, even at high concentrations of nanoparticles.

Rheometric results are shown in Figure 1c. No significant differences were observed between the different bioinks in terms of storage, loss moduli and viscosity. Viscosity decreased under shear strain, showing a shear-thinning behavior.

Viability tests (Figure 2a,b) revealed a good cytocompatibility of doped structures up to a BNTP concentration of 250 μg/mL. Hydrogels doped with 500 μg/mL BNTPs showed lower cell viability. Thus, the highest nanoparticle concentration was excluded. The bare bioink and the one doped with 250 μg/mL BNTPs were tested in the subsequent differentiation experiment.

After 7 days of differentiation, gene expression was compared with respect to GAPDH. Results are shown in Figure 2c. Medium 1 led to better results in comparison with medium 2. Interestingly, the nano-doped bioink promoted a significantly higher expression of *MYOD1*, *MYOG*, *ACTA1* and *CSRP3* (key genes in the early myogenesis phases), with respect to the bare one.

**Conclusion**

The preliminary results reported in this work demonstrate the potential of piezoelectric bioinks for the development of 3D bioprinted skeletal muscle constructs. Future experiments will focus on ultrasound stimulation of such constructs, thus to exploit the piezoelectric nature of the embedded nanoparticles and to achieve an indirect electrical stimulation of the differentiating cells, boosting myogenesis in 3D bioprinted systems.

**Acknowledgement**

This work received both financial and technical support by INAIL, in the framework of the project MIO-PRO.

![Figure 1.](image)

(a) Representative images of 3D bioprinted structures (C2C12 cells-laden Pluronic-alginate hydrogels) at different printing pressure values. (b) SEM images and EDX analysis for the different bioinks. (c) Rheometric characterization of the different bioinks. All measurements were made at 37° C.
Figure 2.
(a) Representative images showing the viability of C2C12 cells embedded in the different hydrogel types, 24 h and 72 h after bioprinting. Live cells are shown in green, dead cells are shown in red. (b) Metabolic activity assessed through the PrestoBlue™ assay of the constructs 24 h and 72 h after bioprinting. (c) Gene expression with respect to GAPDH for myogenesis-relevant genes, through qRT-PCR, for hydrogels doped with 250 ìg/mL of BNTPs and bare ones, and with two different differentiation media. A Tukey post-hoc test was performed. *=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001.

References
Design of injectable and porous hydrogels and their potential as support for skeletal muscle tissue engineering

Louise Griveau1, Emilie Christin2, Marianne Lafont3, Héloïse Le Goff4, Clémence Drouglazet4, Baptiste Robbiani2, Catherine Le Visage3, Pierre Weiss3, Romain Debret1, Vincent Gache2, Jérôme Sohier1

1 CNRS UMR 5305 - UCBL, Université de Lyon, Laboratoire de biologie tissulaire et ingénierie thérapeutique, LBTI, Lyon, FR; 2 CNRS UMR 5310- INSERM U1217 - UCBL, Université de Lyon, Institut NeuroMyogène, INMG, Lyon, FR; 3 INSERM UMR 1229 - Université de Nantes, Regenerative Medicine and Skeleton, RMes, Nantes, FR; 4 CNRS UMR 5510 - INSA, Mateis, Lyon, FR

Introduction

Volumetric muscle loss (VML) resulting from traumatic incidents drastically decreases muscle regeneration capacity and lacks treatments1. Tissue engineering approaches using biomaterials to guide the functional regeneration of tissues and organs are of high potential for VML management. Among biomaterials, hydrogels are promising therapeutic candidates due to their excellent biocompatibility, their similarity to native extracellular matrices (ECM) in terms of water retention and cell adhesion, and injectable potential. However, their regeneration capacity is strongly reliant on the presence of a porosity to allow cell infiltration and efficient vascularization. Therefore, the aims of this study were (1) to find the optimum conditions of a recently developed hydrogel composed of poly-lysine dendrimers (DGL) and NHS-polyethylene glycol (PEG) hydrogel to interact with muscle cell progenitors (myoblasts) and then (2) create a porosity inside the network through a novel biocompatible effervescent approach suitable with its injection. Finally, the ability of these new porous and injectable formulations to sustain myoblast proliferation and differentiation were evaluated for skeletal muscle regeneration purposes.

Experimental Methods

To determine the most suitable environment for myoblasts, dense hydrogels were prepared by mixing various ratios and concentration of DGL (third generation) and PEG-NHS (MW 2kDa), and their mechanical properties were measured in compression. Mouse myoblasts (C2C12) were cultured on 2D hydrogels and their proliferation, spreading and mobility were quantified using image analysis and time-lapse video microscopy. Formation of skeletal muscle cells (myotubes) was appreciated with myosin heavy chain (MHC) antibody staining. To obtain a porosity suitable with injection, acetic acid (Aa) and potassium carbonate (Kc) were dissolved to DGL and PEG solution precursors with a surfactant. Resultant solutions were subsequently injected using a dual-chamber syringe connected to a static mixer and the porosity characterized by image analysis from 200 µm stacks realized by confocal laser scanning microscopy. EPH cytocompatibility toward human dermal fibroblasts was studied along with their biocompatibility in subcutaneous injection in mice. Effervescent porous hydrogels (EPH) ability to sustain muscle cell proliferation in 3D was finally explored by evaluating the behaviour of human primary myoblasts seeded on EPH of various concentrations.

Results and Discussion

As hydrogel stiffness was modulated from 10 to 150kPa (Fig.1A), C2C12 behaviour in proliferative conditions was strongly influenced in terms of cell spreading, morphology and mobility on the support. The optimal behaviour was observed for hydrogels between 50 and 150 kPa (Fig. 1B). Surprisingly, C2C12 subsequent ability to differentiate after 6 days was greatly linked to the DGL/PEG ratios, irrespectively of hydrogels stiffness. Therefore, conditions of interest for myoblasts proliferation and differentiation could be targeted for the engineering of a porosity able to sustain cellular infiltration inside the hydrogel. Through careful optimization of effervescence parameters, we found that with the correct ratio of Aa and Kc, we obtained a spontaneous and interconnected porosity remnant of the
produced CO\textsubscript{2} bubbles entrapment inside simultaneously generated DGL/PEG network (Fig. 1C). Resultant effervescent porous hydrogels (EPH) with pore size of about 200 µm and a porosity of 75 % sustained cellular infiltration and spreading (Fig. 1D). Moreover, EPH were proven biocompatible by subcutaneous injection in mice (Fig. 1E) with extensive vascularization (Fig. 1F) confirming their potential for regeneration purpose.

EPH suitability for myoblasts differentiation was assessed by seeding primary human myoblasts\textsuperscript{2} (phMs) on the top of 2 mm thick EPH cylinders. Formation of skeletal muscle cells (myotubes) was appreciated with myosin heavy chain (MyHC) and α-actinin antibodies staining after 6 and 10 days in serum-depleted medium showing an extensive cell fusion with visible striation (Fig 2A, B and C). As a striking result, spontaneous contractions of phMs were observed and maintained up to 6 days inside EPH. The presence of quiescent satellite cells inside EPH was evaluated with myogenic regulatory factor Pax7, early stage myogenic marker MyoD and cell cycle marker ki67. The presence of quiescent and non-differentiated cells inside EPH was confirmed after 6 and 10 days in differentiation(Fig 2D and E). These results consolidate EPH potential for muscle fibre maturation while preserving a pool of reserve cells\textsuperscript{3}, holding promise for tissue engineering applications.

**Conclusion**

Hence, we describe here a novel porous hydrogel with potential as scalable solution for VML treatments, providing an optimal support for muscle cells progenitors to differentiate into contractile myotubes, while maintaining a pool of undifferentiated, quiescent cells within a swift and straightforward injectable delivery.

**Acknowledgement**

We thank the Agence nationale de la recherche (grant number ANR-17-CE19-0009) and the Centre national de la recherche scientifique for funding.

---

**Figure 1 : Formulation of injectable and porous DGL/PEG hydrogel by effervescence**

A) Various dense DGL/PEG hydrogel stiffness in compression. B) C2C12 confluence 30h post seeding in growth medium on various dense DGL/PEG hydrogels.C) Formation of an interconnected porous structure made by the successful entrapment of effervescently generated CO\textsubscript{2} bubbles in solid hydrogel. D) Study of cytocompatibility using primary human fibroblasts (phalloidin in green, dapi in blue). D) Assessment of in situ injectability by subcutaneous injection in mice showing E) the formation of an interconnected porosity allowing cellular (in purple) and F) vascular infiltration.
Figure 2: Human primary myoblasts inside EPH after 6 days in differentiation

A) Observation of an extensive myotubes formation inside effervescent porous hydrogels (EPH) with myosin heavy chain in red, B and C) Formation of sarcomeres inside myotubes visible with alpha-actinin staining (in grey) showing myotubes maturation. D) Observation of mononucleated Pax 7 positive cells (red arrows), non proliferative (Ki67 negative) and E) not entering differentiation (MyoD negative). Green arrows: (D) Ki67 positive cells (E): myoD positive cells

References
3:45 p.m. – 5:15 p.m.

Track04

ESB-SLABO S | ESB-Latin American Society for Biomaterials, Tissue Engineering and Artificial Organs (SLABO) Joint Symposium: Development of New Biomaterials with Multifunctional Surfaces

Chairs
Ana Paula R. Alves Claro
UNESP, Materials and Technology, GUARATINGUETÁ, BR
J. Miguel Oliveira
University of Minho, 3Bs Research Group, Institute 3Bs, Guimarães, PT
Gregory Reid (YSF)
University of Basel, Department of Biomedical Engineering, Basel, CH

Biomaterials field is growing in the last decades due to the increased life expectancy of the world population. Nowadays, the study of biomaterials has been extended to several areas with a focus on the volume and surface properties. Thus, the discussion of advances in new materials with multifunctional surfaces becomes strategic to our area.
ESB-SLABO S-KL01

New trends in multifunctional surface modification of stable and biodegradable biomedical implants

Julietta Rau

Italian National Research Council (CNR), Institute of the Structure of Matter (ISM), Rome, IT

In a near future, a substantial increase of biomedical implants’ demand is expected due to a dominant demographic phenomenon - population ageing and life expectancy rising. The main requests for implants are good osteointegration and long-term stability. For this purpose, metallic implants are coated with biomimetic functional materials improving their properties, and consequently, their integration with the surrounding hard tissue. In this work, the results obtained for novel biomaterials possessing various functional characteristics designed for coatings on titanium and on biodegradable metal alloys are reported. In the case of biodegradable implants, the focus points are controlling their degradation rate and imparting them with additional bioactivity properties. The developed coating materials are based on substituted calcium phosphates and bioactive glasses of innovative composition, containing trace elements with therapeutic functions, which are involved in natural bone and connective tissue formation. The effect of trace elements on cell functions is dose-dependent and its release is carefully tuned. Various aspects of coatings’ development, among them antibacterial properties, are focused. The coatings’ deposition procedure and corresponding conditions are selected. Comprehensive characterization of the coatings is performed: structural, morphological, mechanical, wetting contact angle, and magnetic properties, electrochemical tests in simulated body fluid, etc. are reported. In vitro bioactivity, microbiology and cell tests results are presented, focused on material-cell interactions, and, in particular, on trace ion influence. The obtained results suggest that novel biomimetic nanostructured implant materials can be particularly relevant for new strategies in tissue replacement and regeneration.
Functionalization of materials for biomedical applications using anodic oxidation

Ana Paula R. Alves Claro

São Paulo State University, Materials and Technology, Guaratinguetá, BR

In recent years, titanium alloys have been studied as substitute materials for bone tissue repair. These materials are considered bioinert, i.e., they do not induce a specific response after implantation in the host. Another class of metallic materials used in the biomedical area, for the production of implants, are biodegradable metals, and their use has progressively increased in the last 5 years. These materials have the advantage that they are eliminated from the body gradually and do not need to be removed using surgical techniques. For these two kinds of materials, it is possible to improve their use, from the functionalization of the surface. Anodic oxidation has a prominent role among the different techniques used to produced well-ordered tube structures in metals surfaces. The size of the nanopores or nanotubes, the thickness of the layer, and the surface roughness could be easily controlled by optimizing the experimental parameters such as voltage, temperature, and time, which will affect positively the biological properties and response of these surfaces. Studies have been indicated the viability of this technique and the increase of the performance of these materials. The use of anodic oxidation on degradable metals even affected their degradation rate with values higher than those verified for materials without surface treatment. In the case of titanium alloys, an increase in cell adhesion and proliferation was verified, which accelerated the osseointegration process.

References
ESB-SLABO S-03

Investigating the potential of gelMA-encapsulated human iPSC-derived chondrocytes for joint surface regeneration

Hannah Agten1,3, Inge Van Hoven2, Samuel Ribeiro Viseu2, Jasper Van Hoorick4, Luis Freitas Mendes1,2, Sandra Van Vlierberghe4, Frank P. Luyten1,2, Veerle Bloemen1,3

1 KU Leuven, Prometheus, Division of Skeletal Tissue Engineering, Leuven, BE; 2 KU Leuven, Skeletal Biology and Engineering Research Center, Leuven, BE; 3 KU Leuven, Materials Technology TC, Campus Group T, Leuven, BE; 4 Ghent University, Polymer Chemistry & Biomaterials Group, Centre of Macromolecular Chemistry, Ghent, BE

Introduction
Joint surface defects in articulating joints have limited healing potential and can extend into the underlying subchondral bone. Osteochondral defects are a strong risk factor to develop osteoarthritis. Tissue engineering strategies therefore aim at a sustainable repair of deep joint surface defects in an early stage. The production of living implantable cell-based 3D constructs is promising, but a lack of suitable cell sources and insufficient process reproducibility and design complexity and have hampered the translation into a clinically relevant product. Recent advances in additive manufacturing have paved the way for extrusion-based bioprinting, an enabling technology that could overcome these limitations by layer-by-layer deposition of bioinks consisting of cells encapsulated in hydrogels.

Experimental Methods
In this study, bioprintable methacrylated gelatin (gelMA) was combined with human iPSC-derived chondrocytes, with the aim of producing cartilaginous tissue analogs. After crosslinking the bioink with the help of Li-TPO photo-initiator at 365 nm in polytetrafluoroethylene moulds, cell-laden gel constructs (4x4x2 mm³) were cultured in chondrogenic medium for up to 4 weeks. In vitro performance was evaluated using histology, immunohistochemistry and unconfined uniaxial compression and compared to controls cultured in standard growth medium. Additionally, constructs were implanted ectopically in nude mice 24h after preparation as well as after 21 days of in vitro culture for in vivo evaluation after 4 weeks.

Results and Discussion
Histology showed a homogeneous cell distribution in the cross-linked hydrogel, with an increasing number of cells over the course of the study, as also confirmed by DNA quantification. Moreover, on day 21, significantly more cells were present in the constructs cultured in differentiation medium as compared to those cultured in standard growth medium. After 21 days in differentiation medium, the cell-laden constructs deposited a sulphated glycosaminoglycans (GAGs) matrix in the hydrogel as documented by Alcian Blue- and Safranin O staining. In addition, these GAGs were uniformly distributed throughout the construct. Immunohistochemistry also indicated the uniform distribution of collagen type II, whereas collagen type I was only present in a thin layer on the outer surface. In growth medium, no relevant deposition of matrix components was observed, with only very limited Safranin O staining in the pericellular space. These findings were confirmed by uniaxial compression tests, indicating no significant changes in stiffness of the constructs in growth medium, as opposed to a 3 to 4–fold increase in the differentiation conditions. Samples implanted in vivo subcutaneously in nude mice generated cartilage-like constructs after 4 weeks, that were more intensely Safranin O positive than those cultured in vitro only. When 21 days of in vitro priming preceded the implantation, we observed more mature matrix formation compared to early implantation.

Conclusion
iPSC-derived chondrocytes encapsulated in gelMA can generate robust cartilage-like tissue analogs in vitro and in vivo. When primed with a potent chondrogenic medium, a more mature type of cartilage is observed. Taken together,
these data suggest that gelMA combined with iPSC-derived chondrocytes is a potent bioink for off-the-shelve bioprinting strategies for joint regeneration.

References

Anand constitutive model of the pure titanium thermoplastic deformation process

Jakub Bańczerowski, Marek Pawlikowski

Warsaw University of Technology, Faculty of Production Engineering/Institute of Mechanics and Printing, Warsaw, PL

Introduction

Pure titanium requires plastic deformation processing in order to increase its strength [1]. This process depends on many parameters such as strain rate, temperature, coefficients of strain hardening and softening and others [2]. To ensure stability and repeatability of this process, constitutive equation must be applied [3]. The goal of the study is to formulate a new constitutive model describing the process. The behavior of the material during deformation indicates its viscoplastic character, which can be described by Anand model [4].

Experimental Methods

CP titanium (Ti) samples were compressed at elevated temperatures (775 K÷875 K) at different strain rates ($\varepsilon=10^{-2}\div10^1$) to the constant strain $\varepsilon=0.6$ in a Gleeble thermomechanical simulator. The data in the form of stress-strain graphs was acquired from the experiment and served to calculate material parameters for this equation.

The Anand model defines strain rate as:

$$\varepsilon_p = A\ast\exp\left(-\frac{Q}{RT}\right)\ast\sinh\left(\xi*(\sigma/s)\right)^{1/m}$$

(1)

where $\varepsilon_p$ [s-1] is strain rate, $\xi$ - stress multiplier, $s$ - material constant characterizing effects of strengthening mechanisms, $m$ - strain rate sensitivity, $Q$ [kJmol$^{-1}$] - activation energy of plastic strain, $R$ - universal gas constant, $T$ [K] - temperature, $\sigma$ [MPa] - flow stress;

Equivalent stress is as follow:

$$\sigma = cs; c<1$$

(2)

where $c=(1/\xi)*\sinh^{-1}\left\{[\varepsilon_p/A*\exp(Q/RT)]^m\right\}$

(3)

The rate of variable $s$ changes is described in the following evolution equation:

$$s_r = h(\sigma, s, T)\varepsilon_p$$

(4)

The function $h$ is associated with dynamic recovery and strain hardening. The specific form of this equation is given:

$$s_r = h_0*(A-(s/sr))^{a}*\text{sign}(1-(s/sr))\varepsilon_p; \quad a>1$$

(5)

where

$$s_g = s_d*A*\exp(QRTn)$$

(6)

Variable $s_g$ is a saturation value of $s$, $h_0$ - hardening/softening constant, $n$ - strain rate sensitivity, $s_d$ - coefficient of deformation resistance.

Analogically we can formulate saturation value of stress $\sigma_g$:

$$\sigma_g = (s_g/\xi)^{A*(\varepsilon_p/\exp(Q/RT))^{m}}*\sinh^{-1}\left\{[\varepsilon_p/A*\exp(Q/RT)]^m\right\}$$

(7)

Finally, we can formulate stress as:

$$\sigma = \sigma_g - [(\sigma_g - c\sigma_0)^{1-a} + (a+1)(ch_0*\sigma_0^{a})^{1/(1-a)}]$$

(8)

where $\sigma_0$ is the initial value of $s$.

To acquire all the constants following procedure was applied. Saturation stress was determined from the stress-strain graphs. The variables $A$, $Q$, $\xi$, $m$, $s$, $n$ were calculated using nonlinear least square method. The $\xi$ parameter is
selected in such a way, that c variable in (2) is less than 1. The constants $ch0, cs0, a$ are determined by the nonlinear square fit from the constant strain rate data.

**Results and Discussion**

Models fitted to data were relatively precise with the R-square equal 0.935. Confidence bounds were 0.95. The model describes much better deformation processes in higher temperatures (875 K).

**Conclusion**

The applied model provides precise fit mostly in plastic deformation zone. Anand model is promising, due to its ability to predict material behavior during both, elastic and plastic deformation. The further work should apply this model into the finite element simulation of the titanium processing in order to find the best input parameters leading to desired material properties.

![Stress-strain graphs and Anand models for the 775 K deformation process](image1)

![Stress-strain graphs and Anand model for the 875 K deformation process](image2)

**References**


Modulation of microenvironment of cells by bioactive surface coatings based on layer-by-layer technique

**Thomas Groth**¹, Reema Anouz¹, Husnia Kindi¹, Yazmin B. Barerra¹, Christian Woelk², Christian Schmelzer³

¹ Martin Luther University Halle-Wittenberg, Institute of Pharmacy, Department Biomedical Materials, HALLE, DE; ² Leipzig University, Institute of Pharmacy, Pharmaceutical Technology, Leipzig, DE; ³ Fraunhofer Institute for Microstructure of Materials and Systems, HALLE, DE

Introduction

Layer-by-Layer technique (LbL) has been widely used to make bioactive and biomimetic multilayer surface coatings and 3D systems. The wide range of polyelectrolytes from synthetic and natural sources as well as complexation conditions can help to tailor the microenvironment of cells towards molecular composition and mechanical properties. Particular interesting is the fact that extracellular matrix (ECM) components, such as proteins, proteoglycans and glycosaminoglycans (GAG) can be used for multilayer formation due to their polyelectrolyte character. In addition, also larger charged entities such as liposomes or lipoplexes can be used for the multilayer formation process. Eventually, LbL provides an elegant tool to mimic the complex composition of ECM by selection of components that address integrins and growth factor receptors to promote growth and differentiation of cells. Further strategies of using LbL can also be based on cross-linking them with metal ions and in situ transfer of drugs or nucleic acids to program cell behavior. We intend here to provide a brief survey on the potential of LbL technique to control differentiation of stem and other cells by selection of ECM components, strategies of cross-linking, uploading of growth factors.

Experimental Methods

Layer-by-layer technique was performed by dip coating glass slides or silicon wafers as model surfaces in polyelectrolyte solutions (polyanions and polycations) until a minimum of 10 layers was achieved. Collagen I and chitosan were used as polycations. Chondroitin sulfates, and hyaluronan either in native form or oxidized by sodium periodate were used as polyanions. Oxidation of GAG was done to permit intrinsic cross-linking with polycations through imine bond formation. Additionally, uploading of growth factor BMP-2 or cross-linking the multilayers with metal ions calcium, cobalt, copper, and iron ions was done. Different types of cells were cultured on multilayers including C2C12 myoblast, the multipotent mouse fibroblast C3H10T1/2 or adipose-derived stem cells (ADSC). Cell culture was done under standard conditions without differentiation factors. Cell adhesion was studied by confocal laser scanning microscopy staining focal adhesions, cell cytoskeleton and nuclei. Cell differentiation was assessed by CLSM staining matrix components, RT-PCR and histochemical staining.

Results and Discussion

Studies with ADSC and C2C12 myoblasts that were cultured on multilayers made from either native or oxidized chondroitin sulfate showed that multilayers prepared from oxidized GAG are not only stiffer than those made of native GAG, but promoted osteogenic differentiation of ADSC to a higher extent. In addition, it was observed that uploading the growth factor (GF) BMP-2 to multilayers made of oxidized GAG inhibited the release of BMP-2 accompanied by stronger spreading of C2C12 myoblasts and significantly more osteogenic differentiation of these cells compared to multilayers made of native GAG. The underlying mechanism of this enhanced activity of BMP-2 on cross-linked multilayers is probably related to a juxtacrine presentation of the GF to the corresponding receptor and a cross-talk to integrin related signaling pathways as found in other studies. Further studies were done with multilayers made of native hyaluronan and chitosan (Chi/HA)5 cross-linked by metal ions Ca²⁺, Co²⁺, Cu²⁺ and Fe³⁺. Most
interestingly, Fe\(^{3+}\) metal ions promoted adhesion and spreading of C3H10T1/2 cells greatly on the low adhesive [Chi/HA]₅ PEM system. The intrinsic cross-linking of multilayers with Cu\(^{2+}\) and Fe\(^{3+}\) ions led to increased metabolic activity in cells after 24 h and induced cell differentiation towards adipocytes in the absence of any additional adipogenic media supplements. We found indications that cross-linking of [Chi/HA]₅ with metal ions multilayers can occur both through coordination chemistry but also Coulomb interaction, particularly for Ca\(^{2+}\) and Fe\(^{3+}\) with carboxylic groups of HA. However, the assume that major effects on cell spreading and differentiation are not based on the minor changes of physicochemical properties of cells, but rather the chemical effects of metal ions taken up by cells.

**Conclusion**

Overall, the studies demonstrate the potential of LbL technique to fabricate multifunctional surface coatings for implant materials or tissue engineering scaffolds that can control the microenvironment of cells by changing chemical cues through the selection of matrix components and uploaded growth factors that correspond to the composition of desired tissues (e.g. chondroitin sulfate, collagen I and BMP-2 for bone). Moreover, intrinsic-cross-linking that represents a biocompatible, non-toxic procedure to cells is not only changing mechanical properties of these coatings, but also controls the release and presentation of growth factors or metal ions permitting cell differentiation without exogenously added factors.
3:45 p.m. – 5:15 p.m.

Track05

N05 | Hydrogels and Smart Biomaterials II

Chairs
Matthew Baker
Maastricht University, Maastricht, NL
Miguel Castilho
University Medical Center Utrecht, Orthopeadics, Utrecht, NL
Giuseppe Guagliano (YSF)
Politecnico di Milano, Department of Chemistry, Materials, and Chemical Engineering "G. Natta", Milan, IT
Glycerylphytates: alternative bioactive natural-based crosslinkers for the fabrication of hydrogel matrices in tissue engineering

Ana Mora-Boza\textsuperscript{1,2}, Andrés J. García\textsuperscript{1}, Julio San Román\textsuperscript{2}, Blanca Vázquez-Lasa\textsuperscript{2}

\textsuperscript{1} Georgia Institute of Technology, Woodruff School of Mechanical Engineering and Petit Institute for Bioengineering and Bioscience, Atlanta, US; \textsuperscript{2} Institute of Polymer Science and Technology (ICTP-CSIC) and CIBER-BBN (Health Institute Carlos III), Spanish National Research Council, Madrid, ES

Introduction
A major objective in regenerative medicine research is the development of bioactive hydrogels to be applied as functional tissue matrices in regenerative medicine. Thus, many efforts are focused on new crosslinking agents and strategies that provide suitable hydrogel matrices for effective tissue regeneration. Here, we established the synthesis and application of a novel family of natural-occurring crosslinkers derived from the antioxidant agent, phytic acid. Phytic acid has attracted much attention in the biomedical field as it is claimed to act as an anticancer agent, as a lipid peroxidation inhibitor, as a preventive agent of calcifications, and as an anti-osteoporotic compound \cite{1}. Our novel crosslinkers, glycerylphytates (G\textsubscript{x}Phy), were obtained through a condensation reaction between phytic acid and glycerol, focusing on the development of two crosslinkers, G\textsubscript{1}Phy and G\textsubscript{3}Phy, that were successfully applied for the preparation of bioactive hydrogel-based matrices using traditional fabrication techniques (e.g. solvent casting) and next generation of biofabrication techniques (e.g. 3D printing and microfluidics), where the choice of an appropriate crosslinker will determine the successful synthesis of effective hydrogel matrices.

Experimental Methods
Glycerylphytate (G\textsubscript{x}Phy) crosslinkers were obtained through a condensation reaction between phytic acid and glycerol. Two G\textsubscript{x}Phy crosslinkers having different content of glyceryl moieties were synthetized, G\textsubscript{1}Phy and G\textsubscript{3}Phy. The condensation reaction and synthesis of phytic acid derivatives were confirmed by \textsuperscript{1}H NMR and \textsuperscript{13}C NMR spectroscopy, elemental analysis, TGA, and ICP-OES. Antioxidant activity was evaluated by Ferrozine and lipid peroxidation assays, and their biological behaviour was assessed on human mesenchymal stromal cells (hMSCs). G\textsubscript{x}Phy compounds were applied as ionic crosslinkers for the preparation of: (i) 2D chitosan hydrogel membranes using solvent casting methodology; (ii) 3D printed multi-layered scaffolds using a dual-step crosslinking strategy; and (iii) bioactive stem cell-laden chitosan-based microgels using droplet-based microfluidics.

Results and Discussion
A novel family of crosslinking agents based on phytic acid, G\textsubscript{x}Phy, were developed. Specifically, we focused on two derivatives, G\textsubscript{1}Phy and G\textsubscript{3}Phy, which incorporated an average of one and three glyceryl moieties, respectively. Both G\textsubscript{x}Phy compounds demonstrated excellent antioxidant activities and improved cytocompatibility in comparison to its precursor \cite{2}. These crosslinking agents are novel at two different levels: G\textsubscript{x}Phy act not only as cytocompatible and natural-occurring crosslinkers with powerful gelation properties, but also as biologically active components of the developed systems in comparison to other traditionally applied crosslinking agents that lack of bioactivity (e.g. TPP, genipin).

G\textsubscript{3}Phy was applied as ionic crosslinker of chitosan membranes, providing improved osteogenic and osteoinductivity potential by increasing calcium deposition, and alkaline phosphatase (ALP) activity on cultured hMSCs in absence of any typical osteoinducer agent \cite{3}. These G\textsubscript{3}Phy-crosslinked membranes provided a suitable environment for hMSC culture and differentiation into osteoblastic lineage, arising as attractive substrates for guided bone regeneration. G\textsubscript{1}Phy was applied for the fabrication of 3D printed scaffolds using a dual crosslinking strategy for natural-based polymer inks based on chitosan and methacrylated gelatin (GelMA). The applied methodology...
consisted of an initial UV photopolymerization step simultaneously to 3D deposition, followed by a post-printing ionic crosslinking treatment with G1Phy. This approach enabled the fabrication of 3D scaffolds with high shape fidelity and resolution, without the collapse of the consecutive printed layers. Our G1Phy ionic crosslinking agent provided adequate swelling and long-term stability properties to the 3D scaffolds, and good in vitro biological performance of 3D printed multi-layered structures on L929 fibroblast cultures, which showed successful results in terms of adhesion, spreading, and proliferation in comparison to TPP [4]. Finally, G1Phy was used for the generation of chitosan-lactate microgels in a flow-focusing microfluidic device via in situ crosslinking reaction for hMSCs encapsulation applications. The incorporation of G1Phy to chitosan-based microcarriers provided them with some remarkable features in terms of enlargement of viability of encapsulated hMSCs and upregulation of paracrine signaling at adverse conditions (e.g. oxidative stress and inflammation) [5].

**Conclusion**

The developed crosslinking agents were efficient in fabrication of hydrogel matrices in different biofabrication strategies, and demonstrated their versatility and suitability for tissue engineering and regenerative medicine fields. We envision that they will have a profound impact on the preparation of natural-derived hydrogels systems.

**Acknowledgement**

The authors thank the Spanish Ministry of Economy, Industry and Competitiveness (project, MAT2017-84277-R) and U.S. National Institutes of Health (R01 AR062368) for financial support. Ana Mora-Boza was financially supported by "Apadrina La Ciencia"-Ford Spain Foundations, “La Caixa” Foundation (ID 100010434, code LCF/BQ/ES16/11570018) and CIBER-BBN (Health Institute Carlos III) Travel grant. B. Vázquez-Lasa is member of the SusPlast platform from CSIC.

**References**

N05-02

Methacryloyl mucin - tannic acid double network hydrogels

Elena Olaret, Brindusa Balanuca, Andra M. Onas, Jana Ghitman, Horia Iovu, Izabela C. Stancu, Andrada Serafim

University Politehnica of Bucharest, Advanced Polymer Materials Group, Bucharest, RO

Introduction

Mucin represents the major component of mucus and contains a long polypeptide backbone on which dense brushes of carbohydrate chains are grafted \[1\]. The complexity of its structure proved to be appealing and mucins have been studied for applications as carriers for bioactive species \[2\], coatings with improved tribological performance \[3,4\] or anti-fouling agents \[5\]. However, its use in the biomedical field is still underexploited.

This work presents a two-step procedure of obtaining double network (DN) systems based on methacrylic anhydride-modified mucin (MuMA) by (1) covalent bonding of MuMA methacrylate groups through radical photo-polymerization, leading to single network (SN) hydrogels and (2) hydrogen bonds (H-bonds) formed between the carboxyl side groups of the protein with the hydroxyl groups of tannic acid (TA).

Experimental Methods

Following the synthesis and characterization of MuMA, various SN stable hydrogels were prepared using reaction media with different pH values. The SN hydrogels were converted into DN systems through incubation in TA solution. The affinity for aqueous media of both SN and DN hydrogels was assessed and the mechanical properties of the obtained systems were evaluated both at macroscale - through rheology and compression tests and microscale - through nanoindentation tests.

Results and Discussion

Results on the characterization of the newly obtained product (MuMA) revealed that (i) the mucin protein structure was not altered by the methacrylation and (ii) MuMA maintains the pH dependency of the pristine mucin leading to different folding of the protein chains, as shown by circular dichroism spectroscopy (fig. 1). Following the photo-polymerization of the double bonds of the methacryloyl groups, DN systems were obtained through the incubation of the obtained SN hydrogels in tannic acid (fig. 2, A). The successful formation of a secondary network was demonstrated for all systems by the noteworthy differences in swelling ability, referring to both the time required for attaining the hydration equilibrium, as well as their swelling content (approx. three times higher in SN vs. DN systems) (fig. 2, B). Furthermore, mechanical tests showed that the supplementary crosslinking leads to more robust systems that require additional effort to be deformed: the SN hydrogels break around a strain of 30 – 40 %, but are able to undergo higher deformation, while the DN systems require a significantly higher stress to be deformed at the same strain value (around 1.5 ÷ 3 fold for a 30% deformation). While the rheological tests showed that the supplementary reinforcement of the hydrogel brought by the presence of H-bonds was not accompanied by the stiffening of any chain parts, the nanoindentation tests revealed an immobilization of the macromolecular chains on the surface of the synthesized materials which might be beneficial for cells adherence.

Conclusion

The registered results demonstrated the ability of MuMA to form robust DN systems with the ability to uptake large amounts of aqueous media without losing their integrity. The high water uptake ability and macro and micro scale mechanical properties, indicate that these systems may find applications in the field of load bearing soft tissue regenerative medicine. Further studies will envisage the stability of these systems in simulated body fluids and their ability to sustain cell adherence and proliferation, using relevant cell cultures.

Acknowledgement
The research was supported through project NanoSHAC within PNCDI III, PN-III-P1-1.1-TE-2019-1161

Figure 1
(A) FTIR spectra of PGM (black), MuMA (blue) and MA (purple) - Inset A.1: deconvoluted spectra of PGM and MuMA in the wavenumber region 1500-1750 cm⁻¹; Inset A.2: deconvoluted spectra of PGM and MuMA in the wavenumber region 850-1200 cm⁻¹; B) CD spectra of PGM and MuMA - inset: magnification in the 250-320 nm region; C) CD spectra of MuMA|MES, MuMA|PBS and MuMA|CB at 4 °C - inset magnification in the 250-320 nm region.

Figure 2
(A) Schematic representation of the synthesized SN and DN systems (B) Swelling kinetics of the synthesized SN and DN systems (C) Equilibrium water content (EWC,%) computed for the obtained hydrogels

References
Photocrosslinkable hydrogels from solubilized extracellular matrix of the amniotic membrane

Inês A. Deus, Catarina A. Custódio, João F. Mano

University of Aveiro, Department of Chemistry, CICECO - Aveiro Institute of Materials, Aveiro, PT

Introduction
Recently, three-dimensional (3D) cell culture platforms have emerged as valuable tools with potential to increase the accuracy of in vitro studies. Inspired by the extracellular matrix (ECM) that compose living tissues, these platforms have been designed to support the attachment and growth of cells, therefore providing reliable data on how they behave and respond to stimulus when within their natural environments\(^1\). Despite the great advances made on the design of such materials, including the development of decellularize matrices and ECM-based hydrogels, recapitulate the complexity and function of human native tissues, while producing cost-effective and safe materials, remains a challenge\(^2\).

Towards this goal, here we propose the use of perinatal tissues as a source of proteins to develop 3D cell culture platforms. Amniotic membrane (AM) is the innermost part of the foetal membrane which surrounds and protects the fetus during gestation. The potential of this tissue has long been recognized in tissue engineering and regenerative medicine applications due to its richness in ECM proteins and other important components, including growth factors and cytokines\(^3\). However, its use for cell culture applications remains quite under explored, probably due to the lack of adequate mechanical properties of the so far described AM-based hydrogels\(^4\). Here, we report the development of a chemically modified AM-based hydrogel which can be cured upon light exposure and tuned according to the intended application.

Experimental Methods
Human placenta was obtained from consenting mothers in a collaboration with the Centro Hospitalar do Baixo Vouga (Aveiro, Portugal). The AM was isolated from the rest of the tissue, decellularized using detergents (sodium dodecyl sulfate (SDS) and Triton X-100) and solubilized with pepsin. Decellularization efficiency was analysed by histomorphological analysis, 4',6-dimidino-2-phenylindole (DAPI) staining and DNA quantification. ECM preservation was also assessed by quantifying collagen and glycosaminoglycans before and after processing. AM methacrylated (AMMA) was produced by reacting the AM-derived ECM with methacrylic anhydride at two different ratios to obtain distinct degrees of modification\(^5\). Thereafter, AMMA hydrogels were prepared with concentrations of 1, 2.5 and 5\% (w/v) and polymerized upon UV light irradiation in the presence of a photoinitiator. The resulting hydrogels were characterized in terms of structure and mechanical properties through cryoSEM, compression tests and rheological analysis. For cell culture experiments, human bone marrow stem cells (hBM-MSCs) were encapsulated and maintained in culture for 7 days. Cell viability, morphology and proliferation were assessed at pre-determined time-points.

Results and Discussion
In this work, a detergent-based protocol was used to decellularized human AM. Histomorphological analysis and DAPI staining revealed no cell nucleus and good retention of key ECM components. These results were corroborated by DNA, collagen and GAGs quantification. After decellularization, AM was digested with pepsin which resulted in a soluble form of the ECM (dAM-derived ECM).

AM-derived ECM was reacted with methacrylic anhydride (MA) to produce a photopolymerizable material. TNBSA was used to confirm functionalization and assess methacrylation degree. Varying the ratios of MA to AM concentration resulted in the production of materials with low (AMMA100) and high degree (AMMA300) of modification.
modification. Robust hydrogels were obtained with concentrations ranging from 1 – 5% (w/v). Developed hydrogels had different porosities and stiffness as revealed by CryoSEM and compression/rheological tests, respectively. No effects were detected in hydrogel’s water content. Together, these results suggest the production of tuneable materials.

Lastly, cell culture experiments were conducted to evaluate the cytotoxicity and cell supporting ability of AMMA hydrogels. As depicted on figure 2, encapsulated hBM-MSCs were able to adhere and proliferate up to 7 days in culture. Live/dead images revealed no cytotoxic effects, while DAPI/Phalloidin staining evidenced good cell-ECM intertalk. MTS assay and DNA content results were consistent suggesting suitable proliferation rates.

**Conclusion**

Here, we demonstrated for the first time that solubilized AM-derived ECM can be successfully modified with photoresponsive groups to produce an easy-to-handle and highly versatile biomaterial. It is important to note, that as fully human, these materials have great potential to improve the accuracy of in vitro studies and possibly accelerate the process of drug development, reduce costs currently associated to late-stage drug failures and reduce or completely eliminate the use of animal models. Additionally, as a xeno-free option this platform may be used to expand cells intended for cellular therapies or to develop tissue-like constructs avoiding the risk of xenogeneic disease transference and reducing the probability of adverse immune responses.

**Acknowledgement**

The authors acknowledge the funding from the European Research Council (ERC) for project Amniogel (957585 ERC-2020-PoC) and the Portuguese Foundation for Science and Technology (FCT) for the project BEAT (PTDC/BTM-MAT/30869/2017). Catarina A. Custódio also acknowledges the FCT for the individual contract CEECIND/02713/2017. This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES.
Figure 2 Results
Decellularization: a) DAPI staining showing lack of cell nucleus; b) Decrease in DNA content; c) Retention of key ECM components collagen and GAGs. B) AMMA hydrogels characterization: a) TNBSA showing different methacrylation degrees; b) Pore size distributions by CryoSEM; c) Storage and loss modulus obtained in rheology tests; Young's Modulus measured by compression tests. C) Cell encapsulation: a) Live/Dead and DAPI/Phall images depicting cell viability and morphology respectively; b) MTS assay and c) DNA quantification revealing significant cell proliferation up to 7 days in culture.

References
A hybrid hydrogel-based 3D in vitro model to unveil mammary stromal-epithelial cell interactions

Patrícia Barros da Silva1,2,3, Ricardo Oliveira1,2,3, Mariana Coelho1,2,3, Sílvia J. Bidarra1,2,4, Sara C. Neves1,2,3, Cristina C. Barrias1,2,4

1 i3S, Instituto de Inovação e Investigação em Saúde, Porto, PT; 2 INEB, Instituto de Engenharia Biomédica, Porto, PT; 3 FEUP, Faculdade de Engenharia da Universidade do Porto, Porto, PT; 4 ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Porto, PT

Introduction
Balanced parenchymal-stromal cells and cell-matrix interactions are central to breast tissue homeostasis and become dysregulated in diseases such as cancer. The breast tissue microenvironment is complex, and in vitro models that recapitulate both its 3D architecture, the direct contact between different cell types, and cell-matrix interactions, are still scarce (Rodrigues, J. et al, 2020). To address this challenge, we designed a hybrid hydrogel system integrating both stromal and parenchymal breast tissue. For the stromal compartment, we built a porous 3D printed alginate scaffold seeded with human mammary fibroblasts (hMF). After hMF colonization, the pores were filled with in situ forming alginate hydrogel (Bidarra, et al, 2016) with mammary epithelial cells (MCF10A) that developed into prototypical mammary gland-like organoids, in direct contact with hMF and their ECM. After co-culture, the whole system could be dissolved under mild conditions, allowing both cell and ECM recovery for downstream analysis, thus proving to be an excellent tool for dissecting stromal-epithelial cell crosstalk in vitro, in both health and disease contexts.

Experimental Methods
Ultra-pure alginate was grafted with integrin-binding (RGD) and protease-sensitive (PVGLIG) peptides and used as scaffolding material (Fonseca, K.B. et al, 2011). Extrusion 3D printing was used to build ionically crosslinked multi-layered porous scaffolds of RGD-alginate, which were freeze-dried and then re-hydrated before hMF seeding. Gel-precursor solutions of RGD/PVGLIG-alginate with MCF10A cells and crosslinking agents (CaCO3, GDL) were added to the pores, forming a hydrogel in situ. In both compartments, cell and ECM morphology/distribution and phenotypic makers were analysed by immunostaining and confocal microscopy of whole-mounted samples. After co-culture, the system was dissolved (EDTA) and cells were recovered, isolated by FACS and analysed by RT-PCR. The ECM retained in the hydrogel was analysed by proteomics.

Results and Discussion
Upon rehydration, the 3D printed scaffolds perfectly fitted the wells of 48 well-plates. The seeded hMF were able to adhere, proliferate and produce endogenous ECM, uniformly colonizing the filaments of the scaffolds, without clogging the pores. The gel precursor mixture of RGD/PVGLIG-alginate with MCF10A cells completely filled the pores, where epithelial cells proliferated and formed acinar-like structures, recapitulating the mammary gland. The modification of alginate with protease-sensitive peptides created a permissive environment for hMF migration/invasion, promoting the direct contact between epithelial organoids, fibroblasts and ECM. To prove the ability to recover both the cells and the ECM after co-culture in 3D, the whole system was dissolved and the retrieved cell populations were isolated by FACS, followed by RT-PCR analysis that confirmed the expression of prototypical phenotypic markers. The ECM retained in the dissolved hydrogel was analysed by proteomics, which allowed the identification of different types of matrix proteins.

Conclusion
This hybrid hydrogel system provides an easy-to-use platform for studying stromal-parenchymal interactions in vitro, recapitulating both an ECM-like 3D microenvironment and heterotypic cell-cell interactions. Significantly, it allows both the cells and their ECM to be recovered under mild conditions for subsequent analysis.

Acknowledgement

ERDF funds (COMPETE2020, POCI), and Portuguese funds (FCT) for ANGIONICHE project (POCI-01-0145-FEDER-028744, PTDC/BTMMAT/28744/2017), FCT for fellowship SFRH/BD/[CB1] 131757/2017 and contract IF/00296/2015.

References


Rheological properties and speeds of the mechanical waves in Elastin-
Like Recombinamers Catalyst-Free Click Gels

Julio Fernández-Fernández¹, Luis Quintanilla-Sierra¹, José C. Rodríguez-Cabello¹

¹ Universidad De Valladolid, GIR BIOFORGE, Valladolid, ES; ² Universidad De Valladolid, GIR BIOFORGE, Valladolid, ES; ³ Universidad De Valladolid, GIR BIOFORGE, Valladolid, ES

Introduction
Hydrogels are water-swollen polymeric materials that keep a distinctive three-dimensional structure and can act as a scaffold as well as mimic the properties of various tissues in the body [1]. Because of that, researchers have been interested on the mechanical properties with a wide range of methods and experiments, such as, rheology to study the viscoelastic behaviour [2], or ultrasounds tests to measure the speed of sound. We show a practicable option to study the viscoelastic models in a wider range of frequencies that a convectional rheometer does and to measure the speed of the mechanical waves by the through transmission technique with small punctual piezoelectric as transducers and receptors. The main objective is to get the rheological models and the speed of the mechanical waves in Elastin-Like Recombinamers Catalyst-Free Click Gels (ELR-CFCGs)[3] from mechanical waves propagation.

Experimental Methods
Elastin-Like Recombinamers[4] are a kind of artificial peptide polymers made by recombinant DNA technologies and combined by VPGXG pentapeptide repeat units where X can be any amino acid except proline. Once they are synthesized by E. coli fermentation, they are chemically functionalized to get a chemical hydrogel by chemical click reaction between them. As regard the methods, the experimental arrangement consists of a punctual piezo transmitter and receptor with the test hydrogels placed in between them. Different electrical waves in the frequency range 10-2000 Hz generated by a function generator are activated to the transmitter to produce compressional waves propagating through the hydrogel reaching the piezoelectric used as receptor; later this device switches mechanical waves into electric signals that are monitored in an oscilloscope.

Results and Discussion
When square waves are applied, the behaviour of these hydrogels shows that at frequencies lower than 100 Hz the elastic behaviour is obvious, but at frequencies higher than 2 kHz the Newtonian liquid behaviour rules. Thanks to mathematical software, these data can be fitted to the Kelvin-Voigt model ($R^2 > 0.9$) indicating a viscoelastic behaviour of these biomaterials. In connection with sinusoidal waves, the through transmission technique reveals a range of mechanical waves velocities between 1220-1900 m/s and a range of Young’s modulus between 2-6 MPa, comparable to values of the human tissues [5].

Conclusion
We showed a new way to characterize the mechanical properties of hydrogel with the use of mechanical waves and we can conclude that cross-linked Elastin-Like Recombinamer hydrogels can replicate accurately the viscoelasticity and stiffness of tissues. These kinds of hydrogel can be used in Tissue Engineering and Regenerative Medicine.

References

Colloidal Gel Supra- assemblies as 3D Dynamic Platforms for Focalized Therapeutics Delivery

Pedro Lavrador, Leandro Gonçalves, Vítor M. Gaspar, João F. Mano

Universidade de Aveiro, Department of Chemistry - CICECO – Aveiro Institute of Materials, Aveiro, PT

Introduction
Colloidal gels (CGs) represent unique biomedical platforms comprised of nanoparticle building blocks that self-assemble via inter-nanoparticle interactions into higher order 3D multifunctional systems.[1] In these modular platforms, because nanoparticles can operate as reservoirs for both hydrophobic and hydrophilic bioactive molecules, the high nanoparticle content of CGs can be leveraged for designing long-term bioactive depots intended for advanced tissue engineering strategies.[2] Such is particularly attractive considering the limited therapeutic efficacy of locally administered nanocarriers due to poor retention at the injection site and the inability to envision standalone administration regimens. Gathering on this, we design a universal CG platform that includes two different systems that first self-assemble into macrosized 3D constructs via electrostatic interactions and can be additionally covalently crosslinked on-demand (Fig. 1): (i) a moldable, injectable single-network CG system (snCG) comprised solely from non-covalent interactions between cationic PLGA-PEI nanoparticles and anionic Zein-hyaluronan (HA) nanogels that can autonomously shed multi-particles upon injection; and (ii) a moldable, injectable and photocrosslinkable CG system assembled from electrostatic interactions between cationic PLGA-PEI-methacrylate nanoparticles and anionic Zein-HA-methacrylate nanogels, that can be on-demand photocrosslinked in situ, generating a tough double network CG (dnCG) with enhanced mechanical robustness and long-term resistant fit-to-shape features. The biomedical versatility of these bioinstructive platforms is demonstrated by loading snCGs with anti-inflammatory Quercetin flavonoid to attenuate pro-inflammatory activated macrophages. Then, dnCGs were also validated as long-term systems for chemo-phototherapeutic platforms for cancer therapy.

Experimental Methods
Cohesive CGs were manufactured by centrifugation of colloidal suspensions of complementary nanoparticle building blocks, i.e., anionic Zein-HA and cationic PLGA-PEI nanoparticles for snCGs, and methacrylated counterparts Zein-HAm and PLGA-PEIm for dnCGs. The resulting colloidal gels were molded into different shapes in in-house produced circular molds. For dnCGs covalent interlinking, the photoinitiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate was added followed by exposure to UV light (E = 3.68 W/cm²). To produce bioactive CGs, Zein-HA nanogels were loaded with Quercetin – snCGs, whereas chemotherapeutics (DOX) and photothermal agents (ICG) were loaded in dnCGs building blocks. For cellular assays prostate cancer cells and LPS activated THP-1 derived macrophages were employed to evaluate the anti-inflammatory and anti-tumoral potential of the different platforms.

Results and Discussion
An optimized nanoprecipitation protocol enabled the successful one-step formulation of surface-engineered cationic PLGA-PEI and anionic Zein-HA nanocarriers with low polydispersity index and oppositely-charged zeta-potential. Such building blocks were combined and compacted under centrifugation, rapidly producing macroscopic nanostructured CG constructs with homogeneous distribution of nanoparticle species throughout the network, as showcased by confocal imaging upon loading of hydrophobic fluorophores (Figure 2). Rheological studies investigating viscosity as a function of shear rate demonstrated that CGs exhibit shear-thinning behavior and fit-to-shape properties. Importantly, dnCGs could permanently retain their shape upon photocrosslinking. This additional crosslinking also improved CGs compressive modulus as assessed by mechanical compressive tests. Moreover, the Page 301 of 2028
Nanoparticulated supra-assembled platforms showed efficient loading of different bioactive molecules (flavonoids, chemo-phototherapeutics), while maintaining their original physicochemical properties (i.e., shape and hydrodynamic size). Upon snCGs direct contact with M1-Macrophages a dampening of the pro-inflammatory response was observed. On the other hand, dual-loaded dnCGs promoted a more pronounced anti-tumoral effect in cancer cells when compared to their single loaded counterparts.

Conclusion
In conclusion, we developed highly modular and nanostructured CG supra-assembled 3D platforms with different physicochemical and mechanical features for focal therapeutics and particles delivery. The two CG systems were first self-assembled and crosslinked by electrostatic interactions that in snCG allowed for autonomous particle shedding from the 3D platform along time. The introduction of a secondary on-demand photocrosslinking led to the manufacture of electrostatic and covalently interlinked double-network CGs capable of better withstanding mechanical loading and resist degradation in physiological environment for longer time. Overall, the different single and double network supra-assemblies demonstrate valuable properties for operating as delivery systems for localized bioinstruction of endogenous microenvironments or for cancer therapy.

Acknowledgement
This work was developed in the scope of project CICECO-Aveiro Institute of Materials (UID/CTM/50011/2019). Work supported by Programa Operacional Competitividade e Internacionalização (POCI), in the component FEDER, and by national funds (OE) through FCT/MCTES and under the scope of project PANGEIA (PTDC/BTM-SAL/30503/2017). Pedro Lavrador acknowledges a PhD fellowship from FCT (SFRH/BD/141834/2018). FCT is also acknowledged by financial support through an individual contract as Junior Researcher attributed to Vítor Gaspar (CEECIND/01410/2018).
Continuous distribution of nanoparticle building blocks within colloidal gels

Figure 2 - Confocal laser scanning microscopy of a colloidal gel with fluorescently-labeled unitary building blocks, DiD-loaded PLGA-PEI nanoparticles (red channel), and DiO-loaded Zein-HA nanogels (green channel), respectively. Orthogonal projections in merged channel showcase near complete overlap (yellow-colored), evidencing homogeneous distribution of both nanoparticle species throughout the colloidal gel network.

References
A Cell-Adherent Polymer Template to create Multiscale Channel Structures and Cell Patterns with a 3D Hydrogel

Adrian Seijas-Gamardo, Amit Chandrakar, Tristan Bodet, Mikkey van der Spoel, Sebastiano Alberganti, Federica Barbugian, Lorenzo Moroni, Richard Hoogenboom, Victor de la Rosa, Paul Wieringa

1 MERLN Institute, Maastricht University, Maastricht, NL; 2 Ghent University, Ghent, BE; 3 Avroxa BVBA, Ghent, BE

Introduction

Native tissues are characterised by 3D structural anisotropy and a distribution of cells which determines the specific cell-cell and cell-extracellular matrix (ECM) interactions that dictate tissue function. The spatial distribution of cells and ECM in tissues is not arbitrary, with interconnected lumen structures and specifically located cell populations creating a fundamental structure-function relationship that determines the role of specific tissues. Therefore, the capability to mimic this 3D environment is key for a correct in vitro construction of tissues and for future tissue engineering applications.

Here we present a templating strategy using a thermally responsive polymer and we show the fabrication, in one step, of a network of interconnected channels within a hydrogel. While other polymers have been previously described for similar applications, including pNIPAM and PnPrOx [1][2], we uniquely show that our template can create a defined 3D polymer scaffold to which cells can adhere, leading to the subsequent formation of a channel network that directly incorporates cells.

This approach is based on a family of bespoke poly(2-oxazoline)s (PAOx) that have been specifically designed to have a uniquely tunable range of lower critical solubility temperature (LCST), above which it remains insoluble and below which it is triggered to dissolve.

Experimental Methods

We characterized the LCST by monitoring polymer solubility via optical transmission. We did this by preparing polymer films, via spin coating a polymer solution on coverslips, and monitoring the water contact angle (WCA) when the polymer and water are maintained at temperatures ranging from 37°C to 5°C.

In addition, we characterized the mechanical and melt rheological properties of the PAOx and, based on this, optimized the production of polymer filaments via fused deposition modelling (FDM) and melt electrowriting (MEW). We were able to generate filament scaffolds ranging in diameter from the millimeter to micron scale. Using an in-house customized microscope stage with temperature control and time lapse stereomicroscopy, we directly monitored the dissolution dynamics of these polymer filaments while being held at 37°C and from 37°C to 5°C, mimicking the transition of the scaffold from a cell incubator to a standard lab refrigerator.

Results and Discussion

We found that each PAOx formulation had a well-defined LCST between 30°C and 5°C, correlating to the theoretical LCST based on the specific formulation.

All polymer formulations retained a WCA above 60 degrees at 37°C and underwent a rapid transition to a WCA below 10°C. We compared the performance of our polymer to pNIPAM, to which cells poorly adhere without bioactivation, and PnPrOx, which has been reported to directly support cell adhesion. Consistent with this behaviour, pNIPAM maintained a low WCA at all temperatures while PnPrOx underwent a similar temperature-dependent transition from >60°C to <10°C, suggesting that our PAOx polymer family is compatible with protein and cell adhesion. Each polymer variant underwent dissolution, which was complete within 15 minutes of reaching the minimum temperature of 5°C. pNIPAM fibers and PnPrOx also displayed the expected thermally-triggered dissolution, but also...
were found to be mechanically weak even when maintained at 37°C. In contrast, our PAOx variants retained robust mechanical properties when maintained above the LCST.

We designed a PDMS fluidic system to use a 1 mm diameter filament within a hydrogel (Fig. 1). A 4 mg/ml collagen pre-gel solution was used to embed the filaments, crosslinked the gel at 37°C, and then triggered to dissolve. We showed the formation of a patent channel that was perfusable with gravity-driven fluid flow, visualized via fluorescence microscopy of a fluorescent nanobeads suspension. Caco-2 epithelial cells were seeded within the 1 mm channel, confirming biocompatibility of the hydrogel platform is maintained and, in parallel, creating simplified 3D in vitro gut model. To explore the use of PAOx microfilaments, we focused on the biomimetic formation of Schwann cell-lined microchannels to guide axon growth. We created a customized support system to facilitate microfiber handling and confirmed that rat Schwann cells (rSCs) could adhere to the polymer fibers. The seeded templates were embedded within collagen and cooled, with rSCs found to localize to the resulting microchannels. These channels were also able to support axon growth from iPSC-derived human sensory neurons.

**Conclusion**

Here we have demonstrated value of this novel templating technology to generate different in vitro models and established its potential for other 3D in vitro model and tissue engineering applications.

**Acknowledgement**

This Project has been founded by the NWO VENI NeuroBeta project.

---

**References**


3:45 p.m. – 5:15 p.m.

Track06

**N06 | Biomaterials for Drug Delivery**

**Chairs**

*Maria Rosa Aguilar*
Consejo Superior de Investigaciones Científicas, Institute of Polymer Science and Technology, Madrid, ES

*Julien Gautrot*
Queen Mary, University of London, School of Engineering and Materials Science, London, GB

*Prasanna Padmanaban (YSF)*
University of Twente, Biomechanical Engineering Department / Technical Medical Centre/
Faculty of Engineering Technology, Enschede, NL
Polycaprolactone-bioactive glass hybrids as a controlled release platform for the delivery of therapeutics in bone tissue engineering

Lukas Gritsch¹, Cédric Bossard¹, Christiane Forestier², Edouard Jallot¹, Henri Granel³, Yohann Wittrant³, Jonathan Lao¹

Université Clermont Auvergne, LPC - Laboratoire de Physique de Clermont, Clermont Ferrand, FR; Université Clermont Auvergne, LMGE - Laboratoire Microorganismes: Génome et Environnement, Clermont Ferrand, FR; INRAE - Institut national de la recherche agronomique (INRAE), Clermont Ferrand, FR

Introduction
Bioactive organic/inorganic hybrids hold promising results as candidates for the fabrication of bone and cartilage tissue engineering (TE) scaffolds: they combine the mineralization potential of bioactive glasses with the processability and mechanical properties of bioresorbable polymers. In addition, they are prepared at low temperature, making them compatible with the design of controlled release technologies. Recently, we developed a polycaprolactone/bioactive glass (PCL/BG) hybrid using calcium alkoxides as calcium source¹. Its superior properties for bone grafts and TE scaffolds were confirmed benchmarking against a sector leader². Fostered by the promising results of this investigation, we explored the possibility to further enhance the intrinsic biological properties of PCL/BG hybrids by loading therapeutics stimulating bone regeneration, for instance by promoting correct differentiation, stimulating mineralization and protecting from the risk of bone infections (osteomyelitis). Two case studies investigating organic molecules will be presented: fisetin, an osteostimulative polyphenol, and rifampicin, a RNA synthesis blocker known for its effectiveness against osteomyelitis³.

Experimental Methods
PCL/BG hybrids were synthesized by sol-gel chemistry following a published protocol¹. The therapeutic agents were blended with the polymer prior to synthesis. Several concentrations were tested and the loading efficacy and release profiles were measured. Ion release was analyzed by ICP-OES while organic molecules were quantified by UV-Vis spectrophotometry. The response of primary osteoblasts was characterized with particular emphasis on their differentiation (alkaline phosphatase activity) and bone-specific gene expression (COL1A1 and RUNX2). The performance of antibacterial hybrids containing rifampicin was assessed. Gram+ Staphylococcus aureus and Gram- Pseudomonas aeruginosa and Escherichia coli were selected due to their high occurrence in bone infections. In parallel, fisetin-loaded samples were also implanted in vivo in a mouse model.

Results and Discussion
The sustained antibiotic release from PCL/BG hybrids was successful. The material gradually delivered 50% of loaded rifampicin within two weeks, inhibiting bacterial growth (Figure 1). In particular, the reduction in colonies of S. aureus, the major strain responsible for osteomyelitis, was more than 99.999%.

PCL/BG hybrids can simultaneously release therapeutic ions to stimulate osteoblast differentiation (silicates) and to enhance apatite formation (calcium), while degrading at a tailored rate that matches bone regrowth. As demonstrated in an in vivo rat model, tissue ingrowth is higher than the one achieved by market competitors. In addition, doping the hybrid with fisetin was confirmed as an effective approach to further improve the already outstanding biological performance of PCL/BG hybrids, increasing tissue ingrowth almost twofold (from 30% to 55%). MicroCT confirms that newly formed bone around fisetin-doped scaffolds is more abundant than around undoped ones, and thus superior to commercial alternatives (Lubbock®, OST Laboratoires, France). Histological analysis confirmed these findings (Figure 2).

Conclusion
Tailored doping of our biomaterial can significantly enhance the response of osteoblasts, strongly inhibit the bacteria responsible for osteomyelitis and offer superior bone regeneration compared to currently commercialized synthetic bone grafts. These results support the development of PCL/BG hybrids into a platform technology for the controlled and sustained release of diverse therapeutic agents, including but not limited to the ones described in this work: therapeutic ions, polyphenols and antibiotics.

Acknowledgement
This work has received funding by the EU under the European Regional Development Fund ("Fonds Européen de Développement Régional" FEDER) and Région Auvergne-Rhône-Alpes under the “pack Ambition Recherche” program (BIOSTEON project) for doping hybrids with polyphenols and received support from the Agence Nationale de la Recherche of the French government through the program “Investissements d’Avenir” (16-IDEX-0001 CAP 20-25) for doping hybrids with antibiotics (Hub Innovergne project).

Figure 1
Agar disk diffusion assays illustrating the inhibition potential of rifampicin-loaded PCL/BG hybrids. Samples clockwise from the bottom are: control, low, medium and high concentration of antibiotic.

Figure 2
(A) MicroCT of bone regeneration around implanted scaffolds and (B) histology of a fisetin-loaded scaffold showing collagen (green), osteoid tissue (pink) and cell nuclei (purple).

References
3D Printed Microneedles as a Modular Platform for Minimally Invasive Therapeutic Delivery

Nikoletta Sargioti1,2,3, Subrata Mondal1, Tanya J. Levingstone1,2, Eoin O'Cearbhalli3, Hellen O. McCarthy4, Nicholas Dunne1,2

1 Dublin City University, School of Mechanical and Manufacturing Engineering, Dublin, IE; 2 Dublin City University, Centre for Medical Engineering Research, Dublin, IE; 3 University College Dublin, School of Mechanical Materials Engineering, Dublin, IE; 4 pHion Therapeutics Ltd, School of Pharmacy Medical Biology Centre, Belfast, GB

Introduction
Various traditional methods are used as drug delivery systems that usually result in pain and poor administration of nucleic acid and drug molecules [1]. A painless micro-sized device capable of delivering drugs easily and efficiently, with no side effects, would present an attractive solution [2]. Additive manufactured microneedle (MN) arrays have been considered as an innovative platform for an optimal and cost-effective design and manufacture, eliminating the traditional system's disadvantages. This research aims to develop 3D-printed 316L stainless steel MN array, with optimal mechanical/biological properties, for disease management and treatment. A computational modelling approach was developed to determine the optimal geometrical features and mechanical properties of the MN arrays. Experimental investigations were also conducted using a Design of Experiment (DoE) approach to validate the computational data and optimise the printing parameters.

Experimental Methods
Individual needles were generated using SolidWorks (DS SolidWorks Corp., USA) software. The 3D model of MN arrays was discretized with tetrahedral elements. Young’s modulus and Poisson’s ratio values of 195 GPa and 0.25 were applied [3]. The compression and penetration studies were conducted using explicit dynamic analysis (ANSYS, Inc., USA). Parametric studies, changing needle height (300, 500, 1,000 μm) and aspect ratio (3:1, 4:1, 5:1), were used to determine the mechanical and geometrical properties for computational simulations. The analysis was completed by adding an external force by a stainless-steel rod (generated using SolidWorks) at a velocity of 3,000 mm/s [4] to a fixed MN array (Figure 1a). A DoE study (Design Expert 11, Stat-Ease, Inc., USA) was used to determine and optimise the influence of printing parameters on the geometric features (needle height, aspect ratio, surface area, and tip diameter) and mechanical properties (compressive strength, yield strength, and Young’s modulus) of MN arrays. The MN arrays were printed using the Direct Metal Laser Melting (Concept Laser Mlab, GE Additive, Germany) process, changing the laser speed (500–700 mm/s), power (35–70 W), and trace width (0.09–0.12 mm) followed by electropolishing [5]. The 3D printed MN arrays were characterised in terms of their morphology and geometry (SEM and Optical microscopy). Mechanical properties under a displacement rate of 5 mm/min were also determined (Figure 1b).

Results and Discussion
The computational study demonstrated an improvement in the compressive strength due to the increase in the needle height (0.95 GPa for 300 μm compared to 1.18 GPa for 1,000 μm) and aspect ratio (1.16 GPa for 3:1 and 1.28 GPa for 5:1). An increase was also observed from the results following the experimental compression testing. The differences between compressive strength from the experimental and computational studies varied from 0.5% to 1.6%, thereby validating the simulation studies. A difference between CAD file and actual (3D printing) dimensions was determined from the micrographs (post-electropolishing), providing a reduction of 30% in needle height and 20% in base diameter (Figure 2a). SEM analysis demonstrated the presence of un-melted particles, which decreased in number after electropolishing (Figure 2b). DoE analysis and optimisation indicated that a high laser power (70 W)
and speed (600 mm/s) could lead to optimal geometrical and mechanical properties without significant effect of trace width (p-value: 0.139) (Figure 2c).

**Conclusion**

The present study demonstrated the successful 3D printing of MN arrays. The variation of the needle height and aspect ratio demonstrated the greatest effect on the mechanical properties for both computational and experimental studies. The difference between simulation and experimental data was calculated <2%, leading to similar results in terms of the mechanical properties. The present study concluded that MN arrays with 1,000 μm height, 4:1 aspect ratio, printed using a laser power of 70 W, speed of 600 mm/s, and trace width 0.12 mm could lead to optimal dimension and mechanical properties. Overall, these findings provided a better understanding of the geometrical features of the MN arrays as drug delivery system. Future work will optimise and develop the post-processing steps to deliver the required structure, performance and cost.

**Acknowledgement**

This work was supported by Science Foundation Ireland 18/EPSRC-CDT/3584 and the Engineering and Physical Sciences Research Council EP/S022635/1.

**References**


miRNA-engineered extracellular vesicles for Regenerative Medicine applications

Hugo M. Fernandes\textsuperscript{1,2}, Alessandra Zonnari\textsuperscript{2}, Ricardo Abreu\textsuperscript{1,4}, Sezin Aday\textsuperscript{2}, Inês Albino\textsuperscript{2}, Ana Branco\textsuperscript{2}, Cátia Seabra\textsuperscript{2}, Marta Barão\textsuperscript{2}, Tânia Barata\textsuperscript{2}, Ermelindo Leal\textsuperscript{2}, Paul Quax\textsuperscript{3}, Margreet de Vries\textsuperscript{3}, Eugénia Carvalho\textsuperscript{2}, Lino Ferreira\textsuperscript{1,2}

\textsuperscript{1} University Coimbra, Faculty of Medicine, Coimbra, PT; \textsuperscript{2} University Coimbra, Center Neuroscience and Cell Biology, Coimbra, PT; \textsuperscript{3} University Leiden, Leiden Medical Center, Leiden, NL; \textsuperscript{4} University Maastricht, Maastricht, NL

Introduction

Extracellular vesicles (EVs) are secreted by virtually all the cells in the body and are key mediators of intercellular communication. EV cargo provides a snapshot of the cell proteome, metabolome and transcriptome and therefore EVs are often used as biomarkers (1). Additionally, EVs can be used as therapeutic agents, both in their native form or modulated using bioengineering strategies. However, in many cases, the bioactivity of native EVs is insufficient to elicit a therapeutic effect (2). To overcome this, we developed a platform capable of modulating native EVs with miRNAs, non-coding RNAs capable of modulating gene function at a post-transcriptional level. We used high-throughput screening approaches to identify miRNAs capable of enhancing the survival of endothelial cells (ECs) under hypoxia (0.1% O\textsubscript{2}) and subsequently, we developed a platform that allowed us to efficiently conjugate a pre-selected miRNA onto EVs. Ultimately, we showed that miRNA-modulated EV can be used to efficiently deliver miRNAs into target cells and improve the functionality of native EVs for a selected therapeutic application, namely the regeneration of diabetic wounds in mice.

Experimental Methods

Selection of the miRNA of interest: A library of 2080 miRNAs was transfected into ECs using RNAiMax and 48 h after transfection, cells were exposed to hypoxia (0.1% O\textsubscript{2}) in the absence of growth factors for a further 48 h. High-content image analysis was performed to identify miRNAs capable of enhancing the survival of ECs and follow-up assays were used to confirm the selected hits. For subsequent validation in vitro, we selected two hits and used RNA-Sequencing to identify their pro-survival mechanism of action. The pro-survival effect was finally confirmed in vivo using a matrigel plug assay using cells transfected with the selected miRNA.

miRNA-modulated EVs: EVs were isolated from human cord blood-derived mononuclear cells and characterized using western blot analysis (identification of EV markers), nanoparticle tracking analysis (particle quantification and size distribution), protein quantification and dynamic light scattering (zeta potential) as previously described by us (3). Following, a selected miRNA, known to enhance the survival of ECs, was transfected into EVs using Exo-Fect\textsuperscript{\textregistered} and qPCR was used to confirm the transfection. Internalization and intracellular trafficking of native and miRNA-modulated EVs was analyzed in ECs, fibroblasts and keratinocytes. Finally, miRNA-modulated EVs were topically administered (2x/day for 10 days) on excisional wounds of STZ-induced diabetic mice.

Results and Discussion

RNA-Sequencing followed by gene ontology analysis showed that the selected pro-survival miRNAs elicited a pro-inflammatory program that ultimately led to enhanced survival of ECs exposed to hypoxia conditions. Furthermore, based on the list of putative targets selected from the RNA-Seq dataset, we were able to identify a set of genes that, upon inhibition with siRNA, pheno-copied the pro-survival effect of the selected miRNA. After unravelling the mechanism of action of the selected miRNA, we tested the pro-survival effect using an in vivo matrigel plug assay. In brief, ECs were transfected for 48 h with the pro-survival and subsequently embedded in a matrigel plug and
transplanted into the back of immunodeficient mice. Our results showed that, compared with miRNA-scramble transfected ECs, ECs transfected with the pro-survival miRNA had a statistically significant increase in survival after 3 days. After confirming the pro-survival effect in vivo, we used ExoFect to transfect the miRNA onto EVs. Upon transfection, our results showed a >30 000-fold increase in miRNA expression compared to native EVs. Moreover, miRNA-modulated EVs were efficiently internalized by all the cells involved in skin wound healing, particularly keratinocytes and ECs, and the colocalization of miRNA-modulated EVs with the endo-lysosomal compartment differed from their native EVs. Compared to their native counterparts, miRNA-modulated EVs showed significantly lower levels of colocalization with endolysosomal compartments suggesting a more efficient escape from this degradation pathway. Finally, we showed that topical administration of miRNA-modulated EVs into the wounds of diabetic mice significantly accelerated the healing kinetics, partially by enhancing wound vascularization and re-epithelization.

**Conclusion**
In conclusion, we showed that the bioactivity of native EVs can be further enhanced by the transfection of pre-selected biomolecules of interest, in this case a pro-survival miRNA identified using high-throughput screening approaches. Importantly, we showed that miRNA-modulated EVs differed from their native counterparts in terms of internalization and this can be explored to enhance their therapeutic efficacy. Importantly, the selection of the biomolecules to be incorporated into EVs can endow them with novel properties that can play a crucial role in Regeneration of damaged and worn-out tissues.

**Acknowledgement**
This work was partially supported by the Portuguese “Fundação para a Ciência e a Tecnologia” (FCT) through the project POCI-01-0145-FEDER-029919, co-funded by COMPETE2020-UE and European project Era@UC (Ref. 669088). RA and MB were supported by a FCT scholarship (SFRH/BD/129317/2017 and 2020.09432.BD, respectively).

A high-throughput screening assay identifies pro-survival miRNAs capable of enhancing the survival of endothelial cells exposed to hypoxia (0.1%O2) in growth factor depleted media (A). In vivo validation of the pro-survival effect of miRNA using a matrigel plug assay (B).
Schematic overview of the modulation strategy (A). After modulation of extracellular vesicles with the selected miRNAs we observed a significant upregulation of the miRNA onto the EV (B). In vivo administration of miRNA-modulated EV accelerated wound closure in a diabetic wound healing mouse model (C), a process partially mediated by enhanced wound vascularization (D).

References
Competitive Binding and Molecular Crowding Regulate the Cytoplasmic Interactome of Non-Viral Polymeric Gene Delivery Vectors

Julien Gautrot, Danyang Li, Fengjing Qu, Lan Chang, Alex Raynold

Queen Mary, University of London, School of Engineering and Materials Science, London, GB

Introduction

Although polycationic vectors display excellent performance in vitro with many cellular systems, their clinical use remains very restricted. To some level, this is due to the poor compatibility of such systems with biological fluids and tissues. In addition, in contrast to the processes controlling the complexation, targeting and uptake of polycationic gene delivery vectors, such as poly(ethylene imine) and poly(dimethylaminoethyl methacrylate), the detailed molecular mechanisms regulating their cytoplasmic dissociation remains poorly understood. Upon cytosolic entry, gene delivery vectors become exposed to a complex, concentrated mixture of molecules and biomacromolecules.

Experimental Methods

To explore cytosolic release mechanisms, we characterised the cytoplasmic interactome associated with a polycationic vector based on poly(dimethylaminoethyl methacrylate) (PDMAEMA) brushes grafted from nanoparticles. To quantify the contribution of different classes of low molar mass molecules and biomacromolecules to RNA release, we used fluorescence microscopy and developed a kinetic model based on competitive binding.

Results and Discussion

Cationic brushes were found to be particularly effective at trapping small RNAs, resulting in high knock down efficiencies. However, how such stable association is disrupted in the cytosol was not clear. We propose that the molecular structure and architecture (in particular the high surface density) of cationic brush-decorated nanoparticles, together with the cytosolic molecular crowding, modulate competitive binding and, in turn, the long term release of RNA. Based on these observations, we chemically designed polymer brushes with improved RNA retention in the cytosol, avoiding burst release, and enabling to achieve long term (at least 10 days) knock down (>70%) with one single transfection.

Conclusion

Our data demonstrate that the complex crowded cytosolic environment regulates RNA desorption from polyplexes via competitive binding. Understanding the mechanism regulating cytosolic dissociation will enable the improved design of cationic vectors for long term gene release and therapeutic efficacy.

Acknowledgement

Funding from the ERC (ProLiCell, 772462) is gratefully acknowledged.

References


Gene editing formulation with spatio-temporal control

Susana Simões¹, Angela Barreira², Francesca Tomatis¹, Miguel Lino¹, Magdalena Gotz³, Stefan Stricker³, Lino Ferreira¹,²

¹ University of Coimbra, Center for Neuroscience and Cell Biology, Cantanhede, PT; ² Faculty of Medicine, University of Coimbra, Coimbra, PT; ³ Helmholtz Zentrum München, Munich, DE

Introduction

Intracellular delivery of biomolecules is extremely useful for the manipulation of cellular processes, cell reprogramming and gene editing. In the last decades, gene editing has shown potential for the treatment of several genetic mutations and offers the promise of treatment for brain diseases, mediated by either silencing or editing disease-specific genes [1]. Gene editing therapeutics based on the clustered regularly interspaced short palindromic repeats (CRISPR) show great promise for treating genetic diseases. CRISPR consists of a DNA-cutting enzyme called Cas9 and a short RNA (sgRNA) that guides the enzyme to a specific region on the genome so that it can cut this region out [2]. Unfortunately, viral delivery methods of CRISPR-Cas9 and sgRNA may cause genomic undesirable side effects because of the prolonged expression of the CRISPR system [3]. In addition, they do not offer temporal and spatial resolution to allow an extra-level of safety. Some non-viral systems were described using gold NP [4]; however, without spatio-temporal control. Photoactivatable CRISPR with a modified Cas9 enzyme that incorporates light-responsive domains or site-specific caging groups as well as photo-activatable gRNAs [5] have been described recently. These systems have limitations because they respond to blue or UV radiation which has relatively low in vivo penetration. Here, we propose a formulation that has the capacity to release intracellularly the Cas9/sgRNA complex with remote control, using near infrared (NIR) light as a trigger. The formulation is based in gold nanorods (AuNRs) conjugated with oligonucleotides that hybridize with part of the sgRNA inserted in the catalytic site of Cas9. Once the formulation is activated by NIR, the heating of the AuNR leads to the de-hybridization of the oligonucleotide and release of the CRISPR system.

Experimental Methods

For proof of concept we designed a sgRNA for GFP knockdown. Cas9/sgRNA-DNA-AuNR were incubated in d2GFP-Hela cells and used for laser-induced release of Cas9/sgRNA complex. The gene editing was evaluated by the GFP knockdown. Then, we evaluated the in vitro gene editing in different brain cell populations (NSC, microglia and neurons). For that the different cell populations was isolated from a transgenic mice Ai9 with a cassette inserted into the Gt(ROSA)26Sor locus with a LoxP-Stop-LoxP-ttdTomato sequence, for the editing we developed two sequences of sgRNA for the deletion of stop sequences and expression of ttdTomato.

Results and Discussion

Different ssDNA sequences were screened for the immobilization of the Cas9/sgRNA complex in the surface of AuNRs, and thus to identify one that could facilitate cell internalization of the formulation as well Cas9/sgRNA delivery after NIR laser activation. We have identified one sequence that allowed high immobilization of Cas9/sgRNA and its intracellular delivery by a short NIR pulse. More than 60% of the cells were knockdown by this approach in less than 3 days. The biological effect of gene editing was maintained for more than 5 days demonstrating that the cut of genomic DNA was precise in the targeted location. In the brain cell population, we observed around 11% of gene editing in NSC, 30% in microglia cells and 18% in neurons. Preliminary in vivo results show a good gene editing expression two weeks after the intracranial injection of the nanoformulation in hippocampus of adult Ai9 mouse brains.

Conclusion
We report for the first time a non-viral gene editing formulation with efficient spatio-temporal control. This strategy may increase the in vivo safety of gene editing systems.

Acknowledgement

The authors would like to thank the financial support of FCT SFRH/BPD/105327/2014, BrainEdition project (PTDC/NAN-MAT/28060/2017), Marie Curie ITN project NanoStem (ref: 721468) and the Twinning project RESETageing (ref. 952266) as well as ERA Chair project ERAatUC (ref. 669088).

References

Small siRNA polyplexes for tumor tissue penetration

Cristina Casadidio¹², Roberta Censi², Piera Di Martino², Wim E. Hennink¹, Tina Vermonden¹

¹ Utrecht University, Pharmaceutics, Utrecht, NL; ² University of Camerino, School of Pharmacy - Drug Delivery Division, Camerino (MC), IT

Introduction
In the recent pharmaceutical scenario, nucleic acids (NA) based therapeutics have shown outstanding applicability in different disease treatments, not least the formulation of mRNA-based vaccines against COVID-19 [1, 2]. In the oncology field, gene therapy is not that far advanced in clinical reality due to NA in vivo instability and low cell transfection efficiencies of naked NA. To overcome these challenges, one of the strategies used is the complexation of anionic NA via electrostatic interactions with cationic polymers, leading to the formation of polyplexes [3].

Experimental Methods
In this study, the synthesis of a methoxypoly(ethylene glycol) poly[2-(dimethylamino)ethyl methacrylate] (mPEG-pDMAEMA) diblock copolymer was optimized via reversible addition-fragmentation chain-transfer (RAFT) polymerization and this polymer was used as polymeric carrier for NA condensation. Particle size and polydispersity index (PDI) of polyplexes were determined by dynamic light scattering (DLS) while ζ-Potential was measured by laser Doppler electrophoresis (LDE) at 37°C in HEPES buffer (20 mM, pH 7.4). Stability studies of polyplexes were performed via agarose gel retardation assay after incubation up to 24h in culture medium supplemented with 10% of fetal bovine serum (FBS). To resemble in vivo conditions, small polyplexes were tracked in vitro using 3D multicellular tumor models (spheroids). For this purpose, homo- and hetero-spheroids of avascular ascites models were optimized by co-culturing ovarian cancer cells with primary mouse embryonic fibroblasts in different ratios (2:5, 1:1 and 5:2). The fibroblasts present in the 3D spheroids were used to mimic the stroma, a tissue composed of cancer associated fibroblasts and extracellular matrix, which might interfere with polyplexes penetration in vivo. Confocal images were recorded to track fluorescently labelled polyplexes at different time points and processed as optically sectioned images [4].

Results and Discussion
As shown in Fig. 1, 80% of PD polymer conversion was achieved after 8h while full conversion was reached only after 24h, leading to high PDI values. Well-defined mPEG-pDMAEMA (PD) diblock copolymers bearing a thiocarbonylthio (CTA) end group were synthesized via fast polymerization up to 8h. The kinetics of polymerization was slowed down after 10h, likely due to loss of CTA active groups. Following the commonly observed controlled radical polymerization kinetics, the average molecular weight (Mn) of PD increased linearly with the conversion, according to GPC and ¹H-NMR measurements. Afterwards, the PD diblock copolymer of 35.2 kDa was used to condense the anionic NA, forming polyplexes in which siRNA polyplexes were compared to pDNA polyplexes. Condensed siRNA polyplexes prepared at N/P charge ratio of 5, showed a size of 25 ± 2 nm in HEPES solution, while pDNA polyplexes were 162 ± 11 nm at N/P 5. Agarose gel electrophoresis revealed stable siRNA polyplexes upon 24h incubation with culture medium plus 10% of FBS, while free siRNA was observed when incubated in presence of heparin (as destabilizing negatively charged molecule). For in vitro experiments, homo and hetero-spheroids with a reproducible size of ~200 mm were developed. To assess the validity of the 3D models, MTS and live/dead assays were performed with monolayer cultures of cancer cells and fibroblasts (at different ratios), proving high levels of cell viability. Real-time penetration studies revealed that siRNA-polyplexes after 24h of incubation were able to reach the core of live-cell spheroids with ~60% of penetration area, while pDNA-polyplexes were mainly located in the rim, reaching only ~30% of penetration. These results proved that the mechanism of polyplexes
penetration into 3D spheroids is size-dependent. Regarding the influence of stroma in the siRNA polyplexes penetration, no significant differences among cancer-associated and hetero-spheroids were recorded (Fig. 2).

**Conclusion**

PD diblock copolymer proved to be a versatile polymeric carrier for delivery of siRNA. For therapeutic purposes, size-tuned siRNA polyplexes have high potential to exploit penetration into tissues and enhance eradication of the tumor mass. The results revealed that the penetration ability of siRNA polyplexes independent of cell type composition of the spheroids. The next steps in our research will focus on the use of these self-assembled siRNA polyplexes as potential tool for local treatment of ovarian cancer.

**Acknowledgement**

Dr. Lies L.A. Fliervoet, Dr. Marcel H.A.M. Fens, Bárbara S. Mesquita, Dr. Sabrina Oliviera and Martina Viola (Department of Pharmaceutics, Utrecht University)

---

**Figure 1.** Synthesis of mPEG-pDMAEMA (PD) diblock polymer via RAFT: (a) one-step synthesis route. Kinetics of PD polymerization: (b) ln([M]₀/[M]) as a function of time measured by ¹H-NMR; (c) conversion as function of reaction time; (d) Mn based on GPC (using PEG standards) and ¹H-NMR.

**Figure 2.** Representation of siRNA polyplexes formulation (top) and penetration (bottom) into 3D multicellular tumor homo- and hetero-spheroids after 24h of incubation.

**References**

Advanced multilayered three-dimensional epithelium-stroma-endothelium in vitro intestinal model to perform reliable drug permeability studies

Maria H. Macedo¹,², Andreia S. Barros¹,², Elena Martínez⁴,⁵, Cristina C. Barrias¹,², Bruno Sarmento¹,³

¹ Universidade do Porto, I3s - Instituto de Investigação e Inovação em Saúde, Porto, PT; ² Universidade do Porto, ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Porto, PT; ³ CESPU - Coopérativa De Ensino Superior Politécnico Universitário, Gandra, PT; ⁴ IBEC - Institute for Bioengineering of Catalonia, Barcelona, ES; ⁵ CIBER-BBN – Consorcio Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina, Madrid, ES

Introduction
The drug development field is constantly growing and the requirement for tools that can speed up the early pharmacokinetic phases, reducing animal experiments and, thus, decreasing time and costs, is unquestionable. In oral delivery, understanding the absorption in the small intestine in an early stage of the drug development process is paramount, since the small intestine is the place where the absorption process mainly occurs. In vitro models, due to their cost-benefit, are routinely used, but current 2D models are too simplistic and have several drawbacks, not representing the native tissue, which impacts permeability outcomes [1,2].

Experimental Methods
A new 3D in vitro model was developed, comprising 3 different layers: the epithelium, with Caco-2 and HT29-MTX cells, mimicking the intestinal enterocytes and the mucus producing goblet cells, respectively; the lamina propria, composed by a collagen layer with human intestinal fibroblasts (HIF) embedded, providing 3D biological support to the epithelium; and an endothelial layer, surrogating the absorptive capillary network, mimicking the last barrier compounds need to cross before systemic circulation. The crosstalk between the different cells present in the model and their impact on the contractile ability of fibroblasts was assessed by dosing the amounts of transforming growth factor beta (TGF-ß), endothelin-1 (ET-1) and platelet derived growth factor (PDGF) present in the cell culture medium and measuring the activity of metalloproteinases (MMPs) 2 and 9.

The developed 3D model was characterized in terms of genetic expression of intestinal differentiation markers and drug transporters. The presence of drug transporters, tight junctions (TJs) and mucin 2 (MUC2) was confirmed by immunostaining. Activity of P-glycoprotein (P-gp) was confirmed with a transport assay with Rhodamine 123 (Rho 123) and permeability assays with 3 model drugs with different absorption profiles were performed and further correlated with in vivo values.

Results and Discussion
The contraction of collagen by fibroblasts is extensively reported and can be a great drawback when using this material to developed platforms to test permeability. It was observed that secretion of higher amounts of TGF-ß and ET-1 by Caco-2 and HPMEC (endothelial) cells into the medium could lead to a higher activity of MMP2 and MMP9 and, therefore, to a higher ability of the fibroblasts to contract the collagen layer in which are embedded, as already reported in the literature [3,4]. Curiously, it was observed that when HT29-MTX cells are included in the model, the fibroblasts are not able to contract the collagen hydrogel and this relates to lower amounts of TGF-ß, ET-1 and MMPs in the medium.

Genetic expression of intestinal markers showed similar levels between the 3D models and the 2D control. Regarding drug transporters, expression of P-gp and Multidrug Resistance Protein 2 (MRP2) was lower in the 3D models when
compared to 2D, a positive result since these transporters are normally overexpressed in the Caco-2 model, leading to an underestimation of the permeability of compounds that are their substrates [5]. The transport assay with Rho 123 confirmed the genetic expression results. In terms of TJs, no differences were observed between the models, although there was a decrease in the transepitelial electrical resistance (TEER) of the 3D models, becoming closer to in vivo values. The secretion of MUC2 was confirmed. In terms of permeability, data obtained for the 3D Co-culture model without the endothelial layer presented levels more similar with in vivo absorption. For the novel 3D model, the addition of the endothelium rendered a decrease in permeability, which was expected, since there is an additional layer hindering absorption. However, it can be important when studying the interaction of compounds with the endothelium.

Conclusion
The presence of HT29-MTX cells in the model may inhibit the secretion of soluble factors by the other cells present in the model, preventing the contraction of collagen by fibroblasts. This finding can be very important when developing models to test permeability, in order to avoid collagen contraction.

The novel 3D Co-culture model showed a better correlation with in vivo results in terms of expression of drug transporters, barrier tightness and, most importantly, permeability outcomes. The addition of the endothelial layer in the developed 3D model lead to a decrease in the permeability of compounds, which was expected having into account there is an additional layer hindering the passage of compounds. The novel 3D model has the potential of speed up the initial phases of drug development, providing more trustable results than the currently used 2D models and, therefore, decreasing animal experiments and contributing to the 3R’s policy.

Acknowledgement
This work was financed by Portuguese funds through FCT – Fundaçã para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior in the framework of the project “Institute for Research and Innovation in Health Sciences” UID/BIM/04293/2019. Maria Helena Macedo would like to thank FCT for financial support (SFRH/BD/131587/2017).

Development of the 3D model
General overview of the procedure followed to obtain the 3D complete model.
### Permeability outcomes

Papp \((x10^{-6} \text{ cm s}^{-1})\) values of (a) Colchicine, (b) Atenolol and (c) Metoprolol in the 2D Co-culture, 3D Co-culture and 3D Complete models at 14 and 21 days. Results are the average of triplicates, and bars represent the standard error deviation (SEM). Statistical differences were calculated using 2-way ANOVA multiple comparisons to compare Papp between each configuration at different time points and to compare between the same configuration at each timepoint (\(p<0.05\) are denoted by *, \(p<0.01\) are denoted by **, \(p<0.001\) are denoted by *** and \(p<0.0001\) are denoted by ****).

### References


Polysaccharide-based emulsifier for lung administration via inhalation

Raquel Gracia1, Marcos Navascuez1, Janire Alkorta1, Marco Marradi2, Natividad Diaz1, Hans-Jürgen Grande1, Iraida Loinaz1, Fernando López-Gállego2, Jordi Llop3, Damien Dupin1

1 CIDETEC, Basque Research and Technology Alliance (BRTA), Donostia-San Sebastian, ES; 2 CIC biomaGUNE, Basque Research and Technology Alliance (BRTA), Donostia-San Sebastian, ES; 3 University of Florence, Department of Chemistry “Ugo Schiff”, Sesto Fiorentino, IT

Introduction
Nanoemulsions are heterogeneous systems based on the dispersion of nanodroplets (< 500nm) of one phase emulsified in a second immiscible phase, resulting very attractive for encapsulation of active molecules in cosmetic and pharmaceutical formulations.1,2 Low molecular weight surfactants commonly used to stabilise emulsions can exhibit undesired cytotoxicity,3 while macromolecular PEGylated synthetic emulsifiers such as Cremophor, poloxamers and Triton X, have recently raised concerns due to Complement Activation-Related Pseudoallergy (CARPA).4,5 Thus, there is an urgent need for sustainable emulsifiers from natural resources. In this work, we systematically investigated the capacity of Dextran polysaccharide (DXT) and Dextran-based single chain nanoparticles (DXT-SCPN) functionalised with methacrylate (MA) groups (DXT-MA and DXT-SCPN-MA, respectively) or carboxylate groups (DXT-COO- and DXT-SCPN-COO-) to stabilise O/W emulsions produced by sonication. The best DXT emulsifier was then used for the encapsulation of FES drug and administered via inhalation to healthy rat to study the residence time of the drug.

Experimental Methods
Each dextran derivative (DXT-R) was dissolved in deionized water in an 8 mL glass vial. To this solution, the oil was added. The relative amounts of DXT-R, water and oil were modified to reach always a final amount of 2g. The emulsion was then formed by sonication (0°C, no stirring) using an UP400S (Hielscher) system at 100% of amplitude and pulse during 4 minutes (400 W) with a H3 sonotrode tip (3 mm diameter, 100 mm length).

Results and Discussion
Pristine DXT did not show any interfacial activity and a clear phase separation was observed after sonication of vegetable oils (such as olive oil and sunflower oil) with 0.5 wt% of an aqueous solution of DXT. On the other hand, stable O/W emulsions were achieved with our four selected stabilisers with the same vegetable oils. As expected, the presence of hydrophobic MA groups resulted in enhanced interfacial activity. Counter-intuitively, highly hydrophilic DXT-COO- and DXT-SCPN-COO- showed sufficient interfacial activities to stabilize O/W emulsions. However, DXT-MA proved to be the most performing stabilizer and the most straightforward to produce. The presence of ester bonds generated during the production of DXT-MA, together with the absence of interfacial activity of the pristine DXT, suggests that lipases or esterases may catalyse their hydrolysis, compromising the stability and offering triggered release. Therefore, we selected Candida Antartica Lipase B from (CALB), an enzyme widely employed in a diversity of biotechnological applications. After 20h incubation, droplet aggregation and coalescence were observed by Laser Diffraction (LD), while creaming effect and phase separation were visually observed after 40h. On the other hand, the same emulsion without the enzyme remained unaltered. These results confirmed that CALB triggers ester bond hydrolysis from DXT-MA, recovering pristine DXT with no interfacial activity, and ultimately compromising the emulsion stability and sustaining the demulsification process. Stability of DXT-MA-stabilized emulsions proved to be poor in PBS. Thus, taking advantage of the presence of MA group, dithiol crosslinker was added to the oil phase resulting in the crosslinking of the DXT-MA adsorbed at the interface. DLS and LD studies in different physiological media showed a stability enhancement of the nanoemulsions.
Then 16α-[18F]Fluorosteradiol (FES) was encapsulated as model cancer drug in the crosslinked nanocarrier at 5wt% in the oil phase. The resulting nanoemulsion with an hydrodynamic diameter of around 230nm was administered via intratracheal injection to healthy rat and compared to the drug alone. The residence time of the drug in the lung was monitored by PET-SPECT after radio-labelling the drug. It was shown that the nanoemulsiones allowed the drug to be retained longer in the lung. While around 60% of the encapsulated drug remained available after 40 min, the free drug was cleared within a few minutes. This result indicates that DXT-MA could be used as nanocarrier to prolong the residence time of hydrophobic drugs in the lungs after intratracheal administration.

**Conclusion**

In conclusion, a minor modification of natural DXT polysaccharide is sufficient to confer interfacial activity and stabilize O/W emulsions. The resulting emulsions are enzyme-responsive thanks to the presence of ester bonds. In addition, improved stability was obtained by crosslinking the DXT-MA adsorbed at the interface. After lung administration, DXT-MA-stabilized emulsions proved to improve drastically the residence time of hydrophobic drug in the lungs. These properties open opportunities for lung infections treatments and lung cancer.

**Acknowledgement**

Financial support for this work was provided by the Basque Government (ELKARTEK/BMG19 ref KK-2019/00015), ELKARTEK/BMG20 ref KK-2020/00010).

**References**


Poly(urethane)s by synthesis: versatile materials for challenging applications in the biomedical field

Gianluca Ciardelli1,2, Susanna Sartori1, Rossella Laurano1, Alessandro Torchio1, Valeria Chiono1,2, Monica Boffito1,2

1 Politecnico di Torino, Department of Mechanical and Aerospace Engineering, Turin, IT; 2 CNR, IPCF, Pisa, IT

Introduction
Poly(urethane)s (PUs) show an enormous diversity of chemical compositions and properties. Their complex chemical structure which typically comprises three monomers (i.e., a diisocyanate, a macrodiol and a chain extender) allows the synthesis of a virtually infinite number of different materials. For instance, both biostable and biodegradable PUs can be obtained by tuning macrodiol chemistry; on the other hand, macrodiol selection can also lead to either thermoplastic or stimuli-responsive water-soluble materials. Another key PU building block is represented by the chain extender which can be exploited to include into the polymer backbone specific moieties, such as cell adhesive motifs (e.g., RGD), enzyme-degradable sequences (e.g., elastase-sensitive Ala-Ala), pendant functionalities available for further functionalization (e.g., amino groups). In this contribution, a plethora of PUs has been designed and processed into many different devices (i.e., 3D scaffolds and hydrogels) to demonstrate the huge potential of customized PUs in properly answering to the demanding and specific requisites for medical device engineering in tissue engineering (TE) and pharmacology.

Experimental Methods
PUs were synthesized through a two step procedure according to well-established protocols in our group. PUs were chemically characterized and their cytocompatibility was evaluated according to ISO rules. Then, thermoplastic PUs synthesized using poly(caprolactone) (PCL) diol and different chain extenders (e.g., N-Boc serinol, 1,4-cyclohexanedicarboxylate, L-Lysine ester) were mechanically, structurally, and thermally characterized to investigate the role of the chain extender in determining PU properties. Finally, PUs were processed into 3D matrices through thermally induced phase separation, surface functionalized with fibronectin, and colonized with rat neonatal cardiomyocytes (CMs). Amphiphilic water-soluble PUs synthesized using Poloxamer 407 (P407) were tested as constituents of thermosensitive hydrogels for the localized delivery of drugs with different wettability (e.g., ibuprofen, ibuprofen sodium salt) and biomolecules (e.g., bovine serum albumin). The same PUs were blended with cyclodextrins (CDs) to design supramolecular (SM) gels for the release of curcumin (Cur). The exposure of amino groups along PU chains was exploited to graft photo-sensitive moieties (e.g., acrylate, norbornene, thiol) to design hydrogels with additional photo-responsiveness. Hydrogels were characterized by tube inverting test and rheology, meanwhile swelling, stability and release profiles of encapsulated payloads were tested in physiological-like conditions (i.e., pH 7.4, 37°C). Hydrogel printability was also tested using a commercial 3D bioprinter.

Results and Discussion
All PUs were successfully synthesized and exhibited biocompatibility. PCL-based PUs showed tunable mechanical properties through a proper chain extender selection (Fig.1A). The elastomeric PU synthesized using L-Lysine ester was processed into 3D scaffolds with mechanical properties replicating those of myocardial tissue (Fig.1B). Surface functionalization was successfully attained through plasma treatment in the presence of acrylic acid followed by fibronectin grafting via carbodiimide chemistry. CMs colonized the scaffolds and exhibited prolonged beating up to 1 month, thus making the developed matrices suitable for cardiac TE, both for regenerative and modelling purposes. Aqueous solutions of P407-based PUs exhibited improved thermal gelation (i.e., faster gelation at lower temperatures) and mechanical properties compared to P407-based ones (Fig.1C). PU gels allowed a prolonged and...
sustained release of different therapeutic agents over time. Injectable and self-healing SM gels were designed at PU and CD concentrations within 1-5 and 8-10 %w/V, respectively (Fig. 1D).\textsuperscript{4} CDs were also exploited to form inclusion complexes with Cur, allowing the encapsulation of this poorly water-soluble drug at high content and providing a protective environment to the drug molecules. Sustained and prolonged release of active Cur was observed up to 4 days. A PU suitable for further grafting of photo-sensitive groups was also synthesized using an amino-group bearing chain extender.\textsuperscript{5} Thermo- and photo-sensitive hydrogels were successfully photo-cured with Vis light and exhibited different mechanical properties depending on the grafted moieties along PU chains. Finally, PU hydrogels turned out to be printable under mild conditions, with good fidelity of the printed structs to the CAD design.

**Conclusion**

PU versatile chemistry can be widely exploited to fabricate both porous structs and injectable formulations for drug delivery and 3D bioprinting. PU biomaterials could thus represent valuable and reliable building blocks in the establishment of new TE and pharmacological strategies, thanks to the possibility to ad-hoc engineer polymers finely matching the highly demanding requisites of different biomedical applications.

**Figure 1**

A) Young Modulus and strain at break values measured through tensile tests for PCL-based PUs differing in their chain extender (i.e., L-lysine ethyl ester, N-Boc Serinol, peptide sequence containing the Ala-Ala motif, 1,4 cyclohexanedimethanol), B) Scanning Electron Microscopy image of a PU1 scaffold fabricated through thermal induced phase separation, C) images showing the sol and gel states of thermo-sensitive PU hydrogels, D) self-healing behavior of supramolecular hydrogels based on PU and CDs (Reproduced from 4 with permission from The Royal Society of Chemistry)

**References**


3:45 p.m. – 5:15 p.m.

Track07

N07 | Biomaterials for Electrical Stimulation

Chairs
Gianni Ciofani
Istituto Italiano di Tecnologia, Smart Bio-Interfaces, Pontedera, IT
Paul D. Dalton
University of Oregon, Knight Campus for Accelerating Scientific Impact, Eugene, US
Liliana Liverani (YSF)
University of Erlangen-Nuremberg, Institute of Biomaterials, Erlangen, DE
Internalization assessment and ultrasonic actuation of piezoelectric microdevices for electrical cell stimulation

Laura Lefaix¹, Andreu Blanquer², Lucie Bacakova², Gonzalo Murillo¹

¹ Microelectronics Institute of Barcelona (IMB-CNMT), Barcelona, ES; ² Institute of Physiology of the Czech Academy of Science, Prague, CZ

Introduction
Piezoelectric nanogenerators (NGs) convert the ambient mechanical energy produced by physical changes into electricity. NGs smaller than a cell can be integrated into microdevices for local cell stimulation. Among them, ZnO nanosheets (NSs) have been proved biocompatible and able to electrically stimulate human osteoblast-like (1) and muscle cells. These microdevices can be remotely activated by ultrasounds and allow a better spatial resolution to treat single cells (2). In this work, millions of reproducible microdevices, incorporating piezoelectric ZnO NSs with volumes below 25 μm³ were manufactured. These fabricated microdevices were later tested to assess their cytocompatibility, electrical effect and position with respect to the cell. Piezoelectric nanogenerators (NGs) convert the ambient mechanical energy produced by physical changes into electricity. NGs smaller than a cell can be integrated into microdevices for local cell stimulation. Among them, ZnO nanosheets (NSs) have been proved biocompatible and able to electrically stimulate human osteoblast-like (1) and muscle cells. These microdevices can be remotely activated by ultrasounds and allow a better spatial resolution to treat single cells (2). In this work, millions of reproducible microdevices, incorporating piezoelectric ZnO NSs with volumes below 25 μm³ were manufactured. These fabricated microdevices were later tested to assess their cytocompatibility, electrical effect and position with respect to the cell.

Experimental Methods
Piezoelectric microdevices were fabricated patterning a silicon wafer with millions of microparticles through microfabrication processes and the hydrothermal synthesis of the ZnO NSs. These microdevices were 'peeled off' and suspended in deionized water, then, sterilized by ethanol. The cytotoxic effect of the microdevices was assessed using a Live/Dead Kit (Invitrogen) on a cell culture of human osteosarcoma cells (Saos-2 cells) after 1 and 7 days culturing both cells and the microdevices (2:1 ratio). The samples were fixed and dehydrated to analyse the position of the microdevices with respect to the cells using SEM (Auriga-40 Carl Zeiss) and confocal laser scanning microscope (CLSM, Leica SP-8 DIVE). Complementary experiments testing the electrical stimulation of cells by measuring intracellular calcium increases through fluorescence intensity (Fluo-4AM) in the confocal laser scanning microscope (CLSM, Leica SP-5) are being performed (1, 3). After culturing together the microdevices with the Saos-2 cells (2:1 ratio) for 24 h, the microdevices are excited using an ultrasonic transducer (OPTEL, 2 MHz and 10 MHz). Images of osteoblasts are captured every 1 s for 15 min.

Results and Discussion
The microfabrication process and the hydrothermal growth provide a great homogeneity and reproducibility for the fabrication of the microdevice. The cytocompatibility results indicated above 90% of live cells on day 1 and above 85% on day 7, without significant differences with negative controls. Three different positions are taken by the microdevice (Figure 1): inside the cell (internalized), outside the cell with the NSs towards the cell membrane (top down) and with the NSs towards the surrounding medium (face up). The results show that 49% of the cells were in contact with one microdevice, 25% with two, and 12% with three microdevices. Among them, the 47% of these cells did not internalize any microdevice. The position of the microdevices could be controlled by functionalizing them. We can validate the electrical stimulation of the cells by exciting these microdevices through ultrasounds, based on
previous works (1). We expect a positive effect on cell differentiation and proliferation in cells that are in contact with microdevices due to the increase in cell activity. We hypothesize that the microdevices with NSs towards the cell membrane could electrically stimulate osteoblasts (1, 3), whereas the internalized microdevices could interfere the intracellular pathways.

**Conclusion**
Simple, cost-effective, technologically-reproducible microdevices with ZnO NSs were fabricated. The newly developed microdevices are cytocompatible on human osteosarcoma cells and can excite the cell electrically in response to ultrasonic actuation. These added suspended microdevices were in close contact with cells, both internalized and outside the cell membrane, which is important to ensure the electric stimulation.

**Acknowledgement**
This project has been financed by La Caixa Junior Leader Retaining (SPARKDUST, No. LCF/BQ/PR19/11700010) and the Spanish Government (EUR2020-112082).

**References**
Conductive resorbable hyaluronic acid-based hydrogels for tissue stimulation and monitoring

Rachel Auzély-Velty², Isabelle Texier¹, Maxime Leprince¹,²

¹ CEA of Grenoble, LETI/DTBS/L2CB, Grenoble, FR; ² University Grenoble Alpes, CERMAV, Grenoble, FR

Introduction
Due to their low elastic modulus, high hydration and electrical properties, conductive hydrogels are suitable candidates for electronics/soft tissue interface. Recently, conductive hydrogels have been developed based on commercial PEDOT:PSS (3,4-poly(ethylenedioxythiophene : poly(styrene) sulfonate) [1]. However, those hydrogels display some limitations for in vivo implantation due to problems of biocompatibility and biodegradability of PSS. In this work, we intended to replace PSS by a modified hyaluronic acid derivative, acting at the same time as the dopant of PEDOT, and as an extracellular-matrix-mimicking building block for the construction of the hydrogel matrix. The resulting new PEDOT-based conductive hydrogel displays enhanced biocompatibility, and biodegradation features. These properties enable the design of soft transient electronic devices, which could avoid explantation surgery.

Experimental Methods
HA was chemically modified in order to achieve a suitable dopant for PEDOT. It was sulfated (HAS) to increase its acidity and global negative charge, and phenylboronic acid (PBA) moieties were introduced to enhance hydrophobic interactions with PEDOT domains. The resulting biopolymer was cross-linked using adipic dihydrazide acid, simultaneously with EDOT polymerization, resulting in a conductive hydrogel (Fig.1). Material electrochemical and mechanical properties were studied through cyclic voltammetry, electrochemical impedance spectroscopy (Fig. 2.), electron microscopy, swelling and rheology characterizations. Hydrogel cytocompatibility and biodegradation rate were investigated as well. Ex-vivo experiments are presently on-going on rat brain slices to assess hydrogel electrical stimulation properties.

Results and Discussion
PEDOT:HAS-PBA material exhibited a high conductivity of 2-3 S/cm, among the highest of PEDOT:biomolecule reported in literature [2]. The conductive hydrogel appeared soft, with an elastic modulus (G’ ≈ 4 kPa) close to that of brain tissue. It displayed a high charge storage capacity of 2.90 ± 0.30 mC/cm², in addition to a low impedance around 650 Ω at 1 kHz (Fig. 2). Degradation study showed a mass loss around 70 % in 56 days when incubated at 37°C with hyaluronidase, making it suitable for midterm in vivo stimulation treatment. In vitro assays demonstrated hydrogel non-cytotoxicity (according to ISO 10993_5 standards). Based on these positive results, PEDOT-HAS-PBA conductive hydrogels are presently being tested for the ex vivo stimulation of brain tissue sections.

Conclusion
The newly-designed conductive PEDOT:HAS-PBA based hydrogel appeared as perfectly biocompatible, biodegradable, and exhibited unprecedented conductive and electrochemical properties among PEDOT:biomaterial-based conductive hydrogels described in the literature. The total control over material synthesis enabled a fine tuning of electrical and mechanical properties, resulting in a wide range of potential applications, from tissue stimulation to conductive medium for electro-sensitive cells, which are presently being investigated.

Acknowledgement
M.L. is supported by CEA through a Ph.D. scholarship and this work is part of the STRETCH research project founded by the French National Research Agency (ANR-18-CE19-0018). LETI-DTBS and CNRS-CERMAV are supported by
the French National Research Agency in the framework of Arcane Labex (CBH-EUR-GS, ANR-17-EURE-0003), and Glyco@Alps “Investissement d’avenir” program (ANR-15-IDEX-02).

Figure 1
a) Scheme of the simultaneous cross-linking of HAS-PBA with adipicdihydrazide (ADH), and EDOT polymerisation; b) Photograph of obtained conductive hydrogel.

Figure 2
a) Cyclic voltammetry of conductive hydrogel at 50 mV/s; b) Bode plot of conductive hydrogel.

References
[1] Yuk et al., Chemical Society Reviews 48.6 (2019): 1642-1667
Creating multifunctional platforms: electroconductive coaxial fibers to electrically stimulate differentiating neurons

Fábio F.F. Garrudo\textsuperscript{1,2}, Diogo E.S. Nogueira\textsuperscript{1,2}, Carlos A.V. Rodrigues\textsuperscript{1,2}, Flávio A. Ferreira\textsuperscript{1,2}, Joaquim M.S. Cabral\textsuperscript{1,2}, Jorge Morgado\textsuperscript{3}, Robert J. Linhardt\textsuperscript{4}, Frederico C. Ferreira\textsuperscript{1,2}

\textsuperscript{1} Instituto Superior Técnico, Universidade de Lisboa, Department of Bioengineering and iBB-Institute for Bioengineering and Biosciences, Lisboa, PT; \textsuperscript{2} Associate Laboratory i4HB - Institute for Health and Bioeconomy, Lisboa, PT; \textsuperscript{3} Instituto Superior Técnico, Universidade de Lisboa, Department of Bioengineering and Instituto de Telecomunicações, Lisboa, PT; \textsuperscript{4} Rensselaer Polytechnic Institute, Department of Chemistry and Chemical Biology, Center for Biotechnology and Interdisciplinary Studies, Troy, US

Introduction
Tissue engineering and regenerative medicine strategies are the best current approaches to cure neurological diseases. Induced pluripotent stem cells (iPSCs) can provide a source of autologous neural stem cells (NSCs) to treat them. The use of scaffolds can assist in NSC transplantation to the target tissue and promote tissue integration and enable a more efficient differentiation of cells to a favorable phenotype.

Coaxial electrospun fibers, composed of 2 layers, make ideal scaffolds for neural applications due to their versatility: 1) tunable fiber diameter and mechanical properties, 2) creation of chemically-independent layers and with different bioactivities, and 3) potential controlled-release of encapsulated substances. In this work we describe the development of coaxial fibers-based platforms, suitable for electrical stimulation of iPSC-driven NSCs (iNSCs).

Experimental Methods
PCL-PANI and PGS solutions were prepared and coaxial fibers were electrospun under the following conditions: shell solution (PCL-PANI) at 3 mL h\textsuperscript{-1}, core solution (PGS) at 1.5 mL h\textsuperscript{-1}, 22.5 kV, 20 cm, 21 \textdegree C, relative humidity of 40 - 50 \%. PCL-PANI and PCL fibers were used as controls. An air-blower was used to force fiber deposition on the collector. Fiber morphology and diameter was evaluated through scanning electron microscopy (SEM). Coaxial structure was evaluated through transmission electron microscopy (TEM) and complementary physico-chemical properties by differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy (FTIR). The following critical fiber properties for neural cell culture were evaluated: electroconductivity (4-probe method), stiffness (mechanical properties), hydrophilicity (contact angle), and in vitro stability/biodegradability (PBS and lipase-solution). To test biocompatibility, iPSCs were differentiated into iNSCs, transferred to the fibers and further differentiated. The effect of electrical stimulation on iNSCs neural fate was also investigated. Cell were stimulated (1 V cm\textsuperscript{-1}, 100 Hz, pDC) for 30 days, and neural profile was evaluated through immunofluorescence (IF) and quantitative polymerase chain reaction (qPCR).

Results and Discussion
The produced coaxial PCL-PANI/PGS fibers have an average diameter of 951±465 nm and an electroconductivity of 0.063±0.029 S cm\textsuperscript{-1}. The mechanical properties (\(\varepsilon\) of 1.3 MPa) and hydrophilicity (38\textdegree) allow NSC differentiation. Coaxial fibers degrade in the presence of a human lipase (72\% weight-loss in 12 hours). Neural differentiation of iPSCs occurs normally on the coaxial fibers, and the application of electrical stimulation improves neural maturation. In particular, we observe the expression of higher levels of synaptophysin (synaptic vesicles), NEF-H (mature neurons), CACNA1C (functional neurons) and VGLUT1 (glutamatergic neurons), accompanied by a decrease in DCX (migratory early neurons), TUBB3 (early-neural marker) and GAD67 (GABAergic neurons).

Conclusion
This study shows that the fibers developed here have potential applications in neural tissue engineering, namely for in vitro platforms for drug screening and disease modelling, and ultimately for direct transplantation of mature and functional neurons into patients’ brains. The coaxial fibers are electroconductive, soft, hydrophilic, able to swell up to two times their weight. These are stable in PBS, being able to maintain significant electroconductivity for up to 28 days. In the presence of lipase, PGS/PCL-PANI core layer PGS degrades completely after 7 days, but the shell layer (electroconductive) degrades slower. iPSCs, after neural induction, were successfully differentiated into neurons on PGS/PCL-PANI coaxial fibers. The electrical stimulation results suggest a shift from an inhibitory to an excitatory neural cell profile during the 30 days of culture. More studies are required to further explain the changes observed on neural differentiation and the mechanisms involved.

Acknowledgement
The authors acknowledge the help of MD Isabel Nogueira for SEM image acquisition, both Dr Paiyz Mikael, BScs Caitlyn Chapman and Pauline Hoffman for their help in the initial electrospinning optimization, Dr Ranodhi Udangawa Keating for her help with coaxial electrospinning, Professor Ana Clara Marques and the PhD student Mário Vale for FTIR data acquisition, Dr Patrizia Paradiso and Professor Rogério Colaço for the mechanical tests, and Dr Evgenia Bekman for the valuable discussion over of the main cellular findings and important for the conclusion of this work. The authors thank Fundação para a Ciência e Tecnologia for funding through iBB (UID/BIO/04565/2021), IT (UIDB/50008/2020), Neuron (PTDC/CTM-CTM/30237/2017), Stimuli2BioScaffold (PTDC/EME-SIS/032554/2017), PORL 2020 through PRECISE (16394) and PhD scholarships (PD/BD/114045/2015 and PD/BD/128376/2017).

General overview of the work
Workflow describing the approach used in the preparation of coaxial fiber-based platforms for the electrical stimulation of neural cells.
Comparison of neural cell profile with or without electrical stimulation

SEM images (A-C) of iNPCs differentiated for 30 days on PGS/PCL-PANI fibers under electrical stimulation. qPCR data (D) for iNPCs cells differentiating for 30 days on the different fibers tested (D) and respective close-up (E) (mean ± sem, n = 3).

References
Melt Electrowriting of Polymers beyond Poly(caprolactone) with Focus on Electroactive Materials

Juliane C. Kade¹, Paul Otto¹, Biranche Tandon¹², Dario Pisignano³⁴, Luana Persano³, Robert Luxenhofer⁵⁶, Paul D. Dalton¹²

¹ University Hospital Würzburg, Department of Functional Materials in Medicine and Dentistry and Bavarian Polymer Institute, Würzburg, DE; ² University of Oregon, Phil and Penny Knight Campus for Accelerating Scientific Impact, Eugene, US; ³ NEST, Istituto Nanoscienze-CNR and Scuola Normale Superiore, Pisa, IT; ⁴ Università di Pisa, Dipartimento di Fisica, Pisa, IT; ⁵ Julius-Maximilians-University Würzburg, Polymer Functional Materials, Chair for Advanced Materials Synthesis, Department of Chemistry and Pharmacy, Würzburg, DE; ⁶ Faculty of Science University of Helsinki, Soft Matter Chemistry, Department Chemistry, and Helsinki Institute of Sustainability Science, Helsinki, FI

Introduction
Additive manufacturing techniques are increasingly used for tissue engineering and regenerative medicine (TERM). Melt Electrowriting (MEW) is an additive manufacturing technique to fabricate scaffolds with fiber diameters in the lower micron range with a need to expand the range beyond the current gold standard poly(caprolactone) [1,2]. We established MEW of electroactive scaffolds based on poly(vinylidene difluoride) (PVDF) [3] and a copolymer (PVDF-Trifluoroethylene; PVDF-TrFE) [4]. These polymers are known for their piezoelectric properties [5], which can be used for stimulating in TERM applications and wearable sensors.

Experimental Methods
A custom-built MEW printer was used to process the PVDF at 190°C and PVDF-TrFE at 170°C with an N₂-pressure of 3.0 and 0.5 bar, respectively. The distance between the print head and collector was set to 4.0±0.4 mm with an applied potential difference of +3.0 - 3.7 kV. The collector temperature was varied between room temperature and 150°C. Processing of PVDF was possible with collector speeds of up to 4000 mm/min. In contrast, sufficient processing parameters of PVDF-TrFE resulted in remarkable low speeds below 100 mm/min. Furthermore, additional printing parameters, like novel nozzle designs, have been investigated.

Results and Discussion
To MEW-process PVDF using a heated collector helped to improve the layering, accuracy and stability of the printed constructs. Furthermore, printing at speeds of 4000 mm/min and heating the collector to temperatures above 100°C does reduce warping of the already processed construct and therefore, enables to print scaffolds with 20 layers at 110°C (Figure 1A). However, at collector temperatures of 120°C or higher, the deposited polymer agglomerates resulting in coalesced dots at the crossing points of the scaffolds (Figure 1B-C) inducing a reproduceable biomimetic design.

Furthermore, the additional parameter of a heated collector was necessary to MEW-process PVDF-TrFE [4]. Due to the rapid solidification of the polymer jet, only collector temperatures of at least 120°C enabled sufficient fiber adherence and resulting fibers (Figure 2) were straight at remarkable low printing speeds. Using XRD, the processed polymer showed an increase in the total crystallinity, as well as a shift from the α- to the piezoelectric β-phase. The electroactive nature of the processed fibers was proven by PFM measurements.

Conclusion
PVDF and PVDF-TrFE were shown to be MEW-processable resulting into scaffolds with relevance to applications where well-resolved 3D printed structures of piezoelectric polymers are of interest, such as biomedical products.

Acknowledgement
We gratefully acknowledge financial support by the Volkswagen Foundation (grant number 93418) and additionally J. C. K. is supported by the Joachim Herz Foundation. Technical assistance of P. Stahlhut and J. Friedlein for SEM imaging is appreciated, while the Zeiss Crossbeam CB 340 SEM was funded by the German Research Foundation (DFG) State Major Instrumentation Programme (INST 105022/S8-1 FUGG).
**Figure 1.**
Showing SEM images of MEW-processed PVDF with a collector speed of 4000 mm/min and at a collector temperature of A) 110°C resulting in 20 layers and B-C) at 120°C with the coalesced dots at the crossing points. B-C) scale bars = 100 µm.

**Figure 2.**
Showing SEM images of MEW-processed PVDF-TrFE fibers at a collector temperature of 120°C and speeds of A) 10 mm/min and B) 40 mm/min.

**References**


The Development and Characterisation of Electroconductive Biomaterial Scaffold for Spinal Cord Injury Repair

Aleksandra Serafin¹, Mario Culebras Rubio¹, Maurice Collins¹, Miguel Oliveira²,³, Aleksandra Serafin¹

¹ University of Limerick, School of Engineering, Limerick, IE; ² University of Minho, Research Institute on Biomaterials, Biodegradables and Biomimetics, Minho, PT; ³ PT Government Associate Laboratory, ICVS/3B’s, Braga, PT

Introduction
Spinal cord injury (SCI) is a debilitating disorder that often results in paralysis at or below the injury site due to the loss of motor and sensory function of the patient and is caused by a primary injury to the spinal cord by means of traumatic contusion or laceration. Annually, 12,000 new cases of SCI are reported in the United States. Due to a lack of effective medical treatments for SCI, the number of patients who suffer from this affliction rises every year [1, 2]. The scientific and medical communities have thus in turn begun to explore tissue engineering (TE) strategies to try and alleviate the medical conundrum that is SCI repair. Neural stem cells and biomolecules have been often been incorporated into the TE scaffolds for this purpose, though limited results have been achieved to date due to the hostile nature of the injured spinal cord environment. Recently, interest in materials that possess electroconductive properties has enabled the production of a new generation of scaffolding materials, which deliver more optimal results in terms of tissue repair than their non-conductive counterparts.

As with the development of any biomaterial, a number of factors must be met to achieve suitability for use in TE strategies, including excellent biocompatibility, biodegradability, and suitability for cells/bioactive factor incorporation whilst facilitating the regeneration of neuronal or axonal tracts in the spine. The physicochemical characterization of this biomaterial includes morphology, degradation profiles, compression tests, electroconductivity, rheology, and biocompatibility studies. In-vitro cytocompatibility studies include Alamar Blue assay and Immuno-fluorescence staining of protein markers on neural stem cells.

In this work, we present the development and subsequent characterization of a new electroconductive biomaterial. The basis of the electroconductive element in this novel biomaterial is conductive polymer nanoparticles (NPs) created by means of a novel mini-emulsion method. Due to its production method, the polypyrrole (PPy) NPs are water-soluble and have the potential to be functionalized in a myriad of ways.

Experimental Methods

Structural analysis: The morphology of the developed NPs was first analyzed using Scanning Electron Microscopy. The NPs were then incorporated into a hydrogel structure to create NP electroconductive scaffolds. Cross-sectional images of NP electroconductive scaffolds were analyzed to evaluate the changes in the morphology and porosity of the scaffolds.

Compression properties: The compression test carried out on the NP electroconductive scaffolds using an in-house compression test facility equipped with a 1 kN load cell. Circular discs, diameter 13.5 mm and thickness 4 mm, were used with a screw speed of 1 mm/min up to 60% strain. The Young’s Modulus of the samples was calculated as the slope in the linear region of normalized stress vs. strain graph.

Conductivity properties: The resistivity of the NP electroconductive scaffolds was measured using an Ohm meter. The conductivity was then calculated as follows:

\[ \sigma = \frac{RA}{l} \]  

Where \( \sigma \) is the conductivity, \( l \) is the sample length, \( A \) is the cross-sectional area and \( R \) is the resistance.

Biocompatibility: Developed NP electroconductive scaffolds were U.V. sterilized and incubated in complete cell...
culture medium (DMEM media supplemented with 1% penicillin-streptomycin, 2% L-Glutamine and 10% FBS) for 72 hr at 37 °C (5% CO₂ incubator) to allow for equilibrium, with the media changed regularly. Subsequently, the media was removed completely and seeded with 0.05x10⁶ cells/scaffold Neural Stem cells in 24-well plates, and incubated overnight to allow for cell attachment to the scaffold. The scaffolds were then incubated for a period of up to 72 hr to perform Alamar Blue and Immunofluorescence assay.

Results and Discussion
The development and characterization of the NP electroconductive scaffold were successfully accomplished. Figure 1 shows the round and stable morphology of the synthetized PPy NPs, while Figure 2 shows the PPy NPs embedded in a gelatin/hyaluronic acid hydrogel blend scaffold, with individual NPs present dispersed and embedded throughout. The developed NP electroconductive scaffold displays adequate mechanical strength comparable to that of the native spinal cord. The conductivity of the scaffolds ranged up to 10⁻⁷ S/cm. Biocompatibility shows that the developed NP electroconductive scaffold facilitates cell proliferation and growth.

Conclusion
From this work, it can be concluded that the developed NPs and subsequent incorporation into NP electroconductive scaffolds show promise as a TE strategy for SCI repair.

References
Metallic Nanostructured Electrodes for *in vivo* and *in vitro* Electrical Stimulation

**Sahba Mobini**¹, María-Ujué González¹, Olga Caballero-Calero¹, Erin E. Patrick², Marisol Martín-González¹, Jorge M. García Martínez¹, Jose M. García-Martín¹

¹ Spanish National Research Council (CSIC), Instituto de Micro y Nanotecnología, IMN-CNMT, Tres Cantos, ES; ² University of Florida, Department of Electrical and Computer Engineering, Gainesville, US

**Introduction**

The use of electricity in interaction with biological systems for therapeutic and diagnostic purposes become a prominent research topic. Electrodes are key components of electrical stimulation and recording devices for *in vivo* and *in vitro* usages. Bioelectrodes should present excellent *electroconductivity* to be able to transfer the electrical current efficiently (charge mobility inside the device). Moreover, they need to provide excellent *electroactivity* to convert electronic current to ionic current at the electrode/extracellular fluid (ECF) interphase. The use of nanostructures in fabrication of bioelectrodes is beneficial, since nanostructures offer superior effective surface area that decreases impedance (Z) and increases charge storage capacity (CSC) of the electrodes. Low Z prevents irreversible faradic reactions that are harmful for biological systems (safe stimulation), and decreases thermal noises that interfere recording bioelectrical signals. Higher CSC ensures effective charge transfer (effective stimulation)¹. Here we developed highly pure metallic nanostructured electrodes from Ti, Au and Pt and characterized their electrochemical properties. Electrical stimulation (ES) modes for both *in vitro* and *in vivo* applications were simulated. Finally, we present the design of an *in vitro* ES platform incorporating nanostructured electrodes.

**Experimental Methods**

Au, Pt and Ti nanocolumnar layers (NCs) (~250 nm thick) have been deposited on glass substrates by means of magnetron sputtering using the glancing angle deposition configuration (GLAD)². Continuous thin films (~30 nm) of the same metals were also grown on glass as control groups with the same technique but using the standard parallel configuration. The microstructure of the electrodes was determined using scanning electron microscopy (SEM) (Fig. 1A) and atomic force microscopy (AFM). Electrochemical characterization was performed using a potentiostat with FRA32M module in a three-electrode setup. A platinum mesh used as a counter electrode and Ag/AgCl was used as reference electrode. Measurements were performed in PBS with a sinusoidal excitation signal of 10 mV in the 0.1-10⁵ Hz range. Cyclic voltammetry (CV) has been performed within the water potential window recommended for Pt (−0.5 to 0.8 V), with 0.1 V/s sweep rate. CSC values were obtained from CV diagrams.

**Results and Discussion**

Electrical impedance spectroscopy revealed that Z values at 1 Hz for Ti and Pt NCs are one order of magnitude smaller than those for Ti and Pt thin films. In the case of Au, the Z of NCs is ~2 times smaller than that of the thin film (Fig. 1B). Generally, lower Z is a favourable characteristic for ES systems (both for *in vivo* implants and *in vitro* setups) because of the increase in safety and efficiency of stimulation and recording. Moreover, Z is also gaining importance recently due to its strong dependence on the size of electrodes. The common strategy for increasing ES resolution in biological systems is reducing the size of electrodes, that consequently increases Z. Reduced Z offers then the possibility of miniaturizing the electrodes without harmful side effects. CSC values are significantly improved when NCs are used: ~200% in case of Ti and Au and ~400% for Pt. We obtained CSC of 1.3 mC/cm² for PtNCs, 4 times higher than the value reported for similar Pt nanostructures fabricated by electrochemical deposition³. CSC is an indicator for the sum of the charges that can be introduced to ECF at the electrode interface. Although larger surface area significantly improves CSC, this electrochemical behaviour highly depends on the intrinsic properties of...
the material. Therefore, larger values are obtained for Pt compared to Au and Ti (Fig. 1C). Finally, when applying ES via voltage-controlled methods, the resultant voltage experienced by the ECF may not be the same as the applied voltage waveform after being filtered by the electrochemical double layer capacitance at the interface. Since the double layer capacitance of NCs electrodes is higher than the one for the thin film, the NCs provide a voltage drop across ECF ($V_{\text{medium}}$) that is much closer to the source signal (Fig. 1D). Considering all those benefits of nanostructures, we developed a miniaturized device\(^ 4\) for \textit{in vitro} ES application that provides safe and effective stimulation in a large voltage range (Fig. 1E).

\textbf{Conclusion}

We characterized electrochemical properties of metallic nanostructured electrodes. We showed a remarkable improvement in charge storage capacity and highly significant reduction in impedance by using nanostructures. Among the three metals we compared, Pt provide the best properties, thus we incorporate it in \textit{in vitro} electrical stimulation device.

\textbf{Acknowledgement}

Funding from the European Commission (Ref. 793102-NeuPES-H2020-MSCA-IF-2017), Comunidad de Madrid (Atracción de Talento Programme, Modalidad-1 Ref. 2019-T1/IND-1335 and project S2018/NMT-4291 TEC2SPACE) and MINECO (project CSIC13-4E-1794) is acknowledged.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{A) SEM images of metallic electrodes Ti, Au and Pt thin films (TF) (top row), and nanocolumns (NC) (bottom row), scale bar =100 nm. B) Bode plots of electrical impedance of metallic thin films and nanostructured electrodes. C) Charge storage capacity of electrodes calculated from cyclic voltammetry tests. D) Simulation of voltage stimulator: an example for use of gold thin film (Au-TF) and nanostructured electrodes (Au-NC). E) Miniaturised \textit{in vitro} electrical stimulation device (MiDES), incorporating nanostructured electrodes.}
\end{figure}

\textbf{References}


Organic Piezoelectric Biomaterials for Electrostimulation: Poly-1-Lactide Films

Sofija Nikolic, Lea Udovc, Mihaela Apetrei, Matjaz Spreitzer, Marija Vukomanovic

Jozef Stefan Institute, Advanced Materials Department, Ljubljana, SI

Introduction
Due to the high perspective of implantable electro-stimulators in achieving reinforced post-operative regeneration their design is constantly being improved. The improvements are mainly associated with advancing materials used for their assembly. So far the highest progress has been achieved by using piezoelectric materials. Piezoelectric scaffolds can generate electrical pulses as a result of deformation caused by body motion (blood circulation, heart beating, muscle tension, migration of cells, etc.). Important advantage of their application for electro-stimulation is lack of electrodes with questionable biocompatibility or lack of external source of electricity, which can result in accumulation of potentially toxic product of electrolysis. However, selection of the piezoelectric material that will meet the biocompatibility requirements in combination with required piezoelectric coefficients is a big challenge.

Experimental Methods
Our work is focused to optimizing piezoelectric properties of poly-1-lactide (PLLA) films using several processing approaches (illustrated in Fig. 1). In thermal drawing pre-melted, amorphous film is clamped and mechanically stretched to specific drawing ratio. The approach enables thick, self-standing films usually formed out of parallel layers, oriented in the drawing direction. On the other hand, in template wetting, the pillar-shaped pores in AAO template membranes are filled with pre-melted polymer. Polymer is drawn by capillary forces during wetting the walls of the pores inside template. After removing the template, polymer forms PLLA tubes. For the case of electrospining, instead of melting, polymer is dissolved in selected solvent and deposited on conducting collector (stationary or rotational). In this case, polymer is stretched by using forces in electric field combined by forces from rotating collector. The polymer forms fibres which could be random of aligned (depend on the type of collector). The morphology of so-formed fibres depends strongly on selected solvents and enables forming largely porous, non-porous and smooth surfaces. Due to the applied stretching, all three approaches enable orientation of PLLA molecules within formed structures. In all there cases we preferentially detected formation of α crystal structure. In films formed by thermal drawing crystallization takes place along with mechanical stretching, while films formed by wetting template and electrospinning usually need additional annealing to crystallize. As a result of molecular orientation and crystallization, films were having piezoelectric nature and they were able to provide voltage signal either after compressing or twisting deformation.

Results and Discussion
Piezo-PLLA films formed in our lab are investigated for interactions with both prokaryote and eukaryote cells. Electro-stimulation of bacteria in presence of piezo-PLLA films (deformed using soft ultrasound), is observed to provide damages in bacterial cell wall. In sessile bacteria we observed both bacteriostatic and bactericidal effects, while bacteria adhered to film surface are intensively detached after stimulation. During interactions with non-adherent mammalian cells (red blood cells, RBCs, isolated from sheep blood), similar damages on cell membranes were not detected. Electro-stimulation did not provide cell haemolysis and morphologically RBCs were keeping innate morphology without showing signs of clotting. Testing interactions with adherent mammalian cells (human skin keratinocytes, HaCaT) revelled effect of electro-stimulation on cell migration, proliferation and defect recovery (Fig. 1).

Conclusion
Page 341 of 2028
Due to its biocompatibility and biodegradability, piezo-PLLA is one of the most perspective candidate for clinical application of organic piezoelectrics in electro-stimulated tissue regeneration and recovery. Different processing routes enable versatility of their applications either as bioactive coatings or as membrane scaffolds.

Acknowledgement
The work has been supported by the Slovenian Research Agency (ARRS) within grants J2-8169, N2-0150 and PR-08338 and research program P2-0091.

Piezoelectric PLLA films obtained using thermal drawing, wetting template and electrospinning
Morphological characteristics of films obtained by different approaches; voltage signal obtained by deforming films in soft ultrasound; effect of electro-stimulation on viability and de-attachment of bacteria from film surface; effect of electro-stimulation to membrane compactness in red blood cells; electro-stimulation -induced migration and defect recovery observed for HaCaT cells.
5:45 p.m. – 6:30 p.m.

Track01

PL1 | Plenary Lecture 1

Chair
Ana Paula Pêgo
Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, PT

PL1-01

Microrobotics and Nanomedicine: Future Directions in Medical Robotics

Bradley J. Nelson

ETH ZURICH, Mechanical and Process Engineering, Zurich, CH

While the futuristic vision of micro and nanorobotics is of intelligent machines that navigate throughout our bodies searching for and destroying disease, we have a long way to go to get there. Progress is being made, though, and the past decade has seen impressive advances in the fabrication, powering, and control of tiny motile devices. Much of our work focuses on creating systems for controlling micro and nanorobots as well as pursuing applications of these devices. As systems such as these enter clinical trials, and as commercial applications of this new technology are realized, radically new therapies and uses will result that have yet to be envisioned.
7:30 p.m. – 8:30 p.m.

Social Activity 2 | YSF Night Out

This years’ YSF night out is unfortunately not taking place face to face in a beautiful bar near the Atlantic Ocean, as we wished for. But that should not prevent us from social gathering and meeting new and old friends from our scientific community!!!

We challenge young researchers (under 40!) to join our online event, where participants will be gathered into groups and play each other, letting us know how much you know about Portugal, the World, and… ESB!

As in any YSF night out, drinks should be a requirement! Due to restrictions in many countries we were not able to send you our famous Port Wine, but we invite you to grab a beer, glass of wine, or any cocktail of your choice (or a mocktail, if you really must…), so that we better feel the informal environment!

The event is free, but registration is required.
Monday, 6 September, 2021

10:30 a.m. – 16:35 pm.

Track08

NANOSTEM Satellite Symposium

Title: New nanomaterials for neural stem cells drug delivery

Brain conditions such as neurodegenerative diseases, stroke and traumatic injuries are a major burden in modern society. Because neural stem cells (NSCs) can differentiate into new neural cells, including neurons, the regulation of their proliferation, differentiation and migration represents a promising regenerative/therapeutic strategy. The hypothesis of NANOSTEM project is that novel nanoparticles (NPs) combined with recent developed pharmaceuticals can be an efficient approach to control the biological activity of NSCs.

During this multidisciplinary symposium 14 Early-Stage Researchers will present their recent findings and advancements within the frame of the NANOSTEM European Project. The topics covered will be ranging from organic and polymer chemistry, biochemistry, molecular biology, neuroscience, and computational science, with particular emphasis on:
1) synthesis of nanomaterials and uploading of the therapeutics
2) evaluation of the permeation and efficacy of the formulations against neural stem cells
3) in vivo studies to assess the toxicity of the formulations developed and their ability to tackle the brain.

About NANOSTEM:

NANOSTEM is a Marie Skłodowska-Curie Innovative Training Network (ITN) funded by the European Commission through the Horizon 2020 Research and Innovation programme (H2020-MSCA-ITN-2017 G.A. 764958). The network is coordinated by Prof Marina Resmini at QMUL and comprises 9 beneficiary teams and 3 associated partners, from 6 different European countries, both from academia and industry.
10:30 a.m. – 11:55 a.m.

Track08

NANOSTEM 01 | Nanogels for drug delivery

Chair
Eirini Epitropaki
University of Birmingham, BIRMINGHAM, GB
NANOSTEM 01-Intro
Introduction to the NANOSTEM Project
Marina Resmini
Queen Mary University of London, London, GB

NANOSTEM 01-KL01
Modulation of stem cells using nanotechnologies
Lino S. Ferreira
University of Coimbra, Coimbra, PT

NANOSTEM 01-03
The Coil-Globule Transition: Thermosensitive Hydration of Acrylamide-based Polymers
Patrick K. Quoika, Monica L. Fernández-Quintero, Anna S. Kamenik, Maren Podewitz, Klaus R. Liedl
University of Innsbruck, Department of General, Inorganic and Theoretical Chemistry University of Innsbruck, Innsbruck, AT

Introduction
Counterintuitively, thermosensitive polymers undergo a phase-transition with a lower critical solution temperature. Accordingly, they form a gel above a certain temperature, while they exist in liquid mixture with the solvent below this temperature. The macroscopic phase behaviour is linked to a conformational change of the polymers: the so-called Coil-Globule transition. To understand their extraordinary phase behaviour, we investigated the conformational dynamics of a selection of acrylamide-based polymers – including thermosensitive and non-thermosensitive ones.

Experimental Methods
We performed molecular dynamics simulations of linear polymer chains with atomistic detail at a wide range of temperatures. With regards to their potential thermosensitivity, we analyzed the conformational ensembles with respect to the Coil-Globule transition and quantified thermodynamics and kinetics of the process. To this end, we computed the equilibrium constants and energetic contributions to the free energy of the transition, such as e.g. the conformational entropy of the polymers, and characterized the conformational ensembles with polymer-specific descriptors. Therewith, we were able to build markov state models of the conformational dynamics, which yield an accurate reconstruction of the kinetics at different temperatures.
Results and Discussion
We succeeded in distinguishing between thermosensitive from non-thermosensitive polymers based on our simulation results. Further, we report distinct hydration profiles for these two classes of polymers and identified the polymer-solvent interactions to be essential for the energetic balance of the transition. We report a clear trend between the lifetime of the hydrogen bonds between the polymer-surface and water and the thermosensitive character of the polymers. Moreover, we were able to resolve the conformational space of the polymers by means of the freely jointed chain model and obtained consistent results with respect to thermodynamics and kinetics of the thermosensitive Coil-Globule transition.

Conclusion
We identified conformational substates that suggest that the thermosensitive Coil-Globule transitions may be a multi-step process. Furthermore, we found that the radius of gyration of these polymers is not an optimal coordinate to resolve the kinetics of this transition, despite it being a valid descriptor in experiments. Ultimately, we conclude that the solvation entropy is decisive for the thermosensitivity of these substances.

Acknowledgement
The computational results presented have been achieved using the HPC infrastructure LEO of the University of Innsbruck and the Vienna Scientific Cluster (VSC). Furthermore, this project has received funding from the European Union’s Horizon 2020 research and innovation program under grant agreement no. 764958.

References
NANOSTEM 01-04

Nanogels for drug delivery: effect of synthetic methodology on the structure of pNIPAm and drug loading

Alena Vdovchenko¹, Amanda Pearce², Mark Freeley¹, Rachel O’Reilly², Marina Resmini¹

¹ Queen Mary University of London, School of Biological and Chemical Sciences, London, GB; ² University of Birmingham, School of Chemistry, Birmingham, GB

Introduction
The proximity of the volume phase transition temperature (VPTT) of poly-N-isopropylacrylamide (pNIPAm) nanogels to physiological temperature governs interest for their use as a drug delivery system.¹ At temperatures below VPTT, pNIPAm exists in a swollen state in an aqueous solution, while an increase in temperature above VPTT leads to the expulsion of water molecules from the polymer matrix. This thermoresponsive behaviour of pNIPAm can be used to control drug release. At the same time, thermostresponsiveness of pNIPAm leads to the possibility of switching the nature of polymerization from homogeneous, conducted below VPTT, to heterogeneous above VPTT.²

Experimental Methods
In the following study, we analysed how synthetic methodology, whether homogeneous or heterogeneous polymerisation, affects the morphology and properties of pNIPAm nanogels.

Results and Discussion
A number of experimental parameters were altered, such as the temperature of polymerisation and the choice of initiator. The obtained nanoparticles were then characterised with a range of techniques, such as dynamic and electrophoretic light scattering, UV-Vis spectroscopy, surface tensiometry, as well as liquid-phase atomic force and transmission electron microscopy.

Conclusion
The morphology and thermal behaviour of nanogels were rationalised to identify potential link with the polymerisation conditions.

References
NANOSTEM 01-05

Protein corona of nanogels and its impact on the air/water interface

Federico Traldi, Marina Resmini

Queen Mary University London, School of Biological and Chemical Sciences, London, GB

Introduction
In the last few decades, nanomaterials have emerged as attracting platforms for drug delivery and imaging. Despite the great potential of nanoparticles in nanomedicine, their application in clinical settings is hindered by the limited knowledge of their interaction with bio-interfaces. When dispersed in biological media, nanomaterials may be coated with proteins in solution and form a "protein corona". Extensive research has undeniably proven that the formation of a protein corona can deeply affect the behaviour of nanoparticles in biological systems. However, the relative limited knowledge of how the properties of nanomaterials drive the formation of the corona poses a serious obstacle to their rational design. New methods to characterise the nanoparticle corona in its biologically relevant environment are hence required to bridge this knowledge gap.

Experimental Methods
In this work we aimed at developing a novel approach towards the characterisation of protein corona of polymeric nanogels based on dynamic surface tensiometry. Nanogels' characteristics, such as low toxic profile, high colloidal stability and potential stimuli-responsive behaviour make them ideal candidates for drug delivery applications. A series of nanogels based on backbone monomer acrylamide (AM) and crosslinker methylenebisacrylamide (MBA) were synthesised via high dilution radical polymerization. To investigate the effect of electrostatic forces on the affinity of nanogels for model proteins, either negative or positive charged co-monomers were incorporated in the polymer matrix. Evaluation of protein corona formation was carried out using dynamic light scattering and surface tensiometry. The latter provides an attractive novel tool for the identification and characterisation of protein corona of nanogels.

Results and Discussion
We showed that dynamic surface tension could be used as a sensitive technique to characterise protein corona, by detecting in situ nanogel-protein interactions. Moreover, impact of protein corona formation on the properties of nanogels in the bulk of the solution as well as at the air/water interface was investigated with the complementary use of dynamic surface tension, dynamic light scattering, and circular dichroism.

Conclusion
These data advance the understanding of the impact of protein corona on the physico-chemical properties of nanoparticles and enable a more effective rational design of nanomaterials for drug delivery application.

References
1:00 p.m. – 2:15 p.m.

Track08

**NANOSTEM 02 | Investigation of nanocarriers as drug delivery systems**

Chair

Roberta Bilardo
Queen Mary University of London, School of Biological and Chemical Sciences, Department of Chemistry, London, GB
NANOSTEM 02-KL01

How do we create an inclusive and diverse research culture

Marina Resmini
Queen Mary University of London, London, GB

NANOSTEM 02-02

Comparison of release profile of nanocapsules measured with dialysis and an innovative sample and separate technique, the NanoDis system

Sonia M. Lombardo¹², Akif E. Türelı¹, Nazende Günday-Türelı¹, Marc Schneider²

¹ MyBiotech GmbH, R&D Pharma, Überherrn, DE; ² Saarland University, Department of Pharmacy, Biopharmaceutics and Pharmaceutical Technology, Saarbrücken, DE

Introduction
Poly(lactic co-glycolic acid) (PLGA) nanoparticles have been of particular interest the last decades due to their good biocompatibility and their reported extended release properties. However, these nanoparticles often suffer from burst release, where a large amount of the encapsulated drug is released after the first hours in release medium [1]. As burst release can lead to sever adverse effects, its good characterization is essential. Nowadays, there is still no standard release tests for nanoformulations. Dialysis techniques are the most used methods in the literature as they are easy to set up. However, these methods suffer from sever drawbacks as the measured release might depend on the permeation kinetic of the drug through the dialysis membrane. If the release is faster than the permeation kinetic, which is often the case for nanoparticles suffering from burst release, the measured release profile is not representative of the actual release happening inside the dialysis bag. The burst release might then be hidden and the release profile easily misinterpreted as an extended release [2].

Experimental Methods
In this study, an innovative sample and separate technique, the NanoDis system, was used to assess the release of PLGA nanocapsules. With this technique, a USP II (paddle) dissolution apparatus coupled with an autosampler, was connected to hollow fiber filters. The nanocapsule formulations were diluted in different release media in sink conditions. At specific time points, the nanocapsules were automatically filtered by tangential flow filtration to efficiently separate the released drug from the nanocapsules. The same formulation was also tested using dialysis, in the same release media.

Results and Discussion
A high burst release could be observed with the NanoDis system after 1 hour, while the release profile measured with dialysis was limited by the permeation kinetic of the free drug. Thus, the NanoDis system showed clear superiority over the dialysis method for the measurement of burst release of our nanocapsule formulation. Next, the release profile of different nanocapsule formulations, prepared with various surfactants and polymeric coatings, were tested using the NanoDis system. Depending on the formulations, different burst release profiles were observed, showing the ability of the NanoDis system to discriminate between formulations.

Conclusion
Page 352 of 2028
In conclusion, the NanoDis system showed to be an interesting technique for the release test of a variety of PLGA nanocapsules and was able to accurately measure their burst release.

Acknowledgement
This work is part of the NANOSTEM project, a Marie Skłodowska-Curie Innovative Training Network (ITN). This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 764958.

References
Targeted drug delivery of Diblock copolymers and drug encapsulation efficiency for neural stem cell proliferation

Eirini Epitropaki, Sam Parkinson, Rachel O'Reilly

University of Birmingham, BIRMINGHAM, GB

Introduction
Neurodegenerative diseases such as ischemic stroke, Alzheimer’s and Parkinson’s can cause cellular loss, which can be tackled with the use of neural stem cells (NSCs). To promote stem cells differentiation into functional neurons, nanomedicine as a non-invasive method presents an opportunity to enable delivery of differentiating agents, while preserving the blood and brain barrier (BBB). The field of nanomedicine has evolved tremendously in the last decade and has contributed to the development of new medical applications. In order for nanoparticles to achieve efficient delivery to the central nervous system (CNS), numerous physico-chemical parameters must be examined. To avoid multiple-step procedures, addition of toxic surfactants, and extensive intermediate purification steps, nanoparticles were synthesized using polymerization induced self-assembly (PISA) in order to create small and functional particle sizes with various charges. Based on the above, the current work aimed to produce amphiphilic nanoparticles with various corona chemistries and different sizes to evaluate their permeation ability through the BBB. To effectively target the CNS, Transferrin was used as protein coating on the nanoparticles in order to increase the permeation rate. In order to promote neural regeneration retinoic acid (RA) was used as a drug and encapsulated with various methods into the nanoparticles and drug uploading and release were evaluated. As the particles are developed as an intravenous carrier, the interaction between the self-assemblies and protein models must be investigated. The effect of the surface charge was explored by introducing the nanoparticles in various biological environments such as cell culture medium and model protein solutions and monitor the interactions occurring.

Experimental Methods
As reported above, polymerization induced self-assembly (PISA) was utilized to create nanoparticles and they were characterized with multiple techniques. Transferrin was attached to the nanoparticles using click chemistry. Drug encapsulation studies entail emulsification with prepared nanoparticles in a solution, emulsification with freeze-dried nanoparticles, and drug uploading during the synthesis of the nanoparticles. Protein solutions were added to the already formed particles in a ratio of 4:1. The nanoparticles were dialyzed into a cell culture medium and explored by dynamic light scattering (DLS).

Results and Discussion
PISA has completely transformed the concept of block copolymer self-assembly by combining polymerization and self-assembly in a single step, eradicating the tedious synthetic procedures. The drug uploading studies exhibited a higher encapsulation ratio during the synthetic uploading approach but also changes the hydrophobicity of the environment and leads to gelation of the reaction and different morphologies. Protein corona evaluation showed only a hard corona formation around the nanoparticles even in higher concentrations for the particles that can form electrostatic interactions with negatively and positively charged proteins.

Conclusion
Small and well-defined nanoparticles were created through PISA and were explored through further experimentation. Protein corona studies exhibited only a hard corona formation and drug uploading of RA showed small efficiency and hydrophilic drug work better with this system.

Acknowledgement
This work was conducted as part of the NANOSTEM project, Marie Sklodowska-Curie Innovative Training Network (ITN) that has received funding from the European Union's Horizon 2020 research and innovation programme. I would like to thank my supervisor, Prof. O'Reilly, for the guidance and patience all these years, Sam Parkinson for the creation of the PISA particles, and Amrita Sikder for all the scientific discussions and her support.

References
NANOSTEM 02-04

Improved Uptake of Extracellular Vesicles in Ischemia

Inês Albino1,2, Miguel Lino1,2, Lino Ferreira1,2,3

1 University of Coimbra, CNC - Centre for Neuroscience and Cell Biology, CIBB-Centre for Innovative Biomedicine and Biotechnology, Coimbra, PT; 2 University of Coimbra, IIIUC-Institute of Interdisciplinary Research, Coimbra, PT; 3 University of Coimbra, Faculty of Medicine, Coimbra, PT

Introduction

Ischemic diseases, characterized by an insufficient blood flow that leads to low oxygen levels and nutrient uptake by cells are a major contributor to both disability and death worldwide. Cell-based therapies have shown promising results in ischemic diseases although the poor survival and/or engraftment hinder their clinical efficacy.1 Extracellular vesicles (EVs), collected from stem cells and their progenitors represent a significant component of their paracrine effect after transplantation in the context of therapeutic angiogenesis.2 However, most of the therapeutic effects reported in the context of ischemic diseases are likely indirect. Advances in engineering the surface of EVs to increase travelling distance and targeting specific cell surface epitopes are needed to maximize their local/direct effects. Here, we propose a therapy based on engineered EVs to improve their targeting to ischemic tissues and enhance their angiogenic potential.

Experimental Methods

EVs secreted by mesenchymal stem cells (MSCs) were isolated by filtration and purified by size exclusion chromatography. In order to increase the targeting ability of EVs, the EV surface was conjugated by click chemistry with a peptide that targets galectin-3, a β-galactosidase-binding lectin highly expressed after ischemia.3 The angiogenic activity of native and engineered EVs was evaluated in human umbilical vein endothelial cells (HUVECs) by a survival assay to hypoxic conditions and by a tube formation assay. Targeting and uptake was tested in HUVECs after preconditioning cells to hypoxia (0.1% O2) during 18h. Uptake was evaluated by high content microscopy and confocal microscopy after 3h and 24h incubation with EVs.

Results and Discussion

Our data supports that EVs can be conjugated with controlled amounts of peptides via click-chemistry without changing significantly the physicochemical properties of EVs. Furthermore, we showed that the conjugation with a targeting peptide promotes the interaction with endothelial cells subjected to hypoxic stimulus, increasing its uptake significantly relatively to native EVs (more than 2 times after 3h and 24h incubation).

Conclusion

In conclusion, the EV surface can be engineered by click-chemistry methods to allow controlled presentation of biomolecules to enhance targeting to ischemic tissues.

Acknowledgement

The authors would like to thank the European Union’s Horizon 2020 research and innovation program under grant agreement 764958 (NANOSTEM), grant agreement 952266 (RESETageing) and QREN-COMPETE funding (Project “NeuroAtlantic”, Ref: EPA_791/2018, which is co-funded by Program Interreg Atlantic Space through European fund for Regional Development for providing financial support to this project.

References

2:30 p.m. – 3:45 p.m.

Track08

NANOSTEM 03 | Nanoparticles and blood-brain barrier - mechanisms of permeation and permeability for successful drug delivery

Chair
**Matteo Puglisi**
Ludwig-Maximilians-Universität München, Department of Physiological Genomics, Planegg, DE
In vitro and in vivo evaluation of fluorescent NIPAM-based nanogels as brain-targeting drug delivery systems

Roberta Bilardo¹, Eleonora Rizzi², Caroline Mysiorek², Marie-Pierre Dehouck², Caroline H. Brennan¹, Marina Resmini¹

¹ Queen Mary University of London, School of Biological and Chemical Sciences, London, GB; ² University of Artois, Faculty of Sciences Jean Perrin, Blood Brain Barrier Laboratory (UR 2465), Lens, FR

Introduction
The delivery of drugs to the brain represents a big challenge, given the efficiency of the blood brain barrier (BBB) in preventing permeation. The use of nanoparticles as drug delivery systems has attracted considerable interest, as their very small size is a significant advantage. Polymeric covalently cross-linked nanogels are considered very promising candidates because their physico-chemical properties can be easily tailored by using specific functional monomers. Moreover, the ability of these nanoparticles to form stable colloids, while retaining a high volume-to-surface ratio, allows remarkable loading capability. Fluorescent tags can be easily incorporated in the nanogels to track the nanoparticles within in vitro and in vivo systems to ensure that the target area is reached.

Experimental Methods
Fluorescent labelling of nanoparticles is a commonly used approach used to investigate their uptake and distribution within in vitro and in vivo models. In this study, the introduction of a fluorophore was performed via covalent binding in order to prevent its leakage from the polymeric matrix. A small library of N-isopropylacrylamide-based nanogels containing a benzofurazan derivative as fluorescent label was synthesised and investigated. Following optimisation of the polymerisation protocols and ¹H-NMR monomer conversion studies, the most promising fluorescent nanogels were studied via dynamic light scattering and UV-vis spectroscopy to determine their size and thermo responsive behaviour, respectively. Moreover, UV-Vis spectroscopy and fluorescence spectroscopy were used to investigate their optical properties and their suitability to be used for tracking purposes in vitro and in vivo. Then, toxicity studies and an evaluation of BBB-permeation were performed in vitro by using a human BBB model. Additionally, both the biocompatibility and the distribution of these nanoparticles were assessed in vivo, in zebrafish up to 7 days post fertilisation.

Results and Discussion
The introduction of up to 1 mol% of the selected fluorescent monomer did not significantly impact the main properties of the nanogels compared to their non-fluorescent counterparts, yet allowing the nanoparticle tracking within the in vitro and in vivo models. In vitro studies revealed that these nanoparticles were non-toxic up to 0.1 mg/mL and able to permeate the BBB, being finally internalised within cells. Finally, successful tracking of these fluorescent nanoparticles was observed also within zebrafish and allowed to evaluate the nanogel localisation different administration routes.

Conclusion
In conclusion, successful tracking of the fluorescent nanogels within the selected in vitro and in vivo models revealed the suitability of the adopted strategy and of the selected polymerisable fluorescent monomer.

Acknowledgement
This work is part of the NANOSTEM project, a Marie Sklodowska Innovative Training Network (ITN). This project has been funded by the European Commission through grant agreement No 764958.
Development of Blood-Brain Barrier assay that allow a higher screening of compounds

Elisa L. Moya\textsuperscript{1,2}, Elodie Vandenhaute\textsuperscript{2}, Sonia Lombardo\textsuperscript{3,4}, Eleonora Rizzi\textsuperscript{1}, Marc Schneider\textsuperscript{4}, Akif E. Türeli\textsuperscript{3}, Nathalie Maubon\textsuperscript{2}, Nazende Günday-Türeli\textsuperscript{3}, Fabien Gosselet\textsuperscript{1}, Marie-Pierre Dehouck\textsuperscript{1}

\textsuperscript{1} University of Artois, The Blood-Brain Barrier Laboratory (LBHE), Lens, FR; \textsuperscript{2} HCS Pharma, Loos, FR; \textsuperscript{3} MyBiotech GmbH, Überherrn, DE; \textsuperscript{4} Saarland University, Saarland, DE

Introduction

Because of the difficulty of drug penetration into the brain due to the blood–brain barrier (BBB), many CNS drug candidates fail in clinical trials. Hence, there is a need to develop effective CNS drugs following strategies for delivery to the brain, by better selecting them as early as possible during the drug discovery process. The use of \textit{in vitro} BBB models has proved useful to evaluate the impact of drugs/compounds toxicity, BBB permeation rates, and molecular transport mechanisms within the brain cells, in academic research, and early-stage drug discovery [1]. However, these studies that require biological material (animal brain or human cells), are time-consuming, involve costly amounts of materials and plastic wastes due to the format of the models. Hence, to adapt to the high yield needed in early stage drug discovery for compound screening, a patented well-established human \textit{in vitro} BBB model [2] developed in 12-well format Transwell (TW) inserts has been miniaturized and automatized in 96-well format. In addition, PLGA nanoparticles coated with surfactants, polysorbate 80 (PS80) and poloxamer 188 (P188), have been shown to be able to cross the BBB [3], and thus, are interesting formulations for brain drug delivery. Hence, these nanoparticles, labelled with a fluorescent dye, and produced with the MicroJet reactor\textsuperscript{®} technology were developed and tested using the \textit{in vitro} BBB models. Their transport and uptake mechanisms were studied.

Experimental Methods

The original BBB \textit{in vitro} model was developed as a co-culture using endothelial cells derived from CD34+ hemetopoietic stem cells in the inserts, and brain pericytes in the bottom wells. Miniaturization was done using 96 TW plates from Corning\textsuperscript{®} and Falcon\textsuperscript{®}. Concerning automation; the robot Multiflo, BioTek Instruments was used for the cell seeding; Caliper (Sciclone(R), Perkin-Elmer) for experimental assays (permeability and immunostainings); and microscope ImageXpress Micro Confocal High-Content Imaging System (Molecular Devices) for Immunostainings and fluorescence visualization.

Model BBB phenotype was analysed; Gene profile expression by qPCR; the expression of junctional protein by immunofluorescence; BBB model paracellular permeability with Sodium Fluorescein (NaF); Efflux pumps functionality; Receptor functionality using a target molecule and inhibitors of transport; Drug brain penetration was assessed for its correlation with human \textit{in vivo} data; Finally, BBB integrity evaluation towards different compounds depending on concentrations.

Moreover, by using the \textit{in vitro} models developed, the BBB impact of surfactants coated PLGA nanoparticles over different concentrations, as well as the endothelium mechanisms of transport by using a set of inhibitors and competitors.

Results and Discussion

Miniaturized and automated replicate was achieved by adapting cell seeding ratio densities to the 96 TW plates surface area. This replicate reproduced the BBB phenotype [4] as the original 12 TW patented model, with low paracellular permeability, presence of junctional proteins, efflux pumps and functional membrane receptors. The model presented high correlation with human \textit{in vivo} data [5] concerning the brain exposure to the drugs. PLGA nanoparticles showed transport through brain endothelium. PS80 and P188 NP uptake were active transport
dependent and their uptake were reduced with the presence of specific competitors and inhibitors for each formulation.

**Conclusion**

Miniaturized BBB *in vitro* model replicate has met all the BBB model reliability criteria [3] to get predictive results, being easier to handle and using automated technology. This offer the opportunity of a higher screening capacity to generate useful numerous BBB permeation data of many CNS compounds or conditions to test at a time, allowing a significant reduction in biological material, plastic-waste, for being extensively used during early-stage drug discovery studies. Moreover, the PLGA nanoparticles PS80 and P188 tested presented an active internalization mediated by receptors. The formulations seemed to have different uptake profiles and affinities depending on the surfactant coated.

**Acknowledgement**

This research project is part of the NANOSTEM project, a Marie Skłodowska-Curie Innovative Training Network (ITN) project which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 764958.

**References**


Tackling Nanogels ability to cross the human Blood-Brain Barrier: application to stroke using an \textit{in vitro} approach

Eleonora Rizzi\textsuperscript{1}, Federico Traldi\textsuperscript{2}, Susana Simões\textsuperscript{3}, Susana Rosa\textsuperscript{3}, Francesca Tomatis\textsuperscript{3}, Lucie Dehouck\textsuperscript{1}, Emmanuel Sevin\textsuperscript{1}, Marie Pierre Dehouck\textsuperscript{1}, Fabien Gosselet\textsuperscript{1}, Lino Ferreira\textsuperscript{3}, Marina Resmini\textsuperscript{2}, Caroline Mysiorek\textsuperscript{1}

\textsuperscript{1} University of Artois, Laboratoire de la Barrière Hémato-Encéphalique (LBHE), Lens, FR; \textsuperscript{2} Queen Mary University of London, School of Biological and Chemical Sciences, London, GB; \textsuperscript{3} University of Coimbra, CNC- Center for Neuroscience and Cell Biology, Coimbra, PT

Introduction
Secondly to the formation of a clot in a brain vessel leading to a shortage of blood flow in the downstream region, the ischemic stroke cause cell damage and death in an area of the brain. As the second leading cause of death and the first cause of long-term disabilities worldwide, ischemic stroke needs urgent treatment and new therapeutic strategies to reduce the infarcted area and decrease the pathological outcomes in the long term. Overall, treatment targeting brain diseases face a hurdle which is the access of brain parenchyma due to the Blood-Brain barrier (BBB)\textsuperscript{1}. The BBB, localized at the level of capillary endothelial cells forming the brain vessels, controls and restricts the passage of cells and molecules to the brain parenchyma to maintain a physiological environment for the correct brain function. In the frame of NANOSTEM, a Marie Curie Action project, we studied the capacity of new nano-formulations to cross the BBB in order to target the neural stem cells and stimulate their differentiation capabilities as a novel therapeutic approach to brain. Among the multiple possible nano-formulations as novel drug delivery systems, nanogels retain some promising characteristics\textsuperscript{2}.

Experimental Methods
Given the importance of cells communications and interactions, we developed a human BBB in vitro model consisting of the main cell types at the level of the BBB namely endothelial cells (ECs), pericytes (PCs) and astrocytes (ACs). Our human BBB \textit{in vitro} model was submitted to oxygen and glucose deprivation (OGD) followed by 24h of reoxygenation (R). The model has been characterized for viability and barrier tightness. Additionally, toxicity, crossing and intracellular accumulation of nanogels were evaluated.

Results and Discussion
The human BBB \textit{in vitro} model, developed in a triple culture setting, based on a patented and well-defined one\textsuperscript{3}, retained the key characteristics for a BBB model: enhanced barrier tightness, expression of tight junction proteins and efflux pumps. At the same time, the model submitted to OGD showed visual presence of discontinuity in the tight junction network, as well as lower cell viability and increased expression of genes related to damage. All these are then recovered after the 24 hours of R. Nanogels were found not toxic in the studied conditions and their rate of crossing was showed to increase proportionally with time.

Conclusion
Overall, in this study we have developed and characterised a human BBB \textit{in vitro} model submitted to OGD and R, mimicking the \textit{in vivo} environment during stroke and reperfusion. Moreover, preliminary data, concerning nanogels toxicity and BBB crossing showed promising results.

Acknowledgement
This work is part of NANOSTEM project, a Marie Skłodowska Innovative Training Network (ITN). This project has been funded by the European Commission through grant agreement No 764958.

Page 362 of 2028
References


Blood-Brain barrier permeation using DNA nanotechnology

Georges Kiriako, Elena Ambrosetti, Joel Spratt, Ana Teixeira

Karolinska Institutet, Medical Biochemistry and Biophysics, Stockholm, SE

Introduction
Receptor-mediated transcytosis (RMT) across the blood-brain barrier (BBB) is a key component in delivering drugs to the brain. Avidity, the accumulated strength of multiple protein-protein interactions, is a major regulator of RMT and has become a key factor in drug design. Previous research has shown that an optimal BBB permeation could be achieved by varying the avidity of nanoparticles. For example, the selectivity of nanoparticles coated with transferrin has been shown to increase with increasing avidity (1). [A1] However, high levels of avidity have been shown to hamper the release of the nanoparticles into the brain parenchyma (2). These results suggest that avidity in ligand/receptor interactions is a key regulator of RMT. However, in these systems, only crude control of avidity levels could be achieved by changing the average surface density of ligands on the surface of nanoparticles.

Experimental Methods
In this study, we used DNA nanostructures as scaffolds to manipulate the avidity and nanoscale spatial distribution of aptamers against the transferrin receptor (TfR). This tool allows for control of nanocluster spatial configuration and composition in the length scale of 100 nm and is unique in its high precision in presentation of aptamer spatial distributions. We combined WAZ, an RNA aptamer specific for the TfR, with both flexible and rigid DNA nanostructures to produce aptamer nanoclusters with precise patterns and densities ranging from 1 to 24 aptamer per nanostructure. We assessed the proper folding of WAZ/DNA nanostructures using agarose gel electrophoresis (AGE) and then applied DNA-Paint, a super-resolution microscopy technique, to evaluate the density and the spatial distribution of WAZ on each design. Moreover, we studied the internalization of WAZ/DNA nanostructures and their subcellular localizations in human brain endothelial cells by confocal microscopy.

Results and Discussion
We verified that the DNA nanostructures are properly folded, purified, and hybridized to the WAZ aptamer. DNA-Paint analyses confirmed that the density and the spatial distribution of WAZ were in agreement with our theoretical designs. We also showed that the permeability of DNA nanostructures, flexible and rigid, correlated to WAZ densities. Higher WAZ densities lead to greater nanocluster internalization. In addition, we showed that WAZ/DNA nanostructures colocalize with endosomal markers and escape lysosomal degradation. Further experiments will determine the optimal density of WAZ needed to maximize cell permeability.

Conclusion
In conclusion, these results open the possibility of using DNA nanostructures in delivering therapeutics across the blood-brain barrier. The approach presented can be used to probe a multitude of ligand/receptor-mediated pathways and has the potential to provide new insights relevant for the development of new types of multimeric biological drugs.

Acknowledgement
This work is part of NANOSTEM, a Marie Skłodowska-Curie Innovative Training Network (ITN) funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No 764958.

References

Delayed post-stroke blood-brain barrier permeability for drug delivery: a pilot cohort study

Sara Bernardo-Castro¹, João A. Sousa¹, Emanuel Martins¹, Sónia Afonso², Tânia Lopes², César Nunes³, Fernando Silva¹, Antero Abrunhosa², Miguel Castelo-Branco²,⁵, Lino Ferreira⁴,⁵, João Sargento-Freitas¹,⁴,⁵

¹ Centro Hospitalar e Universitário de Coimbra, Neurology department, Coimbra, PT; ² Univ Coimbra, Institute for Nuclear Sciences Applied to Health, Coimbra Institute for Biomedical Imaging and Translational Research, Coimbra, PT; ³ Centro Hospitalar e Universitário de Coimbra, Medical Imaging department, Coimbra, PT; ⁴ Univ Coimbra, CNC - Center for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, Coimbra, PT; ⁵ Univ Coimbra, Faculty of Medicine, Coimbra, PT

Introduction
After ischemic stroke, the blood brain barrier (BBB) undergoes a disruption with a consequent increase in its permeability (BBBP).¹ This event follows a time-course progression through the distinct stroke phases with different underlaying mechanisms. Nonetheless, this marked time-sensitive heterogeneities in post-stroke BBBP hamper the use of a universal and standardized treatment.² BBBP has been reported to be open up until one, three and even five weeks post-stroke³ but its true dynamics, particularly beyond the acute phase, are yet unclear. Understanding post-stroke BBBP evolution could allow the design of personalized and targeted biomaterials aiming to either avoid BBB opening or to tackle neurorepair mechanisms using BBBP as a gate to the central nervous system. Quantitative permeability imaging through magnetic resonance imaging (MRI) can help evaluate these dynamics but the lack of longitudinal studies evaluating BBBP beyond the acute stage hamper the possibility of using delayed BBBP as delivery platform in stroke patients. We aim to study the dynamics of the BBBP in the later phases of stroke and thus we present a pilot cohort study on subacute and chronic BBBP of ischemic stroke patients.

Experimental Methods
We included consecutive patients with acute ischemic strokes in the territory of the middle cerebral artery, aged 18 to 80 years. Neuroimaging was performed at the subacute and chronic stages by MRI including BBB permeability assessment by dynamic contrast enhancement (DCE). We manually drew a region of interest (ROI) including the ischemic region (as defined by diffusion weighted imaging). A second ROI was drawn in the brain parenchyma contralateral to the ischemic area with similar shape and anatomical position and used as control for the analysis. Pharmacokinetic modeling was performed automatically for each ROI using the Tofts Model to obtain the volume transfer coefficient (Ktrans) as a surrogate for BBBP. The same ROIs for each patient were placed in the subsequent 3 months follow-up MRI. We performed a two-tailed paired Student’s t-test between the ipsilateral ischemic and contralateral non-ischemic Ktrans values within each group to evaluate permeability in the lesion area, and between the subacute and the chronic phases to evaluate changes over time. Statistical significance was set at p<0.05.

Results and Discussion
We included 10 patients (6 males, 4 females), mean age of 63.5 ± 16.50 years. Subacute imaging was performed at a mean time of 7 ± 8 days and chronic imaging at 90 ± 12 days. Median (IQR) NIHSS admission was of 9 (8). BBBP was significantly increased in the ischemic tissue when compared to the contralateral (0.011 min⁻¹ vs. 0.0085 min⁻¹ p<0.001) at the subacute stage while in the chronic stage (3 months post-stroke) there was no significant differences between ipsilateral and contralateral permeability (0.008 min⁻¹ vs. 0.007 min⁻¹ p=0.83). We found permeability to be significantly higher (0.011 min⁻¹ vs. 0.008 min⁻¹ p=0.008) in the ischemic tissue in the subacute phase when compared to the chronic phase. Recovery mechanisms such as angiogenesis have been reported to occur in the subacute
phase, being related with higher permeability. This will allow the delivery of novel biomaterials taking advantage of the increased BBBP or the potentially overexpressed receptors in the new forming vessels. Moreover, our preliminary data suggests that contrary to what happens at one-month post-stroke in which BBB is still permeable, at 3 months the BBB appears to be repaired and hence, poorly permeable. This situation will hamper the use of biomaterials that aim to use disrupted BBB as vehicle in this stage.

Conclusion
Our findings reinforce the quantitative and biological heterogeneity of the BBBP after stroke, allowing the design of strategies that aim to use the BBB and its permeability as a therapeutic vehicle or target. Our preliminary data shows that BBBP remains increased in the subacute phase allowing its use as a delivery platform while it progressively decreases over time to reach a repaired and poorly permeable BBB at 3 months.

Acknowledgement
This study is funded by the European Union's Horizon 2020 Research and Innovation Program under grant agreement number 764958 (NANOSTEM project).

References
3:50 p.m. – 4:35 p.m.

Track08

NANOSTEM 04 | Nanoparticles for modulation of gene expression

Chair
Federico Traldi
Queen Mary University of London, School of Biological and Chemical Sciences, London, GB
Engineering extracellular vesicles to cross the blood-brain barrier

Francesca Tomatis\textsuperscript{1,2,3}, Susana M.N. Simões\textsuperscript{1,2}, Susana Rosa\textsuperscript{1,2}, Lino Ferreira\textsuperscript{1,2,4}

\textsuperscript{1} University of Coimbra, CNC - Center for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, Cantanhede, PT; \textsuperscript{2} University of Coimbra, IIIUC - Institute of Interdisciplinary Research, Coimbra, PT; \textsuperscript{3} University of Coimbra, PhD Programme in Experimental Biology and Biomedicine, Institute for Interdisciplinary Research (IIIUC), Coimbra, PT; \textsuperscript{4} University of Coimbra, Faculty of Medicine, Coimbra, PT

Introduction
Neurological disorders represent the second cause of death and the first cause of disability globally [1]. Many drugs and nanoformulations have already been developed to treat brain diseases, but few of them reached the clinical application due to the poor blood-brain barrier (BBB) crossing ability. EVs are naturally released lipidic vesicles that carry a cocktail of bioactive molecules (microRNAs, mRNAs, proteins, lipids) from the parental cell and mediate cell-to-cell communication. It has been recently shown that EVs are able to regulate the integrity of the brain vasculature through specific microRNAs [2,3]. For example, neurons secrete exosomes enriched in miR-132, capable of being translocated to endothelial cells and regulate the expression of vascular endothelial cadherin. In addition, there are experimental evidences that EVs may mediate in vivo the transport of proteins through barriers. For example, it has been reported that EVs secreted by red blood cells from PD patients can transfer α-synuclein across the BBB via adsorption mediated transcytosis (AMT)[4]. Using wheat germ agglutinin (WGA), an inducer of AMT, the amount of radiolabeled EVs increased significantly in the brain after intravenous injection in the jugular vein of mice. Despite these progresses, it is relatively unknown what miRNAs in EVs can modulate in the BBB allowing the transport of the EVs through the BBB. Here, we have transfected EVs with a short list of miRNAs to study their influence in the permeability of the BBB. The selected miRNAs were inspired by their role in ageing.

Experimental Methods
In order to identify the possible miRNA candidates for our system, we elaborated information from literature data by combining the information between miRNA changes during aging and miRNAs affecting the BBB. We then loaded the miRNAs on EVs. The EVs were isolated from umbilical cord blood (UCB)-derived plasma through an ultracentrifugation protocol [5], and they were purified from proteins by size-exclusion chromatography (SEC). For the loading purpose, we used the commercially available Exo-Fect transfection reagent, and we purified the samples by Optiprep density gradient (ODG) centrifugation. In order to test our system, we developed a human BBB model in vitro, both in static and dynamic conditions. A co-culture system of human brain vascular pericytes and endothelial cells differentiated from CD34-positive cells of UCB was established. Four days after starting the co-culture, the miRNA-loaded EVs were incubated with the BBB model for 48 h and effects on the endothelial monolayer were assessed by measuring TEER, Pe, and cell viability. Moreover, immunocytochemistry (ICC) was performed to check the modulation of TJs proteins and RT-qPCR helped assessing the gene expression of BBB markers.

Results and Discussion
During the optimization of our BBB model, we saw that four days of co-culture were enough to obtain transendothelial electrical resistance (TEER) values and paracellular permeability coefficient (Pe) similar to the ones already reported in the literature. By performing the data collection and elaboration, we identified some possible miRNAs candidates for our final system, and we tested them in the above-mentioned BBB model. Even though the cell viability was not affected by the incubation with miRNAs-loaded EVs, ICC showed alterations in claudin 5 and zona occludens 1 (ZO-1) expression in terms of intensity of staining and organizations of the TJs.

Conclusion
Therefore, our system represents a promising strategy for temporary BBB opening, useful to increase the ability to reach the brain of therapeutics injected into the bloodstream.

Acknowledgement

The authors would like to express their gratitude to the European Commission for the funding through the Marie Skłodowska-Curie Innovative Training Network “NANOSTEM” (n. 764958), and the Twinning project RESETageing (n. 952266) as well as QREN-COMPETE for the funding through the projects “NeuroAtlantic”, Ref: EAPA_791/2018, which is co-funded by Program Interreg Atlantic Space through European fund for Regional Development and INTERREG V (POCTEP) (0624_2IQBIONEURO_6_E) and FCT (Project entitled “A therapeutic strategy to fight blood-brain-barrier (BBB) ageing”; Ref: PTDC/BTM-SAL/5174/2020).

References

Development of a CRISPRa Ribonucloproteins system to manipulate neurogenic genes in vitro

Matteo Puglisi¹,²,³, Maximilian F. Wiesbeck⁴,⁵, Andrea M. Neuner⁴,⁵, André Mourão⁶, Arie Geerlof⁶, Magdalena Götz¹,²,⁷, Stefan H. Stricker⁴,⁵

¹ Ludwig-Maximilians-Universität München, Department of Physiological Genomics, Planegg, DE; ² Helmholtz Zentrum München, Institute for Stem Cell Research, Planegg, DE; ³ Ludwig-Maximilians-Universität München, Graduate School of Systemic Neurosciences, Planegg, DE; ⁴ Ludwig-Maximilians-Universität München, MCN Junior Research Group, Munich Center for Neurosciences, Planegg, DE; ⁵ Helmholtz Zentrum München, Epigenetic Engineering, Institute of Stem Cell Research, Planegg, DE; ⁶ Helmholtz Zentrum München, Protein Expression and Purification Facility, Neuherberg, DE; ⁷ Ludwig-Maximilians-Universität München, Excellence Cluster of Systems Neurology (SYNERGY), München, DE

Introduction
Acute brain injuries, neurodegenerative diseases and aging are brain pathologies with a great social burden and a shared feature: neuronal loss. In the last decades regenerative medicine came up with different strategies to replace lost neurons including the conversion of local reactive astrocytes into new neurons. A common method to achieve astrocyte reprogramming consists in the forced expression of neurogenic factors via lentiviral or adenoviral vectors. Even if effective, such approaches permanently introduce exogenous genetic material in the host cells, raising safety concerns and possibly preventing the complete maturation of the induced neurons.

Experimental Methods
To address these issues, we decided to develop a transgene-free method for the transient manipulation of gene expression. Particularly, we investigated the potential of the CRISPR activation system in the form of ribonucloproteins (CRISPRa-RNPs) for the activation of endogenous neurogenic genes. The functional unit of our system are synthetic Transcription Factors (sTFs) made by the combination of a custom-made dCas9-VPR protein with neurogenic gene-specific guide RNAs (gRNAs).

Results and Discussion
In a first round of screening, we lipofected murine P19 cells with sTFs to transcriptionally activate a pool of validated neurogenic factors. After selecting the most efficient ones, we proceeded with validation in primary murine astrocytes too, since they are relevant for neuronal replacement therapies.

Conclusion
In conclusion, our results provide a promising starting point for the development of a fast and versatile approach to readily screen and multiplex TFs. The future application of our methods to different cell types, including human ones, will allow its further development and will bring it towards even more clinically relevant applications.
NANOSTEM 04-03

Engineered formulations for gene editing

Angela M. Barrera1,2, Susana Simões1,3, Patricia Pitrez1,3, Lino Ferreira1,2

1 University of Coimbra, CNC - Center for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, UC-Biotech Parque Tecnológico, Cantanhede, PT; 2 University of Coimbra, Faculty of Medicine, Coimbra, PT; 3 University of Coimbra, IIIUC - Institute of Interdisciplinary Research, Coimbra, PT

Introduction
The incidence of age-related diseases has risen dramatically in the recent years, due to an increase in aging population. Several of these diseases are caused by genetic mutations leading to DNA damage and cell proliferation decrease. Gene editing approaches for these kinds of pathologies are limited due to the difficulty to transfect aged cells. CRISPR/cas9 system is a promising tool to find therapeutic strategies to correct gene mutations, due to its specificity and minor off target effects (1). Nevertheless, this technology is mainly delivered with viral vectors; however, these strategies have been described to induce immune reactivity and integration into the genome. Aged cells characteristics such cell cycle arrest and DNA instability make them more difficult to transfect, due to the low proliferation rate and smaller number of dividing cells. For example, Hutchinson-Gilford progeria syndrome (HGPS), a rare, accelerated premature aging disorder resulting from a de novo point mutation in the LMNA gene that leads to the generation and accumulation of progerin, an abnormal form of LMNA. The accumulation of progerin produces nuclear abnormalities that promote accelerated aging, cellular damage, and senescence (2, 3). HGPS is a good model to understand physiological aging (4). The aim of our work is to find strategies to improve transfection and gene editing of aged cells using non-viral formulations.

Experimental Methods
The first step is the validation of the plasmid that contains the sequence of interest to induce the knockout of the LMNA mutation. For that purpose, we validated the transfection strategy in human WT skin fibroblasts using electroporation with the neon transfection system testing different parameters to evaluate the cell viability, and the transfection efficiency, determined by the percentage of GFP positive cells. Subsequently, we did evaluate the best nucleofection parameters in aged cell: HGPS fibroblasts. Progerin decrease percentage was evaluated by immunofluorescence and cell number after treatment was also evaluated.

Results and Discussion
The results showed that physical parameters such as number of pulses, voltage and width have an impact in the cell viability and transfection efficiency. The optimal nucleofection parameters found in WT fibroblasts (voltage: 1400; width: 2 ms and pulses: 2) showed a transfection efficiency of 28.04% and 67.26% of cell viability. These validated conditions were then applied for the transfection of HGPS cells. The results in the aged cell model showed a transfection efficiency of 24.84% and a cell viability of 70.03%, among the GFP+ cells 11.55% were progerin positive, there was a slight tendency to decrease progerin levels with a 92.91% in cells without nucleofection and 83.36% in transfected cells. More experiments will be performed to clarify the gene editing effect.

Conclusion
Our results showed an optimized strategy to transfect cells with substantial viability, moreover aged cells with progerin accumulation can be effectively transfected. This is the first non-viral based system for gene editing strategies in aged cells. These studies will improve significantly age-related diseases therapies and treatment for translational applications.

Acknowledgement
This work has been funded by the Marie Skłodowska-Curie Innovative Training Network “NANOSTEM” (n. 764958), FCT Ageing-model (Ref: 029229), and the RESETageing project, Horizon 2020 (n. 9552266).

References
## Program Tuesday, 7 September, 2021

<table>
<thead>
<tr>
<th>Time</th>
<th>Track01</th>
<th>Track02</th>
<th>Track03</th>
<th>Track04</th>
<th>Track05</th>
<th>Track06</th>
<th>Track07</th>
<th>Track08</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:30</td>
<td>KaG AW01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:30</td>
<td>Jean Lenay Award 2021</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:00</td>
<td>COFFEE BREAK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:30</td>
<td>KO4</td>
<td>KO6</td>
<td>KO7</td>
<td>KO8</td>
<td>KO9</td>
<td>KO10</td>
<td>KO11</td>
<td>KO12</td>
</tr>
<tr>
<td>12:00</td>
<td>Graphene-based Biomaterials</td>
<td>Hybrid Cell-Microbiomaterial 3D Assemblies</td>
<td>Biomaterials: The Roadmap Towards Personalised Strategies in Musculoskeletal Tissue Engineering</td>
<td>ESB-CRS S</td>
<td>ESB-BIOMAT S</td>
<td>N08</td>
<td>N09</td>
<td>AERO P501</td>
</tr>
<tr>
<td>12:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:00</td>
<td>LUNCH BREAK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:30</td>
<td>LS</td>
<td>L5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>YSF-AERoGELs WS</td>
</tr>
<tr>
<td>14:00</td>
<td>Lunch Symposium Rousselot: Biomedical Applications of Galatins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>YSF Educational workshop — “Writing and Publishing”, YSF &amp; “AERoGELs” Joint COST action event</td>
</tr>
<tr>
<td>14:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:00</td>
<td>BREAK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AERO P502</td>
</tr>
<tr>
<td>16:30</td>
<td>KO5</td>
<td>KO8</td>
<td>KO10</td>
<td>KO12</td>
<td>KO14</td>
<td>KO15</td>
<td>KO16</td>
<td>KO17</td>
</tr>
<tr>
<td>17:00</td>
<td>ImmuneResponse Towards Biomaterials</td>
<td>Beyond Bone and Teeth: Bioactive Glasses in Soft Tissue Regeneration</td>
<td>ESB-SIBB 501</td>
<td>ESB-IBBF 5</td>
<td>ESB-BMJ S</td>
<td>N10</td>
<td>N11</td>
<td>AERO S63</td>
</tr>
<tr>
<td>17:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Drug-delivery systems and Nutraceutical food systems</td>
</tr>
<tr>
<td>18:00</td>
<td>PL2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AERO CLOSING</td>
</tr>
<tr>
<td>18:30</td>
<td>Plenary Lecture 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Closing session</td>
</tr>
<tr>
<td>19:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Social 4</td>
</tr>
<tr>
<td>19:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Discover Portuguese Wine Regions with Sogrape</td>
</tr>
</tbody>
</table>
9:30 a.m. – 10:15 a.m.

Track01

KdG AW01 | Klaas de Groot Award 2020

Chair
Luigi Ambrosio
National Research Council, Institute of Polymers, Composites and Biomaterials, Pozzuoli, IT

KdG AW01-01

Keep Learning: Advantages of Age in Science (KLAAS)

Mário A. Barbosa

1 Universidade do Porto, Inst Ciências Biomédicas Abel Salazar, Porto, PT; 2 i3S-Instituto de Investigação e Inovação em Saúde, Porto, PT; 3 INEB-Instituto de Eng Biomédica, Porto, PT

This presentation is a very personal perspective on a research career. Success and failure are intrinsic parts of it. However, the most fascinating aspects of a long-term career in research are related to the endless opportunities to learn. Normally, a professor is regarded as someone who has something important to teach. However, with the pace of new discoveries it is inevitable that most of the things we teach become rapidly outdated. Continuous learning is not only a demand of the profession but also a fascinating opportunity to feel ignorant. It is this feeling that drives new questions.

As a researcher ages, he/she is often confronted with duties that are essential for the running of institutions but which actually deviate the person from his/her passion (assuming that we do research for passion). Mentoring younger colleagues in a research laboratory is an opportunity to learn with them, both as a scientist and as a person. The one-to-one relationship that is established is incomparably richer and more rewarding than classes in a classroom.

In a society in which the percentage of aged citizens is rapidly increasing, the research environment is affected in the same manner as other work environments. More and more people of different ages work together outside and inside academia. Progressively, the concept of active aging appears inadequate to describe the role of older citizens. Productive aging makes a lot more sense. Some misconceptions about the dependence of scientific productivity on age, and the very strong competition for funds, space and research positions, has led to increasing signs of ageism. Age discrimination affects also the younger members of our community, who often have to face prejudices that stifle their talent. “Ageism towards younger and older people is prevalent, unrecognized, unchallenged and has far-reaching consequences for our economies and societies”[1]. A debate on ageing in research is as needed as for other professions[2].

Acknowledgement
Page 374 of 2028
To Klaas de Groot, all the colleagues and students with whom I have learned so much.

References
10:15 a.m. – 11:00 a.m.

Track01

**JL AW02 | Jean Leray Award 2021**

Chair
Sandra Van Vlierberghe
Ghent University, BE

**JL AW02-01**

**Driving complex and synthetic tissue development via volumetric biofabrication technologies**

Riccardo Levato\(^1\,2\,3\)

\(^1\) Utrecht University, Department of Clinical Sciences, 3584CT, NL; \(^2\) University Medical Center Utrecht, Department of Orthopaedics, 3584CT, NL; \(^3\) Regenerative Medicine Center Utrecht, 3584CT, NL

The function of living tissues is intimately linked to their complex architectures. Advances in biofabrication technologies offer unprecedented opportunities to capture salient features of tissue composition and thus guide the maturation of engineered constructs into mimicking functionalities of native organs. In this lecture, the design of novel biofabrication strategies and printable biomaterials to enable the reconstitution of complex 3D structures with precise heterocellular, multi-material and hierarchical composition is discussed. Architectures designed to stimulate the native interaction between multiple (stem) cell types and self-assembled organoids are introduced, with a particular focus on applications in musculoskeletal regeneration and liver tissue engineering. Layerwise hydrogel extrusion and bioprinting, different additive manufacturing technologies, such as melt electrowriting of polymeric microfibers, ceramic plotting and digital light processing lithographic printing, can be combined to create composite, cell-laden constructs that enable integration between engineered hydrogels and hard tissue scaffolds to generate osteochondral grafts. Albeit powerful and versatile, this approach poses relevant limitations on the scalability and production of constructs having clinically relevant size, as well as on the generation of free-form and support free overhanging, porous structures, typically of native anatomy. To overcome these challenges, custom-designed light responsive hydrogels can be sculpted into cell-laden convoluted 3D structures within tens of second, via the development of layerless, volumetric bioprinting approaches inspired by visible light computed tomography. With such nozzle and shear stress-free, highly rapid cell processing approach a variety of hydrogel-based constructs can be assembled into hydrogel-based actuators for potential applications in soft robotics, or as platforms to enhance cell viability and maturation post-printing, including the shaping of large networks of hepatic epithelial organoids into defined 3D perfusable structures which exhibit biosynthetic and metabolic functions. Altogether, the combination of the different
strengths of advanced bioprinting technologies offers new opportunities for the biofabrication of large, clinically-relevant multi-tissue constructs for regenerative medicine and tissue engineering.

Acknowledgement

This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (grant agreement No 949806) and from the Horizon 2020 research and innovation programme under grant agreement No 964497. R.L. acknowledges funding from ReumaNederlands (LLP-12 and LLP22) and from the Dutch Research Council (NWO) under the Idea Generator programme (NWA.1228.192.105).
11:30 a.m. – 1:00 p.m.

Track01

**K04 | Graphene-based Biomaterials**

**Chairs**

**Inês C. Gonçalves**
i3S/INEB, Porto, PT

**Aitor Larrañaga**
University of the Basque Country, Mining-metallurgy engineering and Materials Science, Bilbao, ES

**Anna Fritschen (YSF)**
Technical University of Darmstadt, Department of Mechanical Engineering, BioMedical Printing Technology, Darmstadt, DE
Optical reduction and shaping of graphene-oxide for controlled bone tissue regeneration and bacterial killing

Massimiliano Papi

Università Cattolica del Sacro Cuore, Neuroscience section of Biophysics, Roma, IT

Graphene and graphene oxide (GO) are capable of inducing stem cells differentiation into bone tissue with variable efficacy depending on reductive state of the material. Thus, modulation of osteogenic process and of bone mineral density distribution is theoretically possible by controlling the GO oxidative state. In this study, we laser-printed GO surfaces in order to obtain both a local photo-thermal GO reduction and the formation of nano-wrinkles along precise geometric pattern. Initially, after cells adhered on the surface, stem cells migrated and accumulated on the reduced and wrinkled surface. When the local density of the stem cells on the reduced stripes was high, cells started to proliferate and occupy the oxidized/flat area. The designed surfaces morphology guided stem cell orientation and the reduction accelerated differentiation. Furthermore the reduced sharp nano-wrinkles were able to enhance the GO antibacterial activity against methicillin-resistant Staphylococcus aureus (MRSA), a common cause of prosthetic joints infections. This strategy can offer a revolution in present and future trends of scaffolds design for regenerative medicine. Interestingly, laser printing of graphene oxide can be also applied in GO-based hydrogels to design specific biomimetic patterns able to enhance the antimicrobial efficacy of graphene oxide [1, 2].

References

Collagen/Pristine Graphene as Electroconductive Composite Material for Neuronal Tissue Engineering Applications

Pedro J. Gouveia\textsuperscript{1,4}, Jack Maughan\textsuperscript{2,1,4}, Javier G. Gonzalez\textsuperscript{1,4,5}, James Garcia\textsuperscript{2,3,4}, Liam Leahy\textsuperscript{1,4}, Ian Woods\textsuperscript{1,4}, Jonathan Coleman\textsuperscript{2,3,4}, Fergal J. O’Brien\textsuperscript{1,4,5}

\textsuperscript{1} Royal College of Surgeons in Ireland, Anatomy and Regenerative Medicine, Dublin, IE; \textsuperscript{2} Trinity College Dublin, School of Physics, Dublin, IE; \textsuperscript{3} Trinity College Dublin, Centre for Research on Adaptive Nanostructures and Nanodevices (CRANN), Dublin, IE; \textsuperscript{4} Advanced Materials and BioEngineering Research (AMBER) Centre, Dublin, IE; \textsuperscript{5} Trinity College Dublin, Trinity Centre for Biomedical Engineering, Dublin, IE

Introduction

Commonly used metallic-based electrodes used in neuro-prosthetic platforms, have ideal conductive properties, yet the disparity in physicochemical properties compared to neuronal tissues, leads to deleterious foreign body responses, such as scarring and inflammation \cite{1}. To overcome this, recent electrode designs look to incorporate materials mimicking more closely the physicochemical properties of neuronal tissue \cite{2}. Graphene-based materials show outstanding electroconductive (EC) features for bioelectronic applications \cite{3}. From several graphene forms, pristine graphene (pG) exhibits optimal performance across all physical properties. Despite this, its impact in neuronal tissues is still widely underexplored. Thus, this study builds upon previous work in our group on the development of collagen-pristine graphene (CpG) EC composite biomaterials \cite{4}, and investigates their ability to support neuronal cell growth, interrogate their cellular function through electrical stimuli, and promote neuronal regeneration.

Experimental Methods

Composite suspensions were created by homogenizing type I collagen with varying contents of pG (0 to 60%; m/m). Films were then prepared via room temperature evaporation and physically crosslinked through a dehydrothermal treatment. Characterization of bulk material properties were conducted using white light interferometry (Filmetrics Profilm), tensile tests (Zwick Roelle) (>n=3), and dynamic contact angle measurements. The electrical resistivity of dry composite samples was determined using four-point probe system (>n=3). \textit{In vitro} electrical stimulation was performed with a C-Pace system (IonOptix) with 4-8h pulse (200mV; 0.6-3ms) stimulations. The neuronal cell lineages used include human astrocytes (primary), microglial cells (N9), human neuroblastoma cells (SH-SY5Y) and mouse motor neuron-like cells (NSC34). These were assayed for metabolic activity (AlamarBlue, Invitrogen), immunoprofiled (ELISA, R&D Systems) and immunostained to appropriate antigens (>n=3).

Results and Discussion

Type I collagen/pristine graphene composites were successfully fabricated via dry casting followed by DHT crosslinking. Loading of pG did not impact CpG film thickness (≈32.6µm), when compared to the collagen control (30.7µm). Tensile tests with hydrated CpG films show a significantly reduction in elastic modulus (10%-30%pG; E=0.4MPa), ultimate tensile strength unchanged (UTS≈16MPa) and maximum strain (60%pG ≈ 41%) (Fig. 1A), compared to collagen films (E=0.73MPa; UTS=25.9MPa; MS=41%). Analysis of sheet conductivity shows a relatively high percolation threshold at 50% pG loading (Fig. 1B). The highest conductivity with physically stable films was achieved at 60% loading (1.47S/m), rendering the composite conductive to physiologically relevant levels. Next, assessment of surface contact angles revealed that higher pG loadings lead to significantly higher contact angles (≈74º) (Fig. 1C), denoting a decrease in surface hydrophilicity.

To assess the neurotrophic potential of CpG materials, metabolic activity (Fig 2A) and cellular morphology (Fig 2B) was tested over 3 days of culture with 4 different cell lines (NSC34, SH-SY5Y, N9, and human astrocytes). Generally,
metabolic viability is maintained across the different cell types, with a consistent decrease seen only on astrocytes grown in CpG 60% films. To determine the immunomodulatory potential of CpG films, levels of anti-inflammatory (IL-10) and pro-inflammatory markers (IL-6/IL-1 beta) produced by astrocytes and microglia cells were quantified during 3 days. Astrocytes showed a significant increase in IL-10 production over time, followed by a decrease in IL-6 levels. For microglial cells, similar results were seen with IL-10 levels, while the levels of IL-1β were maintained consistent over time. These results demonstrate that CpG films do not induce a consistent neuroinflammatory response from both immunogenic cell types.

Next, we aimed to demonstrate how CpG composites can harness the regenerative potential of electrical stimulation. Currently there are no standardized protocols to electrically stimulate neuronal growth and maturation in vitro [5], therefore, it is crucial to optimize stimulation patterns to better assess the functional impact of EC materials. Our initial data with neurons (SH-SY5Y) in a standardized electrostimulation system, showed that neurite outgrowth significant increases with higher frequencies (1Hz-x1.25; 10Hz-x1.20), with deleterious effects occurring solely at above 50Hz.

Conclusion
Commonly used materials for neuronal interfaces lack the necessary balance of electrical conductivity and biocompatibility. In response to this, we report a collagen/pristine graphene composite with physiologically relevant levels of conductivity, which is capable of supporting growth and polarizing neuronal cells towards neurotrophic phenotypes. Current project milestones aim in demonstrating the potential of CpG films in harnessing electric stimuli to drive neuronal function and regeneration.

Acknowledgement
We acknowledge the Science Foundation Ireland and AMBER Centre for providing financial support to this study (SFI/12/RC/2278_P2). Additionally, we would like to acknowledge the support given by: Brenton Cavanagh (Royal College of Surgeons in Ireland) in the acquisition of microscopy images and in the development of image processing scripts; Su Hyun In (Royal College of Surgeons in Ireland) in film fabrication and determination of physical properties and cellular viability; the Centre for Microscopy and Analysis (Trinity College Dublin) in the acquisition of surface characterization data through white light interferometry and in the acquisition of SEM data.

Figure 1 – Physicochemical characterization of CpG films composites.
Assessment of the CpG film’s elastic modulus (A.1), ultimate tensile strength (A.2), conductivity (B) and contact angle (C) as a function of pG loading.
Figure 2 - Characterization of neuronal cell viability towards CpG composites.

Assessment of metabolic viability of mouse motor neurons (NSC34), mouse microglia (N9) and human astrocytes (primary cell line) in CpG films at 1 and 3 days after seeding. Images demonstrating general cell morphology of motor neuron cells (B.1), microglia (B.2) and astrocytes (B.3) at 3 days after seeding in conductive CpG films (60%). Cells stained for cytoskeleton (phalloidin; green) and nuclei (blue). Astrocytes were additionally stained for GDAP (red).

References
The combined power of NIR and graphene against drug-resistant bacteria

Patrícia C. Henriques1,2,3, Andreia T. Pereira1,2, Diana Bogas3, Artur M. Pinto3, Fernão D. Magalhães3, Inês C. Gonçalves1,2

1 i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, PT; 2 INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Porto, PT; 3 LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Porto, PT; 4 FEUP - Faculdade de Engenharia, Departamento de Engenharia Metalúrgica e de Materiais, Universidade do Porto, Porto, PT; 5 ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, PT

Introduction

Bacterial adhesion to surfaces is the onset of biofilm formation and a hard problem to tackle, with antibiotics still being the major weapon to fight bacterial infections. The rise of antimicrobial resistance is however a growing concern and a threat to public health, translated into more than 500 000 deaths globally/yr [1]. Light-based approaches, such as photodynamic (PDT) and photothermal (PTT) therapies, offer promising solutions, where development of bacterial resistance is highly unlikely to occur [2]. Graphene-based materials (GBMs) show great potential to be used as antimicrobial agents [3, 4], especially as photosensitizers in light-based therapies, due to their high photothermal conversion efficiency and absorption ability of near-infra red (NIR), particularly in the first biological window (650-950 nm) [5]. Here, few-layer graphene (FLG) and few-layer graphene oxide (FLGO) were explored as stand-alone antibacterial NIR-responsive platforms towards the two major agents responsible for medical infections, S. epidermidis and methicillin-resistant S. aureus. Bacteria response to irradiation with safe low-power NIR LEDs was explored, considering, for the first time, the impact of the oxidation degree of these films made only of GBMs (without polymers or photosensitizers molecules). Surfaces’ mechanisms of action were hypothesized based on surface physicochemical features, changes in temperature, glutathione (GSH) oxidation, and reactive oxygen species (ROS) generation.

Experimental Methods

FLG (5 μm lateral size) was oxidized into FLGO by modified Hummers method, followed by morphological (TEM) and chemical (XPS) characterization. FLG and FLGO films were produced by vacuum filtration of powders’ aqueous suspensions, and characterized before and after NIR irradiation (LED-based source: ~810 nm, 0.150 W/cm²) regarding surface morphology (SEM), chemistry (XPS) and wettability (water contact angle). Films were incubated with S. epidermidis and methicillin-resistant S. aureus (10⁶ CFUs/mL) in medium supplemented with plasma proteins (to simulate in vivo scenario) for 2 h at 37 ºC. Samples were then either kept in the dark (NIR off) or NIR irradiated (NIR on) for 45 min. Polymethylsiloxane reference materials were used as control surfaces. Antimicrobial activity towards adherent bacteria was assessed by live/dead assay (Baclight™ Bacterial Viability Kit) and fluorescence microscopy, and planktonic bacteria were evaluated by CFU counting and metabolic activity (resazurin assay). The mechanism of action was explored considering NIR’s impact on photothermal properties (temperature measurement at surface and on supernatant with a thermocouple microprobe), GSH oxidation (oxidative potential, Ellman’s assay), and photodynamic properties (ROS generation, DCFH-DA assay).

Results and Discussion

Successful oxidation of FLG (3.5% O) into FLGO (~31% O) was achieved, and lateral size of 5 μm confirmed. Films’ surface morphology, chemistry and wettability are kept upon NIR stimulation, with FLG films maintaining the non-
oxidized character and sharp edges exposed, while FLGO films show graphene sheets fusion with a wrinkled-like morphology and similar oxidation degree (31%).

FLG and FLGO films exposure to NIR significantly boosted their bactericidal performance, efficiently killing 90% and 99% of planktonic bacteria (S. epidermidis and methicillin-resistant S. aureus), respectively, and causing the death of 65% and 85% of adherent bacteria, respectively.

Upon irradiation, FLG films act through a combined action of high surface temperature (51.3 °C), ROS overproduction and elevated GSH depletion (92%), with the action of sharp edges contributing positively to the overall antibacterial effect. With higher bactericidal action than FLG but lower production of ROS, FLGO films induce higher surface temperatures (56.0 °C) and GSH depletion (94%) that lead to increased membrane destabilization. The low temperatures reached in the supernatants (39.3 °C for FLG and 42.0 °C for FLGO films) suggest ROS as the major contributor of planktonic bacteria death. Reference materials validate that the observed effects are specific and associated with the presence of the GBMs films.

As such, both surfaces act through a synergistic and interdependent combination of PTT and PDT, with PDT efficacy being enhanced by the films’ ability to oxidize GSH. FLG films are hypothesized to act predominantly through ROS-dependent oxidative stress pathway, while FLGO through a ROS-independent pathway.

**Conclusion**

Stand-alone FLGO films can thus be used as light-activated platforms for the effective killing of bacteria (~99% planktonic bacteria) under low power NIR light exposure (0.150 W/cm²), and through a synergistic photothermal and photodynamic effect. These NIR-activated graphene-based platforms arise as simple and economical disinfection surfaces/systems, with widespread use in medical and non-medical applications, representing an effective and sustainable solution against antimicrobial resistant infections.

**Acknowledgement**


**References**


Graphene oxide, an incentive for ectopic osteoinduction in biopolymer blend composites

George M. Vlasceanu¹,², Mariana Ionita¹

¹ University Politehnica of Bucharest, Faculty of Medical Engineering, Bucharest, RO; ² University Politehnica of Bucharest, Advanced Polymer Materials Group, Bucharest, RO

Introduction
Herein, the synthesis and characterization of novel chitosan-gelatin highly porous scaffold reinforced with graphene oxide, crosslinked with genipin was targeted. This study addresses the potential boost of mineral-independent osteoinduction and osteodifferentiation featured by fish gelatin/chitosan (GCs) hybrids per graphene oxide (GO) reinforcement. Standard GCs formulation was referenced against genipin (Gp) crosslinked blend and 0.5 wt.% loaded GO composite (GCsGp/GO 0.5 wt%).

Experimental Methods
Suitable GO dispersion was ascertained within the biopolymer mix as nanolayers specific signals lack in both FTIR and XRD spectra and the specific spectral features of the polymers persisted with GO load enhancement. Overall, correlations between the GO induced material structuration, crystallinity variations and chemical interaction of the compounds can be correlated with the physical features and bioactivity of each composite formulation.

Results and Discussion
Exploratory mechanical investigations were performed before assessing in vitro osteodifferentiation (against MC 3T3-E1 pre-osteoblasts) and materials' osteoinductivity in vivo (CD1 mice). Specific biological staining revealed collagen formation and Ca²⁺ deposits, validated by µCT. runx2 and opn markers determination stressed on the osteogenic phenotype of the cells populating the implanted materials. The aftereffects of implantation in ex vivo materials were studied from the angle of in situ biomineralization and morphology alterations. X-ray diffraction and Fourier-transform infrared spectroscopy underlined the novel material structuration in the mineralized extracellular matrix.

Conclusion
This data supports the GO bioactivity in osteogenesis mechanisms as being self-sufficient to boost osteoblast differentiation and bone formation in ectopic sites. Hence, the synthesis route of a natural polymer blend/graphene oxide composite material is anticipated to emerge as influential formulation in bone tissue engineering.

Acknowledgement
This work was supported by a grant of the National Authority for Scientific Research and Innovation, Operational Program Competitiveness Axis 1 - Section E, Program co-financed from European Regional Development Fund “Investments for your future” under the project number 154/25.11.2016, P_37_221/2015. The nano-CT experiments were possible due to European Regional Development Fund through Competitiveness Operational Program 2014-2020, Priority axis 1, ID P_36_611, MySMIS code 107066, INOVABIOMED.
Self-assembled hybrid hydrogels based on graphene derivatives and cerium oxide nanoparticles as three-dimensional substrates for neural stem cells

Yurena Polo1, Jon Luzuriaga2, Sergio Gonzalez de Langarica3, Beatriz Pardo-Rodriguez2, Edurne Marin3, Daniel Martínez-Tong4, Gaskon Ibarretxe2, Fernando Unda2, Jose-Ramon Sarasua3, Jose Ramon Pineda2, Aitor Larrañaga3

1 Polimerbio, Donostia-San Sebastian, ES; 2 University of the Basque Country, Cell Biology and Histology, Leioa, ES; 3 University of the Basque Country, Mining-metallurgy engineering and Materials Science, Bilbao, ES; 4 University of the Basque Country, Polymers and advanced materials: Physics, Chemistry and Technology, Donostia-San Sebastian, ES

Introduction
Stem cell-based therapies have attracted considerable attention to treat the injured or diseased nervous system [1]. A major challenge in utilizing stem cells for regenerative therapies is the poor control over the survival, differentiation and functional integration of the transplanted cells. The combination of stem cells with scaffolds has been proposed to overcome this problem. In the present work, graphene-based hydrogels were developed as a substrate for neural stem cells (NSCs). These hydrogels were further functionalized with cerium oxide nanoparticles, thus providing a multifunctional platform for cells that combines the intrinsic physico-chemical, electrical and mechanical cues provided by graphene derivatives [2, 3] with the antioxidant and cytoprotective properties from cerium oxide [4].

Experimental Methods
The graphene/cerium oxide hydrogels were fabricated via the self-assembly of graphene oxide (GO) in the presence of ascorbic acid. Briefly, GO and cerium oxide nanoparticles were mixed in a bath sonicator for 30 min. Afterwards, ascorbic acid (AsA) at various ratios (GO:AsA ratios: 1:1; 1:4 and 1:10) was added and kept at 37 °C for two hours to allow the self-organization of the hybrid systems into hydrogels (Figure 1A). The resulting hydrogels were fully characterized in terms of mechanical properties (rheology), electrical conductivity, antioxidant capacity, morphology (scanning electron microscopy-SEM) and physico-chemical properties (Raman spectroscopy and X-ray diffraction). NSCs were isolated and cultured as previously described [1]. They were subsequently seeded on the hydrogels and fixed for immunostaining at different time-points (1, 3, 7 and 14 days). Several markers (NeuN, DCX, S100B, GFAP, Olig2, Nestin, etc.) were used to distinguish between the various populations found in the central nervous system.

Results and Discussion
The different GO:AsA ratios, as well as the amount of cerium oxide nanoparticles in the hydrogel, allowed us to adjust the morphology and mechanical and electrical properties of the resulting hydrogels, giving rise to a plethora of substrates for cell attachment with tunable features. Increasing the amount of AsA, which acted as a reducing agent, resulted in a more collapsed structure (smaller pore size—Figure 1B) and higher shear modulus and electrical conductivity (data not shown). The incorporation of cerium oxide nanoparticles endow the hydrogels with antioxidant properties but reduced both their shear modulus and electrical conductivity by an order of magnitude (Figure 1C). The studied hydrogels allowed the attachment of NSCs without the need of any further coating of their surface with extracellular matrix adhesion proteins (e.g., laminin). NSCs were able to differentiate towards cells from the nervous system (neurons, astrocytes and oligodendrocytes) when seeded on the hydrogels and a different expression profile was observed depending on the properties of the substrate (Figure 1D).

Conclusion
In this study, a simple and versatile method is presented for the fabrication of graphene-based hydrogels with tunable mechanical, electrical, physico-chemical and morphological properties. They also support cell adhesion and neurodifferentiation of NSCs. A more detailed analysis is being currently conducted to establish the exact parameters that determine the differentiation of NSCs. Accordingly, the formulated substrates constitute a promising tool for future cellular therapies including nerve tissue regeneration.

Acknowledgement

References
**Scaffolds based on decellularized extracellular matrix and graphene oxide: a route to repair the spinal cord injury?**

Daniela M. da Silva¹, Susana Cristina Pinto¹, Joana Patrícia M. Sousa¹, Lina Papadimitriou², Anthi Ranella², Paula Alexandrina A.P. Marques¹, Nathalie B. Barroca¹

¹ University of Aveiro, Department of Mechanical Engineering - Centre for Mechanical Technology and Automation, Aveiro, PT; ² Institute of Electronic Structure and Laser, Foundation for Research and Technology-Hellas (FORTH/IESL), Heraklion, GR

**Introduction**

Each year, 250,000 to 500,000 people suffer from a spinal cord injury (SCI).[1] Due to the limited ability of the central nervous system to repair itself, most of the devastating consequences on mobility and essential physiological functions last for a lifetime. While surgical and rehabilitation measures have improved functional outcomes at a clinical level, a strategy repair that can cure SCI is still inexistent.

At a research level, progresses in the understanding of axon regeneration,[2] cell engraftment [3] and spontaneous circuit reorganization [4] are leading to tantalizing regenerative routes, currently targeted via biological approaches or neuroprosthetics. Biological approaches often rely on proteins-based platforms, owing to their tissue-like mechanics and a remarkable flexibility in designing their chemical and physical properties. Neuroprosthetics, in its turn, intent to restore voluntary motor impulses using epidural or transcutaneous electrical stimulation.[4] Our group aims to design the coming generation of electronic neural interfaces for the electrical neuromodulation to treat the SCI, via an electroconductive platform coupled with an electrical stimulation system. This platform is formed by two building blocks. The first is decellularized extracellular matrix from porcine adipose tissue, that translates into a microenvironment with natural bioactive cues that can both attract and induce cell differentiation required for tissue repair. The second building block is reduced graphene oxide (rGO) used as the electroconductive filler. Seminal work on the in vivo use of rGO for the SCI has shown remarkable effects. When used as a standalone scaffold, it promoted several regenerative features at the injured spinal cord such as axonal and vascular growth after 4 months of implantation.[5]

Before coupling with the electrical stimulation system, these electroconductive platforms must prove cytocompatibility towards neuronal cells.

**Experimental Methods**

To promote electrical conductivity, graphene oxide was thermally reduced prior its incorporation to the decellularized extracellular matrix (adECM). adECM/rGO scaffolds were produced by solid-liquid phase separation followed by freeze-drying varying the rGO content (7.5, 15, 30 and 50 wt %). Cross-linking with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) (1:1 molar ratio) was performed using 0.0033mM of EDC per mg of adECM. Morphological analysis of the scaffolds was performed using scanning electron microscopy and microcomputed tomography. Mechanical characterization was assessed via compressive tests on the hydrated scaffolds. The crosslinking efficiency was quantified by spectrophotometry using 2,4,6-trinitrobenzenesulfonic acid (TNBS) assay that quantifies the free primary amine groups. For the cytocompatibility study the embryonic neural stem cells (NE-4C) was cultured on the adECM/rGO scaffolds. The NE-4C cell line was derived from anterior brain vesicles of p53-deficient early (E9) mouse embryos and can differentiate into both neurons and astrocytes. Differentiation towards neurons and astrocytes was assessed by confocal microscopy. In conjunction with differentiation, synaptogenesis, as a critical aspect of the nervous system...
function, was evaluated. To determine synapse formation, differentiated cells were stained for synaptophysin, an integral membrane glycoprotein that occurs in presynaptic vesicles of neurons.

Results and Discussion

Traditional solid-liquid phase separation followed by freeze-drying successfully led to porous and mechanically compliant scaffolds based on adECM with up to 50 % wt of rGO. Cross-linking via EDC/NHS was effective, particularly for the scaffolds with less rGO (7.5 % wt), wherein a decrease in free amine groups up to 27% was observed.

In culture, all the scaffolds supported the adhesion, survival, and proliferation of cells. Although more cells were initially observed on the scaffolds with more adECM, the differentiation assays on scaffolds with high loading of rGO revealed that the cells were more prone to differentiation when cultured on scaffolds with more rGO. Preliminary results indicated that neural stem cells cultured on these electroconductive platforms expressed synaptophysin, pointing to the possibility of a functional neuronal network forming inside the adECM/rGO scaffolds.

Conclusion

This work demonstrated the use of a simple technology to produce highly elastic and electroconductive scaffolds based on adECM from the adipose tissue and rGO. Cell culture on the porous and mechanically compliant scaffolds revealed the presence of a fully developed complex network of neurons, wherein synaptogenesis is taking place. These adECM/rGO scaffolds appear as a promising route to be further coupled with electrical stimulation to treat the spinal cord injury.

Acknowledgement

This work has been supported by the European Union’s Horizon 2020 Research and Innovation Programme (H2020-FETOPEN-2018-2020, NeuroStimSpinal Project, Grant Agreement No. 829060).

References


Engineering Graphene-based Nanocomposites for Optical Brain Stimulation

Artur Filipe Rodrigues1, Bruno Figueiredo2, Lino Ferreira1,3

1 Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, PT; 2 Graphenest SA, Paradela do Vouga, PT; 3 Faculty of Medicine, University of Coimbra, Coimbra, PT

Introduction
Neurological disorders are one of the world’s leading medical and societal challenges, affecting approximately 2.6 billion people worldwide. The failure of pharmacological therapies has prompted the investigation of alternative treatments such as electrical stimulation to improve brain function in a range of disorders including stroke, epilepsy, migraines, dementia, Alzheimer’s and Parkinson’s diseases [1]. However, significant adverse effects related to device operation and long-term biocompatibility have encouraged the investigation of novel materials and stimulation modalities [2]. Graphene-based materials (GBMs) reunite promising attributes for brain interfaces, including their biocompatibility, flexibility, and high capacitive charge conduction [3]. GBM electrodes were recently shown to generate an electrical response upon light exposure [4], which could capitalize the enhanced spatiotemporal precision of light for deep brain stimulation. In order to circumvent the use of visible light, which poorly penetrates biological tissue, we propose the incorporation of upconversion nanoparticles (UCNP) for wireless transcranial activation using near-infrared (NIR) radiation [5]. We hypothesize that UCNPs will enhance optical activation of graphene-based electrodes with NIR radiation for stimulation of cells, minimizing photothermal effects which could cause cytotoxicity.

Experimental Methods
Graphene nanoplatelets (Graphenest) were screen-printed onto PET substrates and characterised their electrical response to radiation applied at different wavelengths (405, 785, 980 nm) and power densities (0-2 W/cm²) by cyclic voltammetry and electrochemical impedance spectroscopy. Based on these results, a library of UCNPs was generated using Design of Experiments (DoE) to investigate the influence of different host matrices (NaYF4, NaGdF4) and dopants (Yb, Nd, Tm, Er, Sm) in the electrical response of graphene. The optimal UCNP formulation was then covalently attached to graphene nanoplatelets following selective hydroxyl derivatization. This nanocomposite was then characterized with respect to its optoelectronic performance and in vitro biocompatibility.

Results and Discussion
First, we have demonstrated that graphene nanoplatelets responded to different wavelengths of light (UV-Vis-NIR) according to their energy. These results supported the rational design of UCNPs using a DoE approach, in order to improve optical activation by NIR radiation. This approach enabled the optimization of dopant concentrations in order to adjust the optical properties necessary to maximise the electrical response from graphene nanoplatelets. Covalent functionalisation was essential to maintain long-term stability of the composite in physiological milieu.

Conclusion
Graphene-based nanocomposites are biocompatible options for brain interfaces, providing a variety of strategies for cell stimulation. Our results pave the way for minimally invasive optical stimulation of cells, enabled by transcranial NIR radiation.

Acknowledgement
This work was funded by the EU Horizon 2020 programme under the Marie Skłodowska-Curie grant agreement No. 101003413. The authors would also like to thank the financial support from the European Regional Development Fund (FEDER), through the COMPETE 2020 – Operational Programme for Competitiveness and Internationalisation.
and Portuguese national funds via FCT – Fundação para a Ciência e a Tecnologia (under projects PTDC/NAN-MAT/28060/2017 and UID/NEU/04539/2019) and FEDER (CENTRO-01-0145-FEDER-028060).

References
11:30 a.m. – 1:00 p.m.

Track02

S06 | Hybrid Cell-Microbiomaterial 3D Assemblies

Chairs
Zeinab Niloofar Tahmasebi Birgani
MERLN Institute, Maastricht University, Instructive Biomaterials Engineering, Maastricht, NL
Roman Truckenmüller
Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, NL
Max von Witzleben (YSF)
TU Dresden, Centre for Translational Bone, Joint and Soft Tissue Research, Dresden, DE

At this symposium, the participants will learn how to:
– identify the main criteria for designing instructive designer microbiomaterials.
– analyze and interpret the advanced interactions between progenitor cells and therapeutic micron-scale biomaterials.
– apply these principles in developing 3D hybrid microtissues for regenerative medicine applications.
Designing (almost) all-cellular living materials

Mariana B. Oliveira

University of Aveiro, Department of Chemistry, CICECO - Aveiro Institute of Materials, Aveiro, PT

Relevant regenerative therapies are often directed towards the use of biomaterials as main supporting matrices and cue-providers to cells. However, the passage of new biomaterials to the marketplace is subjected to time consuming and costly regulatory processes. Although scaffolding materials are designed to mimic aspects of native extracellular matrix (ECM), cellular presentation and assembly in these structures is mostly man-induced. Scaffold-free strategies allow cells to naturally assemble and form stand-alone structures through cell-cell contact and deposition of their own ECM. Cell sheets are the most widely reported scaffold-free constructs for tissue engineering. Despite having shown outstanding clinical results in the treatment of thin and poorly vascularized tissues, the scaling of these structures into long-term functional constructs capable of filling large three-dimensional (3D) defects is challenging. Multicellular microtissues, mostly with spherical geometry, have also been suggested as building blocks to generate higher order tissues. However, the construction of such constructs often depends on mobility confinement strategies and/or the multistep deposition of multicellular micrometric units, hampering the complete free-form and direct fabrication of versatile shapes.

The use of long cell fibers may allow using knitting/textile, printing and even stitching techniques to position cellular constructs prepared in vitro in favorable architectural configurations, and to build macrometric tissues with spatially controlled compositions. We recently reported the rapid one-step fabrication of centimeter-long cellular fibers made of human mesenchymal stem cells derived from the adipose tissue (hASCs) [1]. Previously reported techniques for the fabrication of scaffold-free cellular fibers consistently required the use of exogenous proteins and sacrificial biomaterials templates. Instead, we described the fabrication of centimeter-long cell-only monotypic and heterotypic fibers, using human hASCs and their combination with endothelial cells. hASCs were seeded onto wettable channels patterned on superhydrophobic surfaces, generating a hanging column setup after upside-down rotation. The self-assembly of cells into fiber-shaped constructs rendered highly malleable structures, able to adapt to complex-shaped defects. Those also exhibited high viability and homogenous thickness distribution. After 7 days of culture into a simplistic ECM-mimetic in vitro model, hASCs fibers were able to sprout and secrete pro-angiogenic, trophic and immunomodulatory factors. The integration of cell fibroids into an ex ovo matrix (CAM assay) was also confirmed, and heterotypic fibers (hASCs+HUVECs) showed pro-angiogenic properties. Such micro-to-macrotissues may be enabling platforms for regenerative medicine, and may find broader application as in vitro screening devices.

Although architectural features of cell-rich structures may be crucial to enable ease of processing into higher-order structures, as well as their in vivo applicability and ease of handling, the functionality of living structures based on mesenchymal stem cells has benefited from cellular conditioning. Mostly, these strategies rely on the administration of soluble factors to boost cellular pro-regenerative function, or to trigger their differentiation into organotypic cell phenotypes. The biophysical guidance of cell response by direct contact with biomaterials has proven promising for the local modulation of adherent stem cells’ response. Nonetheless, the introduction of high masses of biomaterials into cell aggregates may lead to the loss of their extremely cell-rich character and associated advantages. Also, regulatory approval of such structures may be hampered or delayed, especially if materials that have not been previously tested in clinical settings are introduced. We recently demonstrated the fabrication of sheet-like microparticles (~200 nm thickness) made of polymers previously approved by the FDA for implantation purposes, encompassing a vast array of geometries with high fidelity to original patterns (e.g., circles, triangles, squares, hexagons), using a straightforward and scalable method based on platforms showcasing wettability contrast [2].
These structures showed cell-mediated pliability, and their integration in gravity-enforced human adipose-derived stem cells aggregates led to enhanced metabolic activity and prolonged secretion of a relevant pro-angiogenic factor (VEGF). These geometrically controlled ultrathin microparticles may act as valuable elements in the design of “low-biomaterial” living structures with improved therapeutic purposes. Different strategies resorting to scaffold-free and low-biomaterial approaches targeting the design of highly functional cell-dense constructs as regenerative devices will be presented, considering crucial steps towards their ease of manufacturing and (pre-)clinical translation.

Acknowledgement
This work was supported by the Programa Operacional Competitividade e Internacionalização, in the component FEDER, and by national funds (OE) through FCT/MCTES, in the scope of the projects “TranSphera” (PTDC/BTM-ORG/30770/2017) and “CellFi” (PTDC/BTM-ORG/3215/2020). This work was also developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. The individual contract CEECIND/03605/2017 is also acknowledged.

References
S06-02

Inorganic-based partially coating of mammalian cells towards the enhancement of cell viability in suspension conditions

Marta M. Maciel¹², Tiago R. Correia², Vítor M. Gaspar², João M.M. Rodrigues², Insung S. Choi³, João F. Mano²

¹ University of Minho, CEB - Centre of Biological Engineering, Braga, PT; ² University of Aveiro, CICECO - Aveiro Institute of Materials, Aveiro, PT; ³ KAIST- Korea Advanced Institute of Science and Technology, Department of Chemistry, Daejeon, KR

Introduction

Stem cells are one of the most rising and promising research lines in Tissue Engineering and Cell Therapies due to their properties of self-renewal and potency. The available solutions usually involve stem cells in vitro expansion followed by their administration through systemic and local injection. However, most of the failure attempts are related with the cell handling and manipulation processes, thus the optimization of these therapies is a great necessity due to the low rates of cell survival and efficacy of treatment. New strategies are being explored to extend the period of cell survival in suspension conditions. These approaches mostly consist of giving mammalian cells a better support against external stresses for instance by functionalizing the cells to improve the targeting, integrate cells on 3D scaffolds, coating cells to improve their mechanical resistance and protection or by encapsulating them. In fact, single cell encapsulation seems to be one of the most interesting approaches by allowing a better control and programming of cells while improving their viability. It offers a tougher and durable support for cells which is very important regarding cell therapies and the harsh processes they must go through. Inspiration from nature has been leading a variety of fields from science to architecture to study, mimic and find new strategies and materials. Biomineralization is a natural process by which living organisms form minerals from bioorganic molecules and inorganic solids. Silica, as biomaterial, possess a range of characteristics like mechanical strength, chemical inertness, hydrophilic character and biocompatibility that make this material appealing to biomedical applications. Furthermore, silica has an important role in the bone and connective tissue formation, and it can also be easily manipulated using sol-gel methods under mild conditions to obtain new materials with different properties.[1] Fully encapsulated cells using silica were already achieved with promising results against enzymatic attacks although during long time applications cells start to die.[2] Such can be caused by the processing of the materials that can be quite toxic and disruptive for the cell membrane along with cell full entrapment which limits their normal functions. We report an innovative concept and strategy, where silica-based partial coatings (silica backpacks) are formed at the surface of individual stem cells. Based on electrostatic interactions and sol-gel process under mild conditions, only a part of cell surface was successfully engineered with a silica backpack.

Experimental Methods

A 2-step procedure was performed in isolated human adipose derived stem cells (hASCs) while adhered to a petri dish as previously described.[3] After washing, cells were incubated with a new modified chitosan with carnitine prepared in a PBS solution to be used as a primer coating material. Then, a silica-based solution was prepared under mild conditions. The obtained partial-coated cells were characterized by flow cytometry and confocal microscopy. Cell viability was evaluated by the Live/Dead and Apoptosis/Necrosis assay for 21 days while the cells were in suspension during the assay. To prove their potential for injection therapies an assay was carried out using partially coated cells and uncoated (control) cells. They were resuspended in a filtered 2% w/v alginate solution dissolved in PBS solution (pH = 7.4). By using a 1 mL syringe fitted with a 30 G needle and a flow rate of 30 mL h⁻¹, cells were
injected at the same time. Then, they were incubated with Live/Dead solution and their viability was assessed through fluorescence microscopy.

Results and Discussion
Partial coated cells were achieved by a straightforward 2 step procedure under mild conditions and using a new type of primer not toxic for cells (Fig. 1). It is important to highlight that from hASCs used on this study, 57% of cellular population present silica backpacks. These cells can survive longer than 21 days forming larger aggregates compared to pristine cells, which suggests prolonged cell survival and functionality. Injection assays results showed that partially coated cells possess higher viability after extrusion than the uncoated ones, revealing its potential for bioprinting applications and injecting therapies (Fig. 2).

Conclusion
By using a minimal material approach, we developed a new hybrid material: cells partial coated with an inorganic material with potential to overcome viability issues on applications when used in suspension conditions. This minimal material approach can positively influence cell survival at longer term without compromising cell functions. The use of silica in this strategy can also be explored as drug carrier for cell differentiation or drug delivery.

Acknowledgement
The authors would like to acknowledge the financial support from ATLAS (ERC2014-ADG-669858) and FCT through the PhD grant PD/BD/139117/2018 (M. M. Maciel). This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the FCT/MCTES. Image acquisition was performed in the LiM facility of iBiMED, a node of PPBI: POCI-01-0145-FEDER-022122.

Characterization of partially coated cells

Laser-scanning cozmembrane (red channel), and silica (green channel). B - Flow cytometry dot plot analysis of silicified hASCs populations. Around 57% of cells are positive for DID (cell tracker) and FITC (silica marker) meaning cells with partial silica coatings. Cell viability assay at day 21: live cells (blue), dead cells (red) and silica (green). C - Control aggregate; E - Partial coated hASCs aggregates; D and F - Images of control and partial coated cell aggregates, respectively, in 3D perspective.
Partially coated hASCs with high rate of survival after injection

Live/Dead was performed after cell injection, live (green) and dead (red) cells. G - Control cells (uncoated) after injection. Small image presents cells before injection (scale bar: 200 µm); H - Cells with silica backpack (hASCs@SiO2BP) after injection. I - Quantification of Live/Dead post injection. Data is presented as mean ± s.d. N=3. *p < 0.05.

References
Hyaluronic acid-mediated active targeting: cell uptake is ruled by the interplay between polysaccharide molecular weight and nanoparticle production technique

Francesca Della Sala¹, Assunta Borzacchiello¹, Teresa Silvestri², Laura Mayol², Marco Biondi², Luigi Ambrosio¹

¹ National Research Council, Institute of Polymers, Composites & Biomaterials, Naples, IT; ² Università di Napoli Federico II, Dipartimento di Farmacia, Naples, IT

Introduction
Hyaluronic acid (HA) is an anionic naturally occurring polysaccharide, main constituent of the extracellular matrix, able to bind specifically CD44 receptor, which is overexpressed in a wide array of cancer cells. HA, due to its outstanding characteristics, aroused a noteworthy research interest for the engineering of nanoplatforms endowed with active targeting features. HA has been chemically anchored onto many drug-loaded nanodevices, and their enhanced tumor targeting ability assessed. Nevertheless, the need for a chemical reaction poses serious regulatory challenges for FDA approval. Moreover, HA rearrangement and conformation play a cornerstone role in dictating the specific interaction and binding proneness to CD44 receptor, which in turn strongly depends on HA size and molecular weight (MW). In this study, nanoparticles (NPs) based on poly(lactic-co-glycolic acid) (PLGA) have been produced by a modified nanoprecipitation technique. Specifically, NPs were decorated with HA by exploiting polymer self-aggregation driven by a lipophilicity gradient between oil and water phases. NPs were coated with HA at 200, 800 and 1435 kDa, and named HA2, HA8 and H14 correspondingly. The influence of HA MW on uptake was assessed by kinetic internalization studies on CD44-overexpressing breast carcinoma (HS578T) and healthy mouse fibroblast (L929) cells. Furthermore, the results were compared with the numerical simulations obtained with a kinetic internalization model based on a cell membrane adsorption-desorption balance.

Experimental Methods
NPs were produced by nanoprecipitation, forcing a PLGA/F68/F127 solution in acetone (1:0.5:0.5 weight ratio; 5 mL, 3% w/v) through a syringe at 333 µL/min flow rate by a Syringe Pump [1]. The solution was precipitated in an aqueous phase, containing poloxamers as surfactants (1:1 weight ratio) and different amounts of HA, depending on the specific MW. HA NPs were characterised by TEM, PCS, DLS and DSC. Cellular uptake kinetics of fluorescent NPs were obtained by performing, at scheduled time points, cell lysis followed by a spectrofluorimetric assay on the lysate to quantify NP-associated fluorescence. NPs internalization has been modelled a pseudo-chemical equilibrium relation at cell membrane, as mathematical model described previously [2].

Results and Discussion
Spherical NPs with a < 200 nm mean size were obtained (Fig 1A). ZP was < -50 mV in all cases, thereby indicating HA arrangement on NP surface. DSC thermograms run on poloxamers showed endothermic peaks, associated to the melting temperature (Tm), at about 54.9°C, 56.0°C and 55.2°C for F68 and F127 and their 50:50 w/w mixture (Fig 1B), respectively. Endothermic peaks attributable to poloxamers were also identified in all NPs formulations, but at lower temperatures, comprised between 46.1 and 48.3°C. This has been associated to a plasticizing effect of PLGA and HA toward poloxamers. Furthermore, the heat generated during the melting of poloxamers (∆Hm) within NP bulk was drastically lower than the ∆Hm of the raw materials. This suggests the loss of most of poloxamers crystalline regions within the rubbery phase of the PLGA, which is completely amorphous. HA NPs uptake kinetic experiment, compared with the results of numerical simulations, revealed a higher internalization for HA8 compared the other two formulations (Fig 2A). In particular, the HA14 showed, within 72 hours of exposure, a significantly lower uptake...
uptake than the other two formulations. Uptake by cancer cells was promoted for all three formulations, with an increase of approximately 2, 2.3 and 1.3 times for HA2, HA8 and HA14, respectively. Fig 2 B shows \( N_{\text{MAX}} \) values, which expresses the number of NPs that can be internalized by cells after a virtually infinite time, indicating that the uptake of HA2 and HA8 by HS578T cells is 2.5+3.6-fold higher than \( N_{\text{MAX}} \) calculated with L929 cells. In contrast, in the case of HA14, the calculated values of \( N_{\text{MAX}} \) were similar in both cell populations. Cell uptake experiments and model simulations indicated a significant tropism of HA-decorated NPs towards tumour cells and, more interestingly NPs uptake were higher for NPs HA 8, compared HA 2 and HA 14. HA exposure on NP surface is intimately connected to the NPs production technique used, because HA is physically linked, and HA is actually engaged in the interaction with the hydrophilic poloxamer segments. This indicated that segment of HA chain that actually protrudes from a single NP (influenced by NP production technique) and its different arrangement conformation (influence by HA MW) on particle surface affected the cell internalization.

**Conclusion**

Overall, results obtained in this work point at how HA MW, despite being a pivotal project parameter in NP formulation, cannot dictate NP internalization amount and kinetics per se. Rather, cellular uptake outcomes plainly emphasize the importance of the technique used for NP production in dictating the biological interplay between NPs and CD44-overexpressing cells.

**Acknowledgement**

The authors acknowledge the research project "ADVISE DRUGS AND ANTI-TUMORAL VACCINES FROM THE SEA" - POR CAMPANIA FESR 2014-2020 AND WITH THE AXIS 1 OO.SS. 1.2.2 / 1.1 CUP B43D18000240007"

References

Injectable, self-healing colloidal gels for mRNA delivery to stimulate bone regeneration

Lea Andrée1, Rik Oude Egberink2, Marije Heitmeijer1, Fang Yang1, Roland Brock2, Sander C.G. Leeuwenburgh1

1 Radboudumc, Department of Dentistry – Regenerative Biomaterials, Radboud Institute for Molecular Life Sciences, Nijmegen, NL; 2 Radboudumc, Department of Biochemistry, Radboud Institute for Molecular Life Sciences, Nijmegen, NL

Introduction
Bone can be damaged or lost due to a wide variety of factors such as trauma, disease or congenital malformations [1]. Biomaterials, often in combination with proteinaceous growth factors (GFs), are used to stimulate regeneration of damaged bone tissue. Yet, these growth factors need to be administered in supraphysiological amounts due to their rapid clearance, and they are costly to produce [2].

Messenger ribonucleic acid (mRNA) has evolved into a promising new class of bioactive compounds. In contrast to classical GF delivery, mRNA therapy harnesses the cell’s translation machinery to stimulate endogenous protein production. So far, research has mostly focused on complexation of these oligonucleotides for systemic delivery. By comparison, the development of suitable biomaterial carriers for local delivery of osteogenic mRNA has only received little attention. Injectable biomaterials hold great promises for dental applications since they can be administered via minimally invasive procedures and can fill irregularly shaped bone defects. In this research, we focus on colloidal hydrogels based on gelatin nanoparticles (GNPs) as injectable carrier materials for mRNA-nanocomplexes and investigate their mechanical properties and self-healing behaviour in respect to their composition (solid content, GNP type) along with release characteristics and transfection efficiency of mRNA-polyplexes (mRNA-PPs).

Experimental Methods
Oppositely charged GNPs were prepared by coacervation of type A or type B Rousselot gelatin, and characterized by scanning electron microscopy (SEM), dynamic light scattering (DLS) and zeta potential measurement. To investigate gel formation of GNP-based colloidal gels and their self-healing behaviour after shear-induced destruction, we performed rheological time and amplitude sweep measurements. For mRNA delivery we employed non-covalent complexation with the polypeptide PepFect14. Fluorescently labelled mRNA coding for enhanced green fluorescent protein (EGFP) was complexed with PepFect14 into mRNA-polyplexes (mRNA-PPs) and characterized by DLS and zeta potential measurement. Transfection efficiency of mRNA-PPs was assessed in mouse pre-osteoblasts (MC-3T3) by confocal live cell imaging.

Results and Discussion
SEM showed spherical particles with a diameter of 145 ± 10 nm (type A) and 161 ± 14 nm (type B) in dry state. In swollen state diameters were 473 ± 107 nm (type A) and 313 ± 44 nm (type B). Zeta potential measurement of GNPs revealed a surface charge of 18.3 ± 1.3 mV and -12.6 ± 0.8 mV for type A and type B GNPs, respectively. Rheological time sweep measurements confirmed the formation of GNP-based colloidal gels, which displayed a higher storage modulus (G’) with increasing gelatin content (1.2 ± 0.2 kPa, 8.3 ± 0.8 Pa, 31.2 ± 2.1 Pa for 5 w/v%, 10 w/v% and 15 w/v%, respectively). Furthermore, the self-healing behaviour of gels after shear-induced destruction was shown, with up to 84 ± 5 % immediate recovery of G’ for 10 w/v% gels (Fig. 1). Additionally, colloidal gels with a solid content up to 10 w/v% and 15 w/v% could be extruded through needles of 22G and 16G, respectively. mRNA-PPs of mRNA complexed with PepFect14 had a diameter of 84.2 ± 9.7 nm and a Zeta potential of 26.4 ± 5.3 mV. These mRNA-PPs could transfect mouse pre-osteoblasts within two hours as seen by the intracellular
localization of labelled mRNA (Fig. 2A). Furthermore, successful cell transfection and subsequent translation of mRNA was confirmed by cellular expression of EGFP after 24h (Fig. 2B).

**Conclusion**

GNPs are suitable building blocks for the assembly of colloidal gels with favourable self-healing behaviour for application as an injectable carrier material. Using PepFect14, mRNA can be complexed into nanosized mRNA-PPs which get internalized by pre-osteoblastic cells. Moreover, PepFect14 effectively protects mRNA from premature degradation, leading to strong expression of the encoded protein. In the future, we aim to combine these two components to form an injectable gel stimulating endogenous protein expression through mRNA-PP release. We expect the colloidal gel to facilitate controlled release while maintaining the bioactivity of mRNA-PPs. Moreover, the bottom-up assembly of this colloidal gel will allow to tune the properties of the final biomaterial for clinical application.

**Acknowledgement**

The authors would like to thank the Netherlands Organization for Scientific Research (NWO, project 17615) for funding this research.

---

**Figure 1 - Self-healing behaviour of colloidal gel**

Self-healing behaviour of a 10 w/v% colloidal gel made of oppositely charged gelatin nanoparticles after shear-induced gel destruction assessed by monitoring storage modulus (G’) and loss modulus (G’’) as a function of time using rheology. (I) time sweep at 1% strain, (II) network destruction by increasing strain from 1% to 1000%, and (III) recovery at 1% strain.

**Figure 2 - Cell transfection with mRNA-polyplexes**

Transfection efficiency and bioactivity of Cy5-labelled mRNA coding for EGFP complexed with PepFect14 (N/P = 3). (A) Cellular uptake after 2h and (B) expression of encoded protein after 24h in MC-3T3 pre-osteoblasts.

---

**References**


S06-05

Proteomic Characterization and Inter-Donor Variability Evaluation of Human Cell-Assembled Extracellular Matrices

Fabien Kawecki¹, Maude Gluais¹, Stéphane Claverol², Nathalie Dusserre¹, Todd McAllister³, Nicolas L’Heureux¹

¹ University of Bordeaux, BIOTIS U1026 INSERM, Bordeaux, FR; ² University of Bordeaux, Centre de Génomique Fonctionnelle, Plateforme Protéome, Bordeaux, FR; ³ Fountain Therapeutics, Cluver City, US

Introduction

Synthetic scaffolds can be easily produced at a low cost with great control over their geometry and structure. Nevertheless, they can be associated with limitations, such as foreign body reaction and chronic inflammation resulting in material degradation [1,2]. Interestingly, natural materials, such as collagen or fibrin gel, exhibit several advantages at a biological level by facilitating cell-to-matrix interactions [3]. Our research group focuses on a tissue engineering approach that uses Cell-Assembled extracellular Matrix (CAM) sheets for vascular applications. This approach uses the capacity of mesenchymal cells, such as fibroblasts, to assemble in vitro their completely biological endogenously-secreted extracellular matrix (ECM) in the presence of ascorbate and serum [4]. We have been the pioneers to graft this CAM in humans for autologous and allogeneic vascular applications [5]. This CAM has been rolled and fused to produce tissue-engineered blood vessel under a good manufacturing process (GMP) for hemodialysis access. Clinical results demonstrated that the CAM biomaterial could integrate into the native tissue, be remodeled by the host’s cells, and limit infections [5]. The data allowed us to develop an allogeneic strategy of large-scale production for the CAM, which is more adapted for industrial transfer and/or clinical applications. This study aimed to evaluate this variability as well as the three main parameters (the strength, the thickness, and the quantity of hydroxyproline) that drive CAM production in a clinical manufacturing context.

Experimental Methods

To this end, human CAM sheets were produced under GMP condition using different primary dermal fibroblast populations obtained from 21 patients requiring arteriovenous shunt for hemodialysis. The strength, the thickness, and the hydroxyproline content of the CAM sheets were evaluated for each donor. Also, a detailed inventory of the CAM matrisome composition was realized using mass spectrometry. Then, correlations between the CAM parameters and the proteins detected within the CAMs were assessed by computing two-tailed Pearson correlation coefficients.

Results and Discussion

Results demonstrated that the strength, the thickness, and the hydroxyproline content of the CAM sheets varied between donors by 33% (coefficient of variation), 19%, and 24%, respectively. Another objective was to characterize the CAM matrisome to better understand its relation with the CAM properties. The CAM sheet strength showed moderate and strong positive correlations with the CAM thickness and hydroxyproline quantity, respectively. A detailed CAM matrisome characterization was performed by mass spectrometry and confirmed the inter-donor variability in term of CAM protein composition. Data also revealed that the CAM strength correlates with collagen alpha-1(I) chain abundance. The CAM thickness showed strong correlations with fibrillin-1, dermatopontin, and peroxidasin, which are involved in collagen fibril formation and stabilization. In addition, the CAM hydroxyproline quantity intensely correlated with proteoglycans (e.g. decorin) and ECM regulators (e.g. serpin H1) involved in collagen fibril biosynthesis.
Conclusion
Finally, this study formally evaluates the CAM inter-donor variability in a clinical manufacturing context and suggests that the CAM properties are driven by the fibril formation rate of the collagen and its stabilization. Furthermore, the detailed CAM composition characterization identified molecular predictor of the CAM properties and possible targets for improving CAM properties.

Acknowledgement
This work was supported by the European Research Council [Advanced grant number 785908]. F. Kawecki received a post-doctoral fellowship from the “Fondation pour la Recherche Médicale” (FRM) [grant number SPF202004011810]. The CAM tissue production was sponsored by Cytograft Tissue Engineering, Inc., Novato, California, USA. The authors thanks S. A. Garrido, A. Marini, L. M. De la Fuente, M. Maruszewski, W. Wystrychowski, M. Zembala, and L. Cierpka for the support of the clinical trial that made the samples available for this study.

References
Control of dynamic hydrogels via supramolecular engineering: 1,3,5 Benzene tricarboxamide hydrogels as extracellular matrix mimics

Shahzad Hafeez¹, Agustina Aldana¹, Hans Duimel², Clemens V. Blitterswijk¹, Lorenzo Moroni¹, Matthew B. Baker¹

¹ Institute for Technology-Inspired Regenerative Medicine, Department of Complex Tissue Regeneration, Maastricht, NL; ² Maastricht MultiModal Molecular Imaging Institute, M4I, Microscopy Corelab, Maastricht, NL

Introduction
Supramolecular hydrogels provide a biomimetic way to create artificial extracellular matrices for cell culture and biofabrication applications [1][2][3]. Via supramolecular polymerization, we can create fibrous, dynamic, and stimuli-responsive materials [1][4][5]; however, the rational design and control over the properties of the material remain a challenge. Control of the mechanical properties (both static and dynamic) are paramount to the success of supramolecular hydrogels in applications from simple cell culture to injectability and 3D printing. Herein, we develop a series of hydrogelators based on telechelic 1,3,5-benzene tricarboxamide (BTA) polymers, with systematic variations on the hydrophobic sidearms on the self-assembling unit. We hypothesized that the alterations in the hydrophobic domain of the BTA could provide a convenient handle to control the viscoelastic properties of the hydrogels. We then investigated the resultant supramolecular structures, the mechanical properties, and the utility in applications from injectability to 3D printing enabled by these small alterations in the molecular structure.

Experimental Methods
The telechelically functionalized BTA polymers (PEG-20K, variation in side arms from C₆ to C₁₂) were synthesized according to a novel desymmetrization route developed in the lab. The polymers were fully characterized with NMR and GPC, and gel properties were determined via oscillatory rheology. The supramolecular structures of these polymers were probed with a Nile Red fluorescence assay in combination with cryo-SEM and TEM. Cell viability was investigated using ATDC5 chondrocytes, and 3D printing was performed using a CellInk BioX printer and quantified using image analysis.

Results and Discussion
All target polymers were synthesized in good yield and purity, and all systems formed hydrogels with different critical gelation concentrations. We found that the stiffness of the hydrogels produced was not dependent on the side arm substitution, while the viscoelastic properties were highly dependent. By only changing a few atoms, we could tune stress relaxation from 0.2 to 2500 seconds. The relationship between molecular structure and hydrogel properties could be linked to known theory for the polymer physics of worm-like micelles. All samples formed fibrous aggregates in solution, with a tunable hydrophobic pocket upon self-assembly. The hydrogels were capable of self-healing, injectibility, and 3D bioprinting. Their performance as a bioink was tightly linked to the hydrophobic substitution of the BTA. High cell viability (>85%) was found before and after printing.

Conclusion
We have shown a novel approach to controlling the properties of supramolecular hydrogels based on simple molecular engineering. These hydrogels exhibit high cell viability and rationally tunable mechanical properties. By tuning of the hydrogel properties, we were able to create a 3D printable bioink from this system, with biomimetic mechanical properties and fibrous structure. These results enable further rational design of supramolecular biomaterials and show their potential in advanced applications like 3D bioprinting.
Scematics of BTA, cryo-TEM structure, and 3D Printability

References
In situ mineralization of MnO₂ on layer-by-layer polymer capsules to limit the accumulation of cytotoxic by-products resulting from enzymatic reactions

Edurne Marin¹, Juan F. Cadavid-Vargas², Jose R. Sarasua¹, Aitor Larrañaga¹

¹University of the Basque Country (UPV/EHU), Department of Mining-Metallurgy Engineering and Materials Science, POLYMAT, Faculty of Engineering in Bilbao, Bilbao, ES; ²Universidad Nacional de la Plata, INIFTA-CONICET-UNLP, La Plata, AR

Introduction

The use of enzymes has emerged as an attractive strategy in the fabrication of biomedical systems for therapeutic applications thanks to their specificity and efficacy. However, considering their susceptibility to undergo protease degradation and denaturation in body fluids, several encapsulation strategies have been considered to protect them from harsh environmental conditions and maintain their structural integrity [1]. Glucose oxidase (GOx) catalyzes the reaction of the environmental glucose to yield gluconic acid and hydrogen peroxide (H₂O₂). Based on this reaction, a plethora of biomedical systems have been developed including insulin delivery capsules [2], glucose sensors [3], cancer cell targeting capsules [4] or antimicrobial capsules [1]. The cytotoxic H₂O₂ produced in the reaction has been exploited to combat cancerous cells and bacteria (Fig. 1a) [1,4]. However, in many other strategies, the accumulation of H₂O₂ can have a detrimental effect on the surrounding cells/tissues, as well as in the catalytic activity of the enzyme, thus limiting its biomedical applications (Fig. 1b) [2,3].

To scavenge the produced H₂O₂, the use of MnO₂ as antioxidant synthetic enzyme (nanozyme) has been placed in the spotlight of many investigations owing to its unique enzyme mimetic activity and stability.

In this work, inspired by the compartmentalization strategies found at the cellular and subcellular level, we encapsulate GOx into layer-by-layer (LbL) polymer capsules. Their outer layer was further functionalized with an in situ created MnO₂ shell to simultaneously regulate glucose levels and reduce the undesired production of H₂O₂ from the microenvironment (Fig. 1b).

Experimental Methods

Microcapsules were fabricated via the LbL approach following a well-established protocol [5]. GOx was preloaded in the inner cavity of the capsule through a co-precipitation method, to obtain GOx-loaded CaCO₃ spherical microparticles. After the LbL process, using as polyelectrolytes poly (sodium 4-styrenesulfonate) (PSS) and poly(allylamine hydrochloride) (PAH), the MnO₂ layer was created in situ via a mineralization process incubating different KMnO₄ solutions (i.e., 2.5, 5, 10, 20, 50 and 100 mM) with the microparticle. After a morphological and physicochemical analysis of the fabricated capsules by means of scanning electron microscopy (SEM), ζ-potential, UV-vis and X-ray diffraction (XRD), the antioxidant capacity of the MnO₂ layer and the glucose reduction capacity of the GOx was assessed. Finally, the protective effect of the capsules was studied in an in vitro model of human lung fibroblast cells (MRC-5).

Results and Discussion

Capsules loaded with GOx and functionalized with the MnO₂ shell were satisfactorily fabricated via the LbL approach and displayed a spherical morphology with sizes in the range of 3-5 microns (Fig. 2a). The alternate deposition of the layers was confirmed with ζ-potential measurements in which a charge reversal was observed as different polyelectrolytes were incorporated (Fig. 2b). The capsules efficiently scavenged H₂O₂ from solution at biologically relevant concentrations (10-50 µM H₂O₂) (Fig. 2c), particularly at the highest KMnO₄ concentrations studied (i.e., 20, 50 and 100 mM). The activity of the encapsulated enzyme was preserved, as demonstrated by its ability to...
successfully reduce glucose from solution. The presence of naked (i.e., without the external MnO\textsubscript{2} shell) capsules promoted H\textsubscript{2}O\textsubscript{2}-induced cell death at increasing glucose concentrations in the culture media. In contrast, the external MnO\textsubscript{2} shell scavenged the overproduced H\textsubscript{2}O\textsubscript{2}, thus ensuring the survival of MRC-5 cells.

Conclusion
In this work we fabricated polymer capsules via the LbL approach, which were able to reduce the glucose levels from the cellular microenvironment and simultaneously scavenge the undesired H\textsubscript{2}O\textsubscript{2} created from the GOx reaction. These polymer capsules represent a promising strategy to protect enzymes from harsh environmental conditions maintaining their activity, while avoiding cell damage by reducing the effect of undesirable by-product accumulation.

Acknowledgement
Basque Government (GV/EJ) Department of Education, Linguistic Politics and Culture for funding the consolidated research groups project IT-927-16 (UPV/EHU GIC/152).

Fig. 1.
a) Schematic representation of polymer capsules fabricated for cell killing, b) Schematic representation of polymer capsules capable to reduce H\textsubscript{2}O\textsubscript{2} and protect cells from cell death.
Fig. 2.
a) Morphological characterization via SEM of polymer capsules, b) ζ-potential of polymer capsules incubated with different KMnO₄ concentrations, c) H₂O₂ scavenging capacity of polymer capsules at biologically relevant H₂O₂ concentrations (10 µM and 50 µM).

References
11:30 a.m. – 1:00 p.m.

Track03

S07 | Biomaterials: The Roadmap Towards Personalised Strategies in Musculoskeletal Tissue Engineering

Chairs
Nicola Baldini
University of Bologna & Istituto Ortopedico Rizzoli, Bologna, IT
Maurice Collins
University of Limerick, Limerick, IE
Gabriela Graziani
Rizzoli Orthopaedic Institute, Bologna, IT

Participants will have a comprehensive understanding of the latest advances in musculoskeletal engineering which are underpinning advances at the frontiers of materials, biological and medical science. These include new trends in 3D printing/bioprinting for regenerative medicine and tissue models, combination of 3DP and nanoscale materials, vascularization of 3D constructs.

3D Bioprinting has tended to focus on the production of increasingly intricate architectures. However, in achieving this, many concessions are being made regarding the biological aspects of printed materials. Therefore, there is a pressing need for novel engineering approaches to obtain biomimetic inks for future musculoskeletal engineering strategies.
S07-KL01

3D Bioprinting Strategies to Bioengineer Skeletal Muscle Constructs that Accelerate Muscle Function Restoration

Sang Jin Lee

Wake Forest School of Medicine, Wake Forest Institute for Regenerative Medicine, Winston-Salem, US

To achieve rapid skeletal muscle function restoration, many attempts have been made to bioengineer functional muscle constructs by employing physical, biochemical, or biological cues. Currently, 3D bioprinting technologies combined with tissue engineering principles have been developed to offer the creation of biological tissue constructs that mimic the structural, anatomical, and functional features of native tissues or organs. Hence, these technologies have been applied to fabricate an implantable, bioengineered skeletal muscle tissues composed of human primary muscle cells. The bioprinted muscle constructs showed a highly organized multi-layered muscle bundle made by viable, densely packed, and aligned myofiber-like structures. More importantly, these constructs have been implanted in animal models and showed muscle regeneration with the vascularization and host nerve integrity, resulting in the restoration of muscle function. These 3D bioprinted skeletal muscle constructs present a possibility for therapeutic effects to treat the muscle defect injuries and have implications for future translation.

Acknowledgement
This study was supported by the National Institutes of Health (1P41EB023833-346 01) and the Medical Technology Enterprise Consortium (#W81XWH-15-9-0001).
S07-KL02

Biomimetic 3D scaffold-based model for in vitro investigation of bone pathologies

Silvia Fare
Politecnico di Milano, Dept. of Chemistry, Materials and Chemical Engineering, Milan, IT

Among the adults, 0.2% of the total amount of malignant tumors are primary bone tumors, while among children (<15 years old) they make up 5% [1].

Bone microenvironment, built up by stromal tissue, extracellular matrix (ECM), cells and molecules, is influenced and it influences, itself, the tumor development. In particular, the tumor is characterized by a vicious cycle, in which tumor cells, stimulated by bone environmental factors, causes an increase in osteoclastic activity through osteoblastic activity modulation [2]. Consequences consist in a higher bone tissue resorption and in the development of a tumor niche, which should be perfectly reproduced in vitro, in order to identify anti-neoplastic therapies, which remove oncogenic tumor stimuli.

For decades, tumor research is based on 2D in vitro tumor models and animal models to reproduce what happens in the human body to identify and verify new anti-neoplastic therapies’ effectiveness [3]. However, both have intrinsic limitations: 2D in vitro tumor models do not reproduce the microenvironment (3D architecture, cell-cell, and cell-matrix interactions), while animal models have ethical and economic problems and, in addition, they are not so reproducible. Due to these limitations, only 5% of anti-neoplastic drugs, which demonstrated good results during 2D in vitro and in vivo studies, confirmed these results during clinical trials.

For all these reasons, during the last years, 3D in vitro tumor models have been developed to reproduce mechanical, biochemical, and structural bone microenvironment elements, allowing cells to behave as in vivo. The present talk is aimed at describing different approaches for the design of 3D in vitro models based on various polymeric porous scaffolds, in order to mimic structural, physical and mechanical properties of bone tissue. In vitro biological characterization provides appropriate information on the performance of the produced scaffold-based models in mimicking bone tumors.

References
3D Printed Bioabsorbable Scaffold for Repair of Large Area Load Bearing Chondral Defects

Michael Joyce¹,², Mark Lemoine¹,², Lia Blokpoel Ferreras¹,², Claudio Intini¹,³, Arlyng Gonzalez Vazquez¹,², Gayathri Kollamaram¹, Tom Hodgkinson¹,², Daniel J. Kelly¹,², Fergal J. O'Brien¹,²

¹ Royal College of Surgeons in Ireland, Anatomy and Regenerative Medicine, Tissue Engineering Research Group, Dublin, IE; ² Trinity College Dublin, Trinity Center of Biomedical Engineering TCBE, Dublin, IE; ³ Advanced Materials and Bioengineering Research Centre (AMBER), AMBER, Dublin, IE

Introduction
Articular cartilage lacks the ability to regenerate once damaged. Tissue engineering approaches have shown some promise to overcome this limitation by combining appropriate biomaterials, biochemical cues, and stem cells to facilitate environments capable of producing healthy de-novo cartilage. Our group has had success using freeze dried collagen and hyaluronic acid (CHyA) scaffolds to recapitulate the native cellular environment of cartilage.¹,² Combining this scaffold with bone-marrow derived mesenchymal stromal cells (BM-MSC) has shown production of cartilage like tissue that shows an increase in sulphated glycoaminoglycans (sGAG) and other chondrogenic markers over time.¹,² These scaffolds have shown potential in a number of animal models and in human patients but their mechanical properties restrict their use to small joint defects. To overcome the weak compressive modulus of CHyA scaffolds, this study focussed on developing a biomimetic, bioabsorbable scaffold with both mechanical and biochemical properties tailored to the specific requirements of the complex native tissue

Experimental Methods
PCL was extruded through a bioprinter (Allevi USA) to produce a macro-porous scaffold with mechanical properties tailored specifically to mimic articular cartilage. Two different scaffold designs, rectilinear and gyroid were selected for their ideal printability (continuous, non-intersecting toolpaths) and brought forward for mechanical testing. Aqueous CHyA was combined with PCL scaffolds and freeze dried together at -20C. Uniaxial compressive strain was applied cyclically at 0.15mm/s to 20% strain on a Zwick Roell mechanical tester (Zwick Roel, Germany) to determine Young’s compressive modulus. A 3 point bend test was used to determine how much force is required to bend CHyA-PCL scaffolds to the contour of a femoral condyle. This information was used to determine what fixation methods were strong enough to hold CHyA-PCL to the subchondral bone. 3 different osteochondral suture anchors were tested, two cylindrical scaffolds were 3D printed in-house (4, 6mm diameter) and a commercially available option (Twin Fix Ti, Smith and Nephew) were investigated. For in-vitro studies, scaffolds were seeded with BM-MSCs (5X10⁵ per scaffold) on their 5th passage and placed in complete chondrogenic media (CCM). sGAG production, DNA quantification, and histological analysis was performed on samples after 1, 14, and 28 days in culture. Prism software (GraphPad, USA) was used for one-way analysis of variance (ANOVA) of data or a two-tailed T test when appropriate.

Results and Discussion
Both the Rectilinear and Gyroid toolpath designs (Fig 1A) were able to increase the compressive modulus of CHyA scaffolds (Fig 1B) by 3 orders of magnitude which mimics the native mechanical property of articular cartilage. The Gyroid scaffold was more flexible than the Rectilinear scaffold (Fig 1C) which required an additional 6.9N to bend. All anchor systems tested (Fig 1D) were strong enough to hold a Gyroid scaffold in place, but only the commercial option was able to hold the rectilinear scaffold in place. CHyA matrix was successfully incorporated into PCL reinforced scaffolds (Fig 1E) without affecting the mean pore size (Fig 1F). Since the Gyroid was more flexible, and could be paired with numerous fixation options, it was brought forward for in vitro testing.
PCL reinforcement did not inhibit sGAG production on CHyA scaffolds. Cells seeded on PCL-CHyA scaffolds showed an increase in sGAG accumulation over time (Fig 2A) with a significant increase compared to non-reinforced CHyA 28 days after seeding. GAG production was normalized to DNA content to account for variation in cellular proliferation rates (Fig 2D). Only PCL-CHyA scaffolds were able to show an increase in GAG/DNA production overtime. Histological sections stained with Alcian Blue pH 1 (Fig 2B,C,E,F) show successful production of sulphated GAG (sGAG) signifying a high quality extracellular matrix production for the use in cartilage regeneration.

**Conclusion**

In conclusion, the addition of a PCL framework into a CHyA scaffold was able to significantly increase the compressive modulus the CHyA scaffold to more accurately mimic the native mechanical properties of articular cartilage while maintaining biological functionality as seen by cells attaching and depositing sGAG. Preventing the microporous architecture of CHyA from being flattened under physiological loads has previously been a limitation that restricts the effective area size that can be treated in cartilage defects. Reinforcement with a bioabsorbable material allows autologous BM-MSCs to migrate into the scaffold while also serving as a potential attachment point to fix the CHyA scaffold in place to the subchondral bone when implanted in joint defects. Taken all together, these PCL-CHyA reinforced scaffolds show great potential to be used as an off the shelf treatment for large area cartilage defects when combined with micro-fracture surgical technique.

**Acknowledgement**

This research was funded through European Research Council grant 788753, ERC-2017-ADG

---

**Biofabrication and Characterization**

Figure 1) PCL reinforcement provides appropriate mechanical properties for cartilage. (A) Two 3D printed toolpath designs (Rectilinear, Gyroid) were 3D printed for mechanical testing. (B) Compressive Young’s Modulus of scaffolds up to 20% strain at a rate of 0.15mm/s (n=3). (C) Force required to bend scaffolds to physiological range (n=3). (D) Force required to dislodge osteo-suture anchors from a femoral condyle (n=3). (E) SEM image of successful CHyA incorporation. (F) Mean pore diameter measured from histological slices confirming PCL does not affect mean pore size (n=10). (B,C,D**p<0.001).
PCL reinforcement increases sGAG deposition

Figure 2) PCL reinforcement leads to increased sGAG deposition in vitro. (A) Total sGAG deposited from BM-MSC on PCL-ChyA, and ChyA scaffolds (n=9) after 1, 14, and 28 days in culture. (D) PCL-ChyA increases sGAG/DNA accumulation after 28 days (n=9). ChyA scaffolds (B,E) and PCL-ChyA scaffolds (C,F) stained with Alcian Blue pH 1 to stain sGAG (blue) after 28 days in culture. (*p<0.05, ***p<0.001)

References
Complex designs for tissue regeneration: Melt Electrowriting of gradient scaffolds

Małgorzata Włodarczyk-Biegun1,2, Piotr Zieliński1, Magdalena Gladysz1, Anno Hofman1, Timo Rikmanspoel1, Xixi Wu1, Maria Villiou2, Marcus Koch2, Aranzazu del Campo2, Marleen Kamperman1

1 University of Groningen, Groningen, NL; 2 INM - Leibniz Institute for New Materials, Saarbrücken, DE; 3 Silesian University of Technology, Gliwice, PL

Introduction
Melt electrowriting (MEW) is an additive manufacturing technique that uses a high voltage to deposit with unprecedented precision fibers with diameters in the micrometer range. High control over printed structures and flexibility in the designs makes MEW a promising tool to recapitulate the complexity of native hierarchical structures. Therefore, using MEW we attempted to recreate the original architecture of the Human Trabecular Meshwork (HTM). HTM is a network located in the eye which is responsible for maintaining proper pressure in the ocular chamber, and its dysfunctions lead to glaucoma, one of the leading causes of blindness worldwide. HTM is ca. 600 μm thick and is composed of 3 geometrically different layers with pore dimensions between 0.1μm to 25μm.

We are also employing our approach to mimic the stratified structure of the skin and gradients of hard-soft tissue interfaces. The interfacial tissues, such as bone-tendon or bone-ligament connections, are characterized by gradual change of the architecture, accompanied by the gradients in chemical, mechanical, and biological properties, typically occurring over less than 1 mm length.

Experimental Methods
Printing of medical-grade Polycaprolactone (PURASORB PC 12, Mw ~55,000,) and high Mw Polycaprolactone (Sigma, Mw ~ 80,000) was performed with Melt Eletrowriting printer (Spraybase, Ireland and RegenHu, Switzerland). Scaffolds with different designs (varied pore shapes and sizes, different number of layers) were printed and visualized with scanning electron microscopy (SEM) to assess the accuracy of fabrication. The dependency of mechanical properties (in tensile and compression modes) on the scaffold's architecture was investigated. Biological response of fibroblasts and primary HTM cells seeded on different scaffolds was analysed based on SEM imaging and immunofluorescent staining.

Results and Discussion
Four different designs, inspired by the architecture of the native HTM and its three distinctive layers, were successfully printed (see Figure 1). The thickness of the scaffolds, depending on the design, varied between 70 and 500 μm, the average strand size was ca. 10-12 μm, and scaffolds overall porosity was in the range of 70-90%. These geometrical features closely mimic the native architecture of HTM. Mechanical testing revealed the subtle influence of the design on the mechanical properties of the scaffolds.

Primary HTM cells attached to the scaffolds proliferated to form a confluent layer within 7 days of culture and maintained their phenotype throughout the whole time of the cell study. Additionally, the first attempts to obtain scaffolds mimicking different zones in native skin and hard-soft tissue interfaces were made and different bioinspired structures were designed, printed, and visualized.

Conclusion
In conclusion, we have shown that MEW technique is a powerful tool to obtain high precision, small scale biomimetic scaffolds tailored into specific applications. MEW enabled the reconstruction of the multilayer structure of native HTM. Scaffolds permeability to liquid and response of the HTM cells to specific drugs, are currently under investigation. MEW is also a suitable technique to printed hard-soft tissue interfaces and skin models with varying mechanical and biological performance.
We envision that small-scale hierarchical structures printed with high precision will serve in the future as implantable systems or an in-vitro testing model.

MEW printed scaffold
SEM images of the scaffold mimicking the native HTM: top view (left) and side view with 3 morphologically different layers (right).
S07-05

Significance of Geometry in Biomimetic Eardrum Scaffolds

Shivesh Anand¹, Thomas Stoppe², Marcus Neudert², Serena Danti³, Lorenzo Moroni¹, Carlos Mota¹

¹ Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, NL; ² Technische Universität Dresden, Carl Gustav Carus Faculty of Medicine, Otorhinolaryngology, Dresden, DE; ³ University of Pisa, Department of Civil and Industrial Engineering, Research Unit of INSTM, Pisa, IT

Introduction

The human eardrum is a thin, concave membrane located at the end of the outer ear canal. It is responsible for capturing sound waves from the environment and transforming them into mechanical motion [1]. Perforated eardrums are one of the most common ear injuries, resulting in a partial or severe hearing loss due to inept sound conduction. Microsurgical placement of autologous tissue grafts from temporalis fascia or perichondrium have been the conventional approach to repair a damaged eardrum [2]. However, the incongruent tissue properties may impair an optimal hearing restoration. This has often been attributed to the lack of an appropriate geometry and mechano-acoustical characteristics in the recovered membrane [3]. The concurrent sound transmission and stability of a healthy eardrum is facilitated by its unique three-dimensional (3D) architecture comprising radially and circumferentially distributed collagen fibers [4, 5], although a limited knowledge is available on the contribution of these discrete micro-anatomical features. Therefore, this work presents a combination of theoretical and experimental approaches toward understanding the significance of geometry in biomimetic eardrum scaffolds (Figure 1).

Experimental Methods

A Python script was developed for modelling the anatomical architecture of the human eardrum. Three test designs (case I, II and III) along with a plain control were chosen to decouple some of its dominant structural attributes. Theoretical comparisons were performed on COMSOL Multiphysics by simulating the appropriate mechanical and acoustical interaction of the selected cases (Figure 2 (i)). A dual scale strategy combining electrospinning and additive manufacturing was implemented for manufacturing hierarchical scaffolds. A block copolymer of poly(ethylene oxide terephthalate) (PEOT) and poly(butylene terephthalate) (PBT) was employed as the suitable biomaterial. Parametric optimization of the fabrication steps were conducted, following which the constructed scaffolds were characterized mechanically, acoustically and biologically. The mechanical and acoustical responses were evaluated by applying the techniques of macroscopic indentation and laser Doppler vibrometry (LDV), respectively. For the biological assessments, human dermal fibroblasts (NHDFs) and human mesenchymal stromal cells (hMSCs) were seeded independently on the eardrum constructs. The cultured samples were assessed based on their ability to steer the cell distribution and alignment, along with an effective extracellular collagen production.

Results and Discussion

The Python script served as a versatile and robust tool for generating eardrum models with diverse anatomical features. In silico investigation of the chosen designs suggested a geometrical dependency of their mechanical and acoustical responses, where the presence of radially aligned fibers was observed to have a more prominent effect as compared to their circumferential counterparts. The hybrid fabrication strategy was carefully optimized to manufacture biomimetic scaffolds within the dimensions of the native eardrum. The macro-indentation results corroborated the findings of the theoretical simulations, where radially aligned scaffolds were noted to show a Young’s modulus closer to that of the human eardrum (Figure 2 (iii)). The circumferential filaments, on the other hand, were deemed critical for maintaining an optimal resilience and structural integrity. In addition, the LDV measurements demonstrated all the fabricated constructs to be acoustically comparable to the native tissue (Figure...
Finally, the biological studies performed with NHDFs and hMSCs, revealed a favorable influence of 3D hierarchy on cellular alignment and subsequent deposition of extracellular collagen. A higher orientation coherency and collagen production was obtained along the additive manufactured fibers (Figure 2 (ii)).

**Conclusion**

This study highlights the relevance of scaffold geometry for eardrum reconstruction. A radical improvement in the previously reported limits for manufacturing alloplastic eardrum replacements was achieved [4, 5]. Among the investigated micro-anatomical features, the radially-aligned fibers were observed to have a more prominent effect over stiffness, whereas the circumferentially-aligned ones were deemed critical for maintaining the structural integrity of the constructs. Cellular quantifications demonstrated the role of additive manufactured fibers in steering the distribution and alignment of collagen production on electrospun meshes. In conclusion, desirable mechanical, acoustical and biological behavior, analogous to that of the native tissue, can be achieved by manipulating the 3D geometry of eardrum scaffolds.

**Acknowledgement**

This work is a part of the 4NanoEARDRM project, receiving funding from EuroNanoMed III, the ERA-NET Cofund Action on Nanomedicine under Horizon 2020.

---

**References**


11:30 a.m. – 1:00 p.m.

Track04

ESB-CRS S | ESB-Controlled Release Society (CRS) Joint Symposium

Chairs
Carmen Sofia da Rocha Freire
CICECO/University of Aveiro, Aveiro, PT
Hélder Santos
University of Helsinki, Faculty of Pharmacy, Helsinki, FI
Ewelina Cichoń (YSF)
AGH University of Science and Technology, Krakow, PL
ESB-CRS S-KL01

Nanoactuators for Therapy and Diagnosis

Jesús M. de la Fuente

CSIC-Universidad de Zaragoza & CIBER-BBN, Instituto de Nanociencia y Materiales de Aragón, Zaragoza, ES

In the last decades, inorganic nanoparticles have been steadily gaining more attention from scientists from a wide variety of fields such as material science, engineering, physics, or chemistry. The very different properties compared to that of the respective bulk, and thus intriguing characteristics of materials in the nanometre scale, have driven nanoscience to be the centre of many basic and applied research topics. Moreover, a wide variety of recently developed methodologies for their surface functionalization provide these materials with very specific properties such as drug delivery and circulating cancer biomarkers detection. In this talk we describe the synthesis and functionalization of magnetic and gold nanoparticles as therapeutic and diagnosis tools against cancer. Gold nanoprisms (NPRs) have been functionalized with PEG, glucose, cell penetrating peptides, antibodies and/or fluorescent dyes, aiming to enhance NPRs stability, cellular uptake, and imaging capabilities, respectively. Cellular uptake and impact were assayed by a multiparametric investigation on the impact of surface modified NPRs on mice and human primary and transform cell lines. Under NIR illumination, these nanoprobes can cause apoptosis. Moreover, these nanoparticles have also been used for optoacoustic imaging, as well as for tumoral marker detection using a novel type of thermal ELISA and LFIA nanobiosensor using a thermosensitive support.
Tumor-Targeted Nanomedicine Approaches for Enhanced Immunotherapy

Horacio Cabral¹,²,³

¹ The University of Tokyo, Department of Bioengineering, Tokyo, JP; ² The University of Tokyo, Department of Materials Engineering, Tokyo, JP; ³ Innovation Center of Nanomedicine, Kawasaki, JP

Immunotherapy has revolutionized cancer treatment. However, despite the outstanding benefits, the proportion of patients responding to immunotherapies is still low. Nanomedicine has the potential to improve immunotherapy through spatiotemporal control of drug activity and promotion of effective antitumor immunity [1]. Nevertheless, only a few nanomedicines formulations have reached human clinical studies so far, and even fewer systems are being used in the clinic. Among translatable nanomedicines, self-assembled polymeric micelles have shown unique characteristics for dealing with tumor heterogeneity, such as the possibility to control their size in the sub-100 nm range and the possibility to install ligands on their surface to promote tissue and cellular targeting. Polymeric micelles can be designed to enhance antitumor immune responses by various immunopotentiating functions, such as the induction of immunogenic damage to cancer cells, the alteration of the tumor immune microenvironment through the delivery of immunomodulators, the reprogramming or elimination of immunosuppressive cells, and the improvement of the infiltration of antitumor immune cells. Polymeric micelles can be engineered to sense intratumoral stimuli for activating specific immune responses. For example, epirubicin-loaded polymer micelles can induce immunogenic cell death (ICD) to generate a strong immune response against murine models of glioblastoma [3]. The epirubicin-loaded micelles delivered more than 100-fold more drug into brain tumors than free epirubicin for promoting antitumor immunity. The micelles increased the infiltration of cytotoxic CD8+ T cells and M1-like macrophages, turning immunosuppressive glioblastoma into inflammed tumors. Thus, their cytotoxic effects synergized with immune checkpoint blockade elicited by anti-PD1 antibodies, allowing to overcome glioblastoma's resistance to immune checkpoint blockade. Polymeric micelles installed with ligands can be applied for increasing drug levels in tumors to enhance immune signals [4]. We have recently showed that glucose installation on the surface of polymeric micelles facilitated the accumulation into solid tumors by targeting the glucose transporters expressed on the tumor vasculature, which allowed the vascular translocation of the nanomedicines into the tumors [5]. Based on this approach, we designed glucose-installed nanocarriers loaded with anti-PD-L1 antibodies for enhancing the delivery to tumors. The anti-PD-L1 antibody-loaded nanocarriers achieved more than 10-fold higher accumulation in brain tumors compared to native anti-PD-L1 antibodies. Moreover, they eradicated intracranial glioblastoma models after a single intravenous injection. These results indicate that by fostering the intratumoral access of immunomodulating drugs it is possible to sensitize brain tumors to antitumor immunity. Thus, the capability of nanomedicines to exert immunomodulatory functions selectively in tumor and tumor-associated tissues increases the efficiency of the treatments, while avoiding systemic immunosuppressive toxicities and the exacerbation of adverse immune responses.

Acknowledgement
This work was supported by Grants-in-Aid for Scientific Research B (20H04524) from the Japan Society for the Promotion of Science (JSPS). This work was also partially supported by the Project for Cancer Research and Therapeutic Evolution (P-CREATE) (Project No. 16cm0106202h0001) from Japan Agency for Medical Research and Development (AMED).

References
ESB-CRS S-03

Tunable drug release from electrospun scaffolds for tympanic membrane regeneration

**Shivesh Anand**¹, Cemre Günday², Sara Munafò¹,³, Nazende G. Türel³, Serena Danti³, Lorenzo Moroni¹, Carlos Mota¹

¹ Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, NL; ² MyBiotech GmbH, Überherrn, DE; ³ University of Pisa, Department of Civil and Industrial Engineering, Research Unit of INSTM, Pisa, IT

**Introduction**

The human tympanic membrane (TM) is a thin tissue lying at the intersection of outer and middle ear. Its main function is to receive sound vibrations from the outer air and transmit them to the adjoining ossicular chain [1]. TM perforations are the most common damage to the human eardrum, where typical etiologies include microbial infections and acoustic traumas [2]. Among them chronic otitis media, a bacterial inflammation of the middle ear has been reported to be the leading cause for TM injuries in children. Majority of the acute perforations heal without an external stimulus, owing to the inherent regenerative capacity of the native tissue [3]. However, in case of larger chronic injuries induced by recurrent infections, the absence of a tangible support limits the requisite cell migration and proliferation for a self-recovery. Several tissue engineered grafts have been reported to treat the damaged eardrums, although not much attention has been directed towards preventing on-site microbial infections upon transplantation [4]. In this work, we investigate the integration of biofabrication with targeted drug delivery to create antibacterial scaffolds for TM.

**Experimental Methods**

Ciprofloxacin-encapsulated poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles were synthesized with two distinct approaches, namely double-emulsion and nanoprecipitation, by applying an automated microjet reactor technology. A co-electrospinning/electrospraying strategy was implemented to incorporate the drug-loaded nanoparticles within the electrospun mesh of poly(ethylene oxide terephthalate)/poly(butylene terephthalate) (PEOT/PBT) (Figure 1). The resultant scaffolds were evaluated with respect to a simple immersion approach where pre-fabricated PEOT/PBT electrospun meshes were soaked in the nanoparticle colloidal suspension. Furthermore, additional electrospinning modalities were investigated by including the ciprofloxacin in the precursor polymeric blend. This was performed to entrap the drug within the PEOT/PBT nanofibers, which in combination with the simultaneous electrospraying of PLGA nanoparticles allowed the manufacturing of dual loaded TM scaffolds. The chosen modalities were compared based on the surface properties, drug release kinetics, and mechanical response of their corresponding constructs. Preliminary cytocompatibility assessments of ciprofloxacin and PLGA nanoparticles were carried out with human mesenchymal stromal cells (hMSCs).

**Results and Discussion**

Among the two nanoparticle synthesis routes, nanoprecipitation was found more suitable for the applied co-electrospinning/electrospraying strategy [5]. Moreover, unlike the immersion approach, it resulted in a more homogeneous distribution of the deposited nanoparticles (Figure 1). The successful adhesion of the electrosprayed nanoparticles to the PEOT/PBT nanofibers was confirmed using scanning electron microscopy (Figure 2A) and fluorescent labeled PLGA (Figure 2C). Following an initial burst release, the PLGA nanoparticles ensured a controlled delivery of ciprofloxacin for the next 48 hours. On the other hand, the inclusion of ciprofloxacin within the electrospun fibers demonstrated an enhanced release kinetics between day 1 and 7. Therefore, a combination of the two modalities is being optimized to manufacture TM scaffolds with a tunable release profile up to 7 days. Cytocompatibility studies performed with hMSCs revealed no noticeable effect of the antibiotic-encapsulated...
nanoparticles on cellular attachment and proliferation (Figure 2B). Finally, a detrimental influence of ciprofloxacin over the cell viability and metabolic activity assays was noted only for concentrations exceeding 33 µg/mL (Figure 2D).

Conclusion
This work introduces a combination of electrospinning strategies for manufacturing TM scaffolds with a tunable antibacterial response. The implemented modalities have been found more efficient for targeted drug delivery as compared to conventional immersion techniques [5]. Electrospun meshes capable of releasing ciprofloxacin within its minimum inhibitory concentration (0.23 – 1.16 µg/mL) have been fabricated. Preliminary biological studies conducted with hMSCs have shown promising results. Further characterizations of the TM scaffolds along with the validation of their antibacterial activities is currently under investigation.

Acknowledgement
This work is a part of the 4NanoEARDRM project, receiving funding from EuroNanoMed III, the ERA-NET Cofund Action on Nanomedicine under Horizon 2020.

References

ESB-CRS S-04

Breaking the barrier: N-acetyl-cysteine-loaded nanoparticles as a strategy to disrupt bacterial biofilms

Rita M. Pinto¹²³, Claudia Monteiro⁵, Sofia A. Costa Lima¹, Susana Casal¹, Patrick Van Dijck²³, M. Cristina L. Martins⁴⁵, Salette Reis¹, Cláudia Nunes¹

¹ LAQV, REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Porto, PT; ² Laboratory of Molecular Cell Biology Institute of Botany and Microbiology KU Leuven, Leuven, BE; ³ VIB KU Leuven Center for Microbiology, Leuven, BE; ⁴ Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, PT; ⁵ i3S, Instituto de Investigação e Inovação em Saúde INEB, Instituto de Engenharia Biomédica, Universidade do Porto, Porto, PT

Introduction

Bacterial biofilms are nowadays a major health concern due to the escalating figures of antimicrobial resistance. Conventional therapy for implant-associated biofilms usually fails since it is solely focused on the administration of antibiotics. However, the biofilm matrix represents a biological barrier against these drugs, leading to antimicrobial resistance phenomena. A combinatorial therapy that focus on both bacterial cells and the matrix is urgently needed. In this work we developed lipid nanoparticles (LNPs) to encapsulate N-acetyl-cysteine (NAC), which is a mucolytic agent with potential to disrupt the matrix of mature biofilms, promoting the break of this protective barrier [1].

Experimental Methods

The LNPs were produced by the double emulsion technique and optimized by a rational design. The nanoparticles were characterized regarding their hydrodynamic diameter, polydispersity index, and zeta potential. Their biocompatibility was assessed through a hemolysis assay and an in vitro cytotoxicity assay against the L929 fibroblasts cell line. The in vitro antibiofilm efficacy of the developed LNPs was tested against Staphylococcus epidermidis (Gram-positive) and Pseudomonas aeruginosa (Gram-negative) biofilms.

Results and Discussion

The LNPs showed suitable characteristics for intravenous administration and biocompatibility. At the tested concentration, the LNPs were more efficient against Pseudomonas aeruginosa biofilms, with a 50% reduction of biofilm biomass. These results were further confirmed by a biofilm viability assay and by scanning electron microscopy. In a more complex approach, the LNPs were combined with a well-known antibiotic, moxifloxacin. This combination showed a potential synergistic effect between the LNPs and the antibiotic on the reduction of bacterial viability within the biofilms.

Conclusion

Overall, the developed LNPs have a potential therapeutic effect on Pseudomonas aeruginosa biofilms, alone or in combination with antibiotics.

Acknowledgement

RMP and CN are thankful to Fundação para a Ciência e Tecnologia (FCT) for the PhD Grant [SFRH/BD/130319/2017] and the investigator Grant [IF/00293/2015], respectively. This work was supported by FCT through the FCT PhD Programmes and by Programa Operacional Capital Humano (POCH), specifically by the BiotechHealth Programe. The work was supported by UID/QUI/50006/2019 with funding from FCT/MCTES through national fund as well as from the Fund for Scientific Research Flanders (FWO grant WO.009.16N) to PVD. This work is under the framework of the project POCI-01-0145-FEDER-31444, financed by Fundo Europeu de Desenvolvimento Regional (FEDER) - through the COMPETE 2020 - Operacional Programme for Competitiveness and Internationalisation (POCI) - and by national funds through FCT.

Page 427 of 2028
References
ESB-CRS S-05

Chitosan-hybrid micelles loaded with camptothecin inhibit colorectal cancer progression \textit{in vivo}

Andreia Almeida$^{1,2}$, Flávia Castro$^1$, Marlene Lúcio$^3$, Simo Schwartz Jr.$^4$, Bruno Sarmento$^{1,5}$

$^1$ Institute for Research and Innovation in Health, University of Porto, Porto, PT; $^2$ Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, PT; $^3$ Centre of Physics of University of Minho and Porto, University of Minho, Braga, PT; $^4$ Vall d’Hebron Institut de Recerca, Universitat Autònoma de Barcelona, Barcelona, ES; $^5$ Institute for Research and Advanced Training in Health Sciences and Technologies, Porto, PT

Introduction

Colorectal cancer (CRC) is among the cancers with highest prevalence and mortality worldwide. Conventional CRC chemotherapy is hindered by poor local drug bioavailability and solubility, resulting in a reduced therapeutic outcome. To address this inadequacy, we design polymeric micelles, based on a new synthesized chitosan derivative (mPEG-CS-OA), for delivery of camptothecin (CPT), a chemotherapeutic agent [1].

Experimental Methods

Methoxypolyethylene glycol (mPEG) and oleic acid (OA) was grafted on chitosan backbone by carbodiimide reactions, resulting in an amphiphilic copolymer with ability to produce micelles. Copolymer characterization included $^1$H NMR, FTIR, GPC, DSC/TGA, and XRD. Moreover, micelles were prepared by dialysis and characterized by the size, zeta potential and morphology (DLS, ELS, and TEM, respectively). The association efficiency of CPT was determined by HPLC. The cytocompatibility of empty micelles was study in PBMCs, Caco-2, HT29, HCT116 tumor intestinal cell lines as well was the anticancer efficacy of CPT-micelles. The intestinal permeability was studied on Caco-2 model and on a 3D intestinal model developed by our group [2]. The effect of micelles on the metabolic activity and tumor size reduction was studied on a CRC triple co-culture spheroid model [3] and the anticancer therapeutic potential on a HCT116 xenograft model.

Results and Discussion

While empty micelles presented a safe profile during 72 h of incubation with PBMCs, CPT-loaded micelles decreased their metabolic activity at the highest concentration (250 µg/mL). However, free CPT presented a significative reduction on the metabolic activity after 24 h, highlighting the advantage of micelles for CPT delivery to avoid off-target toxicity. Regarding the anticancer efficacy, CPT-loaded micelles were able to significantly decrease the cellular viability, more evidently after 48 and 72 h. Further, CPT-loaded micelles significantly improved CPT permeability across a 3D intestinal model about a 2-fold increase as compared to free drug. CPT-loaded micelles were able to significantly decrease the spheroids’ metabolic activity and their size, demonstrating an active effect on the reductions of an \textit{in vitro} ‘tumor-like structure’. Finally, pharmacodynamic studies using HCT116 xenograft animal model showed a tumor reduction of 45% as compared with the control group (without treatment) and an area under the curve (AUC) significantly lower as compared with all the tested groups (saline, empty micelles and free CPT).

Conclusion

Overall, this study suggests that CPT-loaded micelles improved the CPT intestinal permeability and may be a promising system for chemotherapy, capable of inhibit tumor progression \textit{in vivo}.

Acknowledgement

This work was financed by FCT - Fundação para a Ciência e a Tecnologia/ Ministério da Ciência, Tecnologia e Ensino Superior in the framework of the project “Institute for Research and Innovation in Health Sciences” (UID/BIM/04293/2019) Andreia Almeida (SFRH/BD/118721/2016) acknowledge Fundação para a Ciência e a Tecnologia (FCT), Portugal for financial support.
In vivo efficacy of CPT-loaded micelles

CBA nude mice injected with $1 \times 10^6$ HCT116 cells on the right flank were subcutaneously treated with CPT-loaded micelles, empty micelles, free CPT, CPT i.v. and saline. Animals treated with saline solution were used as control. (A) The values presented were normalized to each tumor values at the first treatment and represented the tumor growth during 30 days; (B) The AUC for all the treatments used, where CPT-loaded micelles presented a lower value; (C) Tumor volume after treatment finished and a 45% tumor reduction was observed for CPT-loaded micelles; (D) Survival rate of animals during 60 days.

References
11:30 a.m. – 1:00 p.m.

Track05


Chairs
Joëlle Amedee
University Bordeaux, Tissue bioengineering Laboratory, Bordeaux, FR
Pedro L. Granja
Instituto de Investigação e Inovação em Saúde (i3S) da Universidade do Porto, Porto, PT
Deyo Maeztu Redin (YSF)
École Nationale Supérieure des Mines de Paris, Paris, France

With Keynote lecture by Prof. Luigi Ambrosio in homage to Pr Charles Baquey – a great biomaterials scientist and friend of the ESB society, passed away at the end of December 2020, this symposium will present the relevant strategies for the development and the design of the future tissue engineered products according to the clinical requirements.
Nano-biocomposites: from tissue repair/regeneration to therapeutic behaviour

Luigi Ambrosio

National Research Council, Institute of Polymers, Composites and Biomaterials, Pozzuoli, IT

The implementation of a personalised therapy together a less invasive surgery for the restoration of human tissues is becoming an appropriate strategy to mitigate costs of the modern health care system and the maintenance of health and quality of life. The current challenge is to design material at the site of surgery with specific behaviours for mimicking the natural structures and delivering appropriate signals to cells promoting tissue repair/regeneration. Selection of a suitable injectable is often based on material characteristics (including mechanical properties, drug release kinetics and degradation) that serve for the specific treatment function. Micro or nano-structured materials in the form of gels, nanoparticles, nano-fibers and nano-composites have gained increasing interest in regenerative medicine because they are able to mimic the physical features of natural extracellular matrix (ECM) at the sub-micro and nano-scale levels. By a careful selection of materials and processing conditions it is possible to finely control characteristic shapes and sizes from micro to sub-micrometric scale and to incorporate bioactive molecules such as proteins or growth factor to develop active platforms to support the repair/regeneration of different tissues. It has been shown that the use of minimally invasive approach such as for bone cement to treat vertebral fracture has significant clinical potential. To achieve the ideal properties of bone filler efforts have been paid to optimize injectable calcium phosphate cements (CPC) which have been recognized as excellent alloplastic material for osseous augmentation/regeneration. The sol-gel synthesis approach appears to be the most suitable route towards performing injectable CPC. Furthermore, injectable hybrid material based on graphene oxide nanosheets and hydroxyapatite prepared by sol-gel approach is described. The presence of GO increases the bioactive and osteogenic properties of materials [1,2]. Within the class of 2D materials, exfoliated black Phosphorus is investigated to evaluate proliferation and the osteogenic differentiation of human pre-osteoblast cells (HOb) and mesenchymal stem (hMSC) cells. In vitro analysis demonstrated that 2D bP induced the proliferation and the osteogenic differentiation of human pre-osteoblast cells (HOb) and mesenchymal stem (hMSC) cells while inhibited the metabolic activity of osteosarcoma cells (SAOS-2) [3].

One more approach is based on the use of antimicrobial injectable materials [4]. It has been demonstrated that some imidazolium, pyridinium and quaternary ammonium ionic liquids (IL) have antimicrobial activity against some different clinically significant bacterial and fungal pathogens. Here, several systems based on IL at different alkyl-chain length incorporated in Hydroxyapatite (HA) through the sol-gel process to obtain an injectable material with simultaneous osteoinductive and antimicrobial behaviours.

Furthermore, as example of technology transfer activity, cellulose superabsorbent hydrogels are discussed as material intended to allow safe and effective weight loss and improvement of glycemic control in overweight and obese people [5]. The hydrogels are designed to mimic the structure and the viscoelastic properties of ingested dietary fibers, while being partially degraded in the last part of the gastrointestinal tract (colon).

Acknowledgement

This work was funded under the PNR Aging program 2012-2018, Project PRIN 2017SZ5WZB Action and Project PRIN 2017CBHCWF Sapient Ministero dell’Università e della Ricerca.

References


ESB-BIOMAT S-02

Mesenchymal stem cell response to disordered bioactive patterns

Yujie Zhang, Christel Chanseau, Emilie Prouve, Murielle Remy, Marie C. Durrieu

University of Bordeaux.fr, CBMN, 33400 Talence, BORDEAUX, FR

Introduction
Stem cells are a major focus in regenerative medicine since they promise to provide unlimited amounts of cells for transplantation. Stem cells within their natural niches in vivo maintain through the lifetime and retain the ability to serve regenerative purposes by making choices for survival, self-renewal, differentiation, quiescence, or apoptosis in a regulated manner. Stem cell behavior patterns are guided by the external signals that a stem cell receives from its local niche. Such cues include soluble growth factors and hormones, contacts from neighboring cells, and also cues from the local extracellular matrix (ECM)\(^1\). In vivo, the cellular microenvironment has a crucial impact on the regulation of cell behavior and functions such as cellular differentiation, proliferation, and migration. One of the challenges confronting cell biologists is to mimic this microenvironment in vitro in order to more efficiently study living cells and model diseases. Adhesion ligands (density, distribution of ligands, sizes of the bioactive patterns) and mechanical properties of extracellular matrix (ECM) play significant roles in directing mesenchymal stem cells (MSCs) behaviors. Patterning of ligands with high degrees of density and spatial control has become a powerful approach for applications in tissue engineering.

Experimental Methods
In this study, we investigate the effects of adhesion and differentiation ligand density and distribution on osteoblastic differentiation of MSCs. Polyethylene terephthalate (PET) substrates were used as a model material to geometrically defined regions of RGD + BMP-2 mimetic peptides. These ECM-derived ligands are under research for the regulation of mesenchymal stem cells’ osteogenic differentiation in a synergistic manner. Briefly, PET was hydrolyzed and oxidized in order to create carboxyl groups on the surface (labeled as “PET-COOH” (Fig.1)). Then, the surfaces were immersed in a solution of EDC (0.2M) + NHS (0.1M) + MES (0.1M) in MilliQ water for activation\(^3\).

Results and Discussion
A smart technique for micropatterning surfaces for cell growth support is described and characterized. This technique allows covering of 2D or 3D surfaces at low cost with controllable size and shape of micropatterns and with a very low time-consuming. This method takes advantage of the random properties of aerosols and the principles of liquid atomization. Parameters of interest were the pressure of atomization air, the flow rate and volume of the atomized liquid, and the distance between the spray nozzle and the surface of the sample. The experimental setup permitted to obtain diameters of spots between 0 μm and 100 μm microns with a maximum surface coverage of 30%.

Conclusion
Bioactive molecular patterns with different densities, different shapes, and sizes were obtained on the PET surface (Fig 1). Different surface coverages of bioactive peptides on PET were obtained(Table 1). Fluorescence microscope and image J were used to evaluate the density of biological peptides (BMP-2=3.1±1 pmol/mm\(^2\) and RGD=2.83±0.6 pmol/mm\(^2\)) (Fig 1e iii). When they were randomly co-distributed on the surface, they promote cell adhesion and osteogenic differentiation, respectively, and further enhance the osteogenic effect (Fig 1e-f). Further works include the investigation of different patterned surfaces (pattern size, surface coverage, pattern shape, and surface grafted with different peptides) impact on the differentiation yield of hMSCs toward osteoblast lineage.
Table 1. The distribution of spots obtained by spraying

<table>
<thead>
<tr>
<th>Air pressure (mbar)</th>
<th>Speed (μL/min)</th>
<th>Time (s)</th>
<th>0-1 μm (%)</th>
<th>1-10 μm (%)</th>
<th>10-20 μm (%)</th>
<th>20-50 μm (%)</th>
<th>50-100 μm (%)</th>
<th>100-200 μm (%)</th>
<th>200-500 μm (%)</th>
<th>500-1000 μm (%)</th>
<th>Average diameter (μm)</th>
<th>Spacing (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>300</td>
<td>12</td>
<td>14</td>
<td>66</td>
<td>13</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>7.6</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>9</td>
<td>9</td>
<td>70</td>
<td>22</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>8</td>
<td>11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The distribution of spots obtained by spraying

Figure 1. Schematic diagram of the project

a) virgin PET; b) Surface activation of COOH onto PET; c) Peptide immobilization onto PET using spray (1 bar; 300 μL/min; 12 s); d) The second peptide immobilization onto PET; e) Schematic representation of the impact of bioactive patterns on MSC differentiation into osteoblastic lineage

References


Nitric oxide-scavenging hydrogels as therapeutics for inhibiting angiogenesis in triple-negative breast cancer

Amir Abdo¹, Sharon Glynn², Pau Farràs³, Abhay Pandit¹

¹ National University of Ireland Galway, CÚRAM, SFI Research Centre for Medical Devices, Galway, IE; ² National University of Ireland Galway, Discipline of Pathology, Lambe Institute for Translational Medicine, School of Medicine, Galway, IE; ³ National University of Ireland Galway, School of Chemistry, Ryan Institute, Galway, IE

Introduction

Triple-negative breast cancer is a highly aggressive malignancy constituting around 10-20 % of breast cancers, with a limited number of treatment options [1]. Within the tumour microenvironment, nitric oxide (NO) orchestrates the angiogenesis and lymphangiogenesis processes, depending on its concentration, duration of exposure and the surrounding tissue [2,3]. More importantly, the surrounding cells' sensitivity to NO varies with its concentrations [2,3], making NO scavenging a potential strategy to bring in homeostasis in these cells. According to its molecular weight (MW), hyaluronic acid (HA) may have pro-or-anti-inflammatory roles in breast cancer. For instance, specific low MW-HA fragments are pro-inflammatory by inducing the production of specific inflammatory cytokines and proteases by tumour-associated macrophages [4]. The specific objectives of this study were: (i) to construct HA hydrogels loaded with different hemin conjugates; (ii) to test their NO-scavenging potential and (iii) to evaluate the cytotoxicity of the hydrogels and assess the downstream effects of NO-scavenging.

Experimental Methods

Hydrogel fabrication: Following the synthesis of several hemin derivatives proved to act as potential NO-scavengers, each type was loaded to 4-arm polyethylene glycol (PEG)-crosslinked HA hydrogel with investigating their physicochemical properties.

NO-scavenging potential: This was evaluated using NO-sensor and luminol/H₂O₂-luminescence reaction in phosphate buffer and culture media for choosing the formulations with optimum functionality and injectibility properties. In vitro testing: The hydrogels' cytotoxicity was tested by measuring the metabolic activity of the triple-negative breast cancer cell line (MDA-MB-231), human umbilical vein endothelial cells and microvascular endothelial cells using alamarBlue™ cell viability assay. Next, NO secreted intracellular levels from 300 μM Diethylenetriamine-NONOate (DETA-NONOate) were measured over 24 hours using Diaminofluorescein-FM diacetate and fluorescence microscopy following the cell culture with different hydrogels. Next, the effects of NO-scavenging by the hydrogels on cell migration were evaluated using wound healing assay and measuring the levels of the secreted Vascular Endothelial Growth Factor A and C using Enzyme-Linked Immunosorbent Assay (ELISA).

Results and Discussion

Hydrogel fabrication: The enhanced activation of HA chains for the crosslinking resulted in more activation of the loaded hemin/hemin conjugate alongside the formation of hydrogels with high storage modulus. This allows further covalent bonding of hemin with the cross-linker to be more protected within the hydrogel matrix. The loading of hemin was confirmed using SEM/EDX (fig. 1).

NO-scavenging potential: Some hydrogel formulations were proven to have different abilities to scavenge NO, depending on the degree of crosslinking within and the loaded compounds’ availability to react with NO (fig. 2a). Moreover, some formulations were able to protect the loaded compounds within the hydrogel matrix against the degrading effects of H₂O₂ (fig. 2b).

In-vitro testing: The hydrogel formulations characterized to be injectable, slowly degrading with the highest efficiency to bind NO, while loaded with the lowest concentrations of the different hemin compounds, were selected for further in vitro testing. Herein, specific formulations loaded with hemin or a hemin conjugate up-to 30 μM were
tested. The loaded hydrogels that maintained the cells' metabolic activity above 80% were chosen for further evaluation against the candidate hydrogels. Next, the intracellular NO was measured over 24 hours following the treatment of cells with DETA-NONOate and culturing with hydrogels loaded with 3.83, 7.66 or 15 μM. The in vitro experiments revealed that specific HA hydrogel formulations could protect the loaded NO-scavenging compounds without influencing their functions on inhibiting the cancer cell migration and quenching the intracellular NO levels while maintaining the injectability of the hydrogel.

**Conclusion**

Injectable hydrogels loaded with different hemin conjugates have been fabricated. The hydrogel formulation can be tuned to maintain the NO-scavenging efficiency by loading the lowest possible NO-scavenger concentrations, mechanical properties and degradability.

**Acknowledgement**

This work has emanated from research conducted with the financial support of Science Foundation Ireland and is co-funded under the European Regional Development Fund under Grant number 13/RC/2073_P2, the College of Engineering and Informatics, National University of Ireland, Galway, Ireland.

**References**


Allysine-mediated cross-linking for the development of elastin-like biomaterials

Tobias Hedtke1,2, Wolfgang Hoehenwarter3, Matthias Menzel1, Jürgen Brinckmann4, Thomas Groth2, Christian E.H. Schmelzer1

1 Fraunhofer Institute for Microstructure of Materials and Systems IMWS, Biological and Macromolecular Materials, Halle (Saale), DE; 2 Martin Luther University Halle-Wittenberg, Biomedical Materials Group, Institute of Pharmacy, Halle (Saale), DE; 3 Leibniz Institute of Plant Biochemistry, Proteome Analytics Research Group, Halle (Saale), DE; 4 University of Lübeck, Institute of Virology and Cell Biology, Lübeck, DE

Introduction

Elastin is a highly cross-linked biopolymer and the main component of elastic fibers in the extracellular matrix. Thus, it is essential for force-bearing connective tissues and organs including large blood vessels, tendons, skin and lung, which it provides with elasticity and resilience. The biosynthesis of elastic fibers is referred to as elastogenesis, a complex multi-step process, in which elastin is assembled from its monomeric precursor tropoelastin by covalent cross-linking involving lysine residues. This step requires their oxidative deamination to α-aminoadipic-δ-semialdehyde by lysyl oxidase (LOX) or LOX-like (LOXL) enzymes. Since elastin production dramatically drops in the early neonatal period and the biopolymer is not renewed throughout life, it is subject to degeneration associated with functional losses of the respective tissues. The development of close-to-native elastin-like biomaterials offers promising treatment options for severe pathological conditions. To comprehend the molecular structure behind its properties, we thoroughly investigated the crosslinking pattern of elastin. These findings helped us to develop elastin-like biomaterials based on its precursor by using enzymatic and non-enzymatic methods to induce allysine-mediated cross-linking in vitro.

Experimental Methods

Mature elastin was isolated from human aorta and skin as published by Schmelzer et al. [1]. Multi-enzyme digestion and subsequent HPLC-MS/MS analyses were used to study the cross-linking pattern on a molecular level using an untargeted bottom-up proteomics approach. Tropoelastin-based biomaterials were obtained by incubating recombinant human tropoelastin with lysyl-oxidase like 2 (LOXL2). Due to the limited availability of this enzyme, we further developed and applied an alternative non-enzymatic approach based on pyrroloquinoline quinone (PQQ). The resulting biomaterials were characterized by HPLC-MS/MS and amino acid analysis for cross-linking structures. Atomic force microscopy, scanning electron microscopy and enzymatic degradation assays were applied to study mechanical properties, microstructure and stability, respectively.

Results and Discussion

In human elastin, several bi- and tetrafucntional cross-linked peptides were identified and revealed an overall randomized cross-linking pattern with respect to the involvement of distinct cross-linking sites. This means that across single tropoelastin monomers distinct lysine residues were cross-linked by different cross-linking entities to different other lysine residues or were not cross-linked at all [2].

The cross-linking of recombinant tropoelastin by LOXL2 resulted in a stable elastic polymer with elastin-like mechanical properties. Cross-linking structures identified by mass spectrometry and a decrease of free lysine found by amino acid analysis indicate a successful transformation of lysine to allysine in vitro. Although the material showed elevated resistance against trypsin, the lysine modification rate was only 13% with respect to mature elastin [3]. PQQ, a soluble cofactor of bacterial oxidoreductases, comprises an ortho-quinone structure like the native cofactor of LOX/LOXL enzymes. The incubation of recombinant tropoelastin with PQQ and divalent copper in solution led to
the formation of a stable and trypsin-resistant biomaterial with a lysine modification rate of 56% with respect to mature elastin. The detection of free allysine and α-aminoadipic acid as well as cross-linked peptides indicate a high efficiency of lysine modification. Amino acid analysis and quantification of cross-linking amino acids gave proof to the PQQ-mediated activation of the cross-linking cascade known from mature elastin. The mean elastic modulus of the resulting biomaterial was in the range of 1-2 MPa and thus comparable to mature elastin.

**Conclusion**

PQQ is a well-suited alternative for the oxidative deamination and subsequent cross-linking of lysine residues for the fabrication of elastin-like biomaterials. With recombinant tropoelastin, a stable biomaterial with close-to-native mechanical properties and an elastin-like cross-linking pattern can be achieved. Beyond that, PQQ may also be considered as a promising alternative over toxic agents for the cross-linking of other protein-based biomaterials.

**Acknowledgement**

The work was supported by the Fraunhofer Internal Programs under Grant No. Attract 069-608203. Prof. Markus Pietzsch (Martin Luther University Halle-Wittenberg) is thanked for providing recombinant human tropoelastin. Dr. Laurent Muller (Collège de France) is thanked for providing recombinant human LOXL2. James McConnell (University of Manchester) is thanked for the assistance with atomic force microscopy and nanoindentation of LOXL2 cross-linked tropoelastin.

Amino acid analysis

This figure indicates the reduction of free lysine residues through the action of PQQ in cross-linked tropoelastin (cTE) when compared to untreated tropoelastin (TE). The highest modification rate with respect to mature human aortic elastin (hAE) was found at an incubation temperature of 50 °C.
The Young’s modulus of three cTE specimens and elastin isolated from bovine ligamentum nuchae were analyzed by atomic force microscopy. The mean modulus of cTE is between 1.0-1.9 MPa and therefore comparable to the Young’s modulus of elastin that was determined to be 1.8 ± 0.3 MPa.

References
Development of a curcumin loaded-NLCs hydrogel system for topical applications

**Rosa Calderon-Jacinto**, Pietro Matricardi, Violeta Rodriguez-Ruiz, Emmanuel Pauthe

1 CY Cergy-Paris University, ERRMECe Laboratory, Biomaterials for Health group, Maison Internationale de la Recherche I MAT, Neuville sur Oise, FR; 2 Sapienza University of Rome, Department of Drug Chemistry and Technologies, Rome, IT

**Introduction**

The balance of prooxidant-antioxidant reactions plays a crucial role in the regulation of skin physiological processes, especially during wound-healing. In standard conditions acute wounds heal in few weeks. However, an uncontrolled oxidative stress may hinder the normal wound-healing process by perpetuating tissue damage. Curcumin is a natural active ingredient known by its capacity to efficiently act at the wound site by controlling the toxicity of radicals and through the activation of cytoprotective signaling pathways in dermal cells, thus contributing to the regeneration of the skin barrier function [1]. However, the efficient topical application of active curcumin at cutaneous tissues is hindered by its low solubility in aqueous systems and the need to avoid its degradation (especially by heat and light) during its formulation.

In this work, Nanostructured Lipid Carriers (NLCs) [2] were developed as an encapsulating drug system aimed to prevent the drawbacks of curcumin stability and to favor its delivery to the skin. This would allow its topical application at the wound site by being incorporated into a dynamic hydrogel matrix.

**Experimental Methods**

NLCs were prepared by the hot homogenization method. Particle size and polydispersity index were determined by Dynamic Light Scattering. Zeta potential was calculated from the electrophoretic mobility obtained from Laser Doppler Micro-electrophoresis.

The amount of loaded curcumin was indirectly and directly determined by HPLC and UV-VIS spectrophotometry. In addition, the antioxidant properties of the formulation were assessed by the ABTS assay. The cytocompatibility of the developed nanocarriers towards human fibroblasts and keratinocytes was studied by the MTT assay and by the Trypan Blue staining method. Moreover, the amount of curcumin released from NLCs was evaluated at different time points after incubation at 37°C in culture media and in PBS.

After the incorporation of the system in a carbopol gel, the flow behaviour of the new composite system was analysed by rheology.

**Results and Discussion**

A stable NLCs formulation composed of negatively charged particles of an average size of about 300 nm was obtained. The curcumin encapsulation efficiency was more than 80% and the preservation of the antioxidant properties during the preparation process was demonstrated.

The viability of dermal cells involved in the wound-healing process was assessed after 24 hours of contact. Curcumin cytotoxicity was decreased while being encapsulated in the NLCs. Developed NLCs were found to be non toxic to fibroblasts and keratinocytes at concentrations below 0.54 mg/mL (containing 10 µM of curcumin) and 1.1 mg/mL (containing 20 µM), respectively. Furthermore, the formulation showed a prolonged curcumin release profile in biological media, reaching a maximum of 75 % in 72 hours.

Finally, NLCs were successfully incorporated into a carbopol hydrogel. Rheological analysis revealed that the mechanical properties of the gel matrix were not disturbed by the addition of NLCs.
Overall, our results show the feasibility of combining a natural compound-loaded NLCs and a hydrogel support into a multiscale platform in order to modify the release of the natural compound.

**Conclusion**

The developed system is highly compatible and promising for a topical application. Most of all, it preserves the antioxidant properties of curcumin and controls its release towards dermal cells. Such behavior has been demonstrated to be a key feature in order to achieve pro wound-healing effects [3]. Currently, the ability to promote cell migration and proliferation in *in-vitro* wound-healing tests is under assessment.

![Curcumin loaded NLCs/hydrogel system developed and its main characteristics](image)

**References**


On the development of new composite hydrogels mimicking vocal-fold histological and mechanical features

Daniel Ferri\textsuperscript{4}, Hamid Yousefi-Mashouf\textsuperscript{2,3}, Lucie Bailly\textsuperscript{2}, Laurent Orgéas\textsuperscript{2}, Jerome Sohier\textsuperscript{1}

\textsuperscript{1} CNRS, UMR 5305 - Laboratoire de biologie tissulaire et ingénierie thérapeutique, Lyon, FR; \textsuperscript{2} University Grenoble Alpes, CNRS, Grenoble INP, 3SR, Grenoble, FR; \textsuperscript{3} University Grenoble Alpes, CNRS, Grenoble INP, GIPSA-lab, Grenoble, FR; \textsuperscript{4} CNRS, UMR5510 MATEIS, Villeurbanne, FR

Introduction

Human vocal folds are soft laryngeal structures able to endure very large and reversible deformations (up to 50\% stretching), despite the stresses they are subjected to during voice production. These outstanding properties are related to their multi-layered structure (epithelium, lamina propria and vocalis muscle) and fibrous microstructures\textsuperscript{1}. Vocal folds can accommodate with many traumas, but severe or cancerous lesions require microsurgical intervention that may induce dysfunctional scarring lesions. Few biomaterials have been envisaged to treat vocal folds’ lesions, mainly because their unique mechanical properties are not clearly understood. A way to better comprehend these properties and pave the way to future clinical applications would be to mimic the fibrillar and elastomeric structure of vocal folds. In this respect, a tailorable mimetic hydrogel (poly-lysine dendrimers (DGL) and polyethylene glycol (PEG-NHS)) could be of interest\textsuperscript{2}. Its association with polycaprolactone (PCL) nanofibers could provide composites of modular mechanical and vibratory properties, close to native tissues. Thus, this work aims at (i) characterizing the organization of collagen and elastin of the human vocal folds, and (ii) reproducing it through the design and mechanical evaluation of different nanofibres/hydrogels composites (nFHC) with tuned hydrogel elasticity, nanofibrillar orientation and mono/multi-layered organization, (iii) comparing their mechanical response with those of native human tissues.

Experimental Methods

Microstructural organization of vocal folds excised from donated bodies was assessed with biphotonic microscopy for collagen and elastin fibres (Zeiss® LSM 780). PCL nanofibres of adjustable orientations (iso- and anisotropic) were processed using a jet-spraying method\textsuperscript{3}. Diameter distribution and orientation of collagen, elastin and PCL fibres (SEM FEI quanta) were analysed using Fiji®. Hydrogels of varying mechanical properties were obtained by mixing DGL (Colcom®) and PEG-NHS aqueous solutions of various concentrations, PEG molecular weights (2 k, 10 k or 40 kDa) and geometry. Different nFHC were prepared by percolation of hydrogel solutions through the nanofibers. Swelling of hydrogels and composites was measured over 48 h. Their mechanical properties were characterized in cyclic tension upon finite strains (BOSE® EF3200). Similar experiments carried out previously with Human vocal folds were used as reference data\textsuperscript{4}.

Results and Discussion

The microstructural analysis of human vocal folds revealed specific organizations of collagen and elastin fibres in the different anatomical layers. Epithelium was devoid of either, few were present in the muscle layer while the lamina propria was rich in both. Within the latter, both elastin and collagen wavy fibers were arranged in an entangled network of antero-posterior orientation, showing density gradients from superficial to deep layers (Fig 1A). Collagen fibers were thicker than elastin ones.

PCL nanofibers in the processed mats were of orientations and diameters similar to collagen and elastin fibers (Fig 1B). Thanks to the very high porosity of nanofibrillar networks (more than 96\%), nanofibers could be impregnated in a continuous DGL/PEG hydrogels matrix to create composites. (Fig 2A). Nanofibers incorporation reduced their swelling, revealing a coherent integration and the effect of stronger PCL fibres on composite properties (Fig 2B).
Mechanically, DGL/PEG hydrogels showed a quasi-linear reversible behavior with negligible residual strains and hysteresis (Fig 2C). Increasing the PEG/DGL ratio augmented elastic modulus (10 to 75kPa in PEG2k at 5% strain), while increase of $\text{PEG}_{\text{MW}}$ resulted in improved elongation at break (from 35% to <90%). Incorporating PCL nanofibers into the hydrogels systematically yielded to non-linear stress-strain responses characterized by a J-shape strain hardening, due to the progressive recruitment and/or reorientation of nanofibers during tension. A typical stress hysteresis was also observed with residual strains during unloading, potentially due to nanofiber rearrangements and plastic deformation. Therefore, through initial fiber orientation and hydrogel composition, it was possible to modulate the composites mechanical properties in terms of stress levels (up to 120 kPa at 10% peak strain), tangent moduli (up to 3 MPa), residual strains (up to 3.5%) and dissipated energy (up to 0.2 J/m$^3$). This parametric adjustability of the nFHC allowed us to conform with the corridor of human vocal folds mechanical properties (Fig 2C), indicating the potential of our approach.

**Conclusion**

Through the design of nFHC, it was possible to provide simple, efficient and highly versatile tools to mimic the structure and mechanical properties of human vocal folds, in view of modelling and better understanding their unique mechano-biological properties. Future work focuses on resulting vibro-mechanical properties of the composites.

**Acknowledgement**

The French ANR (N°17-CE19-0015-01) is acknowledged for financial support.

---

**Figure 1**

Representative picture of a human vocal fold lamina propria where collagen (blue) and elastin (yellow) are visualized respectively through second harmonic generation and autofluorescence, quantification of the fibres diameters and their orientations relative to the epithelium (A). Representative SEM pictures of isotropic and anisotropic PCL nanofibres, quantification of fibers diameters and their orientations (B).
**Figure 2**
Representative pictures DGL/PEG hydrogels/PCL nanofibers composites of homogeneous incorporation (A). Swelling ratio of hydrogels prepared with 2 and 10 kDa PEG at different concentrations (5 and 10 %) and their homogeneous composites obtained with iso- and anisotropic nanofibrillar PCL mats (B). Cyclic tensile properties of DGL/PEG hydrogels prepared with PEG of different molecular weight, shape and concentrations (left) and of homogeneous composites prepared with nanofibrillar PCL mats compared to human vocal folds samples (right) (C).

**References**


Development of 3D-printed hydrogel scaffolds for liver tissue engineering

Nathan Carpentier¹, Lana Van Damme¹, Louis Van der Meeren², Andre Skirtach², Lindsey Devisscher³, Hans Van Vlierberghe⁴, Peter Dubruel¹, Sandra Van Vlierberghe¹

¹ Ghent University, Polymer Chemistry & Biomaterials Group, Centre of Macromolecular Chemistry, Ghent, BE; ² Ghent University, Nano-biotechnology Laboratory, Department of Biotechnology, Faculty of Bioscience Engineering, Ghent, BE; ³ Ghent University, Gut-Liver Immunopharmacology Unit, Dpt Basic and Applied Medical Sciences, Liver Research Center Ghent, Ghent, BE; ⁴ Ghent University, Hepatology Research Unit, Dpt Internal Medicine and Paediatrics, Liver Research Center Ghent, Ghent, BE

Introduction

Annually, millions of people die because of liver failure, while the waiting duration for a donor liver is around 12 months.[1] Herein, we target hybrid indirect 3D-printed scaffolds to serve liver tissue engineering (LTE) applications. The starting materials gelatin and dextran were selected as mimics for the liver extracellular matrix (ECM) and crosslinked using two different chemical crosslinking approaches to explore their effect on the cell response. Methacrylated gelatin (GelMA) served as benchmark. The hydrogel materials were characterized on 2D- as well as on 3D-level.

HepG2 cells were used to assess the in vitro biocompatibility of the developed hydrogels.

Experimental Methods

Thiolated gelatin (GelSH)[2] was combined with norbornene-functionalized dextran (DexNB) followed by a radical step-growth thiol-NB crosslinking. In parallel, oxidized dextran with a degree of oxidation of 80 (Dexox80) and gelatin were crosslinked using a Schiff-base mechanism.

Both materials were characterized on 2D-level to assess the physico-chemical properties such as the swelling, gel fraction and mechanical properties. HepG2 cells were seeded onto 2D-disks to perform an in vitro biocompatibility study based on the metabolic activity using an MTS assay.

3D-hydrogel scaffolds of both materials were developed by indirect 3D-printing[3]. These scaffolds were characterized assessing their gel fraction, swelling ratio, compressive modulus and in vitro biocompatibility. The biocompatibility was assessed using HepG2 cells seeded onto these 3D-scaffolds and an MTS assay and live-dead staining using a fluorescence microscope were performed.

Results and Discussion

On a 2D-level, GelMA and DexNB-GelISH were superior over Dexox80-gel as their crosslinking kinetics were significantly faster and they mimicked natural liver tissue (NLT) to a greater extent with respect to swelling and mechanical properties. The swelling ratio of GelMA and DexNB-GelISH were respectively 9.1 ± 0.5 and 9.6 ± 0.5, which is in line with the swelling of NLT (i.e. 10)[4]. The swelling of Dexox80-gel was significantly higher (16.6 ± 0.9).

Atomic Force Microscopy (AFM) measurements revealed superior microscale mechanical properties of the DexNB-GelISH hydrogel sheets compared to the other materials. DexNB-GelISH exhibited a stiffness of (196 ± 24) kPa, dexox80-gel of (6 ± 5) kPa and GelMA of (291 ± 11) kPa. Healthy NLT exhibits an average surface stiffness of (183 ± 48) kPa. The higher the stiffness, the more the material mimics the ECM of unhealthy cirrhotic liver ((411 ± 63) kPa)[5].

Because of the inferior physico-chemical properties, dexox80-gel was not taken further beyond the 2D-level.

On a 3D-level, DexNB-GelISH scaffolds exhibited a compressive modulus of (4.8 ± 1.6) kPa which is in excellent agreement with that of NLT (i.e. 1 – 5kPa)[7] as compared to GelMA which resulted in a modulus of (8.5 ± 1.9) kPa.
However, the *in vitro* biocompatibility of both materials was comparable based on the MTS assay. The live-dead staining showed that the cells grew more into clusters on the DexNB-GelSH scaffolds compared to the more spread morphology which the cells exhibited on the GelMA material.

**Conclusion**

DexNB-GelSH scaffolds are promising hybrid materials to support LTE as they exhibit similar physico-chemical properties compared to NLT (cfr. microscale stiffness, compressive modulus and swelling ratio), while cell viability and proliferation of the hepatocytes were preserved. Further analyses evaluating the preservation of cell functionality using PCR are ongoing.

In future research, different coating compounds such as laminin and fibronectin will be applied to the scaffolds in order to investigate the effect on cell interactivity. Furthermore, the influence of replacing dextran with the more cell-interactive glycosaminoglycan chondroitin sulfate will be investigated.

**Acknowledgement**

Nathan Carpentier would like to acknowledge the Research Foundation Flanders (FWO) for providing him with an FWO-SB fellowship (3S99321N).

References

11:30 a.m. – 1:00 p.m.

Track06

N08 | Cell-Biomaterial Interactions

Chairs
Nicolas L’Heureux
University of Bordeaux, BioTis - Laboratory for the Bioengineering of Tissues, Bordeaux, FR
Maria Grazia Raucci
National Research Council, Institute of Polymers, Composites and Biomaterials (IPCB-CNR), Naples, IT
Arn Mignon (YSF)
KU Leuven, Engineering Technology, Group T, Leuven, BE
T cell cultures in 3D printed hydrogels for cancer immunotherapy

Eduardo Pérez del Río¹², Sergi Rey³, Jaume Veciana¹², Imma Ratera¹², Miguel Angel Mateos Timoneda⁴, Elisabeth Engel²³, Judith Guasch¹²

¹ Institute of Materials Science of Barcelona (ICMAB-CSIC), Bellaterra, ES; ² Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Bellaterra, ES; ³ Institute for Bioengineering of Catalonia (IBEC), Barcelona, ES; ⁴ Universitat Internacional de Catalunya, Sant Cugat del Valles, ES

Introduction
Adoptive cellular therapy (ACT), a type of personalized cancer immunotherapy, is achieving long-term remissions in late-stage and refractory cancers, especially in haematological malignancies and melanoma.[1, 2] Nevertheless, there are still several limitations, such as producing the large amounts of therapeutic T cells needed for these therapies in a short period of time and in an economically viable manner, which would then persist in the human body. Previously, we presented three-dimensional (3D) poly(ethylene) glycol (PEG) hydrogels covalently combined with heparin to resemble the lymph nodes,[3] as the current expansion systems do not mimic them. In these hydrogels, PEG provided the needed structural and mechanical properties to imitate the lymphatic tissue, whereas heparin was used as an anchor for the cytokine CCL21, which is present in the lymph nodes, and can affect cell migration and proliferation. The 3D structure of the hydrogel in combination with its loading capacity resulted in an increased primary human CD4+ T cell proliferation compared to the state-of-the-art expansion systems consisting of using artificial antigen presenting cells in suspension, i.e. without the presence of a 3D scaffold. Here, we introduce novel 3D printed hydrogels with the objective to improve 3D cell cultures.[4]

Experimental Methods
Hydrogels of previously functionalized heparin with maleimide and thiolated4-arm PEG were printed with a 3D bioprinter Regenhu 3D-Discovery (RegenHu, Switzerland). Then, the 3D printed hydrogels were loaded with a cytokine cocktail and used to culture primary human CD4+ T cells of healthy adult donors. Cell proliferation and differentiation were studied by CFSE stainings and anti-CD45RO/CD62L expression, respectively, through flow cytometry.

Results and Discussion
Initially, the printing process was optimized to demonstrate the capacity of the hydrogels, consisting of covalently linked PEG and heparin to be used as ink material. Thus, we fabricated 3D printed constructs of a few layers (Figure 1), and evaluated them as 3D scaffolds for T cell culturing. Firstly, T cell proliferation was evaluated in cultures of unloaded 3D printed hydrogels with different layers of biomaterial, showing that all of them promoted higher proliferation rates than the standard cell culture suspensions. Moreover, we observed that the higher the layer number of the printed hydrogels, the higher the proliferation achieved. In addition, T cell differentiation was analysed, and we observed that the 3D printed hydrogels promoted phenotypes associated with better clinical outcomes when using T cells to treat certain malignancies through ACT.[5] Additionally, the hydrogels with the highest number of layers were loaded with a cytokine cocktail, which is known to promote T cell homing and proliferation. In this case, the highest proliferation rates were obtained in the cultures that included immobilized cytokines on the 3D printed hydrogels.

Conclusion
We present a new bioink consisting of PEG and heparin covalently linked, that is suitable to produce 3D hydrogels for cell culture. In this case, we observed higher T cell proliferation rates with the 3D printed hydrogels compared to the state-of-the-art culturing conditions in suspension. These results were further improved by the addition of a
cytokine cocktail. Additionally, the 3D printed hydrogels promoted T cell phenotypes that are desirable for ACT in the clinics.

Acknowledgement
The authors are grateful for the financial support received from the Spanish Ministry of Economy and Competitiveness MINECO with the support of the European Regional Development Fund (FEDER) (MAT2016-80826-R, PID2019-105622RB-I00, RTI2018-096320-B-C21, and the “Ramón y Cajal” program (RYC-2017-22614)) and the Networking Research Center on Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN) through the projects “Alycia” (Nr. BBN18PI01) and “Gels4ACT” (Nr. BBN20PIV02). The work was funded as well by the Max Planck Society through the Max Planck Partner Group “Dynamic Biomimetics for Cancer Immunotherapy” in collaboration with the Max Planck for Medical Research (Heidelberg, Germany). IBEC acknowledges the European Commission-ERANET (nAngioderm JTC2018-103) and the Spanish network of cell therapy (TERCEL). ICMAB-CSIC and IBEC acknowledge support from the MINECO through the Severo Ochoa Programme for Centres of Excellence in R&D (SEV-2015-0496 and SEV-2019-0425).

References
Cartilage Tissue Engineering using Bioinspired Growth Factor Immobilization on Microfiber Scaffolds

Madison J. Ainsworth¹, Oliver Lotz²,³, Aaron Gilmour²,³, Mattie van Rijen¹, David R. McKenzie³, Marcela M.M. Bilek²,³, Jos Malda¹,⁴, Behnam Akhavan²,³, Miguel Castilho¹,⁵

¹ University Medical Center Utrecht, Orthopedics, Utrecht, NL; ² University of Sydney, School of Biomedical Engineering, Sydney, AU; ³ University of Sydney, School of Physics, Sydney, AU; ⁴ Utrecht University, Department of Clinical Sciences, Faculty of Veterinary Sciences, Utrecht, NL; ⁵ Eindhoven University of Technology, Department of Biomedical Engineering, Eindhoven, NL

Introduction

Osteoarthritis is one of the most common chronic diseases globally, with 10% and 13% of men and women affected, respectively [1]. Currently, there is no mechanically competent, biologically functional treatment for the end-stage cartilage degeneration it causes. In this study, we hypothesize that the fabrication of well-organized microfiber reinforcing scaffolds [2] with locally, covalently immobilized growth factors could support and guide the formation of new cartilaginous tissue. The addition of such biomolecular cues, particularly transforming growth factor beta 1 (TGFβ1), are crucial for the differentiation and maintenance of cartilage tissue [3]. To create a complex mechanical structure with the necessary biomolecular cues, we combined melt electrowriting (MEW) and atmospheric-pressure plasma jet (APPJ) treatment to produce well-organized microfiber scaffolds with selectively, covalently-immobilized TGFβ1.

Experimental Methods

Poly-ε-caprolactone melt electrowritten scaffolds were fabricated using a 3DDiscovey printer (regenHU), then functionalized using a computer-controlled APPJ device (4.5 kV discharge voltage, 1.9 L/min feed gas flow, 60 mm/s, 5 mm spaced-parallel line trajectory) [4], generating a controlled functionalization pattern (Figure 1i). TGFβ1 was then immobilized onto the MEW scaffolds using submersion in solution (0.01-2 µg/mL TGFβ1 in PBS, 24 hrs, 4°C). Detergent (Tween20/sodium dodecyl sulfate (SDS)) washing steps were undertaken to remove non-covalently bound protein molecules. Characterization of protein immobilization was performed using attenuated total reflection Fourier-transform Infrared (ATR-FTIR) spectroscopy and immunofluorescence detection. In vitro experiments were performed by seeding equine mesenchymal stromal cells (MSCs) (~16x10⁶ cells/mL) into the MEW scaffolds and were cultured for 28 days (Figure 1i). The culture groups consisted of (i) plasma-treated-scaffolds w/ immobilized TGFβ1, (ii) plasma-treated-scaffolds w/o TGFβ1, (iii) untreated-scaffolds w/ TGFβ1 in the culture medium, and (iv) untreated-scaffolds in basal medium. Neo-cartilage formation was quantified with dimethyl methylene blue/picogreen assays for glycosaminoglycan (GAG) production and confirmed with histological analysis.

Results and Discussion

Covalent immobilization of TGFβ1 was achieved using the APPJ-functionalization approach [4]. ATR-FTIR results confirmed a protein signature on the samples following intensive 5% SDS washing and immunofluorescently-labelled TGFβ1 was detected in microfiber scaffolds (following 0.1% Tween20 washing). In vitro analysis demonstrated that GAG production (DNA-normalized) was significantly enhanced in both the immobilized TGFβ1 (i) and TGFβ1 in medium groups (iii), compared to the control groups (ii & iv). This finding was further validated by the heightened production of GAGs and collagen type II, observed in histological sections (Figure 1ii). Additionally, this increase in matrix production was seen to become more pronounced as the concentration of immobilized TGFβ1 increased.

Conclusion
We have demonstrated that APPJ-facilitated covalent immobilization of TGFβ1 retains the growth factor's bioactivity and allows for cellular interactions that stimulate the differentiation of MSCs into the chondrogenic lineage. Our results also demonstrate that the new constructs with locally-immobilized TGFβ1 are able to support neo-cartilage formation. Furthermore, integrating MEW and plasma functionalization within a single printing platform will allow for functionalization during the MEW of microfiber scaffolds for gradient and patterned protein guidance for neo-cartilage formation.

Acknowledgement
This research was funded by the Netherlands Organization for Scientific Research (024.003.013), the EU’s H2020 Marie Skłodowska-Curie RESCUE co-fund (#801540), the Dutch Arthritis Association (LLP-12), the Australian Research Council and an Office of Global Engagement Partnership Collaboration Award between the University of Sydney and Utrecht University. O.L, A.G, B.A and MB gratefully acknowledge research support and facilities access from the School of Aerospace, Mechanical & Mechatronic Engineering, The University of Sydney, NSW, Australia; the Charles Perkins Centre, University of Sydney, NSW, Australia; and the Sydney Nano Institute, University of Sydney, NSW, Australia.

Figure 1
i) Schematic of methodology. The microfiber mesh is first produced using MEW and then functionalized using APPJ. TGFβ1 is then immobilized and MSCs are seeded and cultured for 28 days. ii) Immunohistochemistry collagen type II and safranin-0 stained paraffin sections from time points (TP) 1, 7, 11, 14 and 28. Scale bars = 200 µm.

References
Bridging the gap between the mechanical and metabolic activity in MSCs differentiation

Ana San Felix¹, Sara Trujillo², Matthew Dalby¹, Manuel Salmeron-Sanchez¹

¹ University of Glasgow, Glasgow, GB; ² Polytechnic University of Valencia, Centre for Biomaterials and Tissue Engineering, Valencia, ES

Introduction
Cells are in constant communication with their surroundings. Any stimulus coming from the extracellular matrix (ECM) is sensed by cells, having a direct impact in cell mechanical and metabolic activity [1]. This close relation between cells and the ECM is established by focal adhesions (FAs) through which cells sense the environment, exert forces, pull to deform the matrix and transmit these forces through the cytoskeleton[2]. We hypothesise that to create an adaptive response to ECM signals, cells undergo mechanical and metabolomic changes that lead to specific cell differentiation and traction force exertion [3]. This work aims to understand how cells test the environment from a mechanobiological perspective during their attachment, proliferation, migration and differentiation. This will provide a better understanding on how microenvironmental cues can be used to control cell fate, enhancing studies in multiple fields e.g. tissue regeneration.

Experimental Methods
The proposed system consists on a 2D full-length fibronectin (FN)-based polyethylene glycol (PEG) hydrogels model developed in our group [4]. This system allows to have full control of gel stiffness, varying the amount of PEG, and degradability, by adding protease-sensitive crosslinkers. For optimisation of the system, we used murine L929 fibroblast cultured for 24 hours. Then we started using mesenchymal stem cells (MSCs). To study the forces exerted by the cells we used traction force microscopy (TFM). In this technique the displacement of fluorescent microspheres embedded in the gels is tracked during cell attachment (fig.1 A-B). Using a plug-in of ImageJ these displacements were used to reconstruct the forces exerted (fig.1C) [5]. By adding a myosin II inhibitor (blebbistatin) we studied the implication of cytoskeletal tension in force generation. At the same time, we are using metabolomics to investigate cells metabolic activity during force exertion.

Results and Discussion
After 24 hours, cells pull more on degradable hydrogels compared to non-degradable ones when maintaining same bulk stiffness (8.1 kPa) (fig.2A). When we looked at force exertion on degradable gels with different stiffnesses (2.6 kPa, 8.1 kPa) for 24 hours, cells exerted higher forces on stiffer gels (8.1 kPa) (fig.2B). Also, cells spread more on stiffer surfaces compared with softer ones (fig. 2D). In all cases, when blebbistatin was added to the system, cells were unable to reach the same force magnitude as the control with no treatment (fig.2A-B). However, cells were still able to pull more on degradable gels compared with non-degradable ones (fig.2A). It was interesting to see that after 24 hours, YAP nuclear translocation was increased on stiffer surfaces (25. 7 kPa), even when myosin II was inhibited (fig.2C), what suggests that YAP might not be sensitive to blebbistatin. The metabolic profiles studied have shown different cell metabolic activity after 24 hours and 7 days on degradable gels with different stiffnesses (fig.2E). Cells showed higher metabolic activity in terms of metabolites production after 24 hours compared with time 0 and day 7, where similar metabolic activity was found. Within these first 24 hours, more metabolites were found on stiffer hydrogel. This pattern is not shown after 7 days, where higher metabolic activity was shown on softer surfaces (2.5 kPa).

Conclusion
Matrix degradability and stiffnesses have shown to have an effect in cell response. A direct evidence is through morphological changes, which indicate that further adaptive response might be taking place inside the cell. Furthermore, actin cytoskeleton contraction has great importance in force loading, as it has been shown after blebbistatin addition. Based on these results we can conclude that changes in the biophysical properties of the matrix alter how cells sense their surroundings and hence, their mechanobiological response and fate. Even though these results are promising, further studies need to be carried out to completely fulfill this gap in understanding how cell mechanics are linked to metabolic activity.

Acknowledgement
The authors acknowledge funding from EPSRC through their Scholarship program 2018.

Figure 1. Traction force microscopy
A) Proposed system set up. B) Beads displacement: beads after trypsinisation in red (gel relaxed)/beads before trypsinisation in green (gel stressed). C) Stress map obtained from traction forces applied by the cell.

Figure 2
A) Traction forces exerted by murine L929 fibroblast on 8.1 kPa degradable and non-degradable FN-PEG gels without (red) and with (grey) blebbistatin treatment. B) MSCs traction forces after 24h on degradable gels of different stiffnesses, without (red) and with (grey) blebbistatin treatment. C) MSCs YAP nucleus/cytoplasm location on degradable gels without (red) and with (grey) blebbistatin treatment. D) MSCs average area on degradable gels. E) MSCs aminoacids metabolism on degradable FN-PEG gels after 24 hours and 7 days.

References
Synergizing topographical and chemical cues to control mesenchymal stem cell adhesion, migration and differentiation on biomaterials

Joaquim Minguela, Luís Llanes, Maria Pau Ginebra, Joan Josep Roa, Carles Mas-Moruno

Technical University of Catalonia, Barcelona, ES

Introduction
Topographical and biochemical surface modifications stand out as powerful strategies to direct cell behavior and improve the bioactivity of biomaterials, aiming at achieving better levels of tissue integration [1]. However, the combination of both approaches has rarely been investigated.

Thus, in this communication we report for the first time the combination of micro-patterning and chemical functionalization on the surface of yttria-stabilized tetragonal zirconia polycrystal (Y-TZP), a biomaterial widely used in dentistry, to explore potential synergisms between topographical and biochemical cues and their effects on fine-tuning cell behavior.

In detail, Y-TZP surfaces were treated with ultrashort pulsed direct laser interference patterning (DLIP) with a femtosecond laser to produce linear micro-patterns. Laser patterned surfaces were further functionalized with a novel hybrid peptide containing cell adhesive and osteogenic potential [2].

Experimental Methods
DLIP with a femtosecond Ti:Sapphire laser was employed to produce two periodic grooved patterns with a periodicity of 3 and 10 μm. A platform containing the cell-adhesive RGD and the osteogenic DWIVA peptides was synthesized using standard solid-phase peptide synthesis (SPPS) methods [2] and used to functionalize the grooved surfaces. Topography and surface damage were characterized by confocal laser scanning (CLSM), scanning electron and scanning transmission electron microscopy techniques. Accelerated tests in water steam were carried out to assess hydrothermal degradation resistance. The attachment of the molecule was analyzed through fluorescence CLSM and X-ray photoelectron spectroscopy. Finally, the biological properties of the surfaces were studied with human mesenchymal stem cells (MSCs) evaluating cell adhesion, morphology, migration and differentiation.

Results and Discussion
A dual approach employing peptidic biofunctionalization and laser micro-patterns on dental zirconia was explored, with the aim of providing a flexible tool to improve tissue integration of restorations.

DLIP produced two periodic grooved patterns with a periodicity of 3 and 10 μm. The surface patterns exhibited a high homogeneity and subsurface damage was found in the form of nano-cracks and nano-pores, at the bottom of the valleys. Accelerated tests in water steam showed a slight decrease in hydrothermal degradation resistance; however, the detrimental effects of the laser modification were reverted by a post-laser thermal treatment. The attachment of the molecule was achieved via chemisorption using two units of L-3,4-dihydroxyphenylalanine (L-DOPA) as anchoring units and verified through fluorescence CLSM and X-ray photoelectron spectroscopy, which indicated a higher attachment of the molecules on the micro-patterned surfaces.

MSCs on grooved surfaces displayed an elongated morphology and aligned along the patterns (Figure 1). On these surfaces, migration was greatly enhanced along the grooves, but also highly restricted in the perpendicular direction as compared to flat specimens. After biofunctionalization, cell number and cell area increased and well-developed cell cytoskeletons were observed. However, no effects on cell migration were found for the peptidic platform. Although some osteogenic potential was found in specimens grooved with a periodicity of 10 μm, the largest effects were observed from the biomolecule, which favored upregulation of several genes related to osteoblastic differentiation in all the surfaces.

Page 456 of 2028
Conclusion

In order to improve the tissue integration potential of zirconia surfaces, this work explored the combination of topographical and biochemical modifications. USP-DLIP was successful at producing defined micro-patterns on the surface of zirconia at a low cost in terms of surface damage. The patterns were combined with a dual RGD/DWIVA peptidic platform. Thanks to the L-DOPA anchoring molecule, the peptide-based platform successfully attached to the surface of zirconia in a single step process, avoiding the use of toxic solvents. The effects derived from the two surface modifications could be combined. Notably, the presence of the peptidic platform did not mask the effects derived from topography. While the grooves were mainly associated with contact guidance (cell alignment, elongation and migration) the peptidic platform improved cell adhesion (cell number and spreading) and introduced a certain osteogenic potential to the surface. The combination of surface topographical features obtained by laser patterning and biofunctionalization with organic molecules provides thus a flexible tool to improve MSC functions on Y-TZP surfaces.

Acknowledgement

This work was supported by the Ministerio de Economía, Industria y Competitividad (MAT2017-83905-R, co-funded with FEDER), the Generalitat de Catalunya (2017 SGR-933 and SGR-1165) and the CREATe-Network Project, Horizon 2020 Program of the European Commission (RISE Project Nr. 644013). J. Minguela has been supported by a predoctoral fellowship from the Barcelona Research Center in Multiscale Science and Engineering of the UPC. M-P. Ginebra acknowledges the ICREA Academia Award and J.J. Roa the Serra Hunter program, both from the Generalitat de Catalunya. C. Mas-Moruno thanks the Spanish Government for a Ramon y Cajal grant.

Figure 1

MSC elongation analyzed by immunofluorescence on Y-TZP surfaces grooved with a periodicity of 10 μm.

References


Substrate-driven dynamics and biocues inducing the formation of human bone marrow mesenchymal stem cells spheroids

Valeria Perugini¹, Cheryl Collins¹, Sophia Khan², Matteo Santin¹

¹ University of Brighton, Centre for Regenerative Medicine and Devices/School of Applied Sciences, Brighton, GB;² Tissue Click Ltd, Brighton, GB

Introduction
In vivo, mesenchymal stem cells (MSCs) reside in what is known as the stem cell niche. They are preserved in this microenvironment in an undifferentiated state and prone to migrate towards tissues in response to signals such as growth factors and cytokines thus contributing to their remodelling or healing. It is recognised that the niche preserves the long-term maintenance of the MSC phenotype through the niche basement membrane and through cell-to-cell interactions [1]. However, the pathways of niche formation and MSC migration are not fully understood and it is worth suggesting that unveiling the interactions between the stem cell niche components and MSCs would be the first step towards the controlled handling of MSC in vitro as well as towards the development of suitable carriers for their transplantation.

Previous research has demonstrated the ability of PhenoDrive, a novel synthetic substrate mimicking the main features of the basement membrane, to induce the formation of human bone marrow MSC spheroidal structures similar to those observed in the MSC niche in vivo [2, 3].

The present study unveils, for the first time, the dynamics and biocues driving the migration of human bone marrow MSCs during the process of spheroid formation when cultivated on this basement membrane-mimicking substrate.

Experimental Methods
Phenodrive-Y (Tissue Click Ltd, UK) was used for the coating of tissue culture plates by reconstituting its powder in 75% ethanol at a concentration of 0.1mg/mL. The solution (200µL) was added to the wells of 24-well plates and left to cast in a sterile tissue culture hood overnight.

Frozen human bone marrow MSCs (Lonza), isolated from 2 donors (age: 19 and 24, gender: female and male), were resuspended in 1mL StemPro MSC SFM. Cells were drop-seeded at 4x10⁴/mL, at 37°C, 5% CO₂ and 90% humidity for 30 minutes to allow their adhesion to the substrates. Medium (1mL) was added to each well and MSC were monitored by time-lapse microscopy (Cytosmart, NL) to assess their migration patterns over 3 days with snapshots taken at an interval of 5 minutes. Each well was washed with 3x1mL Phosphate buffer saline (Sigma-Aldrich, UK). 1mL of 3.7% formaldehyde (Sigma-Aldrich,UK) was then added to each well for fixing and left in each well for 15 minutes at room temperature and washed with 3x3mL PBS (Fisher Scientific, UK). MSCs were stained for Integrin beta 1 and DAPI, by the addition of secondary Ab Alexa Fluora 488 and Alexa Fluora 555 (Fisher Scientific, UK). Cells were imaged using Leica SP5 confocal microscope at 20x with an additional 5x or 3x zoom on the control panel. Tissue culture plastic was used as control.

Results and Discussion
Time-lapse microscopy over 3 days showed the migration of MSC to form spheroids. These structures appeared to be in a constant phase of remodelling characterised by single cells joining a spheroid or leaving it to reach an adjacent one or bridging the merging of two spheroids into larger structures (Figures 1 a and b). This process appeared to be driven by a track-and-trace system led by fragments of cell membrane left deposited on to the substrate (Figure 2). These fragments were exploited by the migrating cells as well as by merging spheroids as adhesion cues through the anchoring of their philopodia (Figures 1 a and b, circles; Figure 2 black arrows). At the same time, the migrating cells showed an induced formation of extracellular vesicles by blebbing (Figure 2a, white arrow). These bi-rifrangent
structures were also found on the surface of PhenoDrive and in contact with the philopodia (Figures 2 a and be, black arrows). Immunostaining demonstrated the presence of integrins preferentially at the periphery of the spheroids and structured as clusters at the extremities of philopodia. Likewise, the released membrane vesicles appeared to be positive to integrin immunostaining confirming their plasmalemma origin and suggesting the hypothesis that they can enable the anchorage of these extracellular vesicles onto the substrate (data not shown). This dynamic process was gradually reduced with time as the spheroids became more established and distant from each other.

MSCs seeded on control tissue culture plastic formed typical fibroblast-like colony units where no significant migration process was observed (data not shown).

**Conclusion**

The data of this study show that the biomimetic substrate PhenoDrive, presenting both a nano-topography and bioligands similar to those of the stem cell niche basement membrane, is able to activate a process of migration of MSC through a track-and-trace system based on plasmalemma blebbing that leads to the formation of 3D cellular structures similar to those observed in the MSC niche in vivo. The unveiling of such pathways of morphogenesis also paves the way towards a controlled handling of MSC in vitro as well as towards the development of carriers for their transplantation in clinics.

**Acknowledgement**

This work has been partially funded by the EC Horizon 2020 project, REFINE, contract n. 761104 and by Ms Sophia Khan's MRes.

**References**


**N08-06**

**The effect of magnesium on protein adsorption in biomaterials for bone tissue regeneration**

Andreia Cerqueira¹, Francisco R. Gavilán¹, Iñaki G. Arnáez², Mikel Azkargorta³, Félix Elortza³, Mariló Gurruchaga², Isabel Goñi², Julio Suay¹

¹ Universitat Jaume I, Department of Industrial Systems Engineering and Design, Castellón de la Plana, ES; ² Universidad del País Vasco, Departament of Science and Technology of Polymers, San Sebastián, ES; ³ Basque Research and Technology Alliance (BRTA), CIBERehd, ProteoRed-ISCIII, Proteomics Platform, CIC bioGUNE, Derio, ES

**Introduction**

Magnesium (Mg) is the fourth most abundant element in the human body with important functions in mineral metabolism and osteoblast/osteoclast regulation. For bone substitution, titanium (Ti) is the most widely used material; however, its surface is relatively bioinert. Bioactive degradable coatings present an interesting possibility when added to Ti surfaces to stimulate early regeneration processes. In this way, coatings produced by the sol-gel technique allow the obtainment of materials with controlled release of biomolecules or ions with different biological functions already known. When applied to biomaterials, Mg ion can increase cell adhesion, reduce inflammation, and increase the osteogenic response. Upon implementation, protein adsorption is the first phenomenon to occur. This is dependent on several factors, such as material surface proprieties (e.g. ion incorporation), and it will affect the following biological response. Considering this, the purpose of this study was to develop Mg-doped coatings to be applied on Ti surfaces, characterize the protein layer formed upon exposure to human serum and correlate it with *in vitro* cell responses. The results showed a higher absorption of proteins related to cell adhesion, inflammatory regulation, and bone regeneration on the Mg coatings. It was possible to establish a correlation with *in vitro* results that showed an anti-inflammatory potential as well as an increment in the osteogenic potential and cell adhesion.

**Experimental Methods**

The coatings were obtained employing the sol-gel route using the precursors MTMOS and TEOS (molar ratio of 7:3) and increasing MgCl₂ concentrations (0, 0.5, 1, and 1.5%). The sol-gels were applied to grade 4-Ti substrates (10 mm diameter, 1-mm thick) by dip-coating. The materials were characterized with FT-IR, ²⁹Si-NMR, XRD, and SEM.

The coating degradation was studied by mass loss and the amount of Mg²⁺ released was measured by ICP. Cell adhesion, ALP activity, and gene expression (ALP, TGF-β, OSX, RUNX2, ITGA5, and ITGB1) were measured in MC3T3-E1 osteoblastic cells to evaluate the osteogenic potential. Gene expression (IL-6, TNF-α, TGF-β, and IL-4) and ELISA (TNF-α and TGF-β) to RAW264.7 macrophages were used to understand the inflammatory potential. Surface protein adsorption was evaluated after incubation with human serum for 3h and characterize by nLC-MS/MS.

**Results and Discussion**

The materials were successfully synthesized and the incorporation of Mg did not affect the crosslinking of the sol-gel network and MgCl₂ did not precipitate and formed crystalline structures, indicating that Mg ions are trapped through hydrogen bonding, Van der Waals, or electrostatic forces. However, the material did not present adverse effects on the cells. A controlled release of Mg was observed and as more MgCl₂ was incorporated into the sol-gel network, more the coating degraded, and more Mg²⁺ was liberated. The Mg-doped coatings preferentially adsorbed proteins related to inflammatory responses, cell adhesion, tissue regeneration, and coagulation. In what concerns the inflammatory responses, the reduction in TNF α secretion and the increase in TGF-β and IL-4 gene expression indicates the materials the anti-inflammatory potential of the materials. This was consistent with the increased adsorption of immune-system regulatory proteins (CLUS, CFAH, IC1, and VNTC). Moreover, the Mg-doped materials
showed an increased affinity to the proteins related to cell adhesion (DESP, FILA2, and DSG1) and VTNC, which is correlated with the increment in cell surface area and rearrangement of the cytoskeleton arrangement, and gene expression of integrins (ITGA5 and ITGB1). For osteogenic responses, ALP activity as well as TGF-β, OSX, and RUNX2 gene expression were augmented in Mg-doped materials. Additionally, the preferential adsorption of proteins related to tissue regeneration (CYTA and VTNC) indicates the regenerative potential of these materials. These results allow us to hypothesize that the effects observed on the materials with Mg can not only be dependent on the ion itself as well as in the proteins adsorbed onto the surface.

**Conclusion**
The overall results obtained show that the Mg-doped coatings present anti-inflammatory properties as well as increased cell adhesion and osteogenic responses. It was possible to establish a correlation between the proteins adsorbed onto the materials with the *in vitro* cell responses. The Mg-doped coatings adsorbed more proteins related to inflammatory response regulation, cell adhesion, and tissue regeneration, while decreased the cell inflammatory response and promoted osteogenesis and cell adhesion. Further studies are needed to better understand the way the adsorbed proteins affect well-known responses of materials with Mg.

**Acknowledgement**
This work was supported by MINECO [MAT2017-86043-R; RTC-2017-6147-1], Generalitat Valenciana [GRISOLIAP/2018/091, APOSTD/2020/036, PROMETEO/2020/069], Universitat Jaume I under [UJI-B2017-37, Posdoc/2019/28], the University of the Basque Country under [GIU18/189] and Basque Government under [PRE_2017_2_0044].

**References**
Active biomaterials for the ex-vivo expansion of Hematopoietic Stem Cells: from biointerfaces to functionalised hydrogels

Michaela Petaroudi, Aleixandre Rodrigo-Navarro, Matthew J. Dalby, Manuel Salmeron-Sanchez

University of Glasgow, Centre for the Cellular Microenvironment, Glasgow, GB

Introduction
Hematopoietic Stem Cells (HSCs) constitute a rare cellular population residing in the bone marrow (BM) and have recently gained traction in research due to their significant clinical potential. These multipotent, self-renewing cells have the unique capacity to regenerate the whole hematopoietic system in the event of hematological disorders. This particular property has placed HSCs in the spotlight of experimental hematology, making ex-vivo HSC expansion a significant challenge for the research community. Such an achievement would produce clinically relevant numbers from a small sample of cells that could be used for BM transplantation and other clinical applications. Inspired by the huge clinical significance of HSC transplants, we aim to develop a synthetic platform to support their ex-vivo expansion. To achieve this, our system is designed to mimic both the Mesenchymal Stem Cell (MSC) and HSC compartments of the BM by providing the soluble and mechanical cues to maintain MSCs in a naïve state in long term cultures and support HSC expansion ex-vivo. Our system is based on a biointerface between human MSCs, HSCs, the non-pathogenic genetically engineered bacterial species Lactococcus lactis (L. lactis)¹ NZ9020, and synthetic polyethylene glycol (PEG) hydrogels that can be used in different setups as shown in figure 1. This platform mimics the chemical and mechanical properties of the bone marrow by providing both stem cell types with soluble and adhesion recombinant proteins produced by the bacteria, and structural support presented by the hydrogels. We report a notable HSC expansion in co-cultures with the biofilms and inside the hydrogels. We also show that the biofilms can retain the MSCs in a stem-like, BM-resembling state. Our final goal is to encapsulate the HSCs in the hydrogels and culture them in contact with the biofilm and MSCs to provide a close bone marrow analogue.

Experimental Methods
We genetically engineered non-pathogenic bacterial biofilms (Lactococcus lactis)¹, to produce key human BM niche factors (CXCL12, TPO, VCAM1, and the FN fragment III7−10)². Bacterial engineering was conducted using PCR and Gibson Assembly and was based on the pT2NX plasmid. We also fabricated synthetic PEG hydrogels, crosslinked with Laminin and Fibronectin to mimic the mechanical and architectural properties of the BM. MSCs and HSCs were cultured directly on the biofilms or in hydrogels for phenotype studies. MSC phenotype evaluation was conducted after 14 days of culture on the biofilms and was assessed by staining for Nestin, Stro1, ALCAM, Osteopontin, and Osterix using In Cell Western analysis. The expanded HSC population was measured after 5 days of co-cultures with the bacteria and was compared to the initially seeded number of HSCs, using the HSC markers CD34, CD38 and CD90, by flow cytometry analysis.

Results and Discussion
Our co-cultures of the biointerface and MSCs and HSCs with and without the presence of hydrogels showed no negative effect of the biofilm on either stem cell viability. Our data shows that MSCs cultured on different biofilms retain a naive, BM-resembling phenotype after 14 days of culture with and without the use of hydrogels. At the same time, we show no MSC commitment towards osteogenic differentiation, suggesting that our platform can be used to maintain MSCs in a stem-like state in long-term cultures. Furthermore, our HSC population analysis suggests that the L. lactis-CD34+ cell co-culture system supports HSC phenotype maintenance and can achieve up to 19-fold HSC expansion in 2D experiments compared to Day 0. Finally, we show that the biophysical properties of the hydrogels

Page 462 of 2028
can be tuned to maintain and encourage HSC proliferation. Combined, these results suggest that the bacteria can maintain MSCs in a BM-like state, while also providing HSCs with the necessary signals for their maintenance and expansion. Additionally, the hydrogels are shown to contribute to HSC maintenance. Together, these platforms could be combined to create a representative BM analogue, with bacteria and MSCs supporting HSC maintenance and proliferation, and the hydrogels adding mechanical support. To our knowledge, this is the first attempt to combine a living biointerface and mechanical support provided by hydrogels for BM and HSC niche engineering.

**Conclusion**

Our data suggest that this novel system has a promising potential to control MSC behaviour and induce HSC expansion *ex-vivo*. The tunable growth factor-producing biointerface, MSC layer and hydrogels can be tailored to produce a close analogue of the BM and more accurately mimic the native conditions that support HSC maintenance and expansion.

**Acknowledgement**

This research was supported by UKRI. The authors would like to thank Dr. Oana Dobre for her invaluable contribution to sharing her expertise on the fabrication of the hydrogels used in this work.

**Figure 1.** Overview of the system and the various platforms we can create for different applications. *L. lactis* NZ9002 biofilms have been engineered to produce recombinant CXCL12, TPO, VCAM1 and FN. Human MSCs and HSCs can be seeded on top of the biofilm in order to study changes or maintenance of their phenotype. We aim to closely mimic the BM conditions by encapsulating the HSCs in hydrogels and ultimately add an MSC feeder layer to create a representative BM analogue that presents the appropriate biochemical and mechanical stimuli in order to induce HSC maintenance and expansion.

**References**


N08-08

Engineering ligand mobility in the adhesive crosstalk to control stem cell differentiation

Eva Barcelona-Estaje¹, Matthew J. Dalby¹, Pere Roca-Cusachs², Marco Cantini¹, Manuel Salmeron-Sanchez¹

¹ University of Glasgow, Glasgow, GB; ² Institute for Bioengineering of Catalonia, Barcelona, ES

Introduction

Cell behaviour is influenced by mechanochemical signals, which are largely mediated by interactions with the extracellular matrix (ECM) via integrins, and by interactions between cells through cadherins. Both of these receptors are in contact with the actin cytoskeleton and act as mechanosensors; they also share multiple signalling pathways that ultimately regulate the so called ‘adhesive crosstalk’ and cell fate (1, 2). Due to the mechanosensitivity of these molecules, cells sense the mechanical properties of their surroundings, responding not only to the elasticity of the substrate, but also to its viscosity. Indeed, while viscous interactions have been traditionally ignored, we previously demonstrated that cells actually sense viscosity using the same integrin-mediated mechanotransductive mechanism that they use to sense stiffness (3).

In this work, we address the role of surface viscosity in stem cell fate and, in particular, how it affects the adhesive crosstalk between integrins (RGD receptors) and cadherins (HAVDI-containing proteins) to elucidate stem cell mechanosensing of viscosity. To do this, we use supported lipid bilayers (SLBs) with varying ligand mobility, functionalised with RGD and HAVDI peptides (Figure 1A).

Experimental Methods

Supported lipid bilayers (SLBs) with varying viscosities (Figure 1a) were prepared by following the vesicle fusion method using either DOPC, which presents a more mobile fluid phase in cell culture conditions, or DPPC, which forms a more viscous gel phase bilayer. Functionalised glass was used as a non-mobile control surface. All the surfaces were functionalised with different ratios of RGD and HAVDI.

Human mesenchymal stem cells (hMSCs) were then seeded on these surfaces and parameters such as cell adhesion, protein translocation or expression of transcription factors were investigated via AFM, immunostaining and in-cell western.

Results and Discussion

When SLBs are functionalized with RGD, an increase in cell area and cell adhesion, in particular the strength of detachment of MSCs to the bilayer, are observed with higher viscosities and concentrations of RGD. Also, longer focal adhesions and decreased actin flow are observed when cells are seeded on the higher viscous surfaces with 2% of RGD. On the other hand, when HAVDI is included in the bilayers, cell spreading and strength of adhesion (Figure 1B) are reduced, compared with surfaces that only contain RGD, and actin flow increases (Figure 1C) in an HAVDI concentration dependent manner. Also, when the bilayers present HAVDI, there is a decrease in nuclear translocation of mechanosensitive proteins (i.e. YAP) are observed, showing an altered sensing of viscosity (Figure 1D).

This interplay between viscosity and ligand presentation provokes not only differences in adhesion and mechanosensing, but also in the expression of nuclear lamina and small GTPases activity, which inhibition has shown a differential implication of N-cadherin adhesion in Rac1 activity depending on the viscosity. Finally, differences in early differentiation markers (Figure 1E to G) confirming the effect of viscosity on cell fate. Osteogenesis increases with higher viscosities and concentration of RGD. On the other hand, adipogenesis increases as the mobility of the
substrate does, while chondrogenesis is enhanced on mobile surfaces and with higher (2\%) concentrations of the N-Cadherin adhesion peptide.

**Conclusion**

Our findings reveal that viscosity elicits a mechanotransductive response that ultimately controls stem cell differentiation. Moreover, when cell-cell interactions are activated, hMSCs have a different perception of the mechanical properties of their surroundings, presenting a low viscosity phenotype (i.e. rounder cells, less force of adhesion, higher actin flow or lower YAP nuclear translocation) compared to when only ECM-cell interactions are present. Also, inhibition of small GTPases showed their implication in the regulation of the adhesive crosstalk depending of viscosity. Further investigations on the mechanisms involved in this response will allow us to establish a paradigm to understand and exploit stem cell response to viscous interactions.

**Acknowledgement**

The authors acknowledge funding from EPSRC (EP/P001114/1) and MRC (MR/S005412/1). This work was also funded by a grant from the UK Regenerative Medicine Platform (MR/S005412/1).

References

Three-dimensional ECM-based in vitro model of the liver

Giuseppe Guagliano\(^1\), Manuela Legnardi\(^1\), Lorenzo Sardelli\(^1\), Silvia Dotti\(^2\), Riccardo Villa\(^2\), Livia Visai\(^3,4\), Paola Petrini\(^1\)

\(^1\) Politecnico di Milano, Department of Chemistry, Materials, and Chemical Engineering "G. Natta", Milan, IT; \(^2\) Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna, National Reference Center for Alternative Methods, Welfare and Care of Laboratory Animals, Brescia, IT; \(^3\) University of Pavia, Molecular Medicine Department (DMM), Center for Health Technologies (CHT), UdR INSTM, Pavia, IT; \(^4\) ICS Maugeri, IRCCS, Medicina Clinica-Specialistica, UOR5 LABORATORIO DI NANOTECNOLOGIE, Pavia, IT

Introduction

Liver is the largest organ of the human body; it fulfills hundreds of viable functions, and could be considered as a major crossroad of human physiology. In vitro modelling the different features of this organ has always been considered crucial for investigating both physiological and pathological processes, as well as to support drug development. Structural and functional complexity of the liver, however, represented major boundaries to the realization of accurate models. Recent advantages in biomaterials science, together with the development of new processing techniques allowed to improve the outcomes in this challenge. The availability of tools enabling to grow even different cell types within the same 3D matrix, mimicking their natural microenvironment represents a step forward if compared to classical 2D cell cultures. Alginate is commonly exploited to encapsulate cells in a tailorable 3D environment [1]. Moreover, decellularized liver are commonly used as scaffold to be recellularized, or enzymatically degraded into a viscous solution to encapsulate cells [2]. Decellularized liver-ECM was previously introduced in alginate beads to specifically encapsulate HCCLM3 cells, showing enhanced viability and protein expression if compared to alginate alone [3]. In light of this, this work presents the development of a 3D in vitro model based on liver ECM and alginate to support hepatocytes growth and activity while tuning the rheological properties to meet the ones of human liver.

Experimental Methods

ECM was obtained from porcine (pECM) and bovine (bECM) livers, by a combination of different methods [4]. The decellularization buffer (TRIS-HCl buffer + 1% w/v SDS + 1% v/v Triton X100) was injected in multiple sites of 0.5 cm cubes of liver and then used to submerge the samples while under orbital shaking up to 7 days. Freeze-dried decellularized cubes were grinded into powder of controlled granulometry. Residual immunogenic content after decellularization was quantified through DNeasy Blood and Tissue Kit. Both pECM and bECM protein compounds were characterized through ELISA. Four different alginate hydrogels were produced exploiting internal gelation: alginate 2% w/v, alginate 2% w/v + gelatin 0.8% w/v, alginate 2% w/v + pECM 0.8% w/v, and alginate 2% w/v + bECM 0.8% w/v. Each solution or suspension was obtained in complete medium (EMEM + 10% v/v FBS + 1% v/v P+S + 1% v/v Na pyruvate + 1% v/v L-Gln). Hydrogels were dried in order to determine their water content. Stability was tested in culture conditions (37 °C; 95% humidity; 5% CO\(_2\)) up to 14 days. Each hydrogel was then subjected to rheological analyses. For cell-loaded hydrogels, HepG2 cell suspension was added to the gel-precursor solution, to obtain a final concentration of 2 x 10\(^6\) cells/ml; after cross-linking, gels were submerged with complete medium and incubated in culture condition. After 24, 48, and 96 hours, gels were dissolved using Na-citrate, cells were retrieved, seeded, and tested with MTT assay. Gels were stained with live/dead kit, and subsequently observed at the confocal microscope (CLSM) after 24, 48, and 96 hours of culture.

Results and Discussion

Agarose gel electrophoresis confirmed the presence of a negligible amount of residual DNA in both pECM and bECM, thus validating the decellularization procedure. Alginate allowed to tailor the cross-linking; it has been possible to Page 466 of 2028
easily tune the reagents amount, thus obtaining rheological characteristics reproducing the ones of physiological liver tissue. Additionally, another set of rheological tests has been applied to evaluate the processability of hydrogels via 3D-bioprinting. The possibility to retrieve the cells from the reversible 3D matrix, even after long culture periods, using a nontoxic Ca\(^{2+}\) ions chelator allowed to quantify cell metabolic activity by MTT assay. An improvement of the viability through time resulted in both gels containing pECM and bECM, if compared to other hydrogels. CLSM analyses with live/dead kit not only confirmed the results of MTT assay, but even allowed to observe 3D clusters, similar to those observed in vivo [5].

Conclusion
This study demonstrated the possibility to synergically combine the chemical features of liver-derived ECM with the structural characteristic of an alginate hydrogel, mimicking the in vivo liver microenvironment. The presence of ECM positively impacted cell viability. Ongoing studies will include the bioprinting of the model, aiming to accelerate and standardize its production.

Acknowledgement
Research project funded by the Italian Ministry of Health (Prot. n. 0025345 del 04/12/2020).

References
11:30 a.m. – 1:00 p.m.

Track07

N09 | Biomaterials for Orthopedic Applications III

Chairs
Matteo D'Este
AO Research Institute Davos, Regenerative Orthopedics, Davos Platz, CH
Manuela Gomes
3B's Research Group-University of Minho, I3Bs Research Institute, Guimaraes, PT
Manon Minsart (YSF)
Ghent University, Ghent, BE
Individual, 3D printed CPC scaffolds in maxillofacial surgery – a case study

David Muallah¹, Philipp Sembdner², Stefan Holtzhausen², André Hutsky³, Daniel Ellmann³, Antje Schönberg⁴, Heike Meissner⁴, Matthias C. Schulz⁶, Günter Lauer¹, Lysann M. Kroschwald¹,⁷

¹ Technische Universität Dresden, Department of Oral and Maxillofacial Surgery, Dresden, DE; ² Technische Universität Dresden, Department of Mechanical Engineering, Institute of Machine Elements and Machine Design, Dresden, DE; ³ Organical CAD/CAM, Berlin, DE; ⁴ Zahntechnik Schönberg, Dresden, DE; ⁵ Technische Universität Dresden, Department of Prosthetic Dentistry, Dresden, DE; ⁶ Eberhard Karls Universität Tübingen, Department of Oral and Maxillofacial Surgery, Tübingen, DE; ⁷ Technische Universität Dresden, Centre for Translational Bone, Joint and Soft Tissue Research, Dresden, DE

Introduction
An increasing number of patients opt for dental implants to replace missing teeth. The success of dental implants depends on the quality and quantity of the patient’s bone. Due to long-term edentulism, periodontal disease or trauma, bone augmentation may be necessary before implant insertion. Therefore, autologous bone is considered as the “gold standard” but requires harvesting from a second surgical site (1). Alternatively, synthetic bone substitute materials such as calcium phosphate cement (CPC) are used. CPC is 3D-printable and allows manufacturing of patient-specific structures with varying pore size and geometry (2,3). The scaffold’s pores allow blood, bone and stem cells to migrate and thereby, to integrate the scaffold into the adjacent tissue. Furthermore, scaffolds in maxillofacial surgery have to withstand high forces during the chewing process (4, 5). Thus, their mechanical properties are of high clinical importance. In the present study, we investigated individual, 3D printed CPC scaffolds with different pore sizes and geometry regarding cell infiltration and mechanical properties in vitro. Based on the in vitro findings, we performed bone augmentation in 6 clinical cases (see figure 1). Our findings show the importance of the external and internal structure for individual scaffolds in maxillofacial surgery to fit all patient specific necessities and ensure stability and cell infiltration.

Experimental Methods
Shape and macro porosity of the printed scaffolds were studied by stereo microscopic investigation and scanning electron microscopy (SEM) analysis. Mechanical characterization was done via uniaxial compressive test. Compressive modulus and compressive strength were calculated. For studying the colonization, scaffolds were seeded with hMSC, covered with culture medium and incubated for up to 12 weeks. Live/Dead staining was performed. For the determination of the cell number DNA quantification and LDH activity were evaluated. Furthermore, fluorescence microscopic analyses of colonized scaffolds were performed. Hereinafter, bone augmentation surgery was performed. During the implant bed preparation, biopsies of the augmented tissue were collected to examine the scaffold’s state of degradation.

Results and Discussion
Energy absorption of the six different reference scaffold types is greatly reduced with increasing pore size. This intense decrease is also shown by compressive strength (see figure 2A). Nevertheless, all investigated scaffolds, independently from pore size, showed a higher energy absorption and strength in comparison to the control group (BioOss®). After seeding the scaffolds with hMSCs and incubation for up to 12 weeks, CPC strands were completely covered after 4 weeks. After 12 weeks, the cell number of scaffolds with lower pore size was significantly lower in comparison to the control and scaffolds with higher pore sizes (see figure 2B). According to these results individual scaffolds were printed with the approved pore size of 0.49 mm and a strand distance of 0.82 mm. Energy absorption of all investigated individual scaffolds, independently from shape, showed also a higher energy absorption and...
strength in comparison to the control group (BioOss®). Based on these data, individual CPC scaffolds (see figure 2C) were inserted in 6 different clinical cases (sinus floor elevation and onlay osteoplasty). After the healing period of 9 months, dental implants were inserted. After another 6 months, prosthetic treatment was supplied.

**Conclusion**

As the pore size strongly affects the migration of cells and the mechanical properties of the scaffolds, we demonstrated *in vitro* that scaffolds with a pore size of more than 490 µm could not meet the mechanical requirements of certain cases in the orofacial system (e.g. osteoplasty of the alveolar ridge). Due to its increased infiltration of cells and therefore an accelerated integration, such scaffolds seem to be indicated only for jaw regions with low mechanical stress such as the maxillary sinus.

**Acknowledgement**

The authors of the present study acknowledge funding of the study by the German Federal Ministry for Economic Affairs and Energy (BMWi, ZIM project ZF4379203MC8). The authors thank Diana Jünger (Department of Oral and Maxillofacial Surgery, University Hospital “Carl Gustav Carus”, Technische Universität Dresden) for the excellent technical assistance.

---

**References**


Silicate mediated bone remodelling is modulated by Fe

Amy Li, Joel Turner, Azadeh Rezaei, Gavin Jell

University College London, Division of Surgery and Interventional Sciences, London, GB

Introduction
Despite over 50 years of silicate based Bioactive glass research, and successful clinical applications, it is still unclear how silicate affects the underlying cellular mechanisms involved in bone remodelling. Previous studies have shown that Si inhibits osteoclastogenesis and osteoclast resorption capacity [1]. Considering the known role of sodium silicate in Fe chelation in paper manufacture [2], and the important role of Fe and ROS in osteoclast function (e.g. in the function of the redox iron containing tartrate resistant acid phosphatase (TRAP) enzyme) [3], here we explore a possible mechanism for Silicate mediate bone remodelling, via Fe chelation and reduced ROS availability.

Experimental Methods
To determine if silicate reduced ROS availability a de-esterified 2’, 7’-dichlorofluorescein diacetate (H2DCF-DA) assay was performed based on the protocol described previously [4]. Si and BG solutions were prepared in PBS with the clinically relevant concentrations of Si 2mM, 1mM, 1mM Deferoxamine Mesylate (DFO) as a control iron chelator. The samples were incubated for 5 minutes in dry incubator before being transferred to a black-well plate measure at the fluorescence of excitation 495nm, emission 538nm. Osteoclast formation was determined with a subclone of RAW264.7 osteoclast precursor cell-line with 3ng/RANKL and 20ng/ml controls over 6 days of culture measured by TRAP-5b staining (Acid Phosphatase, Leukocyte (TRAP) Kit- 387A, sigma Aldrich). Osteoclast resorption was determined by primary MF-1 mice osteoclasts over 9 days of culture with addition of 200ng/ml M-CSF and 3ng/ml RANKL and dropping pH to 6.8 at day 7 to initiate resorption (Data not shown).

Results and Discussion
Si (2mM) resulted in complete inhibition osteoclastogenesis (Figure 1). This finding was confirmed in an acellular biochemical ROS study, where Si inhibited ROS production in sodium metasilicate form and Si-BG dissolution ions in a concentration dependant manner (>50% vs 1mM H2O2 alone in the presence of FeCl2 catalyst). This suggests that Si may be inhibiting the Fenton reaction and ROS availability. To investigate this theory in vitro, Fe(10-100µM) was added to Si containing culture which restored osteoclasts formation in Si (2mM) treated cells.

Conclusion
Silicate inhibition of osteoclastogenesis is mediated by Fe concentration. Understanding how Si based biomaterial interact in bone tissue engineering will allow the design of patient specific bone implants.

Acknowledgement
We are grateful to Prof Tim Arnett (Dept of Cell and Developmental Biology, UCL) for help with primary osteoclast cultures. Dr Bala Subramaniyam Ramesh and Mr. Arnold Derbyshire (both Division of Surgery & Interventional Sciences, UCL) are thanked for their invaluable technical expertise. We are grateful to Prof Tim Arnett (Dept of Cell and Developmental Biology, UCL) for help with primary osteoclast cultures. Dr Bala Subramaniyam Ramesh and Mr. Arnold Derbyshire (both Division of Surgery & Interventional Sciences, UCL) are thanked for their invaluable technical expertise.
Figure 1 A. Osteoclast formation determined addition of Si and FeCl₂ under TRAP-5b staining

Figure 1 A. Osteoclast formation determined addition of Si and FeCl₂ under TRAP staining via iron mediated ROS scavenging. (A) TRAP staining of osteoclast RAW 264.7 subclone with (a) control (3 ng/mL RANKL) vs 20ng/ml RANKL. ***p<0.0001, n=6.

Figure 1 B. Osteoclast formation determined addition of Si and FeCl₂ under acellular ROS assay

(B) ROS production (1mM H₂O₂) were scavenged by Si, Si-BG and DFO via addition of 10uM FeCl₂. ***p<0.0001, n=6.

References


Development of a collagen based composite scaffold encapsulating TGF-b1 and mimicking the growth factor footprint of human bone tissue

Chiara Vitale-Brovarone¹², Federica Banche-Niclot¹³, Caterina Licini¹⁴, Giorgia Montalbano¹, Sonia Fiorilli¹², Monica Mattioli-Belmonte⁴

¹ Politecnico di Torino, Department of Applied Science and Technology, Torino, IT; ² National Interuniversity Consortium of Materials Science and Technology, RU Politecnico di Torino, Firenze, IT; ³ Università degli Studi di Torino, Department of Surgical Science, Torino, IT; ⁴ Università Politecnica delle Marche, Department of Clinical and Molecular Sciences (DISCLIMO), Ancona, IT

Introduction
The human bone undergoes a lifelong process, known as bone remodelling, where bone resorption by osteoclasts (OC) and new bone deposition by osteoblasts (OB) sequentially occur and are synergistically coupled. During this process, the resorption of bone portions by OC involves the excretion of enzymes that are able to digest the collagenous fraction causing the release of the growth factors (GFs) stored in the bone matrix [1]. In bone regenerative strategies the controlled release of GFs is one of the main targets to stimulate a successfully regeneration. Recent trends in the drug delivery field have led to an increasing interest in the development of biodegradable particles able to protect and vehicle active agents. In this frame, poly(lactic-co-glycolic) acid (PLGA) is one of the most used polymers for the realization of carriers due to its well-known biocompatibility and biodegradation [2]. The present research work reports the design and characterization of degradable PLGA nanocarriers suitable for the incorporation and subsequent release of Transforming Growth Factor-βeta1 (TGF-β1), a biomolecule that exerts a central role in the bone remodelling process, controlling both OB and OC migration, proliferation and maturation [3]. With the final purpose of designing a biomimetic 3D printed scaffold able to reproduce the natural bone composition and biology, the developed TGF-β1-containing PLGA nanocarriers (PLGA_TGF-β1) were incorporated into a type I collagen formulation. The obtained hybrid formulation was used for 3D printing a nanostructured scaffold mimicking the amount and distribution of TGF-β1 observed in the human bone extracellular matrix (ECM) able to provide a sustained GFs release for supporting bone regeneration.

Experimental Methods
Human bone samples have been processed by Università Politecnica delle Marche to localize and quantify TGF-β1 in human bone ECM performing immunohistochemical and ELISA analyses. TGF-β1 have been encapsulated into PLGA nanoparticles using a double evaporation technique and then, in order to obtain a sustained delivery, the PLGA_TGF-β1 nanoparticles have been dispersed in a type I collagen matrix. A special focus has been given to rheological studies to obtain a formulation suitable for 3D printing applications and 3D structures have been printed through 27G needles using a commercial bioprinter (BioX, CELLINK) equipped with a pneumatic temperature-controlled print-head. To improve the material stability after deposition, a chemical cross-linking using genipin has been used. The nanocarriers and the composite collagenous structures have been fully characterized and the release kinetics of TGF-β1 from both the nanoparticles alone and when embedded into the scaffold have been assessed under physiological conditions up to 28 days. Finally, the localization of TGF-β1 into the developed 3D collagen-based scaffold was evaluated using histochemical analyses.

Results and Discussion
Spherical and water-stable PLGA_TGF-β1 nanocarriers measuring 257±7.3 nm in diameter and with a high encapsulation efficiency (63.9±7.9%) have been successfully synthetized. Release test on the nanocarriers showed...
an initial burst release of about 38% of the incorporated GFs in the first 24 hours likely due to TGF-β1 adsorbed on the particle surface, followed by more gradual release kinetics reaching 97.6% of GFs released after 28 days. The nanocarriers were subsequently combined with type I collagen, obtaining a homogeneous hybrid suspension containing the same amount of TGF-β1 detected by the semi-quantitative evaluation of immunostaining on human bone tissue. After the optimization of the printing parameters and conditions, 10×10×1 mm³ mesh-like scaffolds have been printed. Morphological analyses confirmed the successful reconstruction of collagen fibrils after the incubation at 37°C as well as the uniform distribution and proper embedding of PLGA_TGF-β1 into the collagenous matrix. Furthermore, the resulting scaffolds showed the capability to slow down the TGF-β1 release compared to the PLGA nanocarriers alone, evidencing that their embedding within the collagenous matrix provides a modulatory effect on the biomolecule release. Finally, the histological staining and immunohistochemistry analyses proved that the developed collagenous 3D system incorporating the PLGA_TGF-β1 nanocarriers successfully reproduce the TGF-β1 quantity and its uniform distribution observed in human bone ECM.

**Conclusion**

PLGA nanocarriers able to encapsulate and gradually release TGF-β1 (PLGA_TGF-β1) have been successfully developed as promising tools for the design of devices for bone regeneration. A further goal achieved by this study was the design of a 3D composite scaffold based on type I collagen and PLGA nanoparticles (Coll/PLGA_TGF-β1) able to effectively mimic the bone ECM in terms of composition, TGF-β1 content and uniform localization in the bone tissue. Future studies will aim at investigating the effect of the released TGF-β1 on OB and OC crosstalk.

**Acknowledgement**

This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme, grant agreement No. 681798-BOOST (www.ercprojectboost.eu) and by the Italian Minister of Education, Universities and Research (MIUR), Progetto FARE Ricerca in Italia (GRACE).

**Graphical abstract**

Graphical summary of the main results: A) TGF-β1 staining in human bone ECM; B) Optical images of Coll/PLGA TGF-β1 3D printed scaffold; C) FESEM images of Coll/PLGA_TGF-β1 composite scaffold (yellow arrows collagen, white arrows PLGA nanocarriers); D) TGF-β1 staining in 3D-printed scaffold.

**References**


Bioinspired adhesive proteins as building blocks for liquified platforms with an impact on bone regenerative medicine

Ana R. Pinho, Maria C. Gomes, João F. Mano

Aveiro University, Department of Chemistry, CICECO - Aveiro Institute of Materials, Aveiro, PT

Introduction

Bone is one of the few tissues that regenerate spontaneously. However, pathological situations make healthy bone regeneration impossible, leading patients to painful clinical conditions and associated with expensive and time-consuming treatments. Bone tissue engineering has long sought biocompatible and biodegradable platforms that effectively promotes bone regeneration and easily translated into clinical practice. In this sense, we designed protein-based liquified microplatforms with unique adhesion and spontaneous mineralization inspired by aquatic organisms.

Experimental Methods

Inspired by the adhesive proteins found in mussels (such as Mfp-3), we chemically obtained synthetic adhesive proteins as building blocks to fashion liquified microcapsules by the combination of coaxial electrospray, and the coordination behavior of catechol-like moieties with metals. The adhesion and mineralization properties were evaluated by mechanical tests and exposure to simulated body fluids, respectively. Preliminary cell biocompatibility was also assessed to evaluate this microplatforms as biofactories.

Results and Discussion

By taking advantage of the adhesive properties, we were able to obtain biocompatible and biodegradable microplatforms with great adhesion and autonomous mineralization behavior. With great prospects for bone tissue regeneration, these microfactories provided the desired environment for cell growth and proliferation.

Conclusion

In the end, this study provides an interesting approach to construct liquified microcapsules with biomimetic adhesive and mineralization properties for bone tissue regeneration.

Acknowledgement

This work was supported by the European Research Council grant agreement ERC-2014-ADG-669858 for the project "ATLAS".

References


**N09-05**

**Scaffold-based 3D cellular models mimicking the heterogeneity of osteosarcoma stem cell niche**

**Giada Bassi**, Silvia Panseri, Arianna Rossi, Elisabetta Campodoni, Monica Sandri, Massimiliano Dapporto, Simone Sprio, Anna Tampieri, Monica Montesi

*National Research Council of Italy, Institute of Science and Technology for Ceramics (ISTEC), Faenza (RA), 48018, IT*

**Introduction**

Worldwide cancer remains the second-most common cause of death, despite the advances in prevention, early detection, treatment; among the bone cancer osteosarcoma (OS) is the most common type diagnosed especially in children and young adults. The failure of the osteosarcoma conventional therapies leads to the growing need for novel therapeutic strategies. The lack of specificity for the Cancer Stem Cells (CSCs) population has been recently identified as the main limitation in the current therapies\(^1\). Moreover, the traditional two-dimensional (2D) *in vitro* models, employed in the drug testing and screening as well as in the study of cell and molecular biology, are affected by a poor *in vitro-in vivo* translation ability\(^2\).

To overcome these limitations, this work provides two 3D "tumour engineering" approaches as new tools to address osteosarcoma and improve therapy outcomes. In detail, two different hydroxyapatite-based bone-mimicking scaffolds\(^3,4\) were used to recapitulate aspects of the *in vivo* bone extracellular matrix, together with enriched-CSCs obtained by sarcosphere-forming culture starting from osteosarcoma cell lines (MG63 and SAOS-2)\(^5\).

**Experimental Methods**

**Scaffold synthesis.** Hybrid composite scaffolds obtained by a biomineralization process involving the direct nucleation of Mg-doped hydroxyapatite (MgHA) on self-assembling collagen fibres (MgHA/Coll)\(^3\) and porous hydroxyapatite scaffolds (HA) produced by direct foaming process\(^4\), were used.

**Sarcospheres-forming culture.** The enriched-CSCs were obtained from parental SAOS-2 and MG63 cell lines under specific culture conditions as reported in literature\(^5\), observing sarcospheres formation.

**3D *in vitro* osteosarcoma models.** The scaffolds were seeded with parental cells or sarcospheres on material upper surface for the development of 3D osteosarcoma models. Sarcospheres were supplied with fresh culture medium and factors cocktail every 3 days for a total of 10 days of culture.

A morphological analysis and immunofluorescence and gene expression profile of stemness and niche-related genes in osteosarcoma cell lines and enriched-CSCs cultured in standard 2D conditions *versus* the 3D culture systems has been performed.

**Results and Discussion**

The overall results showed how the CSCs maintained more their stemness phenotype and features when cultured in both MgHA/Coll and HA biomaterials compared to 2D model. The work confirmed that the use of the 3D scaffolds, together with sarcospheres implementation, improved the osteosarcoma stem cell niche microenvironment simulation, providing precise inputs supporting cell-cell and cell-ECM interactions and tumour signalling pathways *in vitro*. Molecular analysis showed slight variances attributable to the intrinsic biological differences of the two osteosarcoma cell lines and to the specific biomimetic scaffold features. However, also *in vivo* intratumoral variability can occur between tumour cells arising from the same mass, leading to the classification of different tumour cell subtypes that show a range of functional and morphological properties and a different molecular profile\(^1\). The extrinsic interactions between tumour cells and stromal microenvironments may be involved in the *in vivo* tumour heterogeneity as a crucial determinant of tumour malignancy\(^1\). Although recognizing the limitations of these simplified
3D osteosarcoma models, they provided a more accurate starting point to understand the cellular and molecular mechanisms involved in cancer cells/biomatrix interactions, particularly in CSCs population.

**Conclusion**

This work highlight the fundamental role of the tumour microenvironment providing the mimicry of osteosarcoma stem cell niche by the use of CSCs together with the biomimetic scaffolds, compared to conventional 2D culture systems. The proposed culture systems represent promising 3D *in vitro* tumour models that could improve the predictivity of preclinical studies and strongly enhance the clinical translation, closing the gap between the drug discovery and the clinical studies with the ultimate goal to be applied in personalized medicine.

**References**

Optimization of calcium phosphate production in a droplet-based microfluidics platform for bone regeneration

Yousra Alaoui Selsouli, Hoon S. Rho, Zeinab Tahmasebi Birgani, Pamela Habibovic

Maastricht university, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, NL

Introduction
Calcium phosphate (CaP) ceramics such as hydroxyapatite (HA) and tricalcium phosphate (TCP) are among the most widely used synthetic bone graft substitutes in the clinic [1]. Nevertheless, bone regenerative potential of most CaPs is not as high as that of natural bone grafts, and therefore, further improvement is required [2]. Microfluidic technology offers attractive tools for improving and accelerating development of new materials with higher throughput or in a more controlled manner than the conventional production methods [3]. Here, we explored the possibilities of a recently developed droplet-microfluidic platform for controlled synthesis of CaP ceramics.

Experimental Methods
CaP ceramics were synthesized inside microdroplets produced by a flow-focusing microfluidic device, which uses a mixture of calcium nitrate and phosphoric acid as water phase, and mineral oil with Span® 80 as oil phase (figure 1a). After the microdroplets were generated and collected, a base (ammonia or sodium hydroxide) was added to increase the pH, resulting in mineralization of CaPs inside of the droplets. The CaP particles were then purified to remove the oil phase by several washing/filtration steps (figure 1b) [4]. CaPs with different properties were produced by controlling the composition of the calcium and phosphate precursor solutions, pH and post-precipitation heat treatment. Upon production, the materials were characterized by x-ray diffraction (XRD), Fourier transformed infrared spectroscopy (FTIR) and scanning electron microscopy (SEM).

Results and Discussion
CaPs were successfully produced in single-emulsions using the microfluidic platform. By controlling processing parameters such as flow rate, microdroplets, and consequently spherical CaP particles with different sizes were synthesized (Figure 1). FTIR analysis of the materials showed that after the purification step, no traces of oil phase were present in the materials (figure 2a). XRD results showed that by varying Ca/P ratio of the precursors, pH conditions and by applying post-precipitation heat treatment, phase-pure CaP particles of monetite, brushite, hydroxyapatite (HA), β-tricalcium phosphate (β-TCP) and β-pyrophosphate (β-CPP) were successfully obtained. An example of the material produced using precursors with a calcium-to-phosphorus ratio of 1 before and after heat treatment is shown in figure 2b. SEM imaging showed that the precipitated particles exhibited a homogeneous morphology (figure 2c).

Conclusion
Here, we showed that the flow-focusing droplet microfluidic device is a suitable platform for synthesizing different CaP ceramics with control over their composition and size. To further explore the possibilities of the platform, we are currently performing a systematic study by varying the individual processing parameters and investigating their effects on the material properties such as their porosity and degradation behavior. Future steps aim at generating a combinatorial library of different CaP-based ceramics by introducing inorganic additives such as strontium, copper and magnesium, which are also present in bone mineral [5].

Acknowledgement
The authors acknowledge financial support by Innovative Research Incentives Scheme Vidi grant (#15604) of the Netherlands Organisation for Scientific Research (NWO). This research has been made possible with the support of the Interreg Vlaanderen/Nederland BIOMAT collaboration and of the Dutch Province of Limburg.

Page 478 of 2028
Figure 1
Production of water-in-oil droplets using flow-focusing microfluidic device (a), mineralization and purification of CaPs and example of the generated CaP particles, scale bar: 10 µm (b).

Figure 2
Characterization of CaPs using FTIR, indicating the presence of a CaP phase and absence of oil traces (a), XRD, presenting the formation of different CaPs, e.g. monetite and β-CPP (b), and SEM, showing different morphologies in different CaPs, scale bar: 5 µm (c).

References
Calcium phosphate bioceramics with highly anisotropic pore structure produced from hollow filaments

Michael Gelinsky¹, Kathleen Schütz¹, Christine Hecker², Thomas Schulze³, Nadine Liebig⁴, Heike Illing-Günther⁴, Yvonne Joseph²

¹ TU Dresden, Centre for Translational Bone, Joint and Soft Tissue Research, Dresden, DE; ² TU Bergakademie Freiberg, Institute for Electronic and Sensor Materials, Freiberg, DE; ³ Thuringian Institute for Textile and Plastics Research, TITK, Rudolstadt, DE; ⁴ Saxon Textile Research Institute, STFI, Chemnitz, DE

Introduction

Most mammalian tissues have highly anisotropic structures, especially those of the musculoskeletal system. Nevertheless, the majority of biomaterials and scaffolds, utilised for example for bone reconstruction and tissue engineering have isotropic properties. Using a combination of textile and ceramic technologies, we have developed calcium phosphate bioceramics with a channel-like pore morphology, partly mimicking the osteon structure of cortical bone (Fig. 1). Such bioceramics can be produced from both hydroxyapatite (HA) and beta-tricalcium phosphate (TCP). We hypothesise that this morphology should be beneficial for the integration in living bone and for vascularisation.

Experimental Methods

As starting materials, composites consisting of cellulose and either HA or TCP powders have been prepared which then were used for wet spinning of continuous hollow filaments. The filaments were dried carefully in climate chambers and afterwards cut into convenient lengths of 0.3-1 m. The hollow fibres were bundled, and the bundles stabilised by sheathing with conventional polymer fibres, using the Kemafil process. Finally, these fibre bundles were impregnated with a calcium phosphate powder slurry and sintered at temperatures > 1000°C so that pure calcium phosphate ceramics remain and all organic components were burned out completely (Fig. 1). Phase composition was analysed using XRD and the morphology was studied by SEM. For cell culture experiments, disc-like samples of convenient lengths were made using a diamond cutting system. Cytocompatibility of the novel bioceramics was investigated both in indirect and direct cell seeding experiments, using the osteosarcoma cell line SAOS-2 and primary human mesenchymal stem cells (hMSC), isolated from bone marrow. Besides cell adhesion and proliferation, the ability for osteogenic differentiation and migration of the cells into the pore channels has been explored, using several methods.

Results and Discussion

After process optimisation and selection of the right cellulose-calcium phosphate ratio of 1:3 well defined and homogenous hollow filaments could be produced by wet spinning in continuous manner. The drying process is challenging as strong forces are generated by the shrinkage of the material but by continuous stretching of the fibres during drying deformation could be avoided. Typical dimensions of the dried hollow fibres are 400-450 µm outer diameter, 90-100 µm wall thickness and 220 µm lumen. Bundling and compaction of the parallel-aligned fibres led to fibre bundles with diameters of commonly ca. 0.8-1.5 cm. For further stabilisation and better handling these bundles were sheathed using polymer fibres applying the Kemafil process. For infiltration of the materials prior to sintering slurries of the same calcium phosphate powders that have been integrated in the respective hollow fibres were prepared and sucked in the voids between the fibres without closing their hollow lumen. Finally, the samples were sintered at different temperatures (1100, 1200 and 1250°C) to investigate the effects on stability, mechanical properties and cell growth. so that all organic residues (the cellulose content of the hollow fibres as well as the material used for sheathing) At this temperatures, all organic residues (the cellulose content of the hollow fibres as
well as the material used for sheathing) are removed completely, leaving behind pure calcium phosphate bioceramics. The sintering led to further reduction of the dimensions — so that the channel-like pores, originating in the hollow fibres, have typical diameters of around 150 µm depending on their initial dimensions and sintering conditions. XRD analyses confirmed the composition being either HA or beta-TCP, depending on the starting materials. Indirect cytotoxicity evaluation revealed good cytocompatibility of both HA and TCP based bioceramics. Applying a dynamic cell seeding method homogenous cell distribution of both sides of disc-shaped samples could be achieved, as well as high seeding efficiency (Fig. 2). SAOS-2 cells proliferated on the samples over cultivation periods of 14 days and a part of the cells migrated into the pore channels. Similar results could be achieved with hMSC, which also could be differentiated towards the osteogenic lineage after addition of the standard pro-osteogenic cell culture supplements.

**Conclusion**

In conclusion, we have demonstrated the applicability of a novel method for manufacturing of highly anisotropic calcium phosphate bioceramics with channel-like pores in large dimensions and quantities, starting of hollow cellulose-based filaments. In further experiments, we will study the biocompatibility of these materials, using suitable bone defect animal models and the suitability for vascular ingrowth into the pore channels.

**Acknowledgement**

We want to thank the German Federal Ministry for Economic Affairs and Energy (BMWi) and the German Federation of Industrial Research Associations (AiF) for funding within the IGF programme (project No. 20610 BR).
Silk-based inks: from molecules to well-organized fibrous scaffolds

Martina Viola1,2, Susanna Piluso1, Marko Mihajlovic2, Jos Malda1, Miguel D. Castilho1, Vermorden Tina2

1 University Medical Center (UMC) Utrecht, Orthopaedics / Surgical Specialties, Utrecht, NL; 2 Utrecht University, Pharmaceutics, Utrecht, NL

Introduction
One open challenge in tissue engineering is to engineer biomaterials that can mimic the hierarchical fibrous structure of the extracellular matrix (ECM) found in living tissues.1, 2 Silk as a material – from its unique mechanical properties to its biological properties – has been continually inspiring materials scientists, biologists and tissue engineers. However, processing silk in well-organized structures that can potentially mimic native like ECM structures is still a challenge. In this study, we aim to investigate the electrohydrodynamic printing potential of a novel Silk Fibroin (SF) ink in order to create well organized and mechanical stable fibrous constructs.

Experimental Methods
Silk fibroin was extracted from Bombyx mori cocoons.3 These cocoons are cut and boiled for 5 minutes in a Na2CO3 solution (0.02M), in this step sericin is removed from the silk filaments, fibroin is isolated and left to dry overnight. Fibroin is then dissolved in a 60°C LiBr solution (9.3 M) for 4h, and subsequently purified by 5 days of dialysis (Fig 1A). The viscosity of the prepared SF solution was evaluated by rheological analysis with a plate-plate geometry (d = 20 mm). The same geometry of the rheometer was used to detect the strength of SF hydrogels (Fig.1B). Then electrohydrodynamic printing of SF solutions was investigated by studying the key instrument parameters (speed of the collector plate, voltage, pressure and distance between the plate and the extruder).4 Post-printing fibers were treated with a solution of sodium phosphate (1M) to induce physical crosslinking. Post-printing accuracy was evaluated analyzing the diameter and the stacking of the fibers with light microscopy.

Results and Discussion
In this work, we have demonstrated the electrohydrodynamic printing of SF in well-organized fibrous structures. To do so, we adjusted the rheological properties of the SF ink by varying the concentration of SF (15% w/v) and the concentration of polyethylene oxide (2% w/v) (Mw: 600-1000 kDa) (Fig. 1B), to obtain straight fibres with reduced fibre diameters, i.e. 10 to 20 μm. Moreover, we successfully produced fibrous scaffolds with a minimum inter- fibre distance of 200 μm (Fig. 2C) and a maximum thickness of approximately 100 μm (up to 15). After printing, the scaffolds were physically crosslinked with a solution of sodium phosphate (1M) ensure their stability in aqueous solutions and sufficient mechanical integrity (Fig. 2D).
We compared this crosslinking method with crosslinking with methanol, used several times in literature.5 We noticed how Na3PO4 crosslinking not only induced greater extensibility and elasticity of the fibres than those crosslinked with methanol, but also greater stability in an aqueous environment.

Conclusion
Overall, the electrohydrodynamic printing of novel SF inks allows the fabrication of uniquely organized fibrous scaffolds with micrometer resolutions. Our next steps will focus on studying the conformational changes of SF when treated with Na3PO4 by Raman spectroscopy. Furthermore, we will analyse the mechanical properties that SF scaffolds take on when treated with Na3PO4. Ultimately, we will apply the generated scaffolds to recreate the organized ECM structure found in native cartilage.

Acknowledgement
This research was financially supported by the Gravitation Program “Materials Driven Regeneration”, funded by the Netherlands Organization for Scientific Research (024.003.013); This project has received funding from the European
Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie COFUND grant agreement No 801540.

Fig. 1
A) Process of extracting fibroin from Bombyx mori cocoons. The cocoons are cut and boiled for 5 minutes in a 0.02M Na$_2$CO$_3$ solution, in this step sericin is removed from the silk filaments, isolating fibroin. Fibroin is left to dry overnight. Fibroin is dissolved in a 60°C LiBr solution for 4h, and subsequently purified by 5 days of dialysis.

B) Strain sweep and frequency sweep of a 15% w/v SF solution with 2% w/v PEO. Initial viscosity of a 15% w/v SF solution with increased PEO.

Fig. 2
C) Electrohydrodynamic printing of SF 15% w/v and PEO 2%w/v (600-1000 kDa). Scaffold size 20 x 20 mm, with 15 layers. Fibre diameter ~10 mm. The diameter of the fibers can be tuned by the speed of the collector plate. D) Crosslinking of SF with Na$_3$PO$_4$ for 40 minutes, to obtain elasticity and resistance. The scaffolds are not soluble in water after crosslinking.

References
The effect of alkali activation on the porosity of 45S5 bioactive glass microspheres

Si Chen¹, Jozef Kraxner¹, Martin Michálek¹, Dušan Galusek¹,²

¹ A. Dubček University of Trenčín, FunGlass, Študentská 2, 911 50 Trenčín, SK; ² VILA – Join Glass Centre of the IIC SAS, TnUAD, FChPT STU, Študentská 2, 911 50 Trenčín, SK

Introduction
Hench et al. developed 45S5 bioactive glass (BG) in 1971. 45S5 BG can form a strong chemical bond to bone tissue [1]. It is widely used in the fabrication of scaffolds for bone tissue engineering, including ceramic/glass scaffolds, or as filler incorporated into composite scaffolds. 45S5 BG is also able to promote osteogenesis and angiogenesis [1]. The porous structure provides the necessary space for cell migration, angiogenesis and tissue growth which is one of the important criteria for bone tissue engineering [2]. Porous structures can be also used as drug carriers in 3D scaffold preparation. Porous/hollow microspheres can be fabricated by flame spheroidization process after alkali activation of 45S5 bioactive glass [3]. In this study, we investigated the effects of three parameters, namely concentration of NaOH solution, activation time, and temperature, on the porosity and pore size of the 45S5 bioactive glass microspheres prepared by flame spheroidization process.

Experimental Methods
45S5 bioactive glass was prepared by conventional melt quenching method. The BG was milled into a powder with a particle size ≤ 40 μm. The powder was dispersed in different concentrations of NaOH solutions (0.5M, 1M, 2M) at different temperatures (25°C, 50°C) with vigorous stirring for different times (0.5h, 1h, 3h). The three mentioned alkali activation process parameters were adjusted by single factor to assess their effect on the textural properties of produced porous/hollow microspheres. The suspensions were further aged at 75 ºC for 24 h. During aging, sodium silicate produced by the alkali activation caused the powder stick into lumps. Through further grinding and sieving, powders with a particle size of 40-80 μm were obtained and further processed by flame synthesis to obtain porous/hollow microspheres. The structural, morphological, chemical, and textural characteristics of the microspheres were analysed using XRD, SEM, Raman and Micro-CT. Microspheres were immersed in simulated body fluid (SBF) to evaluate their bioactive behaviour.

Results and Discussion
BG microspheres with various morphology and porosity were successfully fabricated by flame synthesis. Figure 1 illustrates a significant porosity difference of the microspheres prepared from alkali activated BG treated under different activation conditions.

The XRD patterns (Figure 2) of the glass after alkali activation confirmed the formation of crystalline sodium phosphate hydroxide hydrate (Na₆.33(PO₄)₂(OH)₀.33•24H₂O) and calcium silicate hydrate ((CaO)ₓ•SiO₂•ZH₂O). In addition to the aforementioned components, NaOH might be completely consumed, possibly yielding amorphous hydrated sodium silicate or remain in the sample after alkali activation. The residual of NaOH is going to be analysed by Raman spectroscopy. After flame synthesis, all samples were X-ray amorphous. Calcite (CaCO₃) crystals were formed after 1 day immersion of glass microspheres in SBF. Similar results were reported in the literature related to bioactive glasses and bioactivity measurement [4]. Compared to hydroxy carbonate apatite (HCA, Ca₁₀(PO₄)₃(CO₃)₃(OH)₂), calcite exhibits better osteogenesis and induces faster osteoblast remodelling [5]. After 3 and 7 days of immersion, the calcite diffraction lines almost disappeared and were replaced by the HCA diffraction lines. The 1M-3h-25°C sample showed significantly weaker HCA diffraction lines at 26° and 32° compared to 1M-1h-
50°C. This can be explained by the lower porosity and hence, a lower specific surface area of 1M-3h-25°C compared with 1M-1h-50°C.

**Conclusion**

Porous/hollow microspheres were successfully fabricated from the 45S5 bioactive glass by alkali activation followed by flame synthesis. The parameters of alkali activation significantly affect the porosity of the microspheres. The microspheres exhibited significant bioactivity.

**Acknowledgement**

This work is a part of dissemination activities of the project FunGlass. This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 739566. The authors want to acknowledge Prof. Aldo R. Boccaccini and Assoc. Prof. Enrico Bernardo as the project partners. Financial support of this work by the grants SAS-MOST JRP 2018/02, VEGA 1/0098/19 and VEGA 1/0456/20 is gratefully acknowledged.

**References**

1:15 p.m. – 1:45 p.m.

Track02

Social Activity 3 | Yoga

Prof. Filipa Ribeiro will give 30min yoga class at lunch time.

Filipa Ribeiro

YOGA SHALA MATOSINHOS, Matosinhos, Portugal
1:30 p.m. – 2:15 p.m.

Track01

LS | Lunch Symposium Rousselot: Biomedical Applications of Gelatins

Chairs

Jeff Daelman
Rousselot, Gent, BE
Jeroen Leijten
University of Twente, Enschede, NL

This symposium is held by:

Rousselot
Biomedical

We are proud to co-present a lunch symposium with Dr Jeroen Leijten, University of Twente, Enschede, Netherlands and our scientific expert Dr Jeff Daelman, in which the complexities and potential of gelatins for use in biomedical applications will be discussed.

Session discussion highlights include:

- **The unique properties of Gelatin for 3D bioprinting**: The use of gelatin to stably incorporate nanoparticles into printable bio-inks.
- **The biocompatibility of Gelatin for use in regenerative medicines**: The ability of gelatin to steer the behavior of immune cells such as macrophages towards a regenerative phenotype, or to endow bio-printed cartilage-like tissue constructs with spatially controlled mechanical properties.
- **The advantages of gelatin for use as a bio-adhesive**: The chemical modification of gelatin to act as hemostatic bio-adhesive or implantable energy storage devices

Our expert Dr Jeff Daelman will share insights from our latest research into how a ‘clean’ gelatin can reduce experimental bias and allow for efficient clinical translation.

Program:
Rousselot introduction – Jeff Daelman
Biomedical applications for Gelatins - Jeroen Leijten
Modified gelatins: from bench to patient – Jeff Daelman
Q&A – Jeff Daelman

Page 487 of 2028
LS-01

Rousselot introduction

Jeff Daelman

Rousselot Biomedical, Rousselot BV, Ghent, Belgium

LS-02

Biomedical applications for Gelatins

Jeroen Leijten

University of Twente, Department of Developmental BioEngineering, Enschede, NL

Gelatin has become one of the most commonly used biomaterials for biomedical research. As a naturally-derived biomacromolecule, gelatins offer amongst others cytocompatibility, ease-of-handling, degradability, and cell attachment. Gelatin allows for facile chemical modifications that allow additional forms of crosslinking, which have been explored for numerous purposes including cell delivery, micropatterning, and additive manufacturing. Here, we will discuss some of the novel areas of application of gelatins. This will include chemical modification of gelatin to act as hemostatic bioadhesive or implantable energy storage devices, use of gelatin to stably incorporate nanoparticles into printable bioinks, ability of gelatin to steer the behavior of immune cells such as macrophages towards a regenerative phenotype, or to endow bioprinted cartilage-like tissue constructs with spatially controlled mechanical properties. For all these applications, a clean (e.g., endotoxin-low/free) and batch-to-batch consistent gelatin formulation is required. Indeed, a GMP-certified and FDA/EMA-compliant biomaterial formulation is required to reduce experimental bias and allow for clinical translation. We will therefore highlight the potential effects of these variables in relation to the presented studies.
Modified gelatins: from bench to patient

Jeff Daelman

Rousselot Biomedical, Rousselot BV, Ghent, Belgium

Modified gelatins (e.g. GelMA) have been around for over 2 decades, but despite a steadily growing body of scientific literature and an ever increasing list of patent applications, no clinical application has yet made it to the patient. We believe this is due to a lack of material suitable for use in clinical trials: materials that are consistent in their physical and chemical properties, are GMP certified and have the required level of purity.

Using 2 case studies, we will demonstrate the importance of considering upscaling and material consistency as early in the process as possible.
4:15 p.m. – 5:45 p.m.

Track01

**K05 | Immunoresponse Towards Biomaterials**

**Chairs**

**Mário A. Barbosa**  
University of Porto, PT

**Willem J.M. Mulder**  
Radboud University Nijmegen Medical Center, Department of Internal Medicine, Radboud Institute of Molecular Life Sciences (RIMLS) and Radboud Center for Infectious Diseases (RCI), Nijmegen, NL

**Elisa Piatti (YSF)**  
Politecnico di Torino, Dipartimento di Scienza e Tecnologia dei Materiali (DISAT), Torino, IT
K05-KL01

Mapping the immune and stromal response to biomaterials

Jennifer H. Elisseeff, Christopher Cherry

Johns Hopkins University, Baltimore, US

Biomaterials serve as the basis of implants, tissue engineering scaffolds, and multiple other biomedical therapeutics. New technologies, such as single cell RNA sequencing (scRNAseq), are enabling characterization of the biomaterial response to an unprecedented level of detail, facilitating new discoveries in the complex cellular environment surrounding materials. We performed scRNAseq and integrated data sets from multiple experiments to create a single cell atlas of the biomaterials response. We developed Domino (github.com/chris-cherry/domino), a computational tool which allows for identification of condition specific intercellular signaling patterns connected to transcription factor activation in the biomaterial response defining interactions between immune, fibroblast, and tissue-specific modules with biomaterials-specific communication patterns.
Development of a biomimetic implant with stiffness-dependent immunomodulatory functionality and neurotrophic characteristics for spinal cord injury

Ian U. Woods¹, Cian O'Connor¹, Lisa Frugoli¹, Séan Kerr¹, Martyna Stasiewicz¹, Alan Hibbits¹, Javier G. Gonzalez¹, Adrian Dervan¹, Fergal O’Brien¹,²,³

¹ Royal College of Surgeons, Dept of Anatomy and Regenerative Medicine, Dublin, IE; ² Royal College of Surgeons Ireland, Advanced Materials and Bioengineering Research (AMBER) Centre, Dublin, IE; ³ Trinity College Dublin, Trinity Centre for Biomedical Engineering, Dublin, IE

Introduction

The injured spinal cord (SC) generates a unique and complex pathophysiology which presents a multifaceted challenge for repair¹. Broadly, normally supportive astroglia become reactive following injury and form a glial scar. This scar in turn inhibits recovery by releasing an impenetrable inhibitive extracellular matrix (ECM)², which prevents axonal regrowth. Tissue engineering approaches aim to overcome these barriers by bridging the cyst with a physical neurotrophic substrate optimised for axonal growth. However, reactive astrocytes rapidly encapsulate these scaffolds, acting as a physical barrier to regeneration. Our previous experience in peripheral nerve repair demonstrates that composition and stiffness of scaffolds play an essential role in neural tissue regeneration, directing the behaviour of both axons and supporting cells³. Considering the physicochemical properties of SC tissue, it was hypothesized that comprehensive optimization of the structure, stiffness and composition of biomaterial scaffolds could provide both potent neurotrophic signalling and intrinsic immunomodulatory properties. Scaffolds with intrinsic signalling present an effective alternative to biochemical factors for solutions to the key clinical challenges of astrogliosis, the CNS foreign body response and cross-injury axonal regrowth.

Experimental Methods

Human SHSY-5Y projection neurons, murine NSC-34 motoneurons and human primary astrocytes were cultured for 7 days on poly-L-lysine-coated or ECM-coated (5 µg/ml) coverslips (n=12), or with increasing gradients of Brain Derived Neurotrophic Factor (BDNF). Fluorescence microscopy captured cell morphologies that were digitally analyzed using FIJI. To produce biomimetic scaffolds of varying mechanical stiffnesses, soft (3mg/ml), medium (5 mg/ml) and stiff (10 mg/ml) hyaluronic acid (Hya) hydrogels were prepared, after which the optimal ECM composition was mixed throughout the hydrogel. The physicochemical properties of the scaffolds were then characterized using compressive testing, scanning electron microscopy and histology.

Astrocytes, neurons and primary adult rat dorsal root ganglia (DRGs) were then seeded individually on the HyA scaffolds of low, medium or high stiffness and cultured for 1 - 3 weeks. ELISA analysis was performed on collected medium to examine the release of inflammatory markers IL-6 and IL-10 from the scaffold-resident astrocytes. Immunohistochemical analysis was performed to examine cell morphology, nuclear:cytoplasmic ratio and axonal extension.

Results and Discussion

Neurite outgrowth analysis indicated that the combination of collagen-IV (Coll-IV) and fibronectin (Fn) significantly (p<0.05) synergistically enhanced outgrowth with a similar potency to high concentrations (50 - 100 ng) of BDNF, a potent neurotrophic growth factor. Analysis of astrocyte behaviour on ECM substrates indicated significantly lower expression of GFAP as well as the adoption of more stellate morphologies, associated with a ‘resting’ or less reactive astrocyte phenotype⁴, on Coll-IV/Fn substrates compared to PLL controls.
Astrocytes seeded on HyA scaffolds (Fig. 1, A-F) exhibited significant changes in a manner dependent on both stiffness and composition. Astrocytes cultured in soft HyA scaffolds displayed stellate morphologies and exhibited significant changes in nuclear:cytoplasmic ratio, a marker of astrocyte reactivity, (p<0.001) (G) compared to astrocytes cultured on scaffolds of supraphysiological stiffnesses (10mg/ml). Furthermore, IL-10 production (H), a pro-regenerative, anti-inflammatory cytokine, was significantly upregulated on both softer and Coll-IV/Fn containing scaffolds. These results indicate capacity for scaffolds optimized for the SC environment to provide innate immunomodulation of astrocyte phenotype towards a neurotrophic subtype.

SH-SY5Y projection neurons exhibited significantly improved extension of βIII-tubulin labelled neurites on softer, ECM-functionalized scaffolds and scaffold stiffness exerted significant effects on both the distribution and morphology of their extensions. Finally, adult primary DRGs were used as an ex vivo model of axonal regeneration from mature neurons (Fig. 2 (A-F)) – an essential quality for clinical application. DRGs exhibited significantly enhanced axonal extension on Coll-IV/Fn-functionalized scaffolds while softer scaffolds (3mg/ml), enhanced both lateral and longitudinal axonal extension (p<0.001) (G – I).

**Conclusion**

Coll-IV/Fn functionalized HyA scaffolds trophically support and stimulate axon regeneration in both an in vitro model of neuronal growth and an ex vivo model of SC injury while also promoting adoption of a pro-regenerative phenotype in infiltrating astrocytes. This paper demonstrates that an optimally designed biomimetic scaffold containing endogenous cord proteins can direct essential inflammatory and regenerative processes towards SC repair in a stiffness-dependent manner.

**Acknowledgement**

This study was funded by a joint funding initiative of the Irish Rugby Football Union Charitable Trust (IRFU-CT) and the Advanced Materials and Bioengineering Research (AMBER) Centre through Science Foundation Ireland (SFI/12/RC/2278) and by an Anatomical Society Student Fellowship (C O'C). The authors would like to thank the Advanced Microscopy Lab (AML) at the Centre for Research on Adaptive Nanostructures and Nanodevices (CRANN) for their help with the performance of electron microscopy.

**Figure 1.** Soft ECM-functionalized hyaluronic acid scaffolds mediate astrocyte polarization.

Astrocytes cultured in scaffolds of ranging stiffness with and without Coll-IV/FN functionalization were imaged to characterise individual cytoskeleton distribution (A-F). Nuclear:Cytoplasm ratio analysis also revealed a lower reactive index for astrocytes in soft scaffolds compared stiffer scaffolds (G). Significant differences in the secretion of IL-10 were observed in a stiffness and Coll-IV/FN dependent manner (H).
Figure 2. Soft, biomimetic scaffolds promote axonal regeneration in an ex vivo model of SCI.

DRGs seeded on softer scaffolds exhibited robust neurite outgrowth compared to stiffer substrates (A-C) while ECM-functionalization was shown to exert potent neurotrophic effects (D–F). Analysis of neurite outgrowth into 3mg/ml scaffolds (G) revealed profuse neurite influx (arrowheads) into the scaffold from the DRG body. Quantification of neurite outgrowth across the scaffold surface (H) and longitudinally (I) indicated that stiffness played a significant role in promoting neurite extension from seeded DRGs.

References
Magnesium sulphate-doped fibrinogen scaffolds modulate macrophage polarization

Mafalda Bessa-Gonçalves\textsuperscript{1,2}, Madalena Costa\textsuperscript{1,3}, Judite N. Barbosa\textsuperscript{1,2}, Mário A. Barbosa\textsuperscript{1,2}, Susana G. Santos\textsuperscript{1,2}

\textsuperscript{1} ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, PT; \textsuperscript{2} i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto; INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Porto, PT; \textsuperscript{3} UMIB - Unit for Multidisciplinary Research in Biomedicine, Porto, PT

Introduction
Implanted biomaterials elicit an inflammatory response, where monocytes/macrophages are central players, conditioning inflammation and bone regeneration [1]. Thus, modulating macrophage response, promoting M1 to M2 phenotype switch, is an increasingly explored strategy in biomaterial development [2]. Our previous work showed that extracts of combinatory biomaterials (fibrinogen (Fg) sponges + metallic magnesium) reduced macrophage pro-inflammatory phenotype and stimulated their capacity to promote Mesenchymal Stem/Stromal Cells (MSC) osteogenic differentiation [3]. Magnesium ions (Mg\textsuperscript{2+}) are reported as immunomodulatory, reducing pro-inflammatory macrophage polarization [3,4]. The hypothesis underlying the current work was that the incorporation of Mg\textsuperscript{2+} ions into Fg-3D scaffolds should lead to an improved ability of the latter to modulate macrophage polarization.

Experimental Methods
A Fg solution (from human plasma, Grifols S.A.) and a magnesium sulphate (MgSO\textsubscript{4}) concentrated 200 mM solution were combined to obtain 3D scaffolds of Fg with 0, 10, 25 and 50 mM of MgSO\textsubscript{4} (FgMgSO\textsubscript{4}). The Fg\textsuperscript{+}MgSO\textsubscript{4} solution was cast, freeze-dried and EDC was used as chemically crosslinker [3]. The properties of the scaffolds were evaluated by: scanning electron microscopy (SEM), for structure and porosity; Fourier Transform Infra-Red (FTIR), for functional groups analysis; dynamic mechanical analysis (DMA) for mechanical properties; and electro kinetic analysis (EKA) for zeta potential. Human monocytes were isolated from buffy coats (kindly donated by Serviço de Immunohemoterapia, CHUSJ) and seeded directly on the scaffolds, in presence of M-CSF for macrophage differentiation. Cells were either left unstimulated (M0), or stimulated with LPS+IFN\gamma (M1). Lactate dehydrogenase (LDH) was measured using the CytoTox96® assay. Confocal microscopy was used to analyze M1 (CCR7) and M2 (CD163) polarization markers, and NF-\kappa B p65 nuclear translocation. Human bone marrow MSCs were cultured on FgMgSO\textsubscript{4} (0 and 10 mM) scaffolds, or in presence of macrophage secretome. Osteogenic differentiation was assessed through Alizarin Red staining.

Results and Discussion
Scaffolds of Fg alone or doped with MgSO\textsubscript{4} (10, 25 and 50 mM) presented a 3D network of interconnected pores. MgSO\textsubscript{4} increased the loss and storage modulus of Fg, as well as surface charge. The analysis of extracellular LDH showed a significant decrease in cell viability for macrophages cultured in 25 and 50 mM FgMgSO\textsubscript{4} scaffolds, either in M0 or M1 conditions. Importantly, FgMgSO\textsubscript{4} (10 mM) scaffolds increased the percentage of CD163 positive M2 cells and decreased the percentage of CCR7 positive M1 cells, in comparison with Fg alone and FgMgSO\textsubscript{4} (25 and 50 mM) scaffolds, both in unstimulated (M0) conditions and in response to LPS+IFN\gamma-driven macrophage M1 polarization. Mechanistically, NF-\kappa B p65 nuclear translocation, activated in response to M1 stimulation, was significantly decreased in macrophages cultured in FgMgSO\textsubscript{4} (10 mM) scaffolds, when compared with Fg scaffolds. The secretome of macrophages cultured in Fg and FgMgSO\textsubscript{4} (10 mM) scaffolds, either in M0 or M1 conditions, increased MSC osteogenic differentiation, when compared with secretome of plastic-cultured M0 and M1
macrophages. Also, MSC cultured directly on FgMgSO$_4$ (10 mM) scaffolds showed significantly increased calcium deposits, in comparison with those cultured on Fg scaffolds.

**Conclusion**

Our results show that doping Fg scaffolds with 10 mM of MgSO$_4$ modulates macrophage polarization towards an M2 phenotype and boosts MSC osteogenic differentiation. Thus, Fg scaffolds incorporating Mg$^{2+}$ ions modulate the host macrophage response. Currently, an animal model is being used to assess the *in vivo* behaviour of these scaffolds.

**Acknowledgement**

Grifols SA for donating clinical grade fibrinogen. *Serviço de Ortopedia* and *Serviço de Imunohemoterapia, CHUSJ* for donating bone marrow samples and buffy coats, respectively. Daniela Silva (CEMUP) and Ricardo Vidal (Bioimaging i3S Scientific Platform) for the technical and scientific support with SEM analysis and biomaterials characterization techniques (FTIR, DMA and EKA), respectively. M. Bessa-Gonçalves funded by FCT through PhD fellowship PD/BD/135489/2018.

**References**

Hydrogels releasing sulfated hyaluronan improve impaired skin wound healing in condition of obesity induced diabetes by regulating inflammatory macrophage activities

Sophia Hauck¹, Marta Torregrossa¹, Ainur Kakpenova¹, Paula Zager¹, Norbert Haßler², Sandra Rother²,³, Stephanie Möller³, Matthias Schnabelrauch³, Jan C. Simon¹, Vera Hintze², Sandra Franz¹

¹ University Leipzig, Department of Dermatology, Venerology and Allergology, Leipzig, DE; ² University of Technology Dresden, Institute of Materials Science, Dresden, DE; ³ INNOVENT e. V. Jena, Biomaterials Department, Jena, DE; ⁴ University of California, Department of Cellular and Molecular Medicine, San Diego, US

Introduction
Non-healing wounds are stuck in a chronic inflammatory phase driven by uncontrolled infiltration and activation of macrophages leading to excessive tissue breakdown. Resolution of this unrestrained inflammatory loop represents an unmet challenge in the treatment of non-healing wounds. We have recently shown, that redirecting macrophage activities and resolving inflammation indeed rescues impaired wound healing in mice [1]. However, implementation of controlling macrophage functions in chronic skin wounds by an applicable immunomodulating wound dressing is still lacking [2]. Here, we developed immunomodulatory hydrogels containing sulfated hyaluronan (sHA) as immunoregulatory component for the modulation of inflammatory macrophage activities to control disturbed healing processes in chronic skin wounds. Hyaluronan (HA) is an essential component of the extracellular matrix and known to regulate inflammatory processes. Immunoregulatory properties of HA typically depend on its molecular size with high molecular weight HA (H-HA) being anti-inflammatory while the supposed pro-inflammatory activity of low molecular weight HA (L-HA) is controversially discussed.

Experimental Methods
Using in vitro assays with human and murine macrophages and skin ex vivo cultures, as well as mouse models of acute skin inflammation and of diabetic wound healing we show that immunoregulatory activities of HA can be uncoupled from its molecular size by chemical modification with sulfate groups and that artificially sulfated HA (sHA) possess potent anti-inflammatory activities towards inflammatory activated macrophages in vitro [1;2] and in skin inflammation in vivo which can be translated for the modulation of macrophage-driven impaired wound healing processes in skin.

Results and Discussion
First, we assessed immunoregulatory effects of sHA (48kDa) in comparison to H-HA (1174kDa), L-HA (48kDa) on human inflammatory macrophages (iMa) and analysed underlying molecular mechanisms using global quantitative proteomics, molecular modeling and targeted assays. We observed significant down-regulation of iMa activation by sHA that required rapid uptake of s-HA mediated by CD44, CD36 and Lox1. Induction of anti-oxidative proteins (SOD2, SOD3) and inhibition of transcription factor activation (pNFkB, pSTAT1, IRF5) resulted in reduced expression and release of pro-inflammatory cytokines (TNF, IL-12, MCP-1, IL-6) and upregulation of anti-inflammatory proteins (IL1RN, NMB). We further observed, that sHA interferes increased activation of inflammatory macrophage activities in conditions of an altered metabolic environment as it occurs in obesity, and in particular inhibits inflammasome activation in the macrophages. Skin ex vivo co-culture experiments showed that the reduced iMa activation by sHA had an impact on the induction of pro-inflammatory signals in the skin. We then, investigated the effect of sHA, H-HA and L-HA in the mouse model of imiquimod-induced acute skin inflammation. Administration of sHA reduced signs of skin inflammation (redness, scaling, epidermal thickness, immune cell infiltration) superior to H-HA. Monocytes/macrophages that we isolated from the inflamed tissue of sHA-treated skin showed reduced expression...
of inflammatory cytokines (IL-1b, TNF) while anti-inflammatory signals (IL-10, IL-1RA) were up-regulated. Finally, we designed hyaluronan/collagen (HA/coll)-based hydrogels that allow the continuous release of sHA over a period of at least 1 week. We tested the effect of sHA-releasing hydrogels in comparison to control hydrogels (no sHA release) in skin wounds of diabetic db/db mice, an established model for delayed diabetic wound healing. In a translational approach, we applied all hydrogels at day 3 post-wounding when inflammation in the wounds had already developed. The sHA-releasing hydrogels improved defective tissue repair in the diabetic mice with reduced inflammation and augmented alternative macrophage activation (down-regulation of IL-1b and up-regulation of IL-10, Relma), increased vascularization (up-regulation of VEGF, CD31) and accelearated new tissue formation (up-regulation of EGF, Ki67, granulation tissue).

**Conclusion**

In sum, our data show that sHA modulates pro-inflammatory macrophage activity in favour of pro-resolution functions and has the capability to improve outcome of disturbed skin wound healing.

**Acknowledgement**

This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) project number 59307082 – TRR67subprojects A3, B3, Z3; project FR2671/4-1 to SF; project 420160411 to SR

**References**


Human protein-based sponges for fully xeno-free cell culture platforms

Sara C. Santos, Catarina A. Custódio, João F. Mano

University of Aveiro, CICECO-Aveiro Institute of Materials, Aveiro, PT

Introduction

Human platelet lysates (PL) are a source of growth factors and other bioactive proteins involved in cell growth, proliferation and differentiation processes. Therefore, they have been explored for multiple clinical and tissue engineering (TE) purposes [1,2]. We recently reported a bioactive human PL derivative precursor (PLMA) that can be cured upon light exposure to form a hydrogel with tuneable mechanical properties [2]. Moreover, the synthesized PLMA-derived hydrogels have proven to support distinct human derived cell cultures [2,3,4] that perform important biological processes such as growth, sprouting and migration. Indeed, we also demonstrated that PLMA cell culture platforms provide the controlled release of several growth factors that are essential for cell maintenance. Animal-derived serum is a ubiquitously used supplement in cell culture media. However, there are serious scientific and ethical concerns about the quality and safety as well as animal welfare [1]. Following a humanized and completely xeno-free strategy we propose PL sponges as a support for human 3D cell culture in animal serum free conditions.

Experimental Methods

PL methacryloyl (PLMA) were prepared by reaction of PL with methacrylic anhydride following a previously reported protocol [2]. PLMA hydrogels were formed by irradiating with UV light a solution of 15% (w/v) PLMA containing 0.5% (w/v) of Irgacure 2959. Afterwards, PLMA hydrogels were freeze-dried to produce PLMA sponges. PLMA-based sponges internal structural features were assessed by Scanning Electron Cryomicroscopy (CryoSEM). Pore size and swelling rate analysis of the scaffolds were performed. Human adipose-derived stem cells (hASCs) were seeded on top of PLMA sponges in order to assess the biological performance of such platforms. hASCs were cultured during 14 days with medium supplemented and no supplemented with FBS and tested for cell adhesion, viability, and proliferation at pre-determined time points (3, 7 and 14 days). As a control, a commercially available collagen type I sponge (SpongeCol®) was used.

Results and Discussion

PLMA hydrogels were already described as a platform for cell culture. Therefore, herein we proposed to produce PLMA sponges to be used as a scaffold for 3D human cell culture in a xeno-free environment. CryoSEM images (Fig. 1A) of PLMA sponges show an internal porous structure dependent on the concentration of PLMA. Swelling rate (Fig. 1B) results show complete re-swelling of the scaffolds after about 5 minutes in contact with PBS. Biological assays were performed by seeding of hASCs on top of PLMA sponges followed by culture in medium with and without animal-derived serum supplement. The cells were cultured for 14 days and as shown by fluorescence images of Live/Dead staining (Fig. 1C) hASCs exhibit high cell viability even when cultured in animal serum free conditions. Moreover, DAPI/Phalloidin staining images (Fig.1C) show the ability of cells to spread and form networks with neighboring cells when cultured on top of PLMA sponges. Cell proliferation and viability assays were performed by DNA quantification (Fig.1D) and MTS test (Fig.1E), respectively. Results show an increase in DNA content up to 14 days of cell culture even with no animal serum supplementation meaning that hASCs are able to proliferate when cultured in PLMA sponges. In addition, MTS test results also revealed the ability of hASCs to stay viable and proliferate. SpongeCol®, a commercially available collagen type I based sponge, was used as control in the biological performance tests. Results show that cells were not able to adhere and proliferate when SpongeCol® discs seeded with hASCs were cultured in medium without FBS. Therefore, such results support the idea that PLMA-based sponges have great potential to be used as platforms to support 3D xeno-free cell culture.
Conclusion
Our results showed that PLMA sponges are a promising platform for human 3D cell culture. Of note, they offer the possibility to culture cells avoiding the use of animal derived serum supplements. It is also a platform that could have an autologous origin, being adequate to produce personalized matrices for in vivo applications with no risk of cross-reactivity, immune reaction, or disease transmission.

Acknowledgement
Funding support from Fundação da Ciência e Tecnologia through the project BEAT (PTDC/BTMMAT/30869/2017), the doctoral grant SFRH/BD/144520/2019, the individual contract CEECIND/02713/2017 and CICECO – Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020.

References
[4] Tavares, MT; Santos, SC; Custódio, CA; Farinha, JPS; Baleizão, C; Mano, JF, Platelet Lysates-Based Hydrogels Incorporating Bioactive Mesoporous Silica Nanoparticles for Stem Cell Osteogenic Differentiation, Mater. Today Bio 2021, 9, 100096
Production and assessment of three-dimensional collagen scaffolds with controlled pore alignment and orientation for tissue engineering applications

Huijie L. Zhang, Ruth E. Cameron, Serena M. Best

University of Cambridge, Materials Science and Metallurgy, Cambridge, GB

Introduction

Ice-templating has been used widely in the production of porous collagen scaffolds for tissue engineering. Complex architectures can be achieved through careful design of the temperature profiles during the freezing process and using this technique, it is possible to fabricate biomimetic scaffolds for specific tissue repair applications. However, the random nature of ice nucleation can create issues with processing-structure-property reproducibility [1]. In this work, we describe a method to produce collagen scaffolds with controlled pore alignment and we offer a modelling design toolkit for rapid prediction of pore orientation. The structure of the scaffolds have been characterised using X-ray microtomography (μCT), generic cell studies have been carried out to assess cell migration, and the effects of pore architecture on the mechanical properties are reported.

Experimental Methods

1 wt% collagen in 0.05 M acetic acid was directionally cooled on custom-made device with a copper cold finger, and the structures were imaged and studied by μCT. The scaffolds were chemically crosslinked with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-hydroxysuccinimide (EDC/NHS) at 10% concentration of that reported previously in the literature [2]. The elastic modulus was then measured by compression tests while scaffolds were fully immersed in water, using a 25 N load cell and crosshead speed of 5 mm/min. The scaffolds were tested in two directions in order to study the effects of pore orientation on stiffness, one along the aligned direction of pores and the other one perpendicular to the pore alignment (Fig. 1a). Additionally, a thin film resistance temperature detector was placed on polycarbonate mould as an external heating source to manipulate pore orientation. The heat transfer during the freezing process was predicted by finite element modelling (FEM) via COMSOL. Preliminary cell studies were undertaken using human dermal fibroblasts to assess cell migration within the three-dimensional scaffolds (1x1x1 cm³ cube) and results were recorded on Days 7, 10 and 14.

Results and Discussion

The technique successfully produced scaffolds with very high pore alignment. A small isotropic region was noticed at the base of the sample closest to the cold finger. The average pore size in the bulk of the sample increased with decreasing cold finger cooling rate. Pore sizes that could be produced ranged from 50-160 μm and were confirmed to be suitable for cell migration over a range of different time points. The mechanical test results for samples taken from the aligned regions showed that elastic modulus varied with direction from approximately 1 kPa in the x-y plane (Direction 2) to 8 kPa when samples were loaded in the z direction (Direction 1) (Fig. 1b).

The addition of a heat patch to the sample mould was designed to control the pore orientation in a precise manner, and this was modelled using FEM (Fig. 2a). The predicted growth of the ice freezing front was found to match well with pore orientation as demonstrated by the μCT images (Fig. 2b-c). A heatmap of the z-direction stiffnesses obtained for 10% EDC/NHS crosslinked samples taken from nine different regions is shown in Fig. 2d. The results combined, provide a design toolkit to rapidly achieve specific structures with desired elastic modulus.

Conclusion
The use of a custom-built freezing rig has allowed the production of scaffolds with highly aligned pores, and by additionally adding a heat patch, the pore orientation could be controlled. Using this method, it is possible to recapitulate architectural features of tissues, such as myocardium, nerve tissue and skeletal tissues. The flexibility of this technique for tissue engineering applications was demonstrated by (1) the range of pore sizes were available (50-160 μm) for cell invasion and vascularisation; (2) elastic modulus values in the kPa range, with the potential to match the properties of target tissues.

Acknowledgement

The authors would like to acknowledge the financial support of EPSRC for a joint Fellowship grant EP/N019938/1, Cambridge Trust and Chinese Scholarship Council.

Fig. 1 The mechanical testing of collagen scaffolds with aligned porosity.

(a) schematic diagram of compressing scaffolds (1x1x1 cm³ cube) in two directions: direction 1 is along the y-axis (same as the pore alignment), and direction 2 is along the x-axis (perpendicular to the pore alignment). μCT images were used to represent the aligned pores. (b) the comparison of stress-strain curves of two different pore orientations.

Fig. 2 Toolkit for predicting and characterising the structure of ice-templated collagen scaffolds

(a-b) finite element modelling of heat transfer within collagen slurry at various timepoints. (c) images of 9 samples (1x1x1 cm³ cube) cut from a freeze-dried scaffold (columns 1-3 display images across the sample (x-axis); bottom-top are along the z-axis). (d) elastic modulus heatmap of samples processed using 10% EDC/NHS crosslinking.

References


Combination of QK peptide and proteolytic sequences for the promotion and direction of angiogenesis in Elastin-Like Recombinamer (ELR) hydrogels

Fernando González-Pérez, Arturo Ibáñez-Fonseca, Matilde Alonso, José C. Rodríguez-Cabello

Universidad de Valladolid, Valladolid, ES

Introduction
The development of three-dimensional (3D) systems with the capacity to promote and spatio-temporally direct the cell infiltration and vascularization in biological tissues hold promise in the area of tissue engineering and regenerative medicine (TERM). To this purpose, fast (GTAR) and slow (DRIR) proteolytic sequences sensitive to uPA (urokinase plasminogen activator) enzyme and the QK vascular endothelial growth factor mimetic peptide have been exploited showing their potential activity for maintaining cell signalization for colonization [1] and promoting angiogenesis [2], respectively. Hydrogels based on Elastin-like recombinamers (ELRs) can be exploited in this regard, their bioactiveness can be tailored on-demand allowing to genetically encode proteolytic sequences into their polypeptide backbone as well as to covalently tether bioactive residues such as the QK peptide.

Experimental Methods
A pair of ELRs containing either the GTAR or DRIR sequence was designed and decorated with the QK pro-angiogenic peptide and click catalyst-free crosslinkable domains to produce the intended hydrogels. In vitro studies allowed to determine the effect of QK peptide over endothelial cell morphogenesis, whereas the elastic modulus of the prepared hydrogels was evaluated by rheology techniques. Coaxial binary ELR tubular constructs were designed to assess the ability of the designed ELRs to guide degradation and vascularization in vivo. In detail, scaffolds displaying the DRIR and the GTAR on the outer and inner part of the tube containing the QK peptide in the inner, both and none of them were fabricated. Cell colonization and vascularization was analyzed by histology and immunohistochemistry (IHC) upon subcutaneous implantation in Swiss CDR-1 mice with time.

Results and Discussion
In vitro studies revealed the pro-angiogenic effect of QK peptide on endothelial morphology. Coaxial binary ELR tubular constructs exhibited a marked increase in cellular colonization and degradation on the inner and fast degradable ELR tube, when compared to the outer part with slow cleavage kinetics. Histology and IHC results of in vivo studies further showed the QK peptide effect triggering high vascularization. Combination of both factors, namely proteolytic sequences and QK peptide, revealed a synergistic effect over angiogenesis process in vivo.

Conclusion
ELR hydrogels displaying proteolytic sequences and decorated with the QK peptide have reported to enhance colonization and vascularization in vivo in a pre-designed way. These results pave the way for developing ELR-based scaffolds for TERM applications with spatio-temporally controlled vascularization in the achievement of an optimal tissue repair.

Acknowledgement
Financial support was received from MINECO (PCIN-2015-010, MAT2016-78903-R, FPU 16/04015), Junta de Castilla y León (VA317P18) and Centro en Red de Medicina Regenerativa y Terapia Celular de Castilla y León.

References
4:15 p.m. – 5:45 p.m.

Track02

S08 | Beyond Bone and Teeth: Bioactive Glasses in Soft Tissue Regeneration

Chairs

Janis Locs
Riga Technical University, Riga Biomaterials Innovations and Development Centre, Riga, LV

Enrica Verné
Politecnico di Torino, Scienza Applicata e Tecnologia, Torino, IT

Joana Moreira Marques (YSF)
University of Porto, i3S, Porto, PT

Bioactive glasses represent a revolutionary technology in the healthcare sector. Numerous bioactive glass formulations have found applications to treat bone and dental injuries and, over the years, applications in soft tissue repair and drug delivery have emerged. This symposium is focused on bioactive glass advances based on the controlled release of biologically active ions for applications in soft tissue repair and wound healing, including skin, muscle and nerve tissue regeneration.

Prof. Aldo R. Boccaccini and Prof. Jonathan Massera are well known researchers in the field of bioactive glasses. They will be the keynote speakers in this symposium covering different aspects of the processing and characterisation of bioactive glasses in the field of tissue engineering. They will provide a broad overview of the field, highlighting challenges and opportunities of bioactive glasses in soft tissue regeneration.
Bioactive glasses-based biomaterials with potential in soft tissue engineering

Jonathan Massera

Tampere University, Faculty of Medicine and Health Technology, Tampere, FI

Introduction
Silicate-bioactive glasses have been extensively studied for bone tissue engineering. The “traditional” bioactive glasses (i.e. 45S5 and S53P4) have found space in clinics: from bone filler to ocular implants. The benefit of using bioactive glasses, lies in the ability to tailor the composition to control the dissolution/reaction rate or thermal properties (fiber drawing, scaffold sintering). More importantly, the ease to incorporate ions of therapeutic interest has led to remarkable discoveries. Indeed, control release of strontium, zinc, magnesium, silver, copper or boron, just to cite a few, has enabled to process materials with excellent osteogenesis, angiogenesis or antimicrobial properties [1]. However, while bioactive glasses, in bone related applications, was promising, the difficulties in shaping the grafts into the exact patient’s defect has, to some extent, limited their clinical use. Nevertheless, it appears clear that the unique properties of bioactive glasses are still of interest. Indeed, researchers are constantly studying their potential use in composites or hybrid biomaterials [2].

While many questions remain to be answered to better grasp the full potential of bioactive glasses in hard tissue regeneration, some researchers already devoted their energies to explore new avenues for such uniquely tailorable material. For instance, researchers have explored the potential of bioactive glasses in soft tissue regeneration. For example, tubular phosphate glass fibres have been found to give a promising alternative to end-to-end suturing in facial nerve reconstruction [3]. More recently, borate bioactive glass nanofibers (Mirragen™) has proved to be excellent in treating long-term venous stasis ulcer in diabetic patients who were irresponsible to conventional treatments [4].

These new results are opening new horizons for the use of bioactive glasses in clinics.

RESULTS AND DISCUSSION
In the recent years our work has focused on designing materials that, not only could be applicable to hard tissue engineering but also soft tissue. In order to develop innovative construct; able to not only support, but also trigger, soft tissue regeneration a survey study where bioactive glass (13-93) extract was performed to culture various cell types (adipose stem cells, lung fibroblasts, or uroepithelial cells). The hASCs and the WI fibroblasts remained viable in the extracts. Li and Sr appeared to have positive impact on the cell’s proliferation. However, the high Ca concentration inhibits the proliferation of UE cells.

Tubular structures (PLA/bioactive glasses) were efficiently produced with a controlled porosity (>50%) and pore size ranging from 100 to 400 µm (Fig 1). The glass (13-93) dispersion was found to be homogeneous within the structure. The glass loading (25-35 wt%) was consistent with the target loading. The presence of bioactive glass allowed to prevent acidification of the environment while contributing to the faster degradation of the polymer matrix. Hybrid biomaterials (wood based nanofibrils and bioactive glasses or gelatin/GPTMS/bioactive glasses) were also developed into inks for bioprinting. In this study we demonstrated the possibility to develop an ink that can be printed into the shape of a mandible (Fig.2) while maintaining cell viability. Composites and hybrids could have potential for nerve regeneration, urethra or trachea repair.

Finally, while ions can trigger signalling pathways leading to osteogenesis or angiogenesis, in recent years the use of light to direct tissue healing as gained increasing interest. As such, biophotonic bioactive glasses were develop. While their characterization is only starting, the ability to design material with persistent luminescence in wavelength...
ranging from UV to NIR has high potential in tissue engineering. Indeed, it was shown by Liu et al. that micro-patterned light emission can enable to orientate cells and create anisotropic cell sheets, thus mimicking the complex structure of soft tissue such as muscles, artery, and nervous system [5]

**Conclusion**

Bioactive glasses have been widely studied in hard tissue reconstruction. However, their potential in soft tissue is nowadays being investigating. Bioactive glass, when combined with natural or synthetic polymeric matrices, enables to design materials for a wide range of application (from restoring the nervous system to healing urethra defects). The benefit of ions released from the bioactive glass is undeniable. Combining controlled ion release, biophotonic and tissue engineering may open the door to significant breakthrough in soft tissue regeneration.

**Acknowledgement**

The author would like to acknowledge all the PhD students and Post-Doctoral researcher that took part of the various projects. The funding bodies, i.e., the Academy of Finland, The Jane and Aatos Erkko Foundation and EU-H2020 are acknowledged for their financial support.

---

**References**

S08-KL02

Applications of inorganic materials in soft tissue regeneration: progress and challenges

Aldo R. Boccaccini

University of Erlangen-Nuremberg, Institute of Biomaterials, Erlangen, DE

In 2021 the biomaterials community celebrates 50 years of bioactive glass (BG), the first man-made material capable to bond to tissues, discovered by the late Prof. Larry L. Hench [1]. It is well-known that the traditional applications of BGs have been in bone substitution, for example as bone defect filler, small bone and dental implants and as bioactive coatings for orthopedic and dental applications. Moreover BGs emerged as attractive inorganic materials for bone tissue engineering (TE) given their bone bonding ability and the effect of BG dissolution products on osteogenesis. More recently, BGs have started to be considered in soft TE and wound healing. Such TE applications exploit the biochemical interactions occurring at the interface between BG surfaces and the biological environment, which lead to the (controlled) release of biologically active ions to activate specific cellular pathways [2]. As an example, the effect of selected metallic ions on vascular endothelial growth factor release from stem cells has been demonstrated to indicate the angiogenic potential of BGs.

For applications in soft TE, BGs must be "softened" which is achieved by smart combination of BGs, usually in particulate or fibre form, with suitable biodegradable polymers. Processing techniques for biopolymer scaffolds such as freeze-drying, electrospinning and 3D printing, are commonly applied to develop BG containing scaffolds with suitable mechanical properties, porosity and degradation behaviour for soft tissue repair applications. Typical examples of such composites for a variety of soft TE applications, including wound healing, nerve and muscle regeneration will be discussed.

In the second part of the presentation, applications of BGs (e.g. as mesoporous nanoparticles) in the field of 3D bioprinting (biofabrication), which have been emerging in the last few years, will be introduced. Such applications expand the scope of applications of BGs in TE. In this context, the progress in the development and characterization of cell laden hydrogel-BG scaffolds by 3D bioprinting will be discussed. Examples of such applications will be presented highlighting the novel developments of hydrogel-bioactive glass composites as innovative multimaterial bioinks for cell encapsulation and for biofabrication of TE scaffolds of increasing complexity [3]. The author’s views on the challenges and opportunities for further research in the field will be presented.

References
Restoring bone regeneration in diabetic (hyperglycaemic) environments with HIF mimetics and bioactive glasses

Azadeh Rezaei, Joel Turner, Kaveh Shakib, Gavin Jell

University College London (UCL), Division of Surgery and Interventional Science, London, GB

Introduction
Diabetic patients have an increased risk of fracture and non-union fracture healing\(^1\). Diabetic patients and hyperglycaemic conditions have also been shown to cause an impaired hypoxia inducible factor-1α (HIF-1α) and reduced cellular response to low oxygen conditions (hypoxia)\(^2\). Whilst the HIF pathway is undoubtedly important in angiogenic signalling and restoring the vasculature in bone repair\(^3\), the direct role of HIF-1α on osteoblast behaviour and mineralisation in both normal and hyperglycaemic conditions is unclear. There may also be differing effects between hypoxia and HIF stabilisation. Using a multidisciplinary characterisation approach (biological, ultrastructural and microstructural quantitative techniques), this study investigates the role of hypoxia (1% O\(_2\)) and HIF-stabilisers (CoCl\(_2\) and dimethyloxalylglycine (DMOG), on bone formation, in normal and high glucose in vitro environments. Moreover, the effect of controlled cobalt ion release via cobalt bioactive glasses (CoBGs) on hyperglycaemic nodule formation will be studied with the aim to determine if targeting HIF pathway can promote bone regeneration in diabetic patients.

Experimental Methods
Primary osteoblasts were isolated from neonatal Sprague-Dawley rats and cultured in α-MEM supplemented with 2 mM β-glycerophosphate, 10nM dexamethasone, and 50μg/mL ascorbate. Once confluent, cells were exposed to normal (5.5mM), moderate (25mM) and high (50mM) glucose environments and kept in normoxia (20% O\(_2\)) and hypoxia (1% O\(_2\)) for 21 days. Cells were also treated with CoCl\(_2\) (12.5, 25 and 50μM), DMOG (250,500 and 1000μM) and CoBG conditioned medium (containing 0, 12.5 and 25μM cobalt) in the various glucose conditions. Metabolic activity, proliferation rate, alkaline phosphatase (ALP) activity and vascular endothelial growth factor (VEGF) expression. Bone nodules were characterised using Alizarin Red staining, scanning and transmission electron microscopy (SEM,TEM), Raman spectroscopy and interferometry for quantitative analysis of the nodule size.

Results and Discussion
Moderate and high glucose conditions inhibited nodule formation. Hypoxia (1% O\(_2\)), inhibited nodule formation in all glucose levels. HIF stabilisers (CoCl\(_2\), DMOG and CoBG) and CoBG conditioned media did not prevent nodule formation in normal glucose, and restored nodule formation in hyperglycaemic conditions (as determined by size)(Figure 1. a&b) All HIF stabilisers enhanced HIF-1α expression and VEGF production in moderate and high glucose environments. Glucose level did not have any effect on ALP activity.

Conclusion
A hyperglycaemic bone model was developed that demonstrated that hyperglycaemic environments inhibit bone nodule formation, whilst the use of HIF mimetics (CoCl\(_2\), DMOG and CoBG) restored bone nodule formation in these conditions. This develops our understanding of the role of the HIF pathway in bone mineralisation and the creation of biomaterials or tissue scaffolds designed for patients with impaired bone regeneration due to an impaired HIF-1α pathway.
Figure 1
The effect of best concentrations of HIF mimetics (Co 12.5µM and DMOG 500µM) on nodule formation in low, moderate and high glucose environments. Alizarin Red calcium staining showed that non-toxic range of CoCl_2 and DMOG restore bone formation in moderate and high glucose environments (scale bar is 200nm)

References
Sr-containing Mesoporous Bioactive Glasses Bio-Functionalized with ICOS-Fc: an advanced tool to target osteoporotic fractures

Sonia L. Fiorilli¹, Mattia Pagani¹, Elena Boggio²,³, Casimiro L. Gigliotti²,³, Chiara Dianzani⁴, Carlotta Pontremoli¹, Giorgia Montalbano¹, Umberto Dianzani³, Chiara Vitale-Brovarone¹

¹ Politecnico di Torino, Department of Applied Science and Technology, Turin, IT; ² NOVAICOS s.r.l.s, Novara, IT; ³ Università del Piemonte Orientale, Department of Health Sciences, Novara, IT; ⁴ Università di Torino, Department of Drug Science and Technology, Torino, IT

Introduction

Osteoporotic bone fractures represent a critical clinical issue and need personalized and specific treatments. The development of smart nano-biomaterials able to synergistically combine chemical and biological cues to impart specific therapeutic effects (i.e., pro-osteogenic, anti-clastogenic) can provide novel and effective medical solutions to target compromised bone remodeling. Recently, strontium-containing mesoporous bioactive glasses (Sr-MBGs) proved to exert a role in the activation of both osteoblast (Ob) and osteoclast (Oc) cell signaling pathways, which allows the promotion of osteoblast replication, differentiation, and survival while downregulating osteoclast activities [1]. In this contribution, with the aim to open new perspectives for advanced treatments of osteoporotic fractures, Sr-MBGs have been bio-functionalized with ICOS-Fc, a molecule able to reversibly inhibit Oc activity. The proposed strategy (Figure 1) aims to combine in single device the intrinsic properties of MBGs with the specific effect exerted by ICOS-Fc to deliver a multifunctional platform (i.e., bioactivity, stimulation of osteoblast cells, inhibition of osteoclast activity) for the treatment of osteoporotic fractures.

Experimental Methods

The extracellular portion of human ICOS was cloned as fusion protein to the human IgG1 Fc region, generating ICOS-Fc fusion protein, according to Di Niro et al. [2]. Sr-containing MBGs (10% mol.) were synthesized as reported in ref [3] and post-modified with ((3-aminopropyl)silanetriol to expose surface amino groups able to react with the carboxyl groups present on Fc residue of ICOS-Fc molecule. The Sr-MBGs after ICOS-Fc grafting were fully characterized by scanning electron microscopy, N₂ adsorption-desorption analysis, FT-IR spectroscopy, thermogravimetric analysis. Flow cytometry (Attune NxT, Life Technologies, Carlsbad, CA, USA) was performed to assess the presence of ICOS-Fc molecule on MBG surface and an in house-developed ELISA-Like Assay was performed to prove the retention of ICOS-Fc functionality upon grafting and the binding stability in aqueous medium. Cell Migration Assay were carried out in the Boyden chamber (BD Biosciences, Milan, Italy) migration assay by using ICOSL positive cell lines PC-3 (prostate cancer) and U2OS (osteosarcoma). The ability of grafted ICOS-Fc to inhibit osteoclast differentiation and function was assessed by monitoring the differentiation of monocyte-derived osteoclasts (MDOCs) up to 21 days.

Results and Discussion

N₂ adsorption analysis, FT-IR spectroscopy and flow-cytometry analysis proved the successful grafting of ICOS-Fc on the surface of Sr-containing MBGs, which were also proved to retain the peculiar ability to release osteogenic strontium ions and an excellent bioactivity after functionalization. An ELISA-like assay allowed to confirm that grafted ICOS-Fc molecules were able to bind ICOS-L (the ICOS binding ligand) and evidenced the stability of the surface binding to hydrolysis in aqueous environment up to 21 days. The migration assay demonstrated that, in analogy to the free form of the molecule, PC-3 and U2OS cell (ICOSL positive) migration was affected by ICOS-Fc grafted Sr-MBGs (in a dose dependent manner), at variance with HOS (ICOSL negative) cell migration, confirming the specificity of ICOS-Fc to bind ICOSL. Furthermore, grafted ICOS-Fc was able to strongly inhibit the differentiation of MDOCs.
which after the treatment showed a round shape and a morphology similar to a spindle. The strong inhibitory effect was also proved by the downregulation of DC-STAMP, OSCAR, and NFATc1 expression, as evidenced by real-time PCR after 21 days.

**Conclusion**

The functionality of ICOS-Fc molecule anchored to Sr-containing MBGs was confirmed by a custom-made ELISA-like and the binding stability was assessed up to 21 days of soaking in culture medium. The inhibitory effect of grafted ICOS-Fc on cell migratory activity was demonstrated by using ICOSL positive cell lines and the ability to inhibit osteoclast differentiation and function confirmed (in analogy to the free form of the molecule) by monitoring the differentiation of MDOCs, which revealed a strong inhibitory effect, also proved by the downregulation of Oc differentiation genes.

The obtained results are very promising and pave the way to personalized clinical solutions for bone regeneration of fractures in osteoporotic patients.

**Acknowledgement**

This project has received funding from the European Union’s Horizon 2020 research and innovation program under grant agreement No. 814410.

![Figure 1](image)

**Figure 1**  
Schematic illustration of the effect produced by Sr-containing MBGs functionalized with ICOS-Fc on osteoblast and osteoclast cells

**References**


3D plotted composites consisting of calcium phosphate bone cement and mesoporous bioactive glass with drug delivery function

Richard F. Richter, Tilman Ahlfeld, Michael Gelinsky, Anja Lode

TU Dresden, Centre for Translational Bone, Joint and Soft Tissue Research, Dresden, DE

Introduction
Calcium phosphate bone cements (CPC) and mesoporous bioactive glasses (MBG) are two well studied biomaterial groups widely under investigation concerning their applicability to treat bone defects. Recently, a CPC-MBG composite was developed in our group which showed promising results with respect to cell response in vitro and new bone formation in vivo [1,2]. Unlike conventional powder/liquid cements, the used CPC is a hydrophobic carrier-liquid (cl) based paste; it maintains a good injectability until contact with aqueous environment that allows its application for extrusion-based additive manufacturing techniques like 3D plotting [3]. By variation of the amount of cl, it is possible to prepare CPC-MBG composites that are suitable for 3D plotting as well and the advantage of 3D plotted scaffolds of such composites compared to bulk scaffolds regarding ion release were shown recently [4]. The aim of the present study was to analyse the degradation behavior of different CPC-MBG composites and the influence of the single components in detail. Furthermore, due to their high specific surface area, MBG are promising candidates to be used as carrier system for proteins, growth factors or anti-inflammatory agents. This study therefore aimed to develop a method that allows to load MBG with proteins and incorporate them into CPC pastes without affecting the plottability.

Experimental Methods
CPC and strontium-modified CPC pastes were provided by INNOTERE GmbH, Germany and MBG particles (< 45 µm) were prepared as previously described [5]. Additionally, strontium-modified MBG particles were synthesized by complete substitution of the calcium part with strontium. To study the degradation behavior, mass loss, change of porosity and ion release of 3D plotted scaffolds (Figure 1) made from different CPC-MBG combinations were evaluated and compared to those of 3D plotted scaffolds or samples made of the single components. Afterwards, a protocol for loading MBG particles with proteins was developed. The protein uptake kinetics for different types of MBG and various initial protein concentrations were studied using lysozyme as a model protein. The release of lysozyme from pure MBG and from 3D plotted CPC-MBG scaffolds was investigated and it was verified whether the released lysozyme retained its biological activity. Finally, this developed protocol was transferred to the proangiogenic growth factor VEGF (Vascular Endothelial Growth Factor) and again the release and biological activity were analysed and verified.

Results and Discussion
Based on our previous work it was possible, by varying the amount of cl, to increase the addition of MBG to 16 wt% and maintain the extrudability of the composites. Composites with at least 8 wt% MBG showed a significantly higher initial porosity and significantly greater mass loss after degradation. Regarding the ion release, again composites with a minimum addition of 8 wt% MBG showed a significantly improved release of ions, e.g. Sr²⁺ compared to pure strontium-modified CPC scaffolds.

Additionally, a protocol for loading MBG particles with proteins was successfully developed, that allowed the incorporation of such functionalised MBG particles into CPC pastes without impairing the plottability. The protein
release from plotted scaffolds could be varied by addition of different amounts of protein-laden MBG into the composite (Figure 2) and the released lysozyme from pure MBG and from plotted composite scaffolds maintained its biological activity. Finally, the established protocol for protein loading of MBG was successfully applied to VEGF and the release from MBG and CPC-MBG composites, while retaining the biological activity, was demonstrated.

**Conclusion**

In this study, we showed the development of a functionalised CPC-MBG composite applicable for 3D plotting that allows fabrication of patient-specific implants. Furthermore, the results of ion and protein release showed the high flexibility of this material system and suggest it as a promising material toolbox to treat bone defects.

**Acknowledgement**

This work was funded by the German Research Foundation (DFG) as part of the Collaborative Research Centre SFB/Transregio 79 (subproject M2).

References


4:15 p.m. – 5:45 p.m.

Track03

ESB-SIBB S01 | ESB-Iberian Society for Biomechanics and Biomaterials (SIBB) Joint Session I: Antimicrobial Biomaterials

Chairs
Inês C. Gonçalves
i3S/INEB, Porto, PT
Federico Soria
European Network of Multidisciplinary Research to improve the Urinary Stents, ENIUS Chairman. CA16217 COST Actions, Brussels, BE

Antimicrobial biomaterials Infection is a serious healthcare issue, and the current pandemic times are a huge proof of that. Antimicrobial biomaterials have always been a strong focus of both the Portuguese and Spanish scientific biomaterials community. This symposium highlights different fields of research in this topic, including both coatings and new designs of polymeric and metallic antimicrobial biomaterials, and ranging several biomedical applications, such as the urinary stents presented by our keynote speaker Federico Soria, as well as bone implants, ventilators, and protection masks.
Urinary stents. Side effects in patients. Why do we need to improve them? Biofilm, Coatings and new stent designs.

Federico Soria¹², Julia E. de la Cruz¹

¹ Jesus Uson Minimally Invasive Surgery Centre, Endoscopy, Caceres, ES; ² European Network of Multidisciplinary Research to Improve Urinary Stents. Chairman. CA16217, Brussels, BE

Urinary stents are an essential tool in the urological armamentarium. They have proven their efficacy in maintaining urine drainage, preventing or relieving ureteral or urethral obstruction caused by both intrinsic and extrinsic circumstances of the urinary tract. Indications include urinary calculi, urinary obstructions, benign or malignance strictures, urinary fistulas, retroperitoneal fibrosis and neoplasms. Urinary stents play a fundamental role in pre- and postoperative endourological management of ureteral lithiasis, as well as in reconstructive surgery of the urinary tract. Stents acts as an internal scaffold favouring tissue healing and ensuring urinary drainage (1).

However, its use is not free of side effects and complications that may affect the quality of life of up to 80% of patients with ureteral stents (2). In addition to the impairment of the patients’ quality of life, complications such as incontinence, nocturia, vesicoureteral reflux, haematuria, as well as inflammatory reactions causing obstruction of the urinary tract can also occur. Since urine represents a hostile environment for any biomaterial, the stent surface provides the ideal substrate for bacterial colonization, biofilm development and encrustation (2). Biofilm and encrustation, in addition to causing irritation and inflammation of the urothelium, worsen the symptoms described above, may also cause obstruction, stent malfunction and the appearance of infections with clinical symptoms, resulting in emergency replacement or removal of the stents, the need for antibiotic therapy and even hospitalization (3).

So, where can we focus research to improve stents, reduce their side effects and improve patients’ quality of life? Mainly, the cornerstones for the improvement of urinary stents focus on: 1. Preventing the formation of biofilm and encrustation on the surface of the stents (3); 2. Developing new coatings with antifouling and antibacterial properties like Antimicrobial peptides (AMPs), and coatings which are able to release drugs into the urinary tract (4); 3. Designing new stents that are better adapted to the anatomy and physiology of the urinary tract (5); 4. Providing stents with new properties such as the biodegradability of their biomaterials to avoid the necessary re-operation that is currently mandatory with these medical devices (5).

It is therefore necessary to develop multidisciplinary research groups to advance in the improvement of current urinary stents, as only from an interdisciplinary approach will it be possible to overcome the present limitations in this area of knowledge, which brings together researchers, urologists, bioengineers, patients and industrial partners (4).

Acknowledgement
This article/publication is based upon work from COST Action CA16217, supported by COST (European Cooperation in Science and Technology).www.cost.eu
Polymeric Ureteral stent encrustation

Bladder end of double pigtail ureteral stent encrustation.

Metallic urinary stent obstruction

Urothelial hyperplasia causing recurrent obstruction after ureteral metallic stent placement.

References


Manufacture of open-cell titanium structures with antibacterial properties

Elia Vidal, Elisa Ruperez, Daniel Rodriguez-Rius

Technical University of Catalonia, Dept. Materials Science and Engineering, Sant Adrià del Besòs, ES

Introduction
The biomechanics of the skeletal system require implants and prostheses to support high mechanical loads. For this reason, these implants are usually manufactured with metallic biomaterials (stainless steel, cobalt-chromium alloys or titanium alloys) with high mechanical resistance. These biomaterials, however, can present problems such as stress shielding, due to their high stiffness, which can result in bone resorption and possibly implant failure [1]. Porous titanium structures have attracted considerable attention since they reduce the stress shielding of the implant towards the bone by presenting an apparent elastic modulus lower than that of the bulk material, and which can be modulated depending on the structure generated. The manufacture of such structures, however, is not easy, due to the high reactivity of titanium and its high melting point. Despite this, porous structures have been manufactured using techniques such as space holder, laser sintering or direct ink writing of metallic inks [2]. Another prominent problem in metal implants and prostheses is the possible appearance of infections in the tissues surrounding the implant, either due to a nosocomial infection in surgery or due to the formation of a biofilm on the surface of the biomaterial by opportunistic planktonic bacteria. In orthopedic applications that require a bone-implant junction, the formation of the bacterial biofilm prevents proper osseointegration and can lead to prosthesis failure. One strategy to avoid this is to cover the prosthesis with a layer with antibacterial properties. There are various possibilities to generate antibacterial properties, such as using metal ions such as silver, doping the surface with polymers or peptides such as AMPs, depositing a layer with antifouling effect, or using a layer as a carrier for an antimicrobial agent [3]. Calcium phosphate can be used as a carrier that releases antibacterial drugs.

Experimental Methods
Porous titanium samples were prepared by 3D printing using Pluronic 127 hydrogel as a binder. The samples were sintered at 1250°C under ultra high vacuum in a modified Hobersal tube furnace. Mechanical compression tests were carried out with an MTS Bionix 858 traction-compression testing machine specially designed to work on biomaterials, with the added ability to perform fatigue and torsion tests. This machine was equipped with a 25KN MTS load cell. The equipment was controlled by connection to a PC equipped with a TestStar II® software package. 6 different samples were tested, taking as the maximum value of compressive strength either the moment when a fracture was detected or the moment when the deformation exceeded 33%.

Pulsed electrodeposition was studied as a coating process, in order to manufacture a calcium phosphate (CaP) coating on porous titanium samples in a single step, doped with an antibacterial agent. The electrolytic process was carried out using the titanium sample as cathode and a solution of Ca(NO₃)₂·4H₂O and NH₄H₂PO₄ as electrolyte. Different conditions of temperature (40-60°C), pulse polarity (unipolar-bipolar) and current density (2-5 mA/cm²) were studied. In order to avoid possible infections around the implant, the CaP coating has been used as a platform for the controlled release of drugs. The coating was doped with Chlorhexidine Digluconate (CHX) by codeposition and adsorption.

The physicochemical properties of the coating were determined by SEM/EDS, FIB, XRD and microCT. In vitro studies made it possible to evaluate the antibacterial response to the S. aureus strain.

Results and Discussion
The morphological analysis of the samples showed that different microstructures can be obtained by varying the process parameters (Figure 1).

Page 518 of 2028
During the compression tests, no fracture occurred, so the value of compressive strength was measured when reaching the established deformation limit of 33%. The average value registered has been 235 MPa. This shows that this material is very ductile. An average modulus of elasticity of 7.5GPa was calculated, with a standard deviation of 0.4GPa. In contrast, the incorporation of CHX did not affect the morphology of the coating. Finally, in the in vitro tests, good results of antibacterial activity of the coating were obtained.

**Conclusion**

A promising solution have been shown for the antibacterial biofunctionalization of titanium orthopedic implants manufactured with a porous structure that limits the problem of stress shielding.

**Acknowledgement**

The authors acknowledge the funding of the projects MAT2015-67183-R and RTI2018-098075-B-C21 with the help of the European Regional Development Fund (MINECO/FEDER), SGR 2017 SGR1165 (Generalitat de Catalunya) and Grant AGAUR FI-DRG of EV.

![Figure 1. Reconstruction of a sample by microCT.](image)

References


A wide-spectrum antimicrobial coating to fight multi-species biofilms in ventilator-associated pneumonia infections

Diana Alves, Maria O. Pereira, Susana P. Lopes

University of Minho, Centre of Biological Engineering, Braga, PT

Introduction
Ventilator-associated pneumonia (VAP) is currently one of the top five most common nosocomial infections and it has been associated with prolonged hospitalization and high morbidity and mortality rates [1]. The use of an endotracheal tube (EET) constitutes the major risk factor for the development of this condition, since it is the airway access point for microorganisms. Indeed, microorganisms’ migration along and within the inside of the EET results in a so-called biofilm which, combined with a network of secretions, will be ultimately leaked down to lungs [2]. Biofilm formation is a crucial step in the pathogenesis of VAP, as microbial cells within a biofilm enlace themselves in a self-produced matrix of extracellular polymeric substances, conferring them protection against both antimicrobial treatments and the host immune system. Furthermore, the polymicrobial nature of biofilms formed on the surfaces of ETT has been increasingly addressed [3].

Experimental Methods
This study aimed to design a wide-spectrum antimicrobial coating to impart endotracheal tubes with the ability to prevent the adhesion and subsequent biofilm formation of several microorganisms commonly associated to VAP, without compromising the viability of lung cells. For that, ciprofloxacin (CIP) and chlorhexidine (CHX), two compounds with different action spectra, were co-immobilized on polyvinyl chloride (PVC) surfaces to obtain a dual-drug release antimicrobial coating. Immobilization was performed using a one-step polydopamine (pDA)-based approach, in which PVC substrata were simply immersed on a solution of dopamine dissolved together with CHX and/or CIP, allowing their incorporation throughout the full thickness of the pDA film.

Results and Discussion
The antimicrobial performance of modified surfaces was evaluated against 5 bacterial species (Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, Staphylococcus aureus and Staphylococcus epidermidis) and 1 yeast species (Candida albicans) as individual, dual and triple consortia. Regarding the single-species colonisation of PVC surfaces, results showed that after 24 h of exposure, co-immobilization of CHX and CIP caused a significant reduction (more than 4 Log) in the number of bacterial cells adhered to the surfaces, while being less efficient against the adhesion of C. albicans (0.5 Log). It is worth mentioning that the combination of CIP and CHX in the proposed coating resulted both in a synergetic effect against P. aeruginosa, A. baumannii and C. albicans and in a facilitative effect against all tested species. Coatings were also tested against the dual adhesion of P. aeruginosa in consortium with all tested species. In general, results showed that the antimicrobial effect observed for each species was not compromised by the presence of any other species. Inhibition of C. albicans was actually more effective (approximately 2 Log reduction) when combined with P. aeruginosa. When the antimicrobial coating was exposed to a triple consortium comprised by P. aeruginosa, S. aureus and C. albicans, its antimicrobial effect against bacterial species was not compromised and it was able to inhibit the adhesion of yeast by about 1 Log. In terms of biocompatibility, the amount of CHX and CIP released from the surfaces did not compromise the growth of lung epithelial cells. Surfaces functionalization with CIP and/or CHX was confirmed by SEM, AFM and contact angle measurements.

Conclusion
In conclusion, the combination of CIP and CHX, using dopamine chemistry, results in a coating with antimicrobial activity towards a wide spectrum of microorganisms commonly associated to VAP. Therefore, this approach holds great potential to be applied in EET, proving to be important in the fight against VAP.

Acknowledgement

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte. The authors also acknowledge the support, through the Programa Operacional Competitividade e Internacionalização (COMPETE2020) and by national funds, through the Portuguese Foundation for Science and Technology (FCT), of the POLY-PrevEnTT project (PTDC/BTM-SAL/29841/2017-POCI-01-0145-FEDER-029841).

References
Multifunctional coatings to improve soft tissue integration of metallic implants

Adriana Vilaça¹,², Rui M. Domingues¹,², Hanna Tiainen³, Barbara B. Mendes¹,², Alejandro Barrantes⁴, Rui R. Reis¹,², Manuela E. Gomes¹,², Manuel Gomez-Florit¹,²

¹ University of Minho, 3B's Research Group, Barco, PT; ² ICVS/3B’s-PT Associated Laboratory, Braga/Guimarães, PT; ³ University of Oslo, Department of Biomaterials, Oslo, NO; ⁴ University of Oslo, Oral Research Laboratory, Oslo, NO

Introduction
Metallic implants are widely used in diverse clinical applications to aid in recovery from lesions or to replace native hard tissues.[1] However, the lack of integration of metallic surfaces with soft tissue interfaces causes the occurrence of biomaterial-associated infections, which can trigger a complicated inflammatory response and, ultimately, implant failure. Therefore, these devices would benefit from engineered surfaces with favorable soft tissue integration properties that promote the development of a sufficient biological seal between the metal implants and the surrounding soft tissue, to create effective barriers between internal and external environments preventing bacterial invasion, tissue inflammation, loosening and failure of the implants.[2,3] We hypothesized the development of multifunctional surfaces that mimic the anisotropic organization of soft tissues and, at the same time, favor cell adhesion, modulate the inflammatory response and inhibit bacterial adhesion and/or growth on the implant surfaces would increase implants long-term success (Figure 1).

Experimental Methods
Anisotropic radial nanopatterns were produced on polished titanium (Ti) surfaces by spin coating cellulose nanocrystals (CNC) at high speed. Then, in order to introduce bioactivity, we used the surface chemistry of CNC to sequester bioactive molecules from platelet lysate (PL) on the surfaces. The formation of the protein coating was analyzed by quartz crystal microbalance with dissipation monitoring (QCM-D). The coatings were thoroughly characterized by atomic force microscopy, profilometry, and contact angle measurement. The specific PL proteins sequestered on the CNC surfaces were studied by proteomic analysis. Adhesion, spreading and growth of human gingival fibroblasts was evaluated. Similarly, the anti-inflammatory properties of the nanocoated surfaces were tested using a macrophages polarization assay. Finally, the effect of the nanocoating on adherence and viability of Staphylococcus aureus was examined.

Results and Discussion
We were able to obtain a layer-by-layer-like coating on Ti surfaces showing nanotopographical cues provided by highly-oriented CNC combined with the multiple bioactive molecules from PL [4]. Fibroblasts demonstrated to sense the nanotopography of these surfaces effectively and tended to orient themselves in the same direction of CNC radial orientation up to 14 days, which, at the end, could lead to an organization that mimics the soft tissue around teeth in healthy gingiva inducing integration and regeneration of the implant. As well, we showed that the PL-derived biomolecules effectively inhibited CCR7 expression while increasing CD206 expression on human macrophages, suggesting an enhanced anti-inflammatory response, which could contribute to a controlled performance of inflammatory processes after implant placement. Moreover, the PL nanocoating decreased initial bacterial viability using S. aureus, which could prevent early bacterial colonization on the implant, thus, potentially preventing peri-implantitis.

Conclusion
We identified two complementary strategies that would potentially contribute to development of a multifunctional surface coating offering an improvement on soft tissue integration, a controlled inflammation process and antimicrobial properties to increase dental implant success. Ultimately, this work demonstrates a multifunctional surface design with a potential to improve success rate of dental implants.

**Acknowledgement**

We acknowledge the financial support from International Team for Implantology - ITI Research grant 1306_2018; and from Fundação para a Ciência e e Tecnologia (FCT) for CEECIND/01375/2017.

![Multifunctional surfaces to improve soft tissue integration and reduce bacterial colonization](image)

**References**

Bactericidal action of eugenol-containing essential oils within chitosan-based blended films towards *Staphylococcus aureus* or *Pseudomonas aeruginosa*

Joana C. Antunes, Tânia D. Tavares, Marta A. Teixeira, Marta O. Teixeira, M. Teresa P. Amorim, Natália C. Homem, Helena P. Felgueiras

*University of Minho, Centre for Textile Science and Technology, Guimarães, PT*

**Introduction**

Diabetic foot ulcers (DFUs) are a common complication of patients suffering from diabetes mellitus (DM), a disabling and incurable chronic disorder, highly prevalent worldwide. DFUs often become infected, with *Staphylococcus aureus* and *Pseudomonas aeruginosa* as the most commonly isolated pathogens within DFUs in Portugal [1]. Surgical debridement, wound cleansing, and antibiotic administration, typically precede the application of a wound dressing over the lesion site to protect the wound, fight infection and promote healing. But current therapeutics are ineffective against most DFU-associated infections, particularly due to bacterial resistance to therapeutics, hence the urgency for efficient alternatives. Hence, we proposed to engineer films via solvent casting and phase inversion method from chitosan (CS) and poly (vinyl alcohol) (PVA) blends, polymers widely combined as templates for antimicrobial (AM) action due to their inherent AM activity (CS), flexibility and hydrophilicity (PVA). CS and PVA readily form hydrogen bonds due to a large number of -OH groups from the monomeric units of both polymers. Loading them with the antibacterial cinnamon leaf (CLO; ≈79% eugenol) or clove essential oils (CO; ≈81% eugenol) should reinforce their AM action. Ergo, the main goal of the present study was to explore the potential of CS and essential oil (EO)’s over *S. aureus* and *P. aeruginosa* survival within a CS/PVA matrix for prospective DFU treatments [2]. Very few have explored CS/PVA blended films as EO delivery platforms and none, to the author’s knowledge, has used the proposed approach.

**Experimental Methods**

Detailed methodology can be consulted at Antunes *et al.* [2]. Briefly, EOs, purchased from Folha d’Água (Santo Tirso, Portugal), were selected based on AM testing results obtained elsewhere by the team, against *S. aureus* (ATCC 6538, grown in TS/TSA) and *P. aeruginosa* (ATCC 25853, grown in NB/NA) [3,4], apart from the minimum inhibitory concentration value of CO while incubated with *P. aeruginosa* that was here determined, using a broth microdilution procedure. CS and poly (vinyl alcohol) (PVA) films were prepared (ratio 30/70 w/w; 9 wt%) by the solvent casting and phase inversion method. Films were supplemented with 1 and 10 wt% of EO in relation to total polymeric mass. Representative images of the films’ macroscopic structures were taken, followed by thickness measurements, and degree of swelling (DS, in %) determined similarly as in Felgueiras *et al.* [5]. Chemical structure (through Fourier-transform Infrared spectroscopy with attenuated total reflection, FTIR-ATR), thermal properties [via thermal gravimetric analysis (TGA) and differential scanning calorimeter (DSC)], EO-loading amount (using UV-visible spectroscopy), and, finally AM assays (namely agar diffusion assay and time-kill kinetics) were conducted on unloaded and EO-loaded films.

**Results and Discussion**

Smooth and homogeneous films were obtained. Film thickness and DS tended to increase with EO content, particularly with 10 wt% CLO (* p<0.05). CS/PVA’s spectrum shows peaks from both CS and PVA, and new peaks are absent, thereby suggesting the occurrence of polymer blending. Spectra of EO-loaded films showed a commitment of free -OH groups with increasing EO amount, suggesting that hydrogen bonds are formed between
EO and the polymer chains. With 10% CLO, the peak decreased 55%. CO inclusion within CS/PVA films promoted a 15% reduction in the intensity of this region of the spectra. The film’s thermal stability data reinforced polymer blending and EO entrapment. UV-visible absorbance scans in the 250–320 cm\(^{-1}\) region confirmed the successful uptake of CLO and CO into CS/PVA films, particularly with films loaded with 10 wt% EO that contained 5.30/5.32 times more CLO/CO than films supplemented with 1 wt% EO. AM testing revealed that CS films alone were effective against both bacteria and capable of eradicating all *P. aeruginosa* within the hour (**p<0.001). Still, loaded CS/PVA films showed significantly improved AM traits in relation to unloaded films within 2 h of contact, especially CS/PVA/CLO 10% (*p<0.0001) following incubation with *S. aureus* and CS/PVA/CO 10% (**p<0.001) with *P. aeruginosa* (Figures 1 and 2).

**Conclusion**
This study is a first proof of concept that CLO and CO can be dispersed into CS/PVA films. CLO incorporated 31% more EO than CO, an amount that appeared to be also bound to the polymer chains in a more effective way. Still, both film types showed significant bactericidal effects, particularly CS/PVA/CLO 10% against *S. aureus*, this way paving the way for efficient DFU therapeutics. Future work will be directed towards a balance between AM action of CS and its mechanical hindrance after processing, together with the combination with CLO or CO for an intensified antimicrobial profile against both bacteria.

**Acknowledgement**
This research received funding from the Portuguese Foundation for Science and Technology (FCT) under the scope of the projects PTDC/CTM-TEX/28074/2017 and UID/CTM/00264/2021.
Time-kill curves against *P. aeruginosa*

Time-kill curves of (a) unloaded and EO-loaded films and (c) EOs at film loaded concentration, up to 24 h culture. Positive controls were also conducted (grey line), reaching a maximum value of $= 4.3 \times 10^6$ CFUs/mL after a 24 h culture (data not shown in graphic). *P. aeruginosa* reduction (calculated as log reduction) of (b) the films and (d) EOs in relation to control samples. The elimination of 100% of bacteria was considered as log 6. Results are represented as the mean ± SD ($n=3$). Statistically significant differences can be highlighted, *$p <0.05$, **$p <0.005$, ***$p <0.001$, and ****$p <0.0001$.

References


Metallic nanoparticles synthesized by laser ablation to prevent periimplantitis

Mónica Fernández-Arias\(^1\), Mohamed Boutinguiza\(^1,4\), Jesús del Val\(^1\), Daniel Rodríguez\(^2\), Felipe Arias-González\(^3\), Francisco J. Gil\(^3\), Juan Pou\(^1,4\)

\(^1\) University of Vigo, Applied Physics Department, Vigo, ES; \(^2\) Universitat Politècnica de Catalunya, Materials Science and Metallurgical Engineering Department (Biomaterials, Biomechanics and Tissue Engineering Group), Barcelona, ES; \(^3\) Universitat Internacional de Catalunya, School of dentistry, Barcelona, ES; \(^4\) Sergas-Uvigo, Galicia Sur Health Research Institute (IIS Galicia Sur), Vigo, ES

Introduction

Peri-implantitis is an inflammation of the gingival tissue caused by oral pathogens and it is one of the main causes of dental implant failure [1]. The great potential of some metallic nanoparticles as bactericidal agent could be an additional solution to the existing therapies. In particular, the antibacterial activity of copper and silver nanoparticles is known as potentially effective against different bacterial pathogens [2]. Although the size reduction to the nanoscale is behind these bactericidal properties, other features such as morphology or crystallinity of the particles, have a key role in the bactericidal effect. These features mostly depend on the fabrication method. The laser ablation technique allows to control these features by tuning the processing parameters [3]. In addition, opposite to other chemical based methods of nanoparticle production, laser ablation allows obtaining nanoparticles without any additional reagent that can be harmful to the healthy tissues.

The objective of the present work is to produce silver and copper nanoparticles by laser ablation on titanium substrates and check their effect as bactericidal agent against two common bacteria linked to peri-implantitis: \textit{Lactobacillus Salivarius} and \textit{Staphylococcus aureus}.

Experimental Methods

Silver and copper foils with 99.99% of purity, were used separately as laser ablation targets. Each target was ablated in open air by two different laser sources. In the case of the silver target, a nanosecond laser emitting at a wavelength of 532nm in the nanosecond regime (Green - ns) and an infrared laser (wavelength of 1064nm) emitting pulses in the millisecond range (Infrared - ms) were used. While in the case of copper the same Green - ns laser was used together with a picosecond laser emitting at 1064nm of wavelength (Infrared - ps). In all experiments, commercially pure (cp) titanium discs Grade 2 were used as substrates for collecting the ablated material as shown in Figure 1. Experiments are summarized in Table 1.

Table 1. Summary of laser sources and materials used in the present work

<table>
<thead>
<tr>
<th>Sample</th>
<th>Metal</th>
<th>Laser source</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Silver</td>
<td>Green - ns</td>
</tr>
<tr>
<td>b</td>
<td></td>
<td>Infrared - ms</td>
</tr>
<tr>
<td>c</td>
<td>Copper</td>
<td>Green - ns</td>
</tr>
<tr>
<td>d</td>
<td></td>
<td>Infrared - ps</td>
</tr>
</tbody>
</table>

Size, morphology, crystalline phases and optical properties of the deposited nanoparticles were studied by means of transmission electron microscopy (TEM), high resolution transmission electron microscopy (HRTEM) and UV/VIS absorption spectroscopy. Additionally, scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) on the sectioning layer of the nanocoatings, were used to analyse respectively the morphology and composition of the nanoparticles on the titanium surface.
The antibacterial properties of the nanoparticles were evaluated in dynamic conditions with *Lactobacillus Salivarius*, a common bacteria strain localized in the mouth with an important role in the biofilm formation and with *Staphylococcus aureus*, a Gram positive bacterial strain which is one of the main pathogens responsible for inducing periimplantitis.

**Results and Discussion**

Silver and copper oxide nanoparticles have been synthetized and deposited on cp Ti substrates in one-step process by means of laser ablation in open air. The process, resulted in coatings of nanoparticles with spongy appearance (see Figure 2). The deposited nanoparticles are crystalline and round with diameters from few nanometers to 60 nm. Average diameters are around 15 nm for silver and 5 nm in case of copper nanoparticles. However, the nanoparticles are smaller and more homogeneous in size when the Green - ns laser is used with both metals. Microbiology tests confirm the strong activity of silver and copper nanoparticle coatings. On one hand, the deposited Ag NPs reduce the adhesion of *L. salivarius* around 61% in relation to the cp Ti without NPs. On the other hand, the adhesion of *S. aureus* decreases about 80% in contact with the Cu NPs coating.

**Conclusion**

Feasibility of laser ablation to produce Ag and Cu NPs coatings on Ti by one-step process without any additional chemical compound is demonstrated. The laser source determines the features of the deposited nanoparticles. Bactericidal activity of these coatings against *L. salivarius* and *S. aureus* encourages their use as anti-periimplantitis agent in oral implants.

**Acknowledgement**

This work was partially supported by the EU research project Bluehuman (EAPA_151/2016 Interreg Atlantic Area), Government of Spain (MAT2015-71459-C2-P (MINECO/FEDER, RED2018-102803-T), and Xunta de Galicia (ED431C 2019/23, ED481D 2017/010, ED481B 2016/047-0). The technical staff from CACTI (University of Vigo) is gratefully acknowledged.
Figure 2

Micrographs obtained by scanning electron microscopy (SEM) of the cross-section of copper nanoparticles coating on the Ti disc.

References
**ESB-SIBB S01-07**

*Escherichia* virus MS2, mimic of SARS-CoV-2, inhibition via essential oils-loaded nanofibers: a potential formulation for antiviral individual protection masks

Joana Domingues¹,², Samira Silva¹, Joana C. Antunes¹, Helena P. Felgueiras¹

¹ University of Minho, Centre for Textile Science and Technology (2C2T), University of Minho, Campus de Azurém 4800-058 Guimarães, Portugal., Guimarães, PT; ² University of Minho, Centre of Biological Engineering (CEB), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal, Braga, PT

**Introduction**

In December 2019, a novel strain of coronavirus, SARS-CoV-2, was identified. Infected patients revealed symptoms of fever, cough (dry), sore throat, and fatigue, which began manifesting after 5 days of incubation. Hoping to prevent transmission, many countries adopted a mandatory mask use in closed and open public spaces [1]. Still, most mask options display a passive-like action against COVID-19, only preventing its infiltration but not eliminating it. Essential oils (EOs) are highly abundant concentrated plant extracts, endowed with antiviral, antibacterial, antifungal, anti-inflammatory, anxiolytic, analgesic, and antioxidant properties [2]. EOs have already proved efficiency against SARS-CoV-2, with garlic oil (GLO) being capable of inhibiting the angiotensin-converting enzyme 2 protein, leading SARS-CoV-2 to lose its host receptor and attack the PDB6LU7 protein (its main protease), thus preventing virus maturation and spreading [3]. The purpose of this work is to investigate other EOs for their antiviral potential against the *Escherichia* virus MS2 (a mimic of SARS-CoV-2) and to incorporate the most effective on electrospun nanofibrous mats adaptable for individual three-layer protection masks (as inner layer).

**Experimental Methods**

Twenty EOs selected based on their antimicrobial nature [4] were examined for the first time against the *Escherichia coli* virus MS2 (ATCC 15597-B1). Minimum inhibitory concentrations (MIC) were established using the broth microdilution method. The virus was grown at 2x10⁵ PFUs/mL and combined at 50/50 v/v with the EOs at concentration varying from 732.5 to 85.6 mg/mL in Mueller Hinton Broth (MHB), for 24 h at 37°C and 120 rpm. Polycaprolactone (PCL) and cellulose acetate (CA) were prepared individually at 14 wt% in chloroform/dimethylformamide (DMF) and 10 wt% in acetone/DMF, respectively, and combined at 3:1 ratio. Polymeric solutions were processed via electrospinning with processing parameters being optimized to 24.7 kV (potential difference), 3.2 mL/h (flow rate) and 21 cm (distance to collector). Mats were characterized by thermogravimetry (TGA), differential scanning calorimetry (DSC), dynamometer, goniometer, Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). Loading of the nanofibrous mats was accomplished via physisorption using the terminal -OH groups of the CA as linkers. Mats were loaded with the three most effective EOs against the MS2 virus at 2xMIC concentration, in an alcoholic solution, for 72 h (saturation). Loading amount was determined by UV-visible spectroscopy. In parallel, EOs at MIC were also incorporated within the polymeric solution prior to electrospinning and extruded in the form of EO-loaded nanofibers. Processing parameters were optimized for each of the three selected oils. Surface characterization resorted to TGA, DSC, FTIR and SEM, for both fiber uniformity and EOs presence. Antimicrobial testing via Kirby-Bauer method was conducted for 24 h, to establish the oils diffusion abilities from the mats. Time-kill kinetics (JIS L 1902) studies were conducted for 1, 2, 4, 6, 24, 48 h to verify the activity of the oils while loaded and the overall antiviral features of the engineered mat.

**Results and Discussion**
From the twenty tested oils (listed in [4]), the most effective were the lemongrass (LO), Niaouli (NO) and eucalyptus (ELO) with a MIC of 356.0 mg/mL, 365.2 mg/mL and 586.0 mg/mL, respectively. PCL/CA nanofibrous mats were successfully produced both in the presence and absence of the oils within the polymeric solutions. Uniform, beadless nanofibers were obtained. Mats were characterized as mechanically resilient, to endure movements arising from mask positioning, and hydrophobic in nature, to repel aqueous droplets coming from the exterior. TGA, DSC and FTIR techniques confirmed the homogeneity of the polymeric blends and the oils presence. In the oil-free mats, loading was accomplished after 72h of immersion. In the three tested cases, a loading amount close to MIC was detected. Antimicrobial testing via halo determination, verified the loaded oils diffusion abilities, determining oil liberation from physisorbed surfaces more important. Time-kill kinetics studies of the loaded mats attested to the EOs capability to fight the virus MS2 even when bonded to the nanofibers. Here, EOs-physisorbed surfaces were quicker in their action, while those entrapping the EOs in their polymer matrix retained the antiviral activity of the mat for longer.

**Conclusion**

Data demonstrated the potential of the EOs, LO, NO and ELO, loaded onto PCL/CA nanofibrous mats to work as COVID-19 active barriers in individual protection masks.

**Acknowledgement**

This research received funding from the Portuguese Foundation for Science and Technology (FCT) under the scope of the projects PTDC/CTM-TEX/28074/2017 (POCI-01-0145-FEDER-028074) and UID/CTM/00264/2021. JD also acknowledges FCT for PhD grant 2020.07387.BD.

**References**

ESB-ISBF S | ESB-International Society for Biofabrication (ISBF) Joint Symposium

Chairs
- Michael Gelinsky
  Centre for Translational Bone, Joint and Soft Tissue Research, TU Dresden, DE
- Lorenzo Moroni
  Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, MAASTRICHT, NL
- Shardul Bhusari (YSF)
  INM - Leibniz Institute for New Materials, Dynamic Biomaterials, Saarbruecken, DE

The session will be highly relevant for scientists working in the fields of additive manufacturing, 3D printing and bioprinting/biofabrication, covering both hardware and biomaterial/bioink developments.
Hybrid Fabrication at the Nano- Micro- and Macro-scale

**Paul D. Dalton**¹, Marcy Zenobi-Wong²

¹ University of Oregon, Phil and Penny Knight Campus for Accelerating Scientific Impact, Eugene, US; ² ETH Zürich, Institute for Biomechanics, Zurich, CH

The prospect of fabricating complex tissues and organs in the future is enticing, and long promised. Appreciating the complexity of the challenges ahead also requires an understanding that simple fabrication processes alone will not likely result in an effective outcome. Our understanding of fabricating tissues through tissue engineering and regenerative medicine research highlights the importance of mechanotransduction, cell adhesion and phenotype, bioreactor arrangement, chemotactic cues, and their gradients. The importance of engineering at different lengths - nano-, micro- and macro-scale indicate that future fabrication approaches require complexity that currently is not available. Furthermore, fabrication/bioprinting technologies are developed individually and each has its advantages and disadvantages [1]. Recently there has been a trend towards hybrid fabrication, where different fabrication technologies are combined to minimize or eliminate the disadvantages posed by separate, individual technology [2, 3]. This talk will provide an overview of how different fabrication technologies [4] can pair together to provide an enhanced output and complexity and provide a provocative vision for the future on how hybrid biofabrication will occur.

**Acknowledgement**

We thank the International Society for Biofabrication (ISBF) for providing the opportunity to present this talk.

**References**


ESB-ISBF S-KL02

Bioprinting at the Nano- Micro- and Macro-scale

Marcy Zenobi-Wong¹, Paul D. Dalton²

¹ ETH Zürich, Institute for Biomechanics, Zurich, CH; ² University of Oregon, Phil and Penny Knight Campus for Accelerating Scientific Impact, Eugene, US

The prospect of fabricating complex tissues and organs in the future is enticing, and long promised. Appreciating the complexity of the challenges ahead also requires an understanding that simple fabrication processes alone will not likely result in an effective outcome. Our understanding of fabricating tissues through tissue engineering and regenerative medicine research highlights the importance of mechanotransduction, cell adhesion and phenotype, bioreactor arrangement, chemotactic cues, and their gradients. The importance of engineering at different lengths - nano-, micro- and macro-scale indicate that future fabrication approaches require complexity that currently is not available. Furthermore, fabrication/bioprinting technologies are developed individually and each has its advantages and disadvantages [1]. Recently there has been a trend towards hybrid fabrication, where different fabrication technologies are combined to minimize or eliminate the disadvantages posed by separate, individual technology [2, 3]. This talk will provide an overview of how different fabrication technologies [4] can pair together to provide an enhanced output and complexity and provide a provocative vision for the future on how hybrid biofabrication will occur.

Acknowledgement

We thank the International Society for Biofabrication (ISBF) for providing the opportunity to present this talk.

References

3D printed dynamic scaffolds for nanovibration-driven tissue regeneration

Sandra Camarero-Espinosa\textsuperscript{1,2}, Lorenzo Moroni\textsuperscript{3}

\textsuperscript{1} University of The Basque Country, POLYMAT, Donostia / San Sebastian, ES; \textsuperscript{2} IKERBASQUE, Basque Foundation for Science, Bilbao, ES; \textsuperscript{3} Maastricht University, MERLN, Maastricht, NL

Introduction
Stimulation of cell cultures with physical and electrical cues has potential to modulate multiple cellular functions including migration, differentiation and survival. Despite the great potential shown by in-vitro models, these are generally based on sophisticated systems with compromised potential to be exploited in-vivo, being the main shortcoming the incapability to apply the stimuli “remotely” and on a controlled manner. Here, we report on the fabrication and exploitation of dynamic additive-manufactured scaffolds that are activated on-command via external application of ultrasounds\cite{1}, resulting in a mechanical nanovibration and an electrical signal that is transmitted to the surrounding cells.

Experimental Methods
Scaffolds were produced via 3D printing from pre-formed polymer blends that spontaneously phase-segregated into well-defined structures, including Janus and particles, following the traditional nucleation and spinoidal decomposition model. 3D printed structures of biodegradable polycaprolactone (PCL) and polylactide (PLA) blends behave as ultrasound transducers (acoustic to mechanical) where the PLA and PCL phases represent the active and backing materials, respectively. To introduce piezoelectricity, biorewable and biocompatible cellulose nanocrystals were added to the PLA phase, at different loading ratios. Human bone marrow derived stromal cells (hBMSCs) were cultured in basal or osteogenic media and used at passage 5 for all experiments.

Results and Discussion
Remote stimulation of scaffolds led to a nanovibration and/or electrical signal generated by piezoelectric cellulose nanocrystals. Control over the material composition (polymer blend ratio and loading of nanoparticles) and structure formation allowed us to control the amplitude of the deflection, the pulse of this one and the intensity of the electrical signal.

Culture of hBMSCs on dynamic scaffolds resulted in enhanced cell proliferation, matrix deposition and osteogenic differentiation as measured by PCR and release and deposition of characteristic markers and matrix proteins. Further, we evidenced that the hBMSC response is a consequence of the formation and activation of voltage-gated calcium ion channels.

Conclusion
Here, we showed a novel method to render 3D printed scaffolds dynamic by the use of ultrasound stimulation. Spatial control over the chemistry of the scaffolds and the inclusion of cellulose nanocrystals allowed us to control the amplitude and pulse of the deflection and the electrical signal generated, respectively. Culture of hBMSCs into these dynamic scaffolds results in an enhanced proliferation, matrix deposition and differentiation when the appropriate parameters are selected. Thus, we believe this to be a novel approach to the regeneration of damaged tissues in which scaffolds can be remotely controlled to guide cell function.

Acknowledgement
The authors acknowledge the Texas A&M Health Science Center College of Medicine Institute for Regenerative Medicine at Scott & White who isolated and provided the cells through a grant from NCRR of the NIH (Grant Page 535 of 2028
The authors acknowledge the financial support from the European Commission under the ERC starting grant “Cell Hybridge” of the Horizon2020 framework program (Grant # 637308) and the Marie-Sklowdoska-Curie-Action “PRIUTE” (MSCA-IF, Grant #845488)

Ultrasound-driven scaffold deflection
Ultrasound-driven nanovibration of dynamic scaffolds is transmitted to surrounding cells modulating their phenotype and the matrix deposition

References
ESB-ISBF S-04

Influence of thiolated crosslinker on gel-NB hydrogels: From additive to subtractive manufacturing.

Jasper Van Hoorick\textsuperscript{1,2}, Agnes Dobos\textsuperscript{1,3}, Marica Markovic\textsuperscript{3,4}, Lana Van Damme\textsuperscript{1}, Tom Gheysens\textsuperscript{1}, Peter Gruber\textsuperscript{3,4}, Jürgen Van Erps\textsuperscript{2}, Hugo Thienpont\textsuperscript{2,1}, Peter Dubruel\textsuperscript{1}, Aleksandr Ovsianikov\textsuperscript{3,4}, Sandra Van Vlierberghe\textsuperscript{1}

\textsuperscript{1} Polymer Chemistry and Biomaterials Group, Department of Organic and Macromolecular Chemistry, Ghent University, Ghent, BE; \textsuperscript{2} Brussels Photonics, Department of Applied Physics and Photonics, Flanders Make and Vrije Universiteit Brussel, Brussels, BE; \textsuperscript{3} 3D Printing and Biofabrication Research Group, Institute of Materials Science and Technology, Technische Universität Wien, Vienna, AT; \textsuperscript{4} Austrian Cluster for Tissue Regeneration, www.tissue-regeneration.at, Vienna, AT

Introduction

Gelatin is a very popular material in the fields of tissue engineering and biofabrication as reflected by the high number of publications covering a plethora of chemical modifications (1-3). Especially the widely investigated gelatin-methacryl hydrogels hold an impressive track record (3). While these different chemical modifications allow to tailor the properties of the hydrogel, one very interesting approach is the use of thiol-ene chemistry, as this generates additional control over the hydrogel properties via adjusting the thiol-ene ratio, but also by varying the applied crosslinker itself (4). Additionally, these thiol-ene systems exhibit a higher reactivity along with superior biocompatibility and processability (4-5).

Experimental Methods

In order to exploit this photo-click chemistry, often an ene-functionality is introduced onto gelatin followed by crosslinking in the presence of a multifunctional thiol. In the present work gelatin was modified with norbornene functionalities as previously described [4].

Results and Discussion

To date, very limited research has been performed on the influence of the applied thiolated crosslinker on the final hydrogel properties. Therefore, the present work assesses the influence of different thiolated crosslinkers on the crosslinking kinetics, mechanical properties and biological performance of the hydrogels upon encapsulation of adipose tissue-derived stem cells. Furthermore, the different formulations were processed using two-photon polymerisation which indicated, in addition to differences in processing window and swelling ratio, a previously unreported phenomenon. At high intensities, the laser results in cleavage of the gelatin backbone even in the absence of distinct photo-cleavable functionalities. This can have potential to introduce channels or softer regions in gels to result in zones characterized by different degradation speeds or the formation of blood vessels (Figure 1).

Conclusion

Consequently, the present study can be used to provide guidance towards tailoring the thiol-ene system towards the desired applications.

Acknowledgement

Jasper Van Hoorick and Lana Van Damme were granted an FWO-SB PhD grant provided by the Research Foundation Flanders (FWO, Belgium). The FWO-FWF grant (a bilateral Research foundation Flanders - Austrian Science fund project #I2444N28) is acknowledged for financial support. S. Van Vlierberghhe thanks the FWO for financial support under the form of research grants (G005616N, G0F0516N, FWOKN273, G044516N) as well as Ghent University for funding a starting grant through the Special Research Fund.
Overview of applied thiolated crosslinkers and multiphoton based processing.

References
4D printing of thermoresponsive shape-transforming hydrogel actuators

Daria Podstawczyk

Wrocław University of Science and Technology, Department of Process Engineering and Technology of Polymer and Carbon Materials, Wrocław, PL

Introduction

4D printing has emerged as an important technique for fabricating three-dimensional objects from programmable materials capable of time-dependent reshaping. The 4D approach combines 3D printing with functional shape-memory materials (SMMs) to produce objects that can undergo temporal shape transformation under external stimuli, e.g. temperature, electric/magnetic field, moisture, light, or chemical signal [1,2]. The material returns to its initial configuration as the stimulus ceases. Poly-N-isopropyl acrylamide (PNIPAAm) is the most popular thermosensitive polymer that exhibits temperature-induced shape transforming behavior. Motions of PNIPAAm-based materials originate from their anisotropic nature and the presence of regions of different swelling behaviors within their network. It has been demonstrated that shear generated during printing induces the alignment of the anisotropic fillers along the printing direction [2,3]. The present investigation demonstrates new 4D inks that contain laponite nanoclay, poly(N-isopropylacrylamide) (PNIPAAm), and alginate. The inks were further used for extrusion-based direct printing of shape-morphing structures, whose shape change was activated by a temperature change. This abstract is based on the paper prepared by my group that has a detailed presentation of the results [2].

Experimental Methods

The inks were prepared by mixing aqueous solutions of precursors (NIPAAm, N,N’-methylenebis(acrylamide) (MBA), 2-hydroxy-4-(2-hydroxyethoxy)-2-methylpropioophenone (I2959), poly(ethylene glycol) diacrylate (PEGDA), and sodium alginate) with laponite XLG under stirring at ambient temperature. Before printing, inks were overnight stored at 4°C and then centrifuged to remove air bubbles that could impede extrusion. 3D printing was carried out using a temperature-controlled pneumatic-based extrusion printer (BioX, Cellink). The 3D constructs were designed in CAD software (AutoDesk Inventor) and exported to a file format acceptable by the 3D bioprinter (*.stl). Completed 3D objects (actuators) were cured under UV light for 5 minutes and then soaked in 0.5M CaCl₂ for 24h for PNIPAAm and alginate cross-linking, respectively. The rheological properties of the inks were determined using a parallel-plate rotational rheometer. The printed and cross-linked objects were further characterized in terms of surface/cross-section morphology, swelling behavior, and mechanical stability. The temperature-induced shape transformation was measured by measuring the curvature of the self-folding sample cyclically immersed in cold and hot waters.

Results and Discussion

The 4D inks exhibited elastic behavior as shear storage modulus (G’) was higher than shear loss modulus (G”) within the measured range of frequency from 0.01 Hz to 100 Hz and behaved in a shear-thinning manner. SEM images demonstrated the anisotropic nature of the printed actuators. Under shear stress generated within the nozzle, the nanoclay that is contained in the inks’ network aligns along the deposition direction causing heterogeneous arrangement of the chains. The compositionally tunable hydrogel discs were programmed to exhibit different actuation behaviors at different temperatures. In cold water, the disc actuator self-folded forming a tube in 15 min. When immersed in hot water, the tubular construct exhibited rapid unrolling behavior, followed by the slight curving in the opposite direction. Dually photo-crosslinked PNIPAAm-based structures showed inversed action and rolled up in hot and unfolded in cold waters. The samples were further examined for their cytotoxicity. It was found out that the hydrogels maintain >80% viability of fibroblasts.
Conclusion
The printed actuators exhibited very good actuating performance in response to temperature because of the temperature-dependent asymmetric swelling/shrinkage behavior governed by the anisotropic nature of the composites. The direct printing technique was found to be an excellent tool to introduce both structural and geometrical anisotropy into printed hydrogels.

Acknowledgement
This work was supported by the Polish National Science Centre [grant number 2016/23/D/ST8/01267].

References
4:15 p.m. – 5:15 p.m.

Track05

**ESB-BMJ S | ESB-Biomaterials Journal Joint Session: Meet the Editor Session**

**Chairs**

- **Kam W. Leong**  
  Columbia University, Department of Biomedical Engineering, New York, USA

- **Hai-Quan Mao**  
  Johns Hopkins University, Baltimore, USA

- **Ankur Singh**  
  Georgia Institute of Technology Wallace H Coulter, Department of Biomedical Engineering, Atlanta, USA

- **Ana Paula Pêgo**  
  University of Porto, Instituto de Investigação e Inovação em Saúde (i3S), Porto, PT

*This symposium is held by:*

![Elsevier logo]

The flagship journal Biomaterials invites you to join the "Meet the Editor Session" where you have the opportunity to communicate with editors of the journal and ask questions about being the author, reviewer, or any other questions you have about the journal. The session will feature a panel of Biomaterials editors who will discuss the specific goals and objectives and the recent performance of the journal. Open and moderated discussion will address the process of writing and submitting to the journal, the critical factors that shape editorial decisions on submitted manuscripts etc.
ESB-BMJ S-01

Biomaterials Journal

Abhay Pandit

National University of Ireland Galway, CÚRAM Research Centre for Medical Devices, Galway, IE

ESB-BMJ S-02

How to Review for Biomaterials

Abhay Pandit

National University of Ireland Galway, CÚRAM Research Centre for Medical Devices, Galway, IE

ESB-BMJ S-03

Q/A
4:15 p.m. – 5:45 p.m.

Track06

**N10 | Skin Regeneration and Wound Healing**

**Chairs**

Ana Oliveira  
Catholic University, PT  
Maria Cristina Tanzi  
Politecnico di Milano, Chemistry, Materials and Chemical Engineering, Milano, IT  
Claudia Monteiro (YSF)  
i3S, Instituto de Investigação e Inovação em Saude, Porto, PT
N10-01

Bioprinting as a strategy to fabricate cell-hydrogel-microfibre composite tissue constructs for a potential skin tissue engineering application

Marcin Kotlarz\textsuperscript{1}, Ana Ferreira-Duarte\textsuperscript{1}, Piergiorgio Gentile\textsuperscript{1}, Stephen J. Russell\textsuperscript{2}, Kenneth Dalgarno\textsuperscript{1}

\textsuperscript{1}Newcastle University, School of Engineering, Newcastle upon Tyne, GB; \textsuperscript{2}University of Leeds, School of Design, Leeds, GB

Introduction
Composites offer an opportunity to couple particular benefits of their constituents to achieve unique material properties that can be of extra value in many tissue engineering applications\textsuperscript{1}. Strategies combing hydrogels with fibre-based scaffolds create a chance to develop more biologically and structurally functional tissue constructs. This research investigated the feasibility of a droplet-based bioprinting system called the Reactive Jet Impingement (ReJI) to integrate a cell-laden hydrogel within a fibre-based substrate.

Experimental Methods
Calcium alginate (CaAlg) fibre-based substrate was manufactured in a three-step process of fibre formation, dry-laid web formation and needlepunching. Two nominal print cell densities ($5 \times 10^6$ and $30 \times 10^6$ cell/ml) of neonatal human dermal fibroblasts were embedded in a tricomponent collagen-alginate-fibrin hydrogel and bioprinted using the ReJI system onto the CaAlg mesh. Cell-hydrogel-microfibre composites were assessed \textit{in vitro} using immunofluorescence, SEM, histological analysis, and metabolic activity and DNA content quantification assays.

Results and Discussion
The ReJI system allowed for mid-air processing of low viscosity bio-inks that upon the meet, react to form a hydrogel\textsuperscript{2}. Fibroblasts in the constructs maintained high viability post-printing. Specifically, the lower fibroblast density triggered proliferation within the composite construct whereas the higher fibroblast density accelerated extracellular matrix deposition. Additionally, the fibrous component of the composite was characterised by its high swelling properties and quick calcium ions release. Our results demonstrate that the ReJI process can produce cell-gel-fibre constructs with high cell densities. Future work will consider application of the constructs for wound healing and skin tissue engineering.

Conclusion
The ReJI system is an effective strategy to fabricate cell-hydrogel-microfibre composite tissue constructs.

Acknowledgement
The research was funded by the EPSRC Centre for Doctoral Training in Additive Manufacturing and 3D Printing (EP/L01534X/1) and DePuy Synthes.
a) The schematic fabrication approach of a cell-hydrogel-microfibre composite tissue construct
b) Immunofluorescence staining of fibroblasts in a composite construct on day 3. The blue staining indicates cell nuclei, red F-actin and green vinculin. Scalebar: 50 µm. c) SEM microphotograph of composite constructs showing cell-hydrogel-fibre interactions on day 3. Scalebar: 50 µm.

Figure 2.
(a) Metabolic activity of fibroblasts in a hydrogel printed onto a mesh normalised to the total DNA content per construct. (b) DNA quantification of Neo-NHDF cells in a cell-hydrogel-microfibre constructs. Data is represented as Mean ± SD (n=3); **p < 0.01, *p < 0.05.

References
In vitro effect of ZnO piezoelectric nanogenerators on skin cells

Andreu Blanquer¹, Laura Lefai², Elena Filova¹, Jaume Esteve², Gonzalo Murillo², Lucie Bacakova¹

¹ Institute of Physiology of the Czech Academy of Sciences, Biomaterials and Tissue Engineering, Prague, CZ; ² Institut de Microelectronic de Barcelona, Cerdanyola del Valles, ES

Introduction
Skin wound healing is a complex physiological process involving several cell types, such as keratinocytes, fibroblasts, endothelial cells and immune cells. In some cases, skin regeneration is reduced due to several factors including diabetes and people aging causing chronic wounds [1]. It is estimated that 1-2% of the population would experience a chronic wound during their lifetime and would become a serious problem for health services in developed countries [2]. In recent years, some studies analysed the effect of exogenous electric field to enhance wound healing [3]. Piezoelectric nanogenerators (NGs) are materials which create an inherent electric field when strained. The use of piezoelectric NGs could enhance skin regeneration due to the electrical stimulation of cells involved in wound healing. Here, we demonstrate the in vitro effect of ZnO piezoelectric NGs on the viability, adhesion, proliferation and differentiation of selected cell types involved in skin wound healing.

Experimental Methods
ZnO NGs were synthesized by hydrothermal growth on an AlN thin-film layer. Human keratinocytes and human fibroblasts were used for in vitro analyses on ZnO NGs. Cell viability was analysed after 24 h in culture using Live/Dead Kit (Invitrogen). Initial cell adhesion and cytoskeleton morphology was analysed after 24 h in culture by phalloidin staining of actin stress fibres. Cell proliferation was quantified up to 14 days in culture using resazurin assay (Sigma-Alrich). Differentiation markers of keratinocytes (basal cytokeratin 14 and differentiated cytokeratin 10) and type I collagen synthesis by fibroblasts were analysed by immunofluorescence and visualized with confocal laser scanning microscope (Leica SP8).

Results and Discussion
Results indicated that all cell types tested were able to adhere and grow on ZnO NGs. Cell viability assay showed that ZnO NGs were not cytotoxic. In addition, after 24 h in culture, an increased number of keratinocytes were adhered on ZnO NGs. Both fibroblasts and endothelial cells showed similar morphology on both ZnO NGs and glass coverslips (control). All three cell types were able to proliferate on ZnO NGs reaching a confluent monolayer after 7 days in case of fibroblasts, and 14 days in case of keratinocytes. The quantification of collagen synthesis by fibroblasts indicated 50% increased amount of collagen on ZnO NGs compared to control samples (Figure 1A). Collagen is a main component of the extracellular matrix of skin and plays an important role in skin regeneration. Images of cytokeratin 10 and 14 immunostaining showed differentiated cells positive for cytokeratin 10 on both ZnO NGs (Figure 1B) and control. Keratinocytes positive for cytokeratin 10 were detected in the upper layers of cells in accordance with other researchers that evaluated the presence of cytokeratin in stratified skin [4]. In addition, the quantification of differentiated cells positive for cytokeratin 10 indicated an increased cell differentiation on ZnO NGs. These results indicate a positive effect of piezoelectric ZnO NGs on cell differentiation. We hypothesize that the electric fields generated by the inherent forces produced by adhering cells are responsible for the increased differentiation of skin cell types.

Conclusion
Piezoelectric ZnO NGs were able to support the skin cell adhesion and proliferation and proved to be cytocompatible. Moreover, the enhanced type I collagen synthesis by fibroblasts and the keratinocyte differentiation on ZnO NGs
allow considering them as promising nanomaterials for skin tissue engineering. Future experiments with immune cells and co-culture systems will be performed to elucidate the potential of ZnO NGs on skin wound healing.

Acknowledgement

This project has received funding from the European Union’s Horizon 2020 research and innovation programme (grant agreement No. 101003407-ELECTROSKIN).

Figure 1.
Immunofluorescence staining of collagen (green) and nuclei (blue) in NHDF fibroblasts after 7 days in culture grown on ZnO NGs (A). Cytokeratin immunostaining in HaCaT cells after 14 days in culture grown on ZnO NGs. A representative image of cytokeratin 10 (green), cytokeratin 14 (red) and nuclei (blue) is shown (B). CLSM Leica SP8.

References


N10-03

Plasma-based hydrogels supplemented with commercial fibrinogen for the generation of human skin equivalents

Ignacio Risueño¹, Cristina Quílez¹, Gonzalo de Aranda¹, Sara Martín¹, Nataliya Debera², Leticia Valencia¹, José Luis Jorcano¹, Diego Velasco¹,³

¹ Universidad Carlos III de Madrid, Department of Bioengineering and Aerospace Engineering, Leganés, Madrid, ES; ² Faculty of Engineering University of Porto, Institute of Biomedical Sciences Abel Salazar, Porto, PT; ³ Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, ES

Introduction

Over the last years, advances have been made towards the development and production of in vitro human skin for clinical use and for in vitro toxicology testing [1,2]. Our group has developed engineered human bi-layered skin equivalents using plasma-derived fibrin hydrogels containing primary human fibroblasts (hFBs) and keratinocytes (hKCs) from skin biopsies for different skin tissue engineering applications, although several issues related to the hydrogel stability were found due to the limited concentration of fibrinogen in human plasma (2-4 mg/mL) [3]. Supplementing plasma gels adding commercially available fibrinogen could help to improve the stability and lifespan of the constructs. The objective of this work is to study the changes in the behaviour of commercial fibrinogen-supplemented gels and their suitability for generating organotypic skin cultures.

Experimental Methods

Commercial fibrinogen from human origin was used to supplement human plasma gels with a basal concentration of 1.2 mg/mL of fibrin, increasing their final concentration to 2, 2.5, 3.5 and 4.5 mg/mL. Plasma gels with 1.2 mg/mL of fibrin were used as controls. Gelation kinetics was studied by turbidimetry measuring gel absorbance to light. Hydrogels were completely dried and weighed at time points 1, 7 and 14 for gel degradation study. Fibroblast-mediated matrix contraction was studied by measuring the surface reduction of hydrogels containing 20,000 hFBs/mL. AlamarBlue and MTS assays were performed to measure hFBs viability inside the gels and hKCs proliferation on top of the hydrogels respectively. Organotypic skin cultures were prepared for each condition with hFBs and hKCs using transwell culture plates in the air-liquid interface for epidermal differentiation. After 15 days of culture, hematoxylin/eosin staining and immunofluorescence were performed for structural and biochemical characterization.

Results and Discussion

Increasing fibrin concentration of the hydrogels with commercial fibrinogen did not show changes in gelation kinetics. Hydrogels showed an important weight loss on the first day although it was reduced progressively as fibrin concentration was increased. Fibroblasts-mediated matrix contraction was reduced by more than 70% between the 1.2 mg/mL control gels and the more concentrated fibrin gels (4.5 mg/mL). Fibroblasts embedded in the hydrogels showed a controlled and slower proliferation compared to that of the control plasma hydrogel. Hematoxylin/eosin staining showed both a more stabilized dermal compartment for the plasma/commercial fibrinogen conditions and normal development of a fully differentiated and mature epidermis (Figure1). This was confirmed by the presence of specific epidermal markers such as keratins 5 and 10 and filaggrin; normal hFBs growth was shown by vimentin expression.

Conclusion

The stability of the human plasma-based hydrogels was increased with the addition of commercial fibrinogen, reducing both their degradation and contraction while not disturbing the gelation process. When applied to organotypic skin cultures, these supplemented gels formed a more stable dermal compartment compared to those...
generated only with plasma, but still allowing embedded hFBs to proliferate and the development of a mature and differentiated epidermis on top. This approach could be a very promising alternative to overcome the persisting issues associated with plasma hydrogels and to increase their properties while maintaining the capacity of generating organotypic skin cultures.

Acknowledgement
We kindly thank Angélica Corral and Guillermo Vizcaíno from UC3M for their excellent technical assistance. This work was supported by Programa de Actividades de I+D entre Grupos de Investigación de la Comunidad de Madrid, S2018/BAA-4480, Biopieltec-CM, Programa Estatal de I+D+i Orientada a los Retos de la Sociedad, RTI2018-101627-B-I00, Programa de Apoyo a la Realización de Proyectos Interdisciplinares de I+D para Jóvenes Investigadores de la Universidad Carlos III de Madrid (Project: BIOMASKIN) and Cátedra Fundación Ramón Areces.

Figure 1
Hematoxylin/eosin staining of human skin equivalents after 15 days of culture. The dermal compartment was formed using hydrogels of a) plasma (1.2 mg/ml) and b) plasma supplemented with commercial fibrinogen (3.5 mg/mL).

References
Bioactive nanofibrillated cellulose modulates the chronic wound environment in vitro

Anna Blasi-Romero¹, Carlos Palo-Nieto¹, Corine Sandström⁰, Jonas Lindh¹, Maria Strømme¹, Natalia Ferraz¹

¹ Uppsala University, Department of Materials Science and Engineering, Nanotechnology and Functional materials, Uppsala, SE; ² Swedish University of Agricultural Sciences, Department of Molecular Sciences, Uppsala, SE

Introduction
The number of patients suffering from chronic wounds is reaching epidemic proportions. This translates into high economic costs, estimated to be 2-4% of the health budgets [1], [2] and a significant impact on the patient’s quality of life. Chronic wounds are characterized by an imbalance of the wound equilibrium, with an excess of tissue degradation over the regeneration and healing of the damaged tissue. An abnormal high level of reactive oxygen species (ROS) and matrix metalloproteases (MMPs) are two of the main characteristic factors of a chronic wound environment. Current treatments usually consist of the application of wound dressings that provide a humid environment, absorption of exudate and physical protection of dirt and trauma. It is believed that bioactive dressings that actively target the ROS and MMPs excessive activity could aid in the healing of chronic wounds by restoring the biochemical imbalance typically found in these hard-to-heal wounds [3].

Nanocellulose from wood has emerged as an interesting material for biomedical applications due to its high chemical and physical tuneability and its renewable source. Nanocellulosic materials can be tailored as nanofibers in suspension, hydrogels, aerogels and films. Wood-derived cellulose nanofibrils (CNF) have been described as a promising material for the treatment of wounds [4], [5].

In this work, cysteine was chosen as active molecule to provide CNF with radical scavenging properties and capacity to inhibit the activity of MMP by metal ion capture. Cysteine was covalently incorporated into the nanocellulose fibres and the ability of the functionalized material to modulate the chronic wound environment was investigated in vitro.

Experimental Methods
The cysteine amino acid was covalently incorporated to carboxylated CNF (c-CNF) via EDC/NHS coupling. The chemical structure of the cysteine-functionalized CNF (cys-CNF) was characterized by solid state nuclear magnetic resonance (NMR) spectroscopy and the cysteine content was quantified by elemental analysis. The number of thiol groups was evaluated with the Ellman assay. The radical scavenging capacity of cys-CNF was assayed with the free radical DPPH (1,1-Diphenyl-2-picrylhydrazyl radical). The interaction of cys-CNF with collagenase, chosen as MMP model, was evaluated by quantifying the enzyme activity after incubation with the material using the EnzCheck assay. Moreover, the potential entrapment of the protein within the nanofibers network was investigated. Finally, the safety profile of the cys-CNF was evaluated with human dermal fibroblasts.

Results and Discussion
The solid-state NMR indicated the presence of covalently bound cysteine in cys-CNF and the formation of its oxidized dimer cystine. The elemental analysis of cys-CNF showed that 60 % of the initial carboxyl groups in CNF were substituted by cysteine. Since the potential ability of cys-CNF to modulate the chronic wound environment is expected to depend on the presence of free thiol groups, the material was further characterized in terms of free thiol content by the Ellman assay, which showed that the number of free thiols corresponded to 26% of the total sulfur content in the cys-CNF. The decrease in thiol groups with respect to the total sulfur content may be explained by the formation of the oxidized dimer cystine, seen in the NMR spectrum.
The DPPH study indicated that cysteine endowed CNF with free radical scavenging properties, with cys-CNF showing a concentration-dependent DPPH inhibition (Figure 1a). Thus, demonstrating that the covalent functionalization of the cellulose nanofibers with cysteine is a promising approach to obtain a nanocellulose-based dressing capable of directly reacting with free radicals.

The cys-CNF material was able to inhibit the collagenase activity in a dose-dependent manner (Figure 1b), while the entrapment of the protein within the nanofiber network was not significant. This indicated that the observed decrease in collagenase activity after incubation with cys-CNF was most probably due to the inactivation of the protease, rather than just the entrapment of the protein within the fibril network. The ability of the cys-CNF to chelate zinc ions in solution could explain the inhibition of the collagenase activity since the sequestration of the zinc ions from the MMP active site is expected to be detrimental for the protease activity.

The evaluation of the cys-CNF safety profile indicated that the material did not induce toxic effects on human dermal fibroblasts when tested in concentrations up to 0.5 mg/mL.

**Conclusion**

Cysteine was successfully covalently incorporated into CNF, which endowed the material with radical scavenging properties and the capacity to inhibit the activity of MMP. This bioactivity against two characteristic factors of the chronic wound environment, together with the safety profile evaluated in vitro, are expected to contribute to the recovery of physiological conditions in the chronic wound bed and promote the healing of the tissue.

**Acknowledgement**

The Swedish Research Council (grant number 2018-04613) and Olle Engkvists Stiftelse (grant number 191-419) are acknowledged for the financial support of this work.

---

References


**In Situ Antioxidant Activity of an Enzymatically Crosslinked Sericin Hydrogel for Healing of Chronic Wounds**

Beatriz G. Bernardes¹, Sara Baptista-Silva¹, Sandra Borges¹, Manuela Pintado¹, Pedro L. Granja², Raquel Soares³, Raquel Costa³, Ana L. Oliveira¹

¹ Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, PT; ² Universidade do Porto, i3S - Instituto de Investigação e Inovação em Saúde, Porto, PT; ³ University of Porto, Department of Biomedicine, Faculty of Medicine, Porto, PT

**Introduction**

Chronic wounds are one of the most frequent complications associated to diabetes mellitus, contributing to a high morbidity. Its long healing process is mostly associated with the overproduction of reactive oxygen species (ROS) and may result in ulceration and serious infections [1]. In this context, the choice of the adequate dressing is of great importance and it needs to reflect the requirements of a particular wound according to its stage. Recently, natural-based hydrogel formulations are gaining increasing attention since they can provide control over shape, physical properties and encapsulated cargo, offering the possibility that more advanced products will soon reach the clinic [2]. In this work, a novel *in situ* crosslinked sericin-based hydrogel for wound healing was prepared by a simple methodology using horseradish peroxidase (HRP). The hydrogel was characterized in terms of its thermal stability, fluid interaction and antimicrobial properties. The antioxidant effect was assessed *in vitro* and *in vivo*, after *in situ* application on a diabetic mouse model.

**Experimental Methods**

Silk sericin extracted from *Bombyx mori* cocoons was used to prepare a fast gelling hydrogel by HRP-mediated crosslinking [3]. The hydrogel was characterized by differential scanning calorimetry (DSC) analysis and its transparency evaluated by colorimetry. The hydration degree and the degradation behavior were also assessed. The antimicrobial activity of sericin solution was determined using an inoculum of 0.5 McFarland (1.5 x 10⁸ CFU/mL). Antioxidant potential was quantified *in vitro* by ORAC assay under physiological protease degradation and investigated *in vivo*. An excisional wound-healing model in genetically induced diabetic db/db mice was performed to evaluate the effect of sericin-based hydrogel in chronic wounds. Animals were randomly divided into three experimental groups (n=6 per group) as follows: Control (wounds were left untreated); Tegaderm group (wounds covered with Tegaderm); and sericin hydrogel group (sericin hydrogel covered with Tegaderm). Superoxide dismutase (SOD) and catalase activity were quantified to clarify if sericin hydrogel could affect the endogenous protective mechanisms against oxidative stress. Transmission electron microscopy (TEM) was performed to analyse collagen fibres at the wound bed treated with sericin hydrogel.

**Results and Discussion**

HRP-mediated silk sericin hydrogel was developed with a gelation kinetics of ~2-3 minutes, in agreement to previous rheological study [3]. The hydrogel presented a high degree of transparency. This study evaluates, for the first time, the antioxidant potential by ORAC methodology under physiological protease degradation (3.5 U/mg), up to 24 h at 37 °C. The results showed an increase of antioxidant activity of the sericin hydrogels. This can be related to the availability of the phenolic groups that were previously bound by the crosslinking and became free to react after degradation.

The sericin solution and sericin-based hydrogel formulations did not present antimicrobial activity against important pathogens of wound infection, such as *S. aureus*, *P. aeruginosa* and *E. coli*, since there was no bacterial growth up to 24 h for all strains as well as there was no inhibition halo for hydrogels.
Sericin hydrogel was applied into diabetic wounds to evaluate their in vivo antioxidant behavior. Wounds treated with sericin hydrogel closed at a similar rate when compared to Tegaderm group, although with reduced granulation tissue and decreased wound edge distance and wound thickness, demonstrating that this treatment is more effective in relation to Tegaderm alone. Application of wound dressings of sericin hydrogel could promote a more controlled inflammatory response and deposition of collagen fibers with smaller diameter that could be an advantage to stimulate re-epithelialization, when compared to Tegaderm alone. Oxidative stress and ROS production have been implicated as major contributors in non-healing wounds [4],[5]. The results showed that sericin hydrogel treatment slightly induced two important endogenous antioxidant defenses, SOD and catalase, although without statistical significance. This result was interesting, since the effect of this wound dressing was explored on oxidative damage. It was possible to denote a significant decrease in the content of advanced oxidation proteins products at wound bed, being the sericin hydrogel able to protect wound from oxidative protein damage.

**Conclusion**

Sericin hydrogel is biocompatible and a promising candidate to be applied in diabetic wound healing to protect against oxidative stress. This is of paramount interest to diabetic patients, due to improvements in some of the comorbidities associated with non-healing wounds.

**Acknowledgement**

This work was supported by national funds from Fundação para a Ciência e a Tecnologia (FCT), through project UID/Multi/50016/2019. Sara Baptista-Silva gratefully acknowledges FCT for the research grant (ref. SFRH/BPD/116024/2016). The authors also acknowledge the support of the i3S Scientific Platform HEMS, member of the national infrastructure PPBI - Portuguese Platform of Bioimaging (PPBI-POCI-01-0145-FEDER-022122). Work carried out in the frame of the COST-Action “Advanced Engineering of aeroGels for Environment and Life Sciences” (AERoGELS, ref. CA18125) funded by the European Commission.

**Figure 1**

a) Schematic illustration of in situ application of the sericin hydrogel in a chronic wound; b) Representative images of wound closure (A-F) and histopathological analysis (G-I) in a mouse model of skin wound healing assay; c) TEM images to analyze collagen fibers at wound bed on diabetic animals treated with sericin hydrogel and covered with Tegaderm, with just Tegaderm or untreated wound (control): A) Dermis organization at low magnification; B) The network of collagen fibres; C) Orientation and distribution of collagen fibrils; and D) Cross-section of collagen fibers.

**References**


Curcumin and piperine on cellulose acetate- and cellulose nanocrystalline-containing eletrospun mats for an enhanced antimicrobial action and accelerated clotting time in chronic wound care

Marta A. Teixeira1, Joana C. Antunes1, Shafagh D. Tohidi2, M. Teresa P. Amorim1, Diana P. Ferreira1, Helena P. Felgueiras1

1 University of Minho, Department of Textile Engineering/Centre for Textile Science and Technology (2C2T), Guimarães, PT; 2 University of Minho, Department of Mechanical Engineering/Digital transformation colab (DTX), Guimarães, PT

Introduction
Chronic wounds (CW) are characterized by a poor healing capacity. External stimuli arising from wound dressings can be effective in stimulating healing[1]. To that effect, nanofibrous dressings with a porous structure resembling the extracellular matrix have been engineered via electrospinning, and functionalized with natural extracts, curcumin and piperine, endowed with antimicrobial, anti-inflammatory and antioxidant properties[2]. Various synthetic and natural polymers have been used in the production of polymeric nanofibrous mats via electrospinning. Poly(vinyl alcohol) (PVA) and cellulose derivatives such as, cellulose acetate (CA) and cellulose nanocrystalline (CNC) are polymers well established in this field for their biocompatibility and biodegradability. In combinations, PVA provides excellent electrospinability and mechanical stability, while derivatives confer cellular viability, being that CNC also increase mats’ mechanical resilience[3]. The present work reports the antimicrobial capacity and hemocompatibility of natural extracts when immobilized onto PVA/CA and PVA/CNC crosslinked mats. To this day very little research has been presented on the use of piperine in healing processes.

Experimental Methods
PVA/CA and PVA/CNC mats were produced by electrospinning at 10 wt% at different ratios, 100/0, 90/10 and 80/20 v/v% in 75/25 v/v% acetic acid/distilled water (dH2O) for PVA/CA and dH2O for PVA/CNC. Optimal processing conditions for both polymeric combinations were established at 25 kV, feeding rate of 0.8 mL/h and distance between needle and collector of 18 cm. Crosslinking process was carried out by applying vapor of glutaraldehyde (GA) for 7h at 60°C. Polymers homogeneity and crosslinking effectiveness, along with the mats morphology and porosity, permeability, thermal and mechanical properties, were assessed using scanning electron microscopy, Fourier-transformed infrared spectroscopy (FTIR), air and water vapor permeability testing equipment, thermogravimetry and a dynamometer. Before mats functionalization, minimum inhibitory concentrations for curcumin and piperine were determined using the broth microdilution procedure described by Wiegand et al[4], against Staphylococcus aureus (S. aureus). Mats were functionalized with the natural extracts at 2xMIC by physisorption and the amount bonded was determined by differences in absorbance before and after binding, mapped via UV-Visible spectroscopy. Release characteristics of curcumin and piperine from loaded mats were also investigated by UV–visible spectroscopy at 420 and 342 nm, respectively[5]. Antimicrobial testing via Kirby-Bauer method was conducted for 24 h against S. aureus, to establish the extracts diffusion abilities from the mats. Time-kill kinetics (JIS L 1902) studies were conducted for 1, 2, 4, 6 and 24 h to verify the bactericidal performance of the extracts while loaded. Clotting time was established visually by the loss of movement of re-calculated plasma, following the Lee-White method. Platelet’s adhesion and activation was analysed using brightfield microscopy.

Results and Discussion
PVA/CA and PVA/CNC porous nanofibrous mats were successfully produced via electrospinning. Crosslinking with GA vapor was effective by promoting intermolecular acetal bridges between the -OH in PVA and CA/CNC and the difunctional aldehyde molecule of GA, as confirmed by the presence of a FTIR peak at 1143 cm\(^{-1}\), attributed to the -O-C-O vibration of the acetal group. After crosslinking, the average diameters increased slightly, maintaining appropriated porosity. Permeability index, even on crosslinked mats, registered values above 80%. The cellulose derivatives increased the ultimate tensile strength and the thermal stability of electrospun mats, being most significative after crosslinking. Extracts were found effective against *S. aureus*. Curcumin was the most effective biomolecule, requiring only 7.8-31.2 µg/mL to inhibit its growth, while piperine needed 31.2-125 µg/mL to induce the same effect. Mats were successfully functionalized with natural extracts and a sustained release rate was attained, which was consistent with the time-kill kinetics evaluations against *S. aureus* (elimination of most bacteria after 24 h contact). Diffusion in agar was not as effective, meaning the action of these extracts against bacteria, while loaded, is more important in a dynamic, aqueous environment. These natural extracts were seen to enhance the clotting time and platelets activation compared to the control groups and, therefore, contributing to a potentially quicker wound closure.

**Conclusion**

Data revealed curcumin and piperine as potential biological cues, capable of fighting infection and promoting wound closure, to be used in CW applications.

**Acknowledgement**

This research received funding from the Portuguese Foundation for Science and Technology (FCT) under the scope of the projects PTDC/CTM-TEX/28074/2017 and UID/CTM/00264/2021. MAT also acknowledges FCT for PhD grant SFRH/BD/148930/2019.

**References**

Bioactive glass fibrous scaffold for chronic wound healing

Xingchen Zhao¹, Joel Turner², Dong Zhai³, Chengtie Wu³, Gavin Jell², Julian Jones¹

¹ Imperial College London, Department of Materials, London, GB; ² University College London, Division of Surgery & Interventional Science, Department of Surgical Biotechnology, London, GB; ³ Shanghai Institute of Ceramics, Chinese Academy of Sciences, State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai, CN

Introduction

Inorganic materials are now being applied in wound healing due to their release of therapeutic ions and specific surface affinities to host tissue [1]. Recently, Mirragen (ETS Woundcare, USA), a borosilicate glass fiber mat, received FDA approval for use in diabetic foot ulcers. The mechanism of its success remains unknown. While Mirragen releases therapeutic ions (e.g. borate, silica, calcium, copper, and zinc), its degradation also releases large amounts of Na and K, which interrupt the integration of regenerated tissue [2]. Its fibers are also not uniform, with up to 4 μm in thickness, and only 25% of the glass becomes fiber, while the remaining 75% are beads. Here, we propose a novel 3D borosilicate bioactive glass (BG) scaffold with uniform fiber diameters that mimic the fibers of the extracellular matrix. The fibers have tailorable degradation and ion-release kinetics. In vitro studies on fibroblasts and endothelial cells and in vivo studies on diabetic C57BL/6J mice further verified angiogenic properties and wound healing effects of the scaffolds.

Experimental Methods

Novel 3D ECM-mimic borosilicate BG fibrous scaffolds were prepared through the sol-gel electrospinning approach. Polyvinyl butyral solution was used as a sol-gel binder to maintain the ideal viscosity during electrospinning [3]. Parameters, including voltage, flow rate, air gap distance, and ambient humidity were studied and adjusted corresponding to the change of composition [4]. Characterizations including XRD, FTIR, and SEM were applied. The dissolution of the BG scaffolds was assessed through ICP-AES and in vitro studies investigated the proliferation (CCK-8), migration (Transwell), and gene expression (Elisa) of fibroblasts (human dermal fibroblasts) and endothelial (human umbilical vein endothelial cell) cells. Scaffolds were further applied to diabetic wound models on C57BL/6J mice to investigate their wound healing efficacy in vivo.

Results and Discussion

The BG scaffolds performed a 3D structure (thickness of 0.5-2 mm) and consisted of long and homogeneous fibers with diameters of 180-270 nm Fig.1). This was induced by the branching effect resulting from calcium ion repulsion. XRD and FTIR characterization proved that the scaffold had no sign of crystallization and unreacted compounds. The dissolution profiles revealed a sustained release of therapeutic ions within the ideal concentration range [5]. The wound healing mechanism of the resultant scaffolds was studied in vitro and in vivo. Fig.2 (a-e) shows that dissolution products of borosilicate BG scaffolds induced much higher normalized protein expressions from fibroblasts compared to the control and borate-free scaffolds. Borosilicate BG scaffolds also induced higher migration of endothelial cells compared with the control media solution and borate-free scaffolds (Fig.2(f)). In diabetic C57BL/6J mice, borosilicate BG scaffolds stimulated faster wound closure, higher collagen deposition, and more blood vessel regeneration compared with the control and borate-free scaffolds.

Conclusion

3D ECM-mimic borosilicate BG scaffolds mimic the fibrils in ECM and induce a controllable therapeutic ion release. In vitro and in vivo studies further confirmed the capability of the resultant scaffolds in sprouting cell proliferation, cell
migration, gene expression, collagen deposition, and angiogenesis. All results suggested the promising potential of the resultant scaffolds in chronic wound regeneration.

Fig. 1
Photograph (a) and SEM images (b,c) of electrospun 3D BG. Scale bars are a) 1cm b) 10μm c) 1μm

Fig. 2.
Figure 2. Gene expression of (a) bFGF, (b) VEGF, (c) PDGF, (d) HIF-α, (e) TGF-β of human dermal fibroblasts cultured with conditioned media produced with the dissolution products from BG scaffolds of different compositions of xmol% SiO$_2$-30 mol% CaO-(70-x) mol% B$_2$O$_3$ (x5 in the legend). (f) The number of migrated endothelial cells in a Transwell assay cultured with endothelial cell medium + BG-HDF conditioned medium (1:1).

References
Hyaluronan/collagen-based hydrogels with anti-inflammatory nanoparticles for promoting chronic wound healing

Norbert Halfter, Eva Espinosa-Cano, Albrecht Berg, Stephanie Moeller, Matthias Schnabelrauch, Maria R. Aguilar, Vera Hintze

Technische Universität Dresden, Max Bergmann Center of Biomaterials, Institute of Materials Science, Dresden, DE; Institute of Polymer Science and Technology, Biomaterials Group, Madrid, ES; Networking Biomedical Research Centre in Bioengineering, Biomaterials and Nanomedicine, Madrid, ES; INNOVENT e. V., Biomaterials Department, Jena, DE

Introduction

Novel innovative material concepts are needed to tackle the growing number of chronic wounds arising due to the demographic changes in western civilizations with an increase in multimorbid patients. Using biomaterials based on extracellular matrix (ECM) components is a promising approach, since the ECM conveys fundamental biological functions regarding tissue homeostasis, cellular differentiation and angiogenesis [1]. The most abundant ECM molecules are collagen type I (coll) and proteoglycans. The latter have a protein core with glycosaminoglycans (GAG) bound to it. GAG itself can bind and protect growth factors and affect proliferation, cell adhesion and differentiation [1]. The GAG hyaluronan (HA) is abundantly available and can be chemical derivatized in a controlled and reproducible manner (e. g. (meth)acrylation) [1]. Hence, HA is suitable for use as functional biomaterial with tunable properties. Previously, HA/coll-based hydrogels (HG) successfully enhanced endothelial cell proliferation showing their capability to support the regeneration of vascularized tissue [2]. To further tune the HG for wound healing purpose, e. g. as skin grafts or dressing in particular for non-healing, chronic wounds, positively charged nanoparticles (NP) containing a non-steroidal anti-inflammatory drug reported to show promising anti-inflammatory properties in vitro, were applied on these HG containing negatively charged HA [3].

Experimental Methods

Fabrication of HA/coll-based HG was done as previously by photo-crosslinking reported by Rother et al. [2]. Additionally, cryogels (CG) were made by freezing of the premixed components before photo-crosslinking. NP were synthesized as reported before by Espinosa-Cano et al. via a nanoprecipitation method and for concentration determination or visualization loaded with coumarin-6 [3]. Incorporation of NP into HG was done either via soaking freeze-dried HG or by the addition before photo-crosslinking with different target concentrations. The elastic modulus of the gels was analyzed with a MicroTester (CellScale) and the morphology was studied via scanning electron microscopy (SEM). To determine the NP distribution inside the HG, laser scanning microscopy (LSM) was applied. The release of the NP was determined via fluorescent measurements by incubation of the gels in PBS for up to 7 days at 37 °C. Murine macrophages (RAW264.7) were used to examine cellular interactions with the HG/NP-systems. Next to the cell proliferation with AlamarBlue® assay, the cell viability and the anti-inflammatory activity after LPS stimulation were determined by live/dead staining and by quantifying the nitric oxide (NO) release with Griess reagent kit, respectively. Furthermore, macrophage spreading and the formation of multinucleated cells was evaluated with fluorescence microscopy after staining of actin filaments and nuclei.

Results and Discussion

HG were loaded with different amounts of NP, with only a marginal NP release over 7 d. Depending on the HG fabrication method NP were differentially distributed within the HG as revealed by LSM. While including NP before HG crosslinking and in CG via soaking led to a distribution over the whole cross-section, all other variants were only decorated with NP on the surface. This was confirmed via SEM. CG had a lower elastic modulus than HG but there
was no significant difference when loaded with NP. Cells proliferated well on all HG variants and in particular on CG. Live/dead staining revealed high cell viability over 90% for all variants. All NP containing HG reduced the NO release significantly except for the variant with NP incorporated before crosslinking. This indicates that direct access to NP for cells is an important factor for the anti-inflammatory effects of the NP. In line with the NO release studies, all NP containing gels display small cell areas indicating reduced macrophage differentiation.

**Conclusion**
HA/coll-based HG and NP were successfully combined and were non-toxic to cells. Depending on the NP incorporation method, anti-inflammatory behavior of the NP was observed. Further, this material platform could be modified with additional immunomodulatory molecules, e.g., sulfated HA, to further increase its anti-inflammatory effect. Future experiments need to reveal if this translates into an *in vivo* anti-inflammatory effect in chronic inflammatory wound models.

**Acknowledgement**
Financial support by DFG, project number 59307082 - TRR67, subprojects A3 and Z3. This work was supported by MICINN (Spain) (MAT2017-2017-84277-R) and the training program for Academic Staff (FPU15/06109) of the Spanish Ministry of Education Culture and Sport. M.R. Aguilar is members of the SusPlast platform from CSIC.

**References**
3D printed patches based on exosomes and methacrylated hyaluronic enhance the regeneration of diabetic wound

Alfredo Ronca¹, Ugo D’Amora¹, Barbara Zavan², Luigi Ambrosio¹

¹ National Research Council, Institute of Polymers, Composites and Biomaterials, Napoli, IT; ² University of Ferrara, Dept. of Translational Medicine, Ferrara, IT

Introduction
Chronic diabetic wound causes serious threat to human health due to its long inflammatory phase and the reduced vascularization [1]. Recently, chronic wounds severely deteriorate the life quality of patients due to the lack of effective treatments, which not only increased family burden but even lead to amputations. Numerous novel hydrogel dressings have been developed to relieve the inflammation response, thus accelerating wound healing [2]. Hyaluronic acid (HA)-based biomaterials have been explored for a number of applications in biomedical engineering, particularly as tissue regeneration scaffold. However, its high solubility, high degradation rate and low mechanical properties limit its use for tissue engineering. Chemical modification of HA with subsequent crosslinking is one of the most used methods to improve the mechanical properties also reducing the degradation time in vivo [3]. Crosslinked forms of HA are more robust and provide tunable mechanical properties and degradation rates that are critical in regenerative medicine [4]. Exosomes, nanovesicles enriched on miRNA secreted by cells to exert paracrine communication, if products from mesenchymal stem cells show antinflammatory and immunomodulative action during regenerative processes. In this work a methacrylated HA in combination with stem cell derived exosomes has been developed and characterized as bioink for the realization of 3D printed patches to promote the healing process of diabetic wounds.

Experimental Methods
HA (Mw~340 kDa) was modified to graft photoactive groups by reacting with methacrylic anhydride (ME) in order to obtain methacrylated hyaluronic acid (MEHA). The bioprinting was performed using a slurry containing 2 wt% MEHA and 0.1wt% Irgacure 2959 (Sigma Aldrich) as biocompatible photoinitiator by an In vivo Rokit 3D bioprinter (Rokit Healthcare Inc.). The printability of MEHA based bioinks was optimized by a homogenous UV pre-crosslinking to produce a rectangular porous structure. After printing, the structures were postcured for 15 minutes in order to complete the photocrosslinking process. The morphology of the structures was investigated by scanning electron microscopy (SEM) and dynamic mechanical analysis (DMA) was carried out to study the mechanical properties. EVs isolation was performed from the growth medium MSCGM™ of MSCs. Cell culture supernatants were collected and centrifuged at 4°C for 10 min at 200 g and then for 10 min at 500 g, thawed and spun down vertically at 4°C for 20 min at 2000g and then centrifuged horizontally at 100.000g for 75 min. Finally, the supernatant was discarded and the exosomes pellet was re-suspended in 1 mL of PBS. The isolated EVs were marked with PKH26 (Red Fluorescent Cell Linker Kits MINI26; Sigma-Aldrich Co., St Louis, MO, USA) for 5 min at room temperature in dark room and blocked with fetal bovine serum, according to manufacturer’s instructions. Exosomes were printed within MEHA at a density of 5 × 10¹⁰ cm⁻². Wound healing process has been evaluated following the Peirce model [5].

Results and Discussion
The methacrylation method provides degree of functionalization around 86% as showed by ¹H-NMR analysis. The bioink was investigated in terms of physico-chemical properties to assess the success of the functionalization. DMA was employed to compare mechanical properties of neat MEHA and MEHA/Exosomes 3D structures as a function of material composition. The presence of exosomes did not alter the mechanical properties of MEHA 3D structures that showed Young modulus of about 4kPa. SEM analyses highlighted a well-organized structure with a fiber
diameter of ~200 µm and a porosity of ~700 µm, confirming the capability of retaining the designed 3D structure without collapse. Exosomes were present inside the patches maintaining their biological activity as confirmed from the tests.

**Conclusion**

Modified-HA based hydrogels have gained research attention as UV photocrosslinkable materials to be used as matrix to produce substrates (i.e. patches) and/or scaffolds with interesting physico-chemical and biological properties. In this work, 3D printed MEHA and MEHA/Exosomes structures were realized and characterized from morphological, mechanical and biological point of view. Wound healing ability analysed by means of histological and molecular biology evaluation confirmed that the presence of exosomes reduces the inflammatory event during the first days, improves the granulation tissue quality and the healing of the wound. The collective mechanical and biological performance profile of the MEHA/Exosomes hydrogels strongly suggests that this class of material has great potential as application for diabetic wound.

**Acknowledgement**

The authors would like to thank the POR FESR 2014 - 2020 - ACCORDI REGIONALI DI INSEDIAMENTO E SVILUPPO DELLE IMPRESE - BANDO 2019 IN ATTUAZIONE DELL'ART. 6 DELLA L.R. N. 14 /2014 Sviluppo di approcci PERsonalizzati nel trattamento del Piede Diabetico mediante utilizzo di stampante 3D” (PERPD-3D).

**References**


4:15 p.m. – 5:45 p.m.

Track07

**N11 | Biomaterials for Orthopedic Applications IV**

**Chairs**

*Maria Chatzinikolaidou*
University of Crete, Department of Materials Science and Technology, Heraklion, GR

*Yousra Alaoui Selsouli (YSF)*
Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, NL
Development of a biomaterial scaffold for combined non-viral delivery of both mimics and inhibitors of microRNA activity for accelerated repair of large bone defects

Joanna M. Sadowska¹, Monika Ziminska², John Redmond³, Nicholas Dunne²,³, Helen O. McCarthy², Seth Donahue⁴, Fergal J. O'Brien¹,⁵,⁶

¹ Royal College of Surgeons in Ireland, Department of Anatomy and Regenerative Medicine, Dublin, IE; ² Queen’s University Belfast, School of Pharmacy, Belfast, GB; ³ Dublin City University, School of Mechanical and Manufacturing Engineering, Dublin, IE; ⁴ University of Massachusetts Amherst, Department of Biomedical Engineering, Massachusetts, US; ⁵ Trinity College Dublin, Trinity Centre for Bioengineering, Dublin, IE; ⁶ Advanced Materials and Bioengineering Research Centre, Dublin, IE

Introduction
The treatment of large volume bone defects, which frequently result in delayed or non-union of tissue, remains one of the biggest challenges of modern bone tissue engineering. The use of growth factors such as bone morphogenetic protein-2 (BMP-2) has yielded some success but is expensive and associated with numerous side effects. The delivery of microRNAs (miRs) potentially offers a more attractive alternative inducing the host cells to produce multiple therapeutic proteins with a physiologically relevant release profile and limited side-effects [1-3]. However, identifying a safe and effective microRNA delivery platform remains a key challenge due to their poor stability and challenges with achieving intracellular cytosolic delivery [4]. Here, we investigate the potential of a scaffold-based approach, for sustained delivery of miRs to enhance bone repair. Collagen-hydroxyapatite scaffolds [1,2,4] were coupled with a cell-penetrating peptide [5] to establish a miR-activated scaffold system which uniquely co-delivers both mimics and inhibitors of microRNA activity, i.e miR-26a-mimic [3] and miR-133a-inhibitor [1,2], for bone repair.

Experimental Methods
Collagen-hydroxyapatite scaffolds were fabricated using established freeze-drying techniques [1,2,4], sterilized dehydrothermally and cross-linked with the EDAC/NHS solution. miRs were complexed with a RALA peptide as a non-viral vector [5] and incorporated into scaffolds. The morphology of the scaffolds with miR nanoparticles (NPs) were assessed by scanning electron microscopy (SEM). The distribution of the miR NPs within the scaffold was determined by confocal laser scanning microscope while the release of miR NPs was determined through spectroscopic methods. Human mesenchymal stem cells (hMSC) were seeded on the scaffolds and evaluated in terms of miR-26a-mimic and miR-133a-inhibitor expression, metabolic activity, DNA content, ALP activity and calcium content. Calcium and H&E staining were evaluated at day 28.

Results and Discussion
This study describes the development of a 3D microRNA-activated scaffold system with a novel cell penetrating peptide for controlled and sustained delivery of therapeutic microRNAs for orthopedic applications. The scaffolds developed here effectively co-delivers both microRNA mimics and microRNA inhibitors at the same time. The miRs were homogeneously incorporated into scaffold without affecting its optimal porosity for bone repair (Fig 1A). The scaffolds retained approx. 60% of miR-cargo up to 28 days (Fig 1B) and transfected the hMSCs. Importantly, the dual-delivery miR-26a-mimic/miR-133a-inhibitor scaffold effectively delivered both cargoes to hMSCs. The miR-activated scaffolds enhanced cellular proliferation of approx. 40% (Fig 1C). The miR-26a-mimic and miR-133a-inhibitor scaffolds stimulated ALP activity (Fig 1C) and the mineralization of hMSCs (Fig 1D). The dual-delivery system enhanced osteogenesis to greater extent compared to miR-26a-mimic and miR-133a-inhibitor alone.

Conclusion
This study describes the development of a 3D microRNA-activated scaffold system with a novel cell penetrating peptide for controlled and sustained delivery of therapeutic microRNAs for orthopedic applications. The scaffolds developed here effectively co-delivers both microRNA mimics and microRNA inhibitors at the same time. The miRs were homogeneously incorporated into scaffold without affecting its optimal porosity for bone repair (Fig 1A). The scaffolds retained approx. 60% of miR-cargo up to 28 days (Fig 1B) and transfected the hMSCs. Importantly, the dual-delivery miR-26a-mimic/miR-133a-inhibitor scaffold effectively delivered both cargoes to hMSCs. The miR-activated scaffolds enhanced cellular proliferation of approx. 40% (Fig 1C). The miR-26a-mimic and miR-133a-inhibitor scaffolds stimulated ALP activity (Fig 1C) and the mineralization of hMSCs (Fig 1D). The dual-delivery system enhanced osteogenesis to greater extent compared to miR-26a-mimic and miR-133a-inhibitor alone.
In this study, collagen-hydroxyapatite scaffolds were successfully used as a system for unique co-delivery of therapeutic microRNA mimics and microRNA inhibitors complexed with novel, cell-penetrating peptide. This demonstrates the potential of the scaffolds to be used as next generation of therapeutics for bone regeneration. A novel dual-microRNA scaffold system could be used as a feasible and universal template for delivery of any therapeutic miRs tailoring for a myriad of applications beyond bone repair.

Acknowledgement

National Science Foundation- Science Foundation Ireland (NSF-SFI) US-Ireland R&D Partnership Programme (NSF_17_US_3437). JMS benefits from a Marie Skłodowska-Curie Individual Fellowships from the European Commission through the H2020 project GAMBBa (Project ID: 892389).

References


Simultaneous sterilization and production of medicated scaffolds by supercritical CO$_2$ technology

**Víctor Santos-Rosales**, Beatriz Magariños, Carmen Alvarez-Lorenzo, Carlos A. García-González

Universidade Santiago de Compostela, Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, I+D Farma Group, Facultad de Farmacia, Agrupación Estratégica de Materiales (AeMAT) and Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, ES; Universidade Santiago de Compostela, Departamento de Microbiología y Parasitología, Facultad de Biología, CIBUS, Santiago de Compostela, ES

**Introduction**

The sterilization treatment of medical devices must ensure a sterility assurance level (SAL-6) against bacterial endospores prior to their usage, according to the current legal framework. This scenario represents a major hurdle in the development and commercialization of new generation biomedical product, including scaffolds [1]. Conventional sterilization techniques frequently lead to significant morphological and physicochemical modifications in the treated products. Carbon dioxide under supercritical (sc-) conditions incorporating low contents of hydrogen peroxide is able to inactivate bacterial endospores while preserving the physiochemical properties of the treated biomaterial, emerging as a promising method for scaffold sterilization [2].

On the other hand, the sc-foaming technology allows the manufacturing of drug-loaded scaffolds in absence of organic solvents [3]. The scaffold porous morphology can be modulated in order to match the natural bone tissue characteristics by means of the fine control of the working parameters [4].

In this work, vancomycin-loaded poly(caprolactone) scaffolds were obtained through a simultaneous sterilization and foaming procedure based on scCO$_2$ technology. Mild pressure and temperature conditions along with the addition of H$_2$O$_2$ (1200 ppm) ensured a SAL-6 level against dry bacterial endospores of biological indicators (*Bacillus stearothermophilus*, *Bacillus pumilus* and *Bacillus atrophaeus*).

**Experimental Methods**

Hydrogen peroxide was added in liquid form (1200 ppm) in a high-pressure autoclave (100 mL) containing scaffolds components (PCL, PCL-vancomycin 5 wt.%). The system was heated to 39 ºC and pressurized at 140 bar, maintaining a continuous CO$_2$ flow for 2.5 h. Finally, the system was depressurized at a constant venting rate of 3 bar/min until atmospheric pressure.

The characterization of the scaffolds was performed in physicochemical and morphological terms. Biocompatibility tests were performed and the vancomycin release kinetics were evaluated for 14 days.

**Results and Discussion**

The developed method successfully achieved SAL-6 levels against dry spores of *B. stearothermophilus*, *B. pumilus* and *B. atrophaeus* (Figure 1). These three microorganisms are the biological indicators used in steam and hydrogen peroxide vapor sterilization, radiation sterilization and ethylene oxide or dry heat sterilization, respectively.

Sterile PCL scaffolds loaded with vancomycin with a porous architecture in the 100-600 µm range were obtained, which is coherent for their application in bone tissue regeneration (Figure 2). In addition, scaffolds showed good cytocompatibility and a two-stage vancomycin release pattern suitable for the prophylaxis and treatment of infections at the grafted area.

**Conclusion**

A simultaneous sterilization and manufacture procedure for scaffold manufacturing based on the use of scCO$_2$ technology is presented for the first time. Sterile PCL scaffolds loaded with vancomycin were obtained, meeting the
morphological requirements to be used as bone grafts substitutes while ensuring a sustained release of the drug for over two weeks.

Acknowledgement

This research was funded by Xunta de Galicia [ED431C 2020/17], MCIUN [RTI2018-094131-A-100], Consellería de Sanidade, Servizo Galego de Saúde, Axencia de Coñecemento e Saúde (ACIS, CT850A-G), Agencia Estatal de Investigación [AEI] and FEDER funds. V. Santos-Rosales acknowledges to Xunta de Galicia (Consellería de Cultura, Educación e Ordenación Universitaria) for a predoctoral research fellowship [ED481A-2018/014]. C.A. García-González acknowledges to MINECO for a Ramón y Cajal Fellowship [RYC2014-15239].

Figure 1
Example of turbidity observed in TSB tubes containing spore strips (10^6 spores/strip) after 7 days at the optimal growth temperature. The efficacy of the supercritical CO₂ sterilization is clearly appreciated in (a) B. pumilus and (b) B. stearothermophilus spores, compared to the negative controls (untreated strips).
Figure 2
Sterile PCL scaffolds loaded with vancomycin, processed at 39°C and 140 bar in presence of 1200 ppm of H₂O₂, with detail of the pore structure below. Scale bars 5 mm (black), 100 µm (white).

References
N11-03

Manufacturing and characterisation of PLA bioresorbable warp-knitted spacer fabric scaffolds for bone critical sized defects

Flavia Caronna¹, Nikola Glimpel², Georg-Philipp Paar², Khoa Do³, Thomas Gries², Eimear B. Dolan¹, William Ronan¹

¹ National University of Ireland Galway, Biomechanics Research Centre (BMEC), Biomedical Engineering, School of Engineering, Galway, IE; ² RWTH Aachen University, Institut für Textiltechnik (ITA), Aachen, DE; ³ ITA GmbH, Aachen, DE

Introduction

Critical sized bone defects are one of the most challenging orthopaedic conditions to treat. Such problems, in which bone is unable to heal spontaneously within a patient lifetime, could be successfully tackled by tissue engineering. Biodegradable polymers are very attractive for medical implants applications since they reduce the need for revision surgeries and avoid biocompatibility issues associated with conventional permanent implants (1). In this context, textile technology stands out as versatile and innovative tool for scaffold design, allowing a fine control over the final product architecture and microstructure while scaling easily to large volumes. In parallel, acceptance of in silico trials by regulatory agencies is increasing since they provide a reduction of costs and clinical and pre-clinical trials are both reduced and only required later in the product development.

In this work, 3D polylactide (PLA) scaffolds are fabricated by warp-knitting and their performance is assessed. The degradation behaviour of the PLA yarns during accelerated in vitro degradation tests is reported and is used to calibrate a computational model.

Experimental Methods

Warp-knitted spacer fabrics are manufactured with a double needle-bar Raschel knitting machine (Karl Mayer, Germany). Designed to be suitable for bone tissue engineering applications, the textile features a tricot cover area made of multifilament yarns and a monofilament spacer yarn. To confer dimensional stability to the fabricated textile, heat setting is carried out in a convection oven in constrained conditions at 90, 120, and 150°C for 5 minutes. Following this, fabric thickness, porosity, and pore size are measured from µCT data, and mechanical compression tests are performed. Separately, accelerated degradation of PLA yarns is carried out in vitro in phosphate buffered saline (PBS) at 58.5 °C and pH 7.4 (2). Additional degradation tests including pre-treatment of yarns with different heat setting conditions are also conducted.

A simplified reaction-diffusion model for semicrystalline polymer degradation (3, 4) is implemented in COMSOL Multiphysics® software. The present molecular weight model considers ester bond hydrolysis, acidic autocatalysis, monomer diffusion, and the effects of the crystalline volume phase and porosity on polymer degradation. The model is calibrated based on the in vitro yarn degradation data.

Results and Discussion

The manufactured spacer fabric is shown in Figure 1a. The average textile thickness decreases up to 36% after heat setting (Figure 1b, black) with little change in textile porosity, with a minimum value of 81.5% at 120°C (Figure 1b, brown). A general reduction of pore size is observed after heat setting (Figure 1c), with an improvement in mechanical properties with increasing setting temperature (Figure 1d). Textiles heat set at 150°C exhibit the most suitable physical and mechanical properties for bone tissue regeneration.

Crystallinity evolution during yarn degradation (Figure 2a) suggests that chain scission induced crystallization is taking place. Yarns lose mechanical integrity after 15 days of accelerated degradation, during which the ultimate strength drops up to 87% (Figure 2b), with Young’s modulus remaining approximately constant (Figure 2c). The heat

Page 568 of 2028
setting process affects yarn’s initial properties without substantially affecting its degradation behaviour. Preliminary simulation results are shown in Figure 2d with heterogeneous degradation evident, i.e., the interior degrades faster due to trapped autocatalytic reaction products.

**Conclusion**

The present study shows that bioresorbable warp-knitted scaffolds represent a promising technology in the field of bone tissue regeneration, offering large, interconnected pore spaces (>100-400µm) for cells migration, nutrient supply, metabolic waste removal, and high surface to volume ratio (which favours cell attachment and growth). Stacking of spacer fabric layers has the potential to bridge bone defects of critical size (>2cm).

Scaffold degradation *in vitro* will be studied to explore the impact of autocatalysis on structure resorption and acid release. A computational model will predict the degradation of complex structures from material data inputs, thus simplifying and accelerating the development of polymeric bioresorbable implants.

Ongoing work is focused on the assessment of scaffold biocompatibility (including the effect of manufacturing processes). The performance of knitted PLA scaffolds (cell attachment, proliferation, and differentiation) will be investigated *in vitro*, reproducing mechanobiological cues to mimic the bone *in vivo* microenvironment.

**Acknowledgement**

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the grant agreement No 813869. This publication reflects only the author’s view and the REA is not responsible for any use that may be made of the information it contains.

The authors very much appreciate the scientific contribution of S. Limem and S. Rizik at Tepha Medical, Ind. to the present work.

![Figure 1: Characterisation of the heat-set textile.](image)

(a) µCT image of warp-knitted PLA textile before heat setting. (b) Textile thickness and porosity, (c) pore size distribution, and (d) stress-strain curves from uniaxial compression tests, for different heat setting conditions. Non heat-set textile is labelled as reference (Ref).
Figure 2: Yarns’ degradation results. Accelerated in vitro degradation of non heat-set and heat-set PLA yarns: (a) crystallinity (Xc), (b) ultimate strength (Fmax), (c) Young’s modulus (E). (d) Simulation of molecular weight (Mn) distribution during degradation of a PLA monofilament yarn.

References
Bioinspired bone marrow units: a strategic cell-biomaterial combination in liquified capsules as a new therapeutic approach for bone regeneration

Claudia S. Oliveira, Maria C. Gomes, Sara Nadine, Clara Correia, João F. Mano

University of Aveiro, CICECO-Aveiro Institute of Materials, Aveiro, PT

Introduction
The reconstruction of bone defects has been correlated with several disadvantages, namely patient immobility, prolonged pain, support dependence, and expensive treatment costs. Aiming to circumvent the limitations of the current treatment for these conditions, bone tissue engineering (BTE) has developed implantable biomimetic devices to accelerate the bone regeneration process after implantation (1). However, the majority of BTE approaches are deprived of an integrated strategy of the three co-existing systems of the native bone marrow (BM), namely skeletal, vascular and hematopoietic, essential for bone regeneration and healing. Herein, the novelty was to combine these three systems in liquified compartments as a promising approach to recapitulate and exploit the regenerative properties of the BM microenvironment under in vitro conditions.

Experimental Methods
Mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), and human umbilical vein endothelial cells (HUVECs) were isolated from the umbilical cord. Before the encapsulation setup, aggregates of MSCs with surface-modified polycaprolactone microparticles (µPCL) were performed under osteogenic stimulation for 21 days. At 21 days, multilayered and liquified alginate capsules were produced as described (2,3). In order to recapitulated as close as possible the BM microenvironment, co-culture capsules (aggregates of MSCs, HSCs, and HUVECs) and co-culture (aggregates of MSCs and HUVECs) were maintained in static and dynamic culture systems (spinning flasks) for 7 days. After seven days in culture, the microenvironment of the capsules was analysed.

Results and Discussion
The dynamic system promoted an increase in cell viability, proliferation and allowed more complex cellular interactions and connections when compared to the static condition (Figure 1). The expression of bone formation markers such as osteopontin, osteocalcin, bone morphogenetic protein-2, type IV collagen, fibronectin were observed in both types of capsules, but much higher in the dynamic system compared to static, and more significant in the presence of HSCs (Figure 2). The presence of angiogenic factors, namely vascular endothelial growth factor (VEGF) was also observed with significant amounts.

Conclusion
Considering the repair of bone fracture a complex biological mechanism that requires the recruitment of stem cells from the bone marrow (BM), this microenvironment and its components become a powerful candidate to improve bone regeneration. In fact, our results evidence the superior biological outcome resulting from the direct crosstalk between the BM cell components in the tri-coculture capsules. This privileged three dimensional (3D) microenvironment strategically combined with cells in liquified capsules provides ideal support for cell viability, secretion of osteo key components, and vasculogenic factors, essential steps involved with new bone tissue formation and regeneration. The recapitulation of the BM microenvironment under in vitro conditions may provide an advanced platform of fundamental importance for bone regeneration applications.

Acknowledgement
The authors acknowledge the financial support from the Portuguese Foundation for Science and Technology (FCT) through the CIRCUS project (PTDC/BTM-MAT/31064/2017), “CICECO-Aveiro Institute of Materials” project (UIDB/50011/2020 & UIDP/50011/2020) and financed by national funds through the FCT/MEC and, when appropriate, co-financed by FEDER under the PT2020 Partnership Agreement. The authors also acknowledge funding from the European Research Council (ERC) through the project “ATLAS” (ERC-2014-ADG-669858), an Advanced Grant awarded to Professor João F. Mano.

References
A Numerical Investigation of Different 3D Bone Scaffold Geometry for Bone Tissue Regeneration

Amedeo F. Bonatti¹,², Subrata Mondal³,⁴, Carmelo De Maria¹,², Kenneth Dalgarno⁵, Maria Chatzinikolaidou⁶, Aurora De Acutis¹,², Giovanni Vozzi¹,², David MacManus³,⁴, Nicholas Dunne³,⁴

¹ University of Pisa, Research Center E. Piaggio, Pisa, IT; ² University of Pisa, Department of Information Engineering, Pisa, IT; ³ Dublin City University, School of Mechanical and Manufacturing Engineering, Dublin, IE; ⁴ Dublin City University, Centre for Medical Engineering Research, Dublin, IE; ⁵ Newcastle University, School of Engineering, Newcastle upon Tyne, GB; ⁶ Foundation for Research and Technology Hellas (F.O.R.T.H)-IESL, Heraklion, GR

Introduction
Mechanical stimulation plays a key role in enhancing osteogenic differentiation of stem cells in the physiological bone environment [1]. To better replicate this behaviour in vitro, several studies have shown that stimulation by flow perfusion and cyclic mechanical compression enhances the bone cell mechano-transduction [2], [3]. In this context, a computational study can help optimize the stimulation transmitted to cells by evaluating the mechanical load and flow shear stresses. Here, we present an optimization procedure of a 3D printed bone scaffold design based on finite element (FE) simulation approach. A preliminary FE analysis on three different scaffold designs was implemented to investigate the optimal scaffold geometry, orientation, and loading condition for cyclic mechanical stimulation. From this analysis, the best performing geometry and loading direction was selected to be further analysed in a more complex FE model considering the fluid-structure interaction (FSI). These combined simulations can represent a tool to achieve information not only about the optimal scaffold design, but also about the overall mechanical stress and wall shear stresses (WSS) due to fluid flow acting on the cells for bone tissue regeneration in vitro.

Experimental Methods
Three different scaffold geometries were investigated (Fig. 1): (a) a cylindrical scaffold of 5 mm diameter, 1 mm height, and with a line internal infill (geometry 1); (b) a cylindrical scaffold of 5 mm diameter, 1 mm height, and with four vertical pores (geometry 2); (c) a rectangular scaffold with sides of 5 mm and 1 mm height, with infill lines at 45° (geometry 3). This last geometry represents a unit slice of the final scaffold, whose height was further optimized through simulation. For all geometries the internal porosity was 50%. A linear, elastic, homogeneous, and isotropic mechanical behavior was defined for the scaffold material (Young modulus: 2.52 GPa, Poisson ratio: 0.3, density: 1,200 kg/m³). All three geometries were meshed with tetrahedral element, with an element size of 0.4 mm. Regarding the boundary conditions, to model the mechanical loading phase during in vitro stimulation, a boundary condition with a prescribed displacement of 200 µm was chosen for the loaded boundary, while the opposite boundary was set to fixed constraint. These boundary settings allowed to simulate the loading condition as applied in bioreactors such as the CellScale MTX. The load was applied to the top surface of the scaffold for geometry 1 and 2, while for geometry 3 to the scaffold side (Fig. 1). The other boundaries were set to free displacement. Furthermore, a parametric optimization was completed to optimize the scaffold thickness in terms of maximum compressive strength and stiffness. Finally, a computational fluid dynamic and FSI model was developed to investigate the WSS under fluid perfusion. Regarding the material properties, a dynamic viscosity of 1.4 mPa*s was considered for the fluid. Ten noded tetrahedral elements were considered for the discretisation of the scaffold, with an edge length of 0.5-2 mm. In the CFD model, a constant velocity of 100 µm/s was applied at the inlet while a zero-pressure condition was applied at the outlet. The scaffold surface was assumed as a non-slip wall boundary.

Results and Discussion
Data from the preliminary simulations were expressed in terms of the total displacement inside the scaffold, which represents a measure of the portion of stimulation that the cells ‘feel’ \textit{in vitro}. Geometry 1 did not show any relevant displacement in the central struts of the scaffold, since all the loading was sustained by the external walls (Fig. 1). For geometry 2 and geometry 3, the displacement field was more uniform, so seeded cells can virtually feel the mechanical load due to stimulation.

Furthermore, since geometry 3 can be more easily seeded during the experiments, it was chosen to be further evaluated through simulations. In particular, the parametric optimization of geometry 3 resulted in a 5 mm thickness, which demonstrates stresses well within the range of maximum yield strength of 42.6 MPa.

This geometry was further evaluated through a FSI simulation. Preliminary results of the FSI analysis indicated that 70% of total scaffold surface area were evident to levels of WSS, which were deemed adequate to stimulate bone differentiation [2]. However, the combined loading of fluid perfusion and mechanical compression might amplify the WSS at the scaffold surface.

**Conclusion**

The present study showed that the displacement field is more uniform inside the pores in case of geometry 2 and geometry 3 as compared to geometry 1. However, geometry 3 (rectangular scaffold with infill lines at 45°) demonstrated better agreement in terms of cell seeding. The study also concluded that understanding the behavior of mechanical stimulation under different loading conditions can be useful to the researchers in terms of design and optimisation of 3D scaffolds.

**Acknowledgement**

This project has received funding from the European Union’s Horizon 2020 research and innovation program under grant agreement No. 814410.

![Fig. 1](image-url)

\textbf{Fig. 1}
The first row represents the different scaffold geometries: (A) the cylindrical scaffold with line infill, (B) the cylindrical scaffold with vertical pores, and (C) the rectangular scaffold with 45° line infill. The second row shows the results for the three geometries in terms of the total displacement.

**References**

N11-06

Malleolus Fractures Treated with Bioresorbable Magnesium-based Compression Screws ZX00: First in man study

Patrick Holweg, Martin Ornig, Paul Puchwein, Andreas Leithner, Franz Seibert

Medical University Graz, Austria, Department of orthopaedics and trauma, Graz, AT

Introduction
Over the last decade, the development of new and alternative materials in trauma care using bioresorbable metals has increased, concentrating mainly on magnesium (Mg) alloys. No prospective clinical trial has investigated the clinical long-term results of lean bioresorbable screws made of Mg, calcium (Ca) and zinc (Zn), without any rare earth elements (REE), denoted as ZX00. This prospective cohort study investigated this bioresorbable Mg-alloy in compression screws for medial malleolar fracture and the patient-related outcome regarding function and complication. Questions/Purposes: (1) Do Mg-alloy (ZX00) compression screws stabilize medial malleolar fracture fragments in patients? (2) Do patients recover their ankle mobility? (3) Does the degradation rate of Mg-alloy screw correlate with the bone healing? (4) Are the Mg alloy screws completely resorbed after one year?

Experimental Methods
Twenty patients with isolated, bimalleolar, or trimalleolar ankle fractures were recruited and treated with bioresorbable Mg alloy (ZX00) compression screws. Patient-reported outcome measures (PROMs) including visual analog scale (VAS) for pain, American Orthopaedic Foot & Ankle Society (AOFAS) scales and the presence of complications (adverse events) during one year of follow-up were used. The functional outcomes were analyzed through the range of motion (ROM) of the ankle joint. Fracture reduction and the bioresorbability of the screws were evaluated using several plane radiographs. Quantitative data were analyzed using general linear models (GLM) for repeated measures.

Results and Discussion
All fractures were stabilized with 2 screws without any screw failures. The difference in the range of motion of the talocrural joint between the treated and the non-treated site decreased from 39°±12° after 2 weeks to 7°±13° and 2°±11° after 24 weeks and 52 weeks, respectively. The within-subject effect of this difference in the one-year follow-up is statistically significant (p<0.001). A complete consolidation of all fractures was achieved after 12 weeks with all patients, even with one patient suffering from osteoporosis, demonstrating that the radiolucent areas attributed to gas formation did not affect clinical or functional outcomes. After one-year follow-up, in 17 patients (85%), the head of screws was completely resorbed without adverse reactions.

Conclusion
Bioresorbable Mg alloy (ZX00) compression screws prove to be an excellent and safe alternative compared to titanium (Ti) screws while avoiding a second, costly and unnecessary implant removal operation.
Fracture fixation with ZX00 screws
Anteroposterior ankle radiographs of a 29-year-old male patient with a medial malleolar fracture (A) reduced by two Mg-based screws. After 6 weeks (B), a small fracture line is still visible; small radiolucent zones within the bone surrounding the screws. These radiolucent zones resulting from the degradation of the screws decreased after 24 (C) and 52 weeks (D).
Introduction
A major challenge in the development of scaffolds for oral and maxillofacial surgery includes the engineering of materials that simultaneously promote hard (bone) and soft (mucosa) tissue regeneration. The scaffold should support the growth and differentiation of the relevant cell populations, directing cellular interactions, as well as promoting the formation and maintenance of extracellular matrix heterogeneity. This bimodal material must also exhibit a gradation in mechanical properties that mimics the native insertion site and must be biodegradable at a relevant time scale, in order to be gradually replaced by the host tissue.

Recently, our group has developed a Sr-hybrid system, composed of Sr-doped HAp microspheres, delivered in an alginate vehicle crosslinked with Sr (1). This material has already shown to be capable of promoting bone regeneration and possess an immunomodulatory effect (2, 3). Based on these findings, we herein propose an innovative bimodal injectable biomaterial, composed of a bottom layer (bone-like layer - BL) to induce bone regeneration, and a top layer (mucosa-like layer - ML) for periodontal tissue integration.

The bimodal material is further enriched with either human decellularized fetal membranes (dFMs) particles and/or platelet-rich plasma (PRP), due to their potential for regenerative medicine since it is known they contain a vast number of interesting growth, angiogenic and immunomodulatory factors.

Experimental Methods
Both biomaterial layers include as a vehicle a 3.5% (w/v) ultrapure sodium alginate solution being its in situ gelation promoted by the addition of Sr carbonate and Glucone-δ-lactone, biochemical enriched with decellularized human amniotic and chorionic membrane lyophylyzed particles (1:1 ratio) in a concentration of 20 mg/mL. BL was mechanically reinforced with hydroxyapatite Sr-rich microspheres (35% w) with a diameter between 500-560 μm. Fetal membranes were isolated from placental tissue, and followed a previously optimized decellularization protocol. PRP was isolated from whole human blood. The dFMs were characterized by histology, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM). Furthermore, the bimodal biomaterial was mechanically and structurally characterized by dynamic mechanical analysis (DMA), and micro-computed tomography (m-CT), respectively. Cell viability, metabolic activity, proliferation and differentiation of gingival fibroblasts (hGFs) and human mesenchymal stem cells (hMSCs) were investigated. Moreover, a proteomic profile of the cellular response was performed using a transwell experimental design, where the biomaterial was placed on the top compartment and cells seeded on the bottom.

Results and Discussion
A fully functional bimodal biomaterial was obtained, with a uniform and stable interface, even when agitation or compressive forces were applied. dFMs were found to be sucessfully decellularized, while mainting their tissue architecture and were homogenously distributed within the system. PRP was easily dissolved into the hydrogel. Both cell types exhibited an increase in cell number and viability, when in the presence of dFMs. dFMs seem to lead to the formation of cell agglomerates, suggesting them as potential anchoring sites. An increase in mineralization sites...
was observed in the hMSCs for formulations containing dFMs. Proteomic analysis revealed that ML incorporating FMs increase the hGF ECM remodeling potential, particularly when combined with PRP. FMs seem to have an immunomodulatory effect through the regulation of both IL-7 and IFN-\(\gamma\). Concerning the hMSCs response, an enrichment in the ECM-related proteins was observed in the Sr-hybrid containing FMs, particularly when PRP was also present. Moreover, hMSCs differentiation seems to be particularly increased in the formulation containing both FMs and PRP, what is in accordance with the cell culture assays.

**Conclusion**

The bimodal material promoted hGFs (ML-layer) and hMSCs cell growth and hMSCs osteogenic differentiation (BL-layer). This effect is more evident when combining dFMs and PRP, suggesting they act in a synergistic way. The results obtained indicate that the developed bimodal material provides a promising multifunctional approach for maxillofacial regeneration.

**Acknowledgement**

Dr Mário Oliveira from the Obstetrics and Gynecology Department, Infante D. Pedro Hospital, Aveiro, Portugal, for providing the fetal membranes and Centro Hospitalar Universitário de São João for buffy coats donation. Norte Portugal Regional Operational Programme (NORTE 2020) in the framework of the project “Bioengineered Therapies for Infectious Diseases and Tissue Regeneration” (NORTE-01-0145- FEDER-000012).

**References**

Bioinspired spin-assembled multilayered freestanding films with adhesive and bioactive properties

Joana Moreira¹,², Ana C. Vale¹,², Ricardo A. Pires¹,², Gabriela Botelho³, Rui L. Reis¹,², Natália M. Alves¹,²

¹ 3Bs Research Group, I3Bs—Research Institute on Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho, Guimarães, PT; ² ICVS/3B's, PT Government Associate Laboratory, Braga/Guimarães, PT; ³ Department of Chemistry, Campus de Gualtar, University of Minho, Braga, PT; ⁴ University of Minho, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, Guimarães, PT

Introduction

On the design of new biomaterials, research often finds inspiration in the multifunctional materials present in nature. Two examples suitable for the design of freestanding films are the robust nacre’s structure and the water-resistant adhesive proteins secreted by marine mussels (MAPs).

Nacre in a natural laminate composite, which composes the inner layer of mollusc shells, with remarkable high fracture toughness and energy absorption properties owing to unique lamellar and staggered organic-inorganic structure with multiple-length sizes, composed of 95 wt% aragonite and 5 wt% organic material.

MAPs overexpress the amino acid 3,4-dihydroxy-L-phenylalanine (DOPA), which has a critical role in the wet adhesion due to the presence of catechol groups since they are able to form strong covalent and noncovalent bonds with distinct inorganic/organic/metallic surfaces and even biomacromolecules. We stress the novelty of this work, as nacre-inspired freestanding films were successfully developed through spin-assembly LbL (SA-LbL) combining catechol-modified natural polymers, CHIcat, and HAcat, and BGs. The main goal of this work was to study the potential of these nanostructured FS membranes for guided bone regeneration.

Experimental Methods

Bioinspired freestanding films were produced through the SA-LbL technique, composed of natural polymers (chitosan and hyaluronic acid) and bioactive glass nanoparticles (BGs), which were crosslinked with genipin to improve their stability in a wet environment. Both polymers were modified with catechol groups to increase the adhesiveness of the films. Six different films were produced: controls (CTR) with 50 and 100 tetralayers (T), BGs (BG) containing films with 50 and 100T, catechol-containing (Cat) films with 50T, and catechol and BGs (CatBG) films with 50T. Their physicochemical (SEM, TGA, WCA, swelling, and degradation tests) and mechanical properties (DMA and AFM tests) were characterized in detail, as well as their bioactive and possible cytotoxicity effects under physiological-simulated conditions.

Results and Discussion

Catechol-containing films led to the construction of thinner films, moreover, the combination of catechol-modified polymers with BGs showed some porosity and revealed a more compact structure. Although both films with 100 T were slightly thicker than their 50 T counterparts, increasing the layer number did not have a significant impact on the film thickness.

Similar WCA values were obtained for all BGNPs-containing films, meaning that the differences in BGs’ content did not have an impact on the films' wettability, and the genipin crosslinking of the films decreased the WCA of both surfaces, becoming more hydrophilic.
BG100 presented a higher weight loss, due to the higher amount of BGNPs that could be released and dissolved from the films. A lower weight loss was observed for the catechol-containing films, being CatBG50 the lowest, suggesting that the catechol incorporation increased the film stability. The inclusion of catechol groups resulted in increased film stiffness and, when BGNPs were also combined, the highest E’ values were achieved. Unsurprisingly, higher adhesion values were shown for both films containing catechol-modified polymers, Cat50 and CatBG50. Preliminary biological assays indicated that the use of either catechol-modified polymers or BGNPs by itself did not affect negatively cell viability. On the other hand, the combination of BG with catechol groups in CatBG50 led to a higher metabolic activity compared to the other tested film formulation, adhesion, and proliferation similar to CTR50. Summarily, CatBG50 presented the advantage of a lower weight loss rate, while maintaining its bioactive character. Moreover, the stiffness and roughness increase and water uptake decrease also contributed to an enhanced cellular behavior, since cells tend to prefer stiffer and lower hydration surfaces.

**Conclusion**

These bioactive LbL freestanding films that combine good adhesion with improved mechanical properties, and also presented hydrophilic surfaces, reduced biodegradability, decreased swelling, and promoted higher cellular metabolic activity, could be an alternative strategy for guided hard tissue regeneration.

**Acknowledgement**

The authors acknowledge the Portuguese Foundation for Science and Technology (FCT) and the European program FEDER/FEEI for the financial support through projects PTDC/BTM-MAT/28123/2017 and PTDC/NAN-MAT/31036/2017. FCT for the financial support through the exploratory project MIT-EXPL/BIO/0089/2017.
Dense drug-eluting biodegradable metals: Processing, drug release kinetics, and antibacterial properties

Aliya Sharipova¹, Olga Bakina², Aleksandr Lozhkomyov², Marat Lerner², Elazar Gutmanas¹, Alejandro Sosnik¹

¹ Technion - Israel Institute of Technology, Materials Science and Engineering, Haifa, IL; ² Institute of Strength Physics and Materials Science, Russian Academy of Science, Tomsk, RU

Introduction

Biodegradable biomaterials have been extensively investigated in bone fracture fixation and scaffolding as they eliminate the need for a second surgical intervention for implant removal. In addition, biodegradable materials will allow complete tissue regeneration without the risk of chronic inflammation and stress shielding. In addition, biodegradation can be capitalized on to locally release drugs. Drug loading into the implant bulk requires low processing temperatures, which often restricts the biomaterial choice to biodegradable polymers or ceramics. However, polymers are weak and ceramics are brittle for load-bearing applications. To unlock the potential of biodegradable metals (e.g., Mg, Fe, Zn) for local drug delivery and bone scaffolding, an advanced cold sintering process (CS) for fabricating metals at ambient temperatures can be employed.

In recent studies, we showed the feasibility of drug loading and release from bulk Fe-Ag nanocomposites [1]. In the present work, we focused on improving the mechanical properties of drug-loaded bulk biodegradable metals and describe the processing, degradation, mechanical, antibacterial, and cell compatibility properties of vancomycin hydrochloride (VH)-loaded Fe-based biomaterials.

Experimental Methods

Fe and Fe-Fe₂O₃ (5, 10 vol.% of Fe₂O₃) powder blends were prepared from carbonyl Fe powders (5-9 μm) and Fe₂O₃ nanopowders (50 nm) by manual mixing in mortar. Here, Fe₂O₃ nanoparticles were utilized as phase increasing mechanical and corrosion properties of Fe [2]. VH was utilized as a model drug in this study. VH powder was manually mixed with Fe and Fe-Fe₂O₃ blends in a mortar to prepare Fe-VH and Fe-Fe₂O₃-VH blends. Then, powders with and without the drug were cold sintered/high pressure consolidated in high-speed tool steel die at pressure 2.5 GPa (Fig. 1), room temperature to obtain dense drug-carrying samples [1].

Materials were characterized by high resolution scanning electron microscopy (HR-SEM) with energy dispersive spectroscopy (EDS), by using the focused ion beam (FIB) slicing technique and Avizo software for 3D-reconstruction of samples microstructure. The mechanical properties were tested in compression. Degradation behavior in vitro was studied employing immersion test in modified Hank’s solution [3] for periods up to 8 weeks. Drug release in vitro was analyzed by a zone of inhibition (ZOI) technique on VH-sensitive Staphylococcus aureus (S. aureus) [4]. The drug release profile was studied in modified Hank’s solution by UV-spectrophotometry. Finally, the compatibility of drug-eluting metals was studied in vitro on human smooth muscle cells (hSMC).

Results and Discussion

The proposed processing approach allows loading of antibiotics and antitumor drugs during fabrication of bulk metallic samples. Near dense, 90 to 95% of theoretical density, Fe and Fe-Fe₂O₃ samples with 1-5 wt.% VH loaded into system of interconnected pores were prepared by cold sintering (Fig. 2a). Plastic deformation of metallic particles induced by hydrostatic pressure exceeding shear strength of particles (by Hertz model) [4], enables particles sintering. Thus, drug phase was encapsulated between sintered metallic particles, which was verified at 3D-reconstructions of samples microstructure.
VH-loaded Fe-Fe₂O₃ samples also showed high compressive ultimate strength (600 MPa) with almost no change after the loading of 1 wt.% drug. The VH release and analysis of surface microstructure showed that the initial release (first 1-2 weeks) was due to drug dissolution from the material surface and the system of open pores adjacent to outer layers. Then, the release slowed down and was associated to biodegradation. This observation correlated well with results of ZOI analysis (Fig. 2b) showing a decrease in antibacterial activity of bulk samples after ~2 weeks. Finally, preliminary in vitro culture test on hSMC line showed that Fe and Fe-Fe₂O₃ samples loaded with VH are not cytotoxic.

**Conclusion**

In this work on the example of Fe and Fe-Fe₂O₃, we present the capabilities of advanced cold sintering/high-pressure consolidation technique to encapsulate drug into bulk metal-based materials. Cold sintering is a simple and versatile method that can be implemented with different small-molecule drugs and active macromolecules (e.g., proteins). Our findings demonstrate the promise of this strategy in local delivery of bioactive substances at bone healing sites via degradation of temporary metallic implants.

**Acknowledgement**

The work was supported by the Ministry of Science & Technology, Israel (grant 3-16574), and by Russian Foundation for Basic Research (grant 19-53-06006).

**Fig. 1**

Fabrication of dense drug-eluting metals by CS [1].

**Fig. 2**

Fe-10Fe₂O₃ loaded with 1 wt.% vancomycin. (a) HR-SEM micrograph of the surface and (b) inhibition zone (tested against S. aureus) after incubation for 7 days, at 37 °C

**References**


6:00 p.m. – 6:45 p.m.

Track01

PL2 | Plenary Lecture 2

Chair
Pedro L. Granja
Instituto de Investigação e Inovação em Saúde (i3S) da Universidade do Porto, Porto, PT

PL2-01

Engineering Mucosal Barriers in Health and Disease: From Organoids to Organs – on – Chips

Linda Griffith

Massachusetts Institute of Technology, School of Engineering Teaching Innovation Chair of Biological and Mechanical Engineering, Cambridge, US

Mucosal barriers are the gateways to all internal organs, serving to transport oxygen, nutrients, and waste and at the same time performing enormous feats of protection against infection and other hazardous insults. The explosion of interest in the human microbiome – especially but not only that in the gut – has driven new interest in building human mucosal barrier models. This talk will highlight the development of synthetic “one size fits all” biomaterial hydrogels to engineer complex 3D epithelial-stromal-immune mucosal barriers, with a focus on the endometrium and the gut in health and disease. A central feature of the approach is defining a parameter space capturing physical, biochemical, and cell-specific features. Semi-empirical exploration of the parameter space provides efficient definition of hydrogel formulations that foster both initial cell survival and proliferation and subsequent differentiated function. While some phenotypic functions are adequately captured in 3D static culture, complex functions involving microbiome-mucosa interactions or vascular-mucosa interactions require incorporation into micro- or meso-fluidic devices. Examples will emphasize how these approaches can be used to model chronic inflammatory diseases, especially endometriosis and Crohn’s disease.
6:45 p.m. – 7:45 p.m.

Track01

Social Activity 4 | Discover Portuguese Wine Regions with Sogrape

Chair
Meriem Lamghari
Raquel M. Gonçalves

i3S - Institute for Research and Innovation in Health, University of Porto, Porto, PT; INEB - Institute of Biomedical Engineering, Porto, PT; ICBAS - Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, PT

Ligia Marques

Sogrape Distribuição, S.A., Porto, Portugal

Ligia Marques from Sogrape will give a seminar about portuguese wine.

About Sogrape
"Founded in 1942 by Fernando Van Zeller Guedes, Sogrape was born to demonstrate the quality of Portuguese wines to the world. From a single winery in the Douro Valley focused on the production of Mateus Rose, this family company has become global, with presence in more than 120 markets, owning c. 1,600 hectares of vineyards in Portugal, Spain, Chile, Argentina and New Zealand. Moved by the purpose of bringing Friendship and Happiness to everyone it touches through its wonderful wines, the family spirit and the peak performing team culture lived at Sogrape are key in the successful path that it has been building for almost 80 years. Under the leadership of Fernando da Cunha Guedes, current President and 3rd generation of the founding family, the leading company in Portugal aims to spread Sograpiness through the world and be affirmed as a catalyst for positive societal change, respecting the limits of the planet in the construction of a more sustainable and inclusive future."
Tuesday, 7 September, 2021

10:15 a.m. – 18:30 pm.

Track08

ESB-AERoGELS COST Action Joint Symposium

Title: Symposium on aerogels for biomedical applications

Aerogels are unique nanostructured porous materials with special properties adapted to fit certain advanced applications. Namely, aerogel-based materials can be used in biomedical applications as drug carriers, synthetic bone grafts for regenerative medicine and advanced wound dressings. Advances on aerogels and other nanostructured materials in these fields need to assemble expertise from interdisciplinary and intersectorial domains. Technological developments in terms of novel designs, processes, modelling tools, characterization techniques and uses for aerogels should be aligned to boost the progresses on the topic. This symposium aims at assembling and integrating the most recent scientific-technological knowledge in aerogels and other nanostructured materials on a wide range of fundamental topics and particularly applied in biomedical applications.

About AERoGELS COST Action:
AERoGELS COST Action brings together the knowledge on research and technology of aerogels at the international level from more than 200 institutions from academia, industry and regulatory experts. In AERoGELS Action, the use of aerogels is mainly focused on life sciences (pharma, biomedical, food) and environmental (treatment and removal of pollutants, energy management, life cycle analysis) applications.
For more information: https://cost-aerogels.eu/
10:15 a.m. – 11:45 a.m.

Track08

**AERO S01 | Wound materials-based dressings and processing**

*Chairs*

**Ana L. Torres**  
i3S/INEB, Porto, PT

**Rosana Simón**  
Universidade de Vigo, Vigo, ES
Functional Bacterial Nanocellulose Materials

Anna Roig, Cristina Mira, Soledad Roig-Sanchez, Anna Laromaine

Institut de Ciència de Materials de Barcelona (ICMAB-CSIC), Campus UAB, Bellaterra, ES

Introduction
Nanocelluloses and their nanocomposites are gathering increased interest due to the enhanced properties arising from the combination of the nanocellulose with the functionalities characteristic of inorganic nanoparticles. Bacterial nanoellulose (BC) is an animal and donor-free natural polymer presenting some inherent characteristics that are highly regarded in the healthcare sector - purity, biocompatibility, mechanical resistance, thermal stability and a fibrilar microstructure similar to that of the collagen extra-cellular matter.

Experimental Methods
BC hydrogels were synthesized by Komagataeibacter xylinus grown on Hestrin-Schramm medium. K. xylinus cultures were culture for 3 d under static conditions at 30 °C to obtain BC fleeces that were sterilized by autoclaving.

Results and Discussion
I will describe some strategies to control BC topography [1] and microstructuration during the microbial cellulose biosynthetic process (Figure 1A) and then show routes to attain multi-nanoparticle millefeuilles [2] or patterning a BC film with nanoparticles (Figure 1B)

Conclusion
Several approaches to expand the scope, versatility and biomedical uses of bacterial cellulose by simple and scalable processes will be presented.

Acknowledgement

References
Alginate aerogel capsules obtained by prilling in tandem with supercritical drying for wound healing applications

Pasquale Del Gaudio1, Carlos A. García-González2, Renata Adami3, Chiara Amante1, Chiara De Soricellis1, Paola Russo1, Rita P. Aquino1

1 University of Salerno, Department of Pharmacy, Fisciano, IT; 2 University of Santiago de Compostela, Department of Farmacología, Farmacia y Tecnología Farmacéutica, Santiago de Compostela, ES; 3 University of Salerno, Department of Industrial Engineering, Fisciano, IT

Introduction
The incidence of chronic wounds is continuously growing due to the incessant population ageing and its related problems: cardiovascular diseases, diabetes and bedsores. One of the main drawbacks related to the healing of chronic wounds is the lack of an “ideal dressing” able to promote healing process [1]. Ideal wound dressings, in fact, should have a proper adhesion to the wound site, able to fit completely the wound bed, and easy to apply and remove. Moreover, they should provide a good absorbance of exudates without drying excessively the wound; as well as to control the delivery of active pharmaceutical ingredients (APIs). Aerogels in form of in-situ gelling formulations could represent an alternative to conventional dressing thanks to their ability to gel only on the lesion, when in contact with wound fluids, and not on the perilesional skin, thus avoiding a traumatic removal [2]. In this work the possibility to produce polysaccharide-based aerogels in form of beads, with an inner hollow, able to load hydrophobic APIs and to enhance the exudate uptake has been explored using inverse gelation prilling in tandem with supercritical CO2 drying.

Experimental Methods
Manufacturing of core-shell loaded-particles was conducted using alginate solutions at different concentrations (1.50% - 2.25%, (w/v)) to form the shell of the gel beads and an (O/W/O) emulsion was used to form the core. Drug-loaded microparticles were obtained adding ketoprofen lysinate (1% - 25% (w/w)) to the polymer solution. The obtained gel-beads were collected in a 0.3M CaCl2 aqueous or ethanolic solution and maintained under gentle stirring during 5 minutes before being rinsed and collected in water or absolute ethanol, respectively. Previously at the supercritical CO2 drying process, a solvent exchange was carried twice in order to eliminate the possible traces of water. The sc-CO2 drying was carried out at 40ºC and at a CO2 flow rate of 7 g/min during the first hour and at 5 g/min during the rest of the process. Different assays were carried out at 120 bar during 210 minutes and 140 bar during 270 minutes for the production of aerogels capsules.

Results and Discussion
Preparation of O/W/O emulsion was conducted using different concentrations of aqueous CaCl2 solutions to determine the appropriate concentration able to produce the inverse gelation at the nozzle. AI tools [3] were used to optimize CaCl2 concentration (set at 13.4 g/L) and prilling operating variables to produce spherical droplets without nozzle blocking. Homogeneous single droplet core-shell particles with an alginate thin shell were obtained with alginate concentrations between 1.75% and 2.25% (w/v), whereas non-optimized conditions lead to the formation of non-spherical particles inhomogeneous distribution of the oily phase into the shell (figure 1). The supercritical drying of the optimized particles led to aerogels with high porosity (89.9% - 95.3%) specific surface area BET specific surface area (Sb), between 2.50 and 27.5 m2/g and pore volumes ranging between 0.02 and 0.30 cm3/g. SEM images of aerogels showed that sphericity of the beads was retained during the drying process, and they presented an alginate porous layer about 30 µm thick (figure 2). Fluid uptake ability of the aerogel capsules was evaluated using simulated wound fluid. Aerogels capsules were able
to uptake up to 10 folds their weight in SWF. Gelling rate was very fast for all formulations with a total gelling time reduced to 6 seconds for aerogels with thinner alginate layer.

**Conclusion**

In the present work it was developed a robust manufacturing method for the production of core-shell beads by prilling technique in tandem with supercritical drying for the production of aerogels. The obtained optimized gel beads produced by prilling showed a single spherical core encapsulated into an alginate thin shell and had a size > 1 mm. These optimized aerogels capsules showed high surface areas and porosities. Furthermore, aerogels were able to absorb high amount of exudate and easily handled improving in this manner the patient compliance during its direct spreading on a wound. Moreover, the properties of the alginate layer strongly promoted the fast uptake of high amounts of exudate, moving from aerogel to a soft hydrogel, in less than 10 seconds to total gelation. Although those are preliminary studies such results suggest that alginate aerogel capsules could be administered as a self-consistent dosage forms in the treatment of both acute and chronic wounds.

**Figure 1**
Alginate core-shell beads produced by prilling: beads produced with optimized parameters (a) and non-optimized parameters (b-c).

**Figure 2**
Alginate aerogel capsules produced by prilling and supercritical drying: single aerogel particles (a), cracked shell (b) and cross layer porous structure (c).

**References**


Alginate and Hyaluronic Acid Aerogels as Novel Materials for Wound Dressing applications

Tamara Athamneh¹, Drew Lawrence², Pavel Gurikov³, Irina Smirnova²

¹ The Hashemite University, Department of Pharmaceutics and Pharmaceutical Technology, Zarqa, JO; ² Hamburg University of Technology, Institute of Thermal Separation Processes, Hamburg, DE; ³ Hamburg University of Technology, Laboratory for Development and Modelling of Novel Nanoporous Materials, Hamburg, DE

Introduction

The optimal wound dressing material should be able to absorb wound exudates while keeping the wound moist, insulate the wound to keep it at an appropriate biological temperature, allow gases to permeate through the dressing to allow for oxygen exchange and exhibit desirable mechanical characteristics so they are easy to handle but remain stable for extended periods of time [1]. In the case of medicated wound dressings, the dressing material preferred to have a high inner surface area to allow for loading and sustained release of the drug [1]. Thus, aerogels present a suitable option for wound dressing, specifically, bio-based aerogels, because of their high stability, low toxicity, non-allergenic characteristics and good biological performance [2]. In the present study alginate (Alg) and hyaluronic acid (HA) and alginate/hyaluronic acid (Alg/HA) aerogels were produced via drying the chemically or physically cross-linked gels with supercritical carbon dioxide and evaluated as potential wound dressing materials.

Experimental Methods

The physically cross-linked gels were prepared from 2 % solution (HA or Alg) in distilled water. The gelation was triggered by the addition of 6 % D-glucono-d-lactone (GDL), which reduced the polymeric solution pH to 2.5 in 24 hours. The solutions were left overnight to allow gelation and solvent exchange with ethanol was conducted in the next day with sequence of ethanol concentrations: 75 %, 90 %, 100 %. To prepare the chemically cross-linked gel, 2 % Alg or Alg/HA solution were prepared. Calcium carbonate was added to the polymeric solutions to achieve a ratio of 0.1825g CaCO₃ per 1 g Alg. The required mass of GDL was measured and added to achieve 6 % GDL in the final solution. The solution was stirred for 10 seconds before being drawn into a syringe and dispensed into the final containers for gelation. The solvent exchange was conducted as the physically cross-linked gel. Finally, all hydrogel samples were dried using supercritical carbon dioxide at 50°C and 120 bar. The samples were dried for three hours before beginning depressurization, which was performed at a maximum rate of 2 bar/min. Samples were then stored in airtight containers in a desiccator to avoid any effect of moisture in the air.

The prepared aerogel formulations were evaluated for suitability as novel wound dressing materials with high capacity for absorption of wound exudates, by testing density, BET surface area, absorption capacity, and compressive young’s modulus.

Results and Discussion

All the prepared aerogel showed promising results, with superior results for the hybrid aerogel consisting of calcium cross-linked alginate and hyaluronic acid which showed an absorptivity of over 1500 % (Figure 1) and a compressive Young’s modulus of 1.49 MPa. This aerogel also achieved a BET surface area over 400 m²/g and a low density less than 0.1 g/cm³.

Based on the absorptivity test, the calcium-crosslinked aerogels were found to have potential to be used as wound dressing material. The physically cross-linked Alg and HA showed superior absorptivity at the first 30 min, but after that and due to the absence of strong cross-linking mechanism, the aerogel started to dissolve. The ratio of Alg to HA can be manipulated to control the mechanical characteristics depending on the desired application of the aerogel.
Conclusion

Besides the well-known advantages of HA on wound healing, the hybridization of Alg with HA hybrid showed a synergistic effect with a very high absorptivity, high BET surface area, very low density and superior porosity. Making this aerogel an effective candidate for the absorption of wound exudates, isolation of the wound area, and possibly the delivery of antimicrobial or growth agents.

![Figure 1](image)

**Figure 1**

Figure 1. Absorptivity of aerogels using phosphate buffer saline of physically cross-linked alginate aerogel (Alg-Phys), physically crosslinked hyaluronic acid aerogel (HA-Phys), calcium alginate aerogel (Alg-Ca) and calcium alginate-hyaluronic acid aerogel (Alg/HA). (n=3, ± s.d.)

References


Mid-term stability of starch-based aerogels upon storage

Victor Santos-Rosales¹, Gerardo Alvarez-Rivera², Ameya Rege³, Carlos A. García-González¹

¹ Universidade Santiago de Compostela, Pharmacology, Pharmacy and Pharmaceutical Technology, Santiago de Compostela, ES; ² Laboratory of Foodmics, Institute of Food Science, Madrid, ES; ³ Department of Aerogels and Aerogel Composites, Institute of Material Research, German Aerospace Center (DLR), Cologne, DE

Introduction
Starch aerogels are attractive materials for biomedical applications because of their low density and high open porosity coupled with high surface areas. However, the lack of macropores in conventionally manufactured polysaccharide aerogels is a limitation to their use as scaffolds for regenerative medicine [1]. Moreover, the evaluation of the stability under storage of polysaccharide aerogels is critical for biomedical purposes and scarcely studied so far.

In this work, a new macropore population (1-2 μm) well integrated into the starch aerogel backbone was successfully conferred to the aerogel structure by the incorporation of zein. The obtained dual-porous aerogels were evaluated in terms of composition as well as morphological, textural, and mechanical properties. Moreover, stability of aerogels was evaluated at mid-term mimicking the zone II (25ºC, 65 % RH) according to the International Council for Harmonization guidelines. Zein incorporation showed a preventive effect on the morphological changes during the storage period while induced remarkable changes in the mechanical performance [2].

Experimental Methods
Cylindrical aerogel specimens were obtained by a previously reported procedure [1]. A series of starch-aqueous dispersions (10% w/w) with varying ratios of zein as the porogen (Figure 1; Z0-Z4). After the solvent exchange (gel-alcogel transition) and zein leaching, a supercritical drying process (6 g/min, 40ºC, 130 bar) was performed for 4 h. Aerogel probes were collected and characterized in morphological, textural, and mechanical terms. Zein residues were quantitatively determined by high-resolution tandem-mass spectrometry analysis.

Results and Discussion
Starch aerogels with a novel macropore population were obtained with the presented method (Figure 2). Zein residues in the 3-24% range were found in the aerogel products, affecting the entire properties of the aerogel. For instance, aerogels with lower residues (Z1, Z2) were lighter and more porous than the unmodified starch aerogels (Z0), whilst those with higher residues (Z3, Z4) behaved the opposite way. Zein presence strongly influenced the stiffness of the aerogels, although specimens exhibited a good strain memory during the cyclic loading tests.

The exposition to storage conditions induced remarkable morphological and textural changes on the aerogels, but the mechanical behavior remained unaltered. Aerogels with higher zein residues (Z3, Z4) preserved better their features after 3 months of storage.

Conclusion
Highly porous starch aerogels (85-92%) with integrated macropores in the mesoporous starch backbone were obtained, encouraging their use as scaffolds for tissue engineering. Zein incorporation induced remarkable changes in the mechanical performance of the aerogels. The storage period (1 and 3 months) induced morphological modifications, but the presence of zein had a preventive effect on it.

Acknowledgement
This research was funded by Xunta de Galicia [ED431F 2016/010], MCIUN [RTI2018-094131-A-I00], Agencia Estatal de Investigación [AEI] and FEDER funds. V. Santos-Rosales acknowledges to Xunta de Galicia (Consellería de Cultura, Educación e Ordenación Universitaria) for a predoctoral research fellowship [ED481A-2018/014]. C.A.
García-González acknowledges to MINECO for a Ramón y Cajal Fellowship [RYC2014-15239]. Work carried out in the frame of the COST Action CA18125 “Advanced Engineering and Research of aeroGels for Environment and Life Sciences” (AERoGELS) and funded by the European Commission.

Figure 1. Macroscopic view of the manufactured starch-based aerogels with increasing zein contents (from Z0 to Z4).

Figure 2. SEM images of representative starch aerogels modified with zein (larger pores) after (A) performing the sc-drying and (B) 3 months of storage.

References


AERO S01-04

Integrated process of supercritical extraction of hemp seed flour and impregnation on starch xerogel and aerogel

Ivana Z. Lukic¹, Jelena M. Pajnik², Stoja L. Milovanovic¹

¹ University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, RS; ² University of Belgrade, Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, RS

Introduction

Hemp seed flour, a by-product of oil production, is a good source of polyunsaturated essential fatty acids, linoleic (omega-6) and α-linolenic (omega-3), with the balanced ratio between 2:1 and 3:1, which is considered optimal for healthy human nutrition. Beside, flour is rich in other bioactive compounds from the polyphenol group, vitamin E, amino acids and minerals, with well-established health benefits including an anti-allergenic, anti-inflammatory, anti-microbial, and cardioprotective effects which make extract obtained from hemp seed flour suitable for the wide range of possible applications, such as nutraceutical, biomedical and pharmaceutical [1,2]. Prolonged shelf-life, stability and bioavailability of bioactive components could be accomplished by its impregnation on/in a polymeric carrier. Aerogels, a special class of porous materials, appear to be promising candidates for this purpose, having open pore structure and high surface area thus enabling high amount of extract to be loaded [3]. Among them, starch aerogels are biodegradable, biocompatible and obtained from renewable resources in an environmentally friendly manner. Therefore, they can be used as carriers for drug delivery systems. Excellent technique for loading of natural bioactive compounds from plant materials into the polymeric carriers with minimal extract losses is integrated process accomplished by merging the supercritical fluid extraction (SFE) and supercritical solvent impregnation (SSI) into the one process. The aim of this study was to prepare starch gels and functionalize them with hemp seed flour extract using integrated SFE-SSI process.

Experimental Methods

Starch gels were prepared starting from hydrogel formed from an aqueous solution of cornstarch (1:10 w/v) which was stirred for 3 h at 95 °C. After replacement of water with acetone by successive increase of its concentration until 100% was achieved, acetogel was formed and further subjected to drying. Aerogels were prepared by supercritical CO₂ (scCO₂) drying at 45 °C and 10 MPa, using combined static and dynamic drying mode. For comparison, a part of acetogel was dried in the oven at 60 °C to obtain xerogel. Integrated SFE-SSI process was performed on laboratory scale unit (High Pressure Extraction Adsorption (HPEA) 500, Eurotechnica, Germany) at 60 °C and 30 MPa for 4 h. Detailed equipment description is presented elsewhere [4].

Results and Discussion

Starch aerogels prepared by scCO₂ drying at 45 °C and 10 MPa, using combined static and dynamic drying mode, are highly porous material with porosity determined to be 81% and low density of 273 kg/m³. On the other hand, porosity of xerogel was only 10%. Scanning electron microscopy (SEM) analysis also confirmed that air drying was not able to preserve the morphology of wet gels contrary to scCO₂-drying which prevented the collapse of the pores. The amounts of impregnated hemp seed flour extracts using integrated SFE-SSI process were 9.2 and 29.8% for xerogel and aerogel, respectively, showing the great influence of the textural properties of carrier on its loading capacity. The presence of the extract of hemp seed flour on the surface of impregnated samples was confirmed by FTIR analysis. Release study revealed initial faster release of extract into PBS solution from xerogel due to the extract position mainly on material surface, while in the case of aerogel extract was distributed through whole carrier matrix.

Conclusion
Starch gels were successfully impregnated with hemp seed flour extract using the integrated SFE-SSI process at 30 MPa and 60 °C. It was shown that impregnation efficiency is highly dependent on morphology and textural properties of polymeric carrier.

Acknowledgement
This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-9/2021-14/200135 and 451-03-9/2021-14/200287). Work was carried out in the frame of the COST-Action "Advanced Engineering of aeroGels for Environment and Life Sciences" (AERoGELS, Ref. CA18125) funded by the European Commission.

References
11:45 a.m. – 12:15 p.m.

Track08

AERO PS01 | Coffee-Break & Poster session 01

Pre-recorded video presentation (presenting authors do not have to attend this session). Questions could be made in the chat.
Impregnation of starch aerogels with eugenol in supercritical carbon dioxide

Jelena M. Pajnik¹, Ivana Z. Lukic², Stoja L. Milovanovic²

¹ University of Belgrade, Innovation Center of the Faculty of Technology and Metallurgy, Karnegijeva 4, Belgrade, RS; ² University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, Belgrade, RS; ³ University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, Belgrade, RS

Introduction
Despite the fact that aerogels are well known for decades, they are being still considered as emerging ones due to high technological advancement and fast development of new methods for fabrication of versatile aerogels. Supercritical CO₂-assisted drying enables utilization of an environmentally friendly medium for preparation of aerogels with different morphologies by simple variation in operating conditions (e.g. pressure). One of the polymers that was reported for aerogels preparation is starch, a low-cost polysaccharide obtained from renewable resources. As it is biodegradable and highly biocompatible, it can be successfully applied in various fields, including pharmaceutical and biomedical. Beside use of supercritical CO₂ for aerogel preparation, this study was aimed to investigate possibility of supercritical solvent impregnation (SSI), for loading of starch aerogels with eugenol.

Experimental Methods
A starch hydrogel was formed from an aqueous solution (cornstarch to water mass ratio 1:10) at 95 °C. Afterwards, water was gradually replaced with acetone during several days to form an acetogel. Starch acetogels were dried at 45 °C and pressures of 10 and 20 MPa. Scanning Electron Microscopy (SEM) was used to evaluate textural properties of the aerogels. Obtained aerogels were impregnated with eugenol at 35 °C, 20 MPa during 5 and 18 h in a high pressure view cell using green medium, supercritical carbon dioxide. Aerogels before and after the SSI were analyzed by Fourier transform infrared (FTIR) spectroscopy.

Results and Discussion
Porosity of the aerogel dried at 10 MPa was 82%, while increased pressure (20 MPa) led to the formation of less porous structure (48%). The difference in morphology of the starch aerogels was clearly visible on SEM images. Morphological properties of aerogels greatly influenced the SSI (Figure 1). The amounts of loaded eugenol were in the range of 25.3 - 31% and 2.8 – 7%, for aerogels dried at 10 and 20 MPa, respectively. FTIR analyses confirmed presence of eugenol on the surface of the impregnated starch aerogels. Desorption tests of eugenol from the impregnated aerogels in PBS showed controlled release profile.

Conclusion
Proposed SSI technique was found effective in impregnation of starch aerogels with eugenol. Given that eugenol has proven antioxidant, anti-inflammatory, and antimicrobial activity, obtained results indicate various possible application of eugenol loaded starch aerogels.

Acknowledgement
Acknowledgements: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-9/2021-14/200287 and 451-03-9/2021-14/200135). Work was carried out in the frame of the COST-Action "Advanced Engineering of aeroGels for Environment and Life Sciences" (AERoGELS, Ref. CA18125) funded by the European Commission.
SSI of starch aerogels with eugenol at 35 °C and 200 bar
Sol-gel Derived Carbon Microspheres by Continuous Ultrasonic Spray Pyrolysis

Anna Liisa Peikolainen¹, Mai Uibu², Alvo Aabloo¹

¹ University of Tartu, Institute of Technology, Tartu, EE; ² Tallinn University of Technology, Department of Materials and Environmental Technology, Tallinn, EE

Introduction
Sol-gel derived materials are unique type of porous materials because their structure and porosity can be tailored according to its application by the choice of precursors and synthetic conditions. In addition to synthesis of monolithic materials, preparation of sol-gel materials in the powder form via bottom-up routes have been introduced, such as inverse emulsion polymerization [1], electrospraying [2], inkjet printing of microspheres [3]. Here, the fabrication of sol-gel derived carbon microspheres via ultrasonic spray pyrolysis is reported. Ultrasonic spray pyrolysis has been used for fabrication of spherical carbon particles from organic salts. [4] Synthesis of carbon from a colloidal solution via ultrasonic spray pyrolysis, enables to reduce the number of steps in the fabrication process compared to abovementioned methods while enabling continuous synthesis of carbon particles.

Experimental Methods
Aqueous 5-methylresorcinol-formaldehyde solution with Na₂CO₃ as a catalyst with predetermined concentrations was prepared and stirred vigorously at 25°C for 24 h. The aerosol droplets of colloidal solution were generated with ultrasonic nebulizer and transported through a tubular furnace (MTF 12/38/400, Carbolite) at temperature 900°C by a flow of N₂ (0.025 l/min). The carbon particles formed were trapped into water reservoir and then collected by centrifugation.

The particles obtained were investigated using scanning electron microscopy (SEM) (TM-3000, Hitachi). The elemental composition was analysed using energy-dispersive X-ray spectroscopy (EDX) (a Hitachi TM-3000 SEM that was coupled with a SwiftED 3000 EDX analyser, Oxford Instruments). The porosity of the carbon microspheres was determined using N₂ sorption analysis (KELVIN 1042 Sorptometer, Costech Microanalytical SC).

Results and Discussion
Sol-gel derived carbon microspheres obtained were with the maximum particle diameter of 3 µm (Figure 1). The spheres consisted of 95.5 atomic% of carbon which is a high level of carbonization during rapid pyrolysis in flow. Specific surface area calculated based on Brunauer-Emmet-Teller theory was 12 m²/g, and a total pore volume the material reached 137 mm³/g.

Conclusion
The results of elemental analysis, the specific surface area of 12 m²/g and total pore volume of 137 mm³/g suggest that ultrasonic spray pyrolysis enables to produce fine carbon powder for applications where moderate specific surface area is required.

Acknowledgement
This work was supported by Estonian Research Council grants (PUT1696, PRG1084). This project has received funding from European Union’s Horizon 2020 research and innovation programme under grant agreement 857263.
Figure 1 SEM image of sol-gel derived carbon microspheres
Carbon microspheres obtained from 5-methylresorcinol-formaldehyde sol via ultrasonic spray pyrolysis at 900°C

References
**C.elegans** as animal model for cardiac arrhythmia - the case of Polypyrrole Nanoparticles

**Sumithra Y. Srinivasan**¹, Nuria B. Cases², Dmytro Kukhtar³, Pilar A. Illera⁴, Julián C. Madrigal³, Mayte M. Zoccola⁴, Rosalba Inés F. García⁴, Anna Laromaine¹

¹ Universitat de Autonoma de Barcelona, Institut de Ciència de Materials de Barcelona (ICMAB), Bellaterra, ES; ² ALBA Synchrotron, Cerdanyola del Vallès, ES; ³ Hospital Duran i Reynals, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Barcelona, ES; ⁴ Universidad de Valladolid, Instituto de Biología y Genética Molecular (IBGM), Valladolid, ES

**Introduction**

Cardiac arrhythmia (CA) is characterized by irregular beating of the heart cells, caused by improper propagation of cardiac impulse among cardiac cells, prevalent in 90% of CVD patients [1]. Conducting polypyrrole nanoparticles (Ppy NPs) attempts to regulate the electrical impulse propagation across cardiac cells [2]. In our present work, we aim to study the potential of Ppy nanoparticles in synchronizing irregular heart rhythm. In order to evaluate it, we use Caenorhabditis elegans as a small animal model. *C. elegans* are worms with 60% genetic homology to humans, optically transparent, exhibiting short life span and fast reproduction cycle. Notably, the pharynx of *C. elegans* is a continuously pumping organ, the molecular mechanism of which is similar to humans´ cardiac pumping [3]. We are exploring *c.elegans*´ pharynx as a model system for cardiac arrhythmia, to test the potential of Ppy in regulating irregular pumping. Some studies have shown that Ppy has the ability to enhance transient Ca$^{2+}$ signal conduction across voltage gated calcium channels [2].

**Experimental Methods**

Ppy NPs were synthesized reproducibly using chemical oxidative polymerisation method [4]. Physicochemical characterizations, size and morphology and electrical conductivity were measured for the NPs. Ppy NPs were orally administered to *C. elegans* and systemic toxicity and pharynx pumping measurements were carried out. Racepinephrine and propranolol were used as positive and negative control CA drugs, respectively. We employed nanopore sequencing to elucidate the genes that are affected upon Ppy exposure. Further, calcium measurements and electrophysiological measurements from the pharynx are under progress.

**Results and Discussion**

Ppy NPs were found to be spherical with ≈100 nm diameter and a conductivity of ≈0.8 S/cm. We found that Ppy NPs do not show any adverse toxic effects through initial toxicity studies survival %, body length and reproduction. Ppy NPs causes an increase in pharynx pumping rate in *C. elegans*, similar to the positive control racepinephrine. Whereas, propranolol shows a decrease in the pumping rate. We further studied effect of the controls and Ppy NPs on pumping rate through different parameters such as duration time and pumping rate after excretion. Genetic studies to understand which genes are affected upon Ppy exposure, and how those genes can play a role in CA are currently under progress. Taking forward, we are also planning to evaluate cardiac contraction in the cells, we aim to study the transient Ca$^{2+}$ conduction across the voltage-gated calcium channels to understand the exact mechanism of Ppy in changing the pumping rate through calcium imaging in cells as well as *C. elegans*.

**Conclusion**

Application of Ppy in cardiovascular treatments is still at its beginning stage and the molecular mechanism of conducting materials in regulating cardiac synchronization is poorly studied. We believe that our initial screening, as well as genetic and molecular studies with an *in-vivo* small animal model *C. elegans* can greatly accelerate the progress of this material for cardiac therapies.
Acknowledgement
We thank DOC-FAM, European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 754397 for the financial support, funding from Spanish Ministry of Science and Innovation through the RTI2018-096273-B-I00 project, and the ‘Severo Ochoa’ Programme for Centres of Excellence in R&D (CEX2019-000917-S). The Generalitat de Catalunya project, 2017SGR765, is also acknowledged. The authors also express their gratitude to the technical services of ICMAB (Electron microscopy, Soft materials and Spectroscopic techniques laboratory). The authors participate in the CSIC Interdisciplinary Platform for Sustainable Plastics towards a Circular Economy, SUSPLAST and in the Aerogels COST ACTION (CA18125). This work has been performed within the framework of the doctoral program in materials science of UAB.

C. elegans as animal model for cardiac arrhythmia - the case of polypyrrole nanoparticles
A schematic representation showing similarities between human heart and C. elegans’ pharynx, and the exposure system of C. elegans and Ppy NPs.

References
AERO PS01-04

Bacterial Nanocellulose-Polypyrrole (BC-Ppy) composites as scaffolds for cardiac resynchronization

Sumithra Y. Srinivasan, Osnat Zapata, Mariano Campoy, Anna Laromaine

Universitat de Autonoma de Barcelona, Institut de Ciència de Materials de Barcelona, Barcelona, ES

Introduction
Myocardial Infarction (MI) is a major cause of heart failures. The ischemic muscle death impairs electrical impulse propagation, causing abnormalities in heart rhythm, resulting in cardiac arrhythmia and conduction disorders [1]. Biomaterial scaffolds for cardiac tissue engineering is therefore an active subject of interest. Conducting polymers are able to communicate cardiac impulse across the tissue, facilitating synchronization. Especially, Ppy is the most popular candidate among CPs for tissue engineering applications owing to its easy synthesis, aqueous stability and biocompatibility [2]. In order to improve the structural properties, we have designed a nanocellulose-py composite scaffold. Cellulose is the most abundant bio-polymer with excellent biocompatibility. Bacterial nanocellulose (BNC) displays high purity compared to plant derived cellulose. The fibrous structure of BNC mimics the ECM environment and BNC films are flexible with high mechanical strength to withstand chronic shear stress, making them ideal as cardiac scaffolds [3]. Herein, we have designed and will be investigating cardiac cell attachment and contractions on BNC-Ppy scaffolds.

Experimental Methods
BNC were produced from the bacterial strain Komagataeibacter xylinus (K. xylinus) [4] and BC-Ppy via in-situ oxidative polymerization. The scaffolds were characterized by FT-IR, SEM, TEM, TGA and 4-probe keithly instrument to study their size, structure, morphology and conductive properties.

Results and Discussion
BNC-Ppy scaffolds were successfully synthesized and characterized, wherein the fiber diameter was found to be ≈ 70 nm and that of Ppy NPs were found to be ≈ 85 nm. The conductivity of BC-Ppy films were found to be proportional to the concentration of initial monomer added, and was ≈ 2 S/cm. In-vitro cell culture experiments with BNC-Ppy scaffolds are at the initial stage studying the cell attachment and morphology of cells seeded on scaffolds. Following this, we plan to evaluate the contraction of cells seeded with and without scaffolds and in the presence and absence of external stimulation.

Conclusion
Only a handful studies have shown the efficacy of composites with Ppy as cardiac tissue engineering scaffolds and BNC has not been explored as an effective composite material for this purpose till date. Considering the potential and properties of these materials, BC-Ppy is a promising cardiac tissue engineering scaffold material. To the best of our knowledge, this will be a first of its kind study the use of BNC-Ppy as a scaffold for cardiac cells.

Acknowledgement
We thank DOC-FAM, European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 754397 for the financial support, funding from Spanish Ministry of Science and Innovation through the RTI2018-096273-B-I00 project, and the ‘Severo Ochoa’ Programme for Centres of Excellence in R&D (CEX2019-000917-S). The Generalitat de Catalunya project, 2017SGR765, is also acknowledged. The authors also express their gratitude to the technical services of ICMAB (Electron microscopy, Soft materials and Spectroscopic techniques laboratory). The authors participate in the CSIC Interdisciplinary Platform for Sustainable Plastics towards a Circular Economy, SUSPLAST and in the Aerogels COST ACTION (CA 18125). This work has been performed within the framework of the doctoral program in materials science of UAB.
Bacterial Nanocellulose-Polypyrrole (BC-Ppy) composites as scaffolds for cardiac resynchronization

Applicability of BC-Ppy composites as scaffold materials for cardiac tissue engineering.

References
Development of lignin-based aerogels

Mihkel Koel, Piia Jõul, Tiit Lukk

Tallinn University of Technology, Department of Chemistry and Biotechnology, Tallinn, EE

Introduction
The conversion of lignin to higher-value products has been a topic of interest for many years. The idea of substituting phenol in phenol-formaldehyde (PF) resins and resorcinol in resorcinol-formaldehyde (RF) aerogels leads to new, bio-based lignin materials. The low reactivity and heterogeneity of lignin are challenges that can be overcome by lignin modification or cleavage.

Lignin that is cleaved from the lignocellulosic materials and dissolved by organic solvents is known as organosolv lignin. Usually, organosolv lignin is closer in structure to natural lignin, compared to other technical lignins. Lignin can be separated from lignocellulosic biomass with molten salts (ionic liquids and deep eutectic solvents). This kind of treatment is increasing the reactivity of lignin.

The preparation of aerogels from lignin is derived from its phenolic character of chemical structure; it is possible to substitute resorcinol with lignin and use the polycondensation of lignin and formaldehyde for aerogel production. Resulting aerogel is versatile material for value-added applications such as insulation and adsorber materials and raw material for carbon aerogels which can be used as super-absorber or materials for supercapacitors.

Experimental Methods
In current study, organosolv lignin extracted from bleached chemi-thermomechanical aspen pulp (BCTMP) using ethanol and hydrochloric acid was utilized as one of the precursors for the preparation of hydrogels with different lignin/5-methylresorcinol (5-MR) weight ratios to obtain homogeneous lignin-5-MR-formaldehyde (F) hydrogel. After the solvent exchange step, the resulting gel was atmospherically, supercritically or freeze-dried.

Results and Discussion
Chemical and physical properties of obtained xerogels, aerogels and cryogels were evaluated to determine the proper lignin/5-MR weight ratio and the maximum percent of the 5-MR, which could be replaced with BCTMP organosolv lignin.

Conclusion
The heterogeneous and complicated phenolic-rich structure of lignin what differs among lignins of different origin results development lignin-based materials complicated. Chemical extraction / treatment of lignin with molten salts is challenging way to get homogenous and uniform porous aerogels.

Acknowledgement
This study was carried out in the framework of the COST Action “Advanced Engineering and Research on aeroGels for Environment and Life Sciences” (AERoGELS, ref. CA18125) funded by the European Commission; and was supported by ERDF and Estonian Research Council via project RESTA11.
12:15 p.m. – 1:30 p.m.

Track08

AERO S02 | 3D-scaffolds-based aerogels for tissue regeneration

Chairs
Silvia J. Bidarra
i3S, Bioengineered 3D Microenvironments, Porto, PT
Ana Oliveira
Catholic University, PT
AERO S02-KL02

Fabrication of bi-functional drug-laden 3D printed aerogel-based scaffold from antibacterial and biocompatible RGD-AMP modified silk fibroin-silica hybrid ink

Yan Demidov, Hajar H. Maleki

University of Cologne, Institute of Inorganic Chemistry, Köln, DE

Introduction

Scaffold-mediated tissue engineering has become a golden solution for the regeneration of damaged bone tissues that lack self-regeneration capability. A successful scaffold in bone tissue engineering comprised of a multitude of suitable biological, microarchitectural, and mechanical properties that are acting as different signaling cues for the cells to mediate the new tissue formation. Therefore, careful designing of bioactive scaffold macro and microstructures in multiple length scales and biophysical properties fulfilling the tissue repair demands are highly required yet very challenging to achieve.

Experimental Methods

In our study, we have synthesized an antibacterial and biocompatible gel-based ink and processed it as three-dimensional (3D) hybrid aerogel based scaffolds with exploiting from the novel yet simple chemical approaches of sol-gel processing and self-assembly approaches with a controlled microstructural design e.g. micro-extrusion based 3D printing and unidirectional freeze casting approaches [2]. As the main constituent of our biocompatible scaffold of this study, we used the silk fibroin (SF) extracted from B. mori cocoon [3]. However, to increase the cell responsivity and bactericidal efficiency, thiol-ended antibacterial, and cell adhesive peptide sequences (SH-AMP-RGD) have been conjugated to the SF polymer through a covalent attachment using a spacer molecule (Fig. 1). This was followed by processing the AMP-RGD-SF as a 3D printable hydrogel. In the next step, the hybrid hydrogel was successfully assessed in terms of printability and viscosity parameters and 3D printed into the construct with interconnected porous structure with hierarchically organized porosity and a combination of several useful properties.

Results and Discussion

Thanks to the covalent linkage of the antibacterial peptide to the SF, the scaffold indicates potent bactericidal efficiency toward gram-positive and negative bacteria. Besides, for the sake of osteoconductivity and osteogenicity, and mechanical strength, we loaded the hydrogel with inorganic components such as 3D silica nanostructures so that SF and silica could intertwine together through parallel sol-gel and self-assembly processing to support the mechanical structure in the final scaffold.

Conclusion

Finally, this study supports the promise of 3D printed anti-infective silica-SF-based hybrid aerogel constructs for repairing the bone defect.
AMP-RGD modified silk fibroin silica aerogel based scaffold designed by a synergistic combination of sol-gel reaction, self-assembly, and 3D printing approaches

References
Textural and biological evaluation of 3D-printed alginate-hydroxyapatite aerogel scaffolds

Ana Iglesias-Mejuto, Carlos A. García-González

Universidade de Santiago de Compostela, Department of Pharmacology, Pharmacy and Pharmaceutical Technology, I + D Farma Group (GI-1645), Faculty of Pharmacy, and Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, ES

Introduction

3D-printing technology allows the automated and reproducible fabrication of functional, scalable and customized 3D-structures for personalized medicine with high-precision (Boga et al., 2018). Alginate is a natural polysaccharide commonly employed as bioink in 3D-printing due to its biocompatibility, biodegradability, non-immunogenicity and low cost. However, alginate based structures usually lack bioactivity and should be combined with other admixtures for bone tissue engineering. Hydroxyapatite (HA) is an excellent bone substitute because it is bioactive and osteoconductive (Luo et al., 2018). Gel scaffolds prepared with well-defined internal structure and dual-interconnected porosity is essential for tissue engineering and feasible by 3D-printing (Boga et al., 2018). An adequate extraction method of the solvent of a gel is the supercritical CO$_2$-assisted drying as long as it preserves the internal gel structure in terms of its porosity and specific surface area (García-González et al., 2015). In this work, 3D-printed alginate aerogel scaffolds were obtained by the novel combination of 3D-printing and supercritical CO$_2$ drying and evaluated regarding their textural and biological properties aimed to personalized bone regenerative medicine applications.

Experimental Methods

Scaffolds of dimensions 20x20x1 mm were printed from different alginate- HA solutions with a grid pattern and 3 layers. After the printing process, all scaffolds were put directly in contact with CaCl$_2$ aqueous solutions for gelation. The 3D-aerogels were obtained after solvent exchange to ethanol and supercritical drying (120 bar, 40 °C, 3h). The effect of alginate (6-10 wt.%), CaCl$_2$ (0.1-1M) and HA (8-24 wt.%) concentration was evaluated on the textural parameters of the final scaffold (specific surface area, total porosity, mean pore diameter) by BET and SEM analyses. Biological properties (cell viability and adhesion) were assessed in BALB cells after 24 and 48h and the bioactivity tests were performed in simulated body fluid (SBF) at different time periods.

Results and Discussion

Homogeneous 3D-structures formed by filaments arranged in layers with alginate concentrations in the 6-10 wt.% range and HA concentration in the 8-24 wt.% range were successfully obtained after crosslinking with CaCl$_2$ concentrations in the 0.1-1 M range (Fig. 1). The highest BET surface area (438 m$^2$/g), pore size (22 nm) and total pore volume (3.14 cm$^3$/g) were found in aerogels obtained with an alginate concentration of 6 wt.%. Nevertheless, the BET analysis of alginate/HA aerogel scaffolds has shown an important decrease in the surface area and total pore volume while the pore diameter is almost maintained with respect to scaffolds fabricated only with alginate. Overall, the 3D-structure and porosity of all structures were maintained after all processing steps, with mesopores and macropores clearly recognized in all aerogel types (Fig. 1). Furthermore, excellent results on BALB cells viability were found for all aerogel formulations, without observing statistical significance (t-test, p >0.05) with respect to the positive controls, thus confirming the lack of toxicity or other negative effect on the normal cell environment. Finally, cell attachment (Fig. 2) and spreading in terms of nuclear staining was revealed with a proliferation tendency observed in all aerogels formulations, since more nuclei were stained after culturing scaffolds for 13 days than after
6 days. This feature indicated that scaffolds were suitable for tissue growth and able to promote cell adhesion (Fig. 2) and proliferation, as well as the cell colonization of the aerogel scaffold.

**Conclusion**

Macroporous alginate-HA aerogel scaffolds printed in a precise and customized way were obtained by the technological combination of 3D printing and supercritical drying method. Alginate, HA and CaCl₂ concentrations determined the scaffold end nanostructure. The cell viability tests revealed no toxicity effect or negative impact caused by the thus manufactured structures while adhesion assays show cell attachment and spreading with a proliferation tendency throughout the days.

**Acknowledgement**

Work supported by Xunta de Galicia [ED431C 2020/17], MCIUN [RTI2018-094131-A-I00], Agencia Estatal de Investigación [AEI] and FEDER funds. A.I.-M. acknowledges to Xunta de Galicia for her predoctoral research fellowship [ED481A-2020/104].
Fig. 2: Confocal microscopy image of stained MSCs seeded on aerogel scaffold and cultured for 6 days.
Scaffold composition: Alginate 6 wt%, CaCl₂ 1 M.

References
Ethyl cellulose composites with polylactic acid in tissue engineering

Gabrijela Horvat, Klara Žvab, Zeljko Knez, Zoran Novak

University of Maribor, Faculty of Chemistry and Chemical Engineering, Maribor, SI

Introduction
Aerogels and foams are promising materials for bone implants since they could be used as scaffolds for mimicking the natural tissue. Those materials show interconnectivity of pores and are hence interesting materials for cell growth. Additionally, their porous structure and high surface areas enable the incorporation of active agents such as anti-inflammatory agents and antibiotics [1,2,3]. Mechanical properties of polysaccharide aerogels are however poor, leading to a development of new, polymer based composites with polylactic acid (PLA). From a mechanical engineering point of view, moduli of elasticity of implant material and bone materials should be as close as possible (e.g. modulus of elasticity for bone material is around 18 GPa). [4]. Materials, prepared from PLA can reach modulus of elasticity up to 14 GPa, which is very similar to bone. PLA degradation rate in a biological environment is slow, and the combination of polysaccharide aerogels with PLA may result in final biodegradable material with desired mechanical properties and suitable degradation rate for new bone formation.

Experimental Methods
Ethyl cellulose (EC) and polylactic acid (PLA) composites were prepared by dissolving raw materials. Different concentrations of both solutions were prepared and then combined in various ratios in order to obtain composites of various compositions regarding EC and PLA content. Composites were dried by supercritical CO_2 and final materials were characterised by nitrogen adsorption analysis, SEM, DSC-TGA analysis, DMA and swelling. Headspace GC method was used in order to determine the residuals of organic solvents in final materials.

Results and Discussion
Prepared aerogels showed good mechanical properties, however, their surface area was rather low. The surface area decreased by increasing the PLA content, leaving material polymer-like. Supercritical drying of materials showed to be a good method for eliminating the organic solvents from composites as there were no residuals above the toxic level.

Conclusion
The preparation of EC and PLA composites is a promising method for the formulation of bone scaffold materials. Various combinations of EC and PLA gives materials with various properties, however it is noteworthy that the addition of PLA drastically increases the mechanical strenght and elastic modulus of prepared scaffolds. EC-PLA composites are completely biodegradable and biocompatible and there is no residuals of organic solvents.

References
AERO S02-03

In vitro and in vivo biocompatibility and biodistribution screening of aerogel products

Gabor N. Szeman¹, Jozsef Kalmar²

¹ University of Debrecen, Department of Molecular Biotechnology and Microbiology, Debrecen, HU; ² University of Debrecen, Department of Inorganic and Analytical Chemistry, Debrecen, HU

Introduction

Development of aerogel products raises the need for fast and effective evaluation of their biointeractions from the cellular level up to the ecological level. Here we propose a methodological pipeline for fast, yet effective screening of broad-range biological interactions. The experimental setup was optimized for the usage of limited quantities of aerogel samples gained from the early stages of experimental production. Parallel data can be obtained from the very same sample, regarding effects on cellular viability, cell-cycle, cellular surface interactions and genotoxicity. Furthermore, quantitative data can be acquired about ecological interactions using just milligrams of aerogel. In vitro data then will be used to set the in vivo tests on mice for biocompatibility/toxicity and biodistribution. Migration of aerogel particles on lymphatic pathways can be characterized in the same set of experiments reducing the number of animals. The proposed workflow of combined methods can reduce the required efforts for testing in the terms of labour and resources.

Experimental Methods

96-well microtiter plates were used for observing the effects of increasing concentrations of aerogel microparticles (10-100μm) on cultured human keratocyte HaCat cell line and human colorectal carcinoma CaCO2 cell line. Cellular responses were monitored via long-term (one week), non-invasive near-infrared (940nm) time-lapse microscopy inside a CO2 incubator under standard cell breeding conditions. Acquired image sequences were analyzed via customized algorithms of NIH ImageJ software. [1-3] Dynamic cellular parameters like size, motility, uniformity, cell-cycle, divisions were quantified on the single cell and population level against time. The same microtiter plates used for imaging were used for MTT cellular viability tests for determination of average living cell number. In the animal experiments 10 (5M/5F) C3H/HeJ mice were injected intraperitoneally with aerogel microparticles suspended in phosphate-buffered saline solution. After one and two weeks the animals were sacrificed and subjected to autopsy for macroscopic investigation and organ harvesting for histopathological examination. Liver, spleen, kidneys were fixed and embedded in paraffin blocks for hematoxylin-eosin staining and immunohistochemistry. Thymus and parathyrmhical lymphatic nodes were also collected for the evaluation of the aerogel microparticles distribution on lymphatic pathways. [4] Ecological and environmental interactions were investigated using custom-built hardware for time-lapse macroscopic image acquisition of 10 parallel capillary (100μl natural water) containing Paramecium Caudatum model organisms in the presence of Hg-specific aerogel absorbent. [5]

Results and Discussion

Silica-based, silica-gelatin hybrid and silica-casein hybrid aerogels did not cause cytotoxicity in a wide concentration range, although the physical presence of the particles interfered with the cellular migration on the breeding substrate surface. [1-3] Smaller particles about 10 μm are often collected by the cells due to the negative charges of the aerogel, similar to the breeding surface. The size discrimination of the cellular surface attached particles can be used to estimate the strength of cell-aerogel binding. Also, in the case of gelatin-containing aerogel, significant positive chemotaxis, chemotraction were observed while cells migrated toward large chunks of aerogel. [1, 2] Analysis of dynamic functional-morphology parameters of the cells suggest that these aerogels elongated the cell-cycle due to their mechanical presence. The MTT viability test results exhibited a good endpoint correlation with these results.
The animal experiments outlined the non-toxic nature of hybrid aerogel microparticles at low concentration (52 mg per kilogram body weight), while high dose (104 mg kg⁻¹) induced low to moderate hepatotoxicity. Fluorescently labelled silica-gelatin aerogel particles were found in the parathymical nodes, suggesting the spread of these particles in lymphatic pathways. The immunohistochemical staining of CD68 and CD163 demonstrated the activation of monocytes / macrophages by aerogel microparticles. In the ecological tests, Hg(II)-binding aerogels (0.1 mg/ml) protected the _Paramecium Caudatum_ up to 500 ppb Hg(II) concentration. [5]

**Conclusion**

Data obtained from image sequences, viability test and genotoxicity test justified the sensitivity of the experimental methods in the evaluation of cell-aerogel interactions. _Paramecium_-based ecological tests indicated the non-toxic nature of aerogels under natural conditions and also exhibited their potential in mercury-detoxification.

**Acknowledgement**

The research has been financially supported by the National Research, Development and Innovation Office, Hungarian Science Foundation (OTKA: FK_17-124571). J.Kalmár is grateful for the János Bolyai Research Scholarship of the Hungarian Academy of Sciences and for the New National Excellence Program (ÚNKP-20-5 Bolyai+).

![Biocompatible silica-gelatin hybrid aerogels covalently labeled with fluorescein](image)

**Biocompatible silica-gelatin hybrid aerogels covalently labeled with fluorescein**

**References**


1:30 p.m. – 2:30 p.m.

Track08

YSF-AERoGELS WS | YSF Educational workshop – “Writing and Publishing”
YSF & “AERoGELS” Joint COST action event

Chairs
Carlos A. García-González
University of Santiago de Compostela, Pharmacology, Pharmacy and Pharmaceutical Technology Dept., Santiago de Compostela, ES
Nicola Contessi Negrini
Imperial College London, London, GB

Co-Chair
Joana Barros
INEB/i3S, Porto, PT

This workshop aims at sharing tips and tricks on “Effective graphical abstract and covers” by Maria Southall (Executive Editor Biomaterials Science, Royal Society of Chemistry); Uta Goebel will, then, give her advices on how to proceed “From data selection to the best graphical representation” (Editor-in-Chief Advanced Healthcare Materials, Wiley). Christine Horejs will finally provide highly useful guidelines on “Assembling figure panels” by (Chief Editor Nature Reviews Materials, Nature). This session will end with an open discussion, with Q&A to the invited speakers.
YSF-AERoGELS WS-01

Welcome

Carlos A. García-González, Nicola Contessi

University of Santiago de Compostela, Pharmacology, Pharmacy and Pharmaceutical Technology Dept., Santiago de Compostela, ES

YSF-AERoGELS WS-02

Effective graphical abstract and cover

Maria Southall

Royal Society of Chemistry, Publishing, Cambridge, GB

YSF-AERoGELS WS-03

From data selection to the best graphical representation

Uta Goebel

Wiley, Hoboken, US

YSF-AERoGELS WS-04

Assembling figure panels

Christine Horejs

Nature, London, GB

YSF-AERoGELS WS-05

Open discussion

Q&A to Speakers
2:30 p.m. – 4:00 p.m.

Track08

**AERO InvF | Early-career investigators Forum**

**Chairs**

**Tamara K. Athamneh**  
The Hashemite University, Department of Pharmaceutics and Pharmaceutical Technology, Zarqa, JO

**Joana Barros**  
INEB/i3S, Porto, PT
AERO InvF-01

Structure and properties of silk fibroin based aerogel particles prepared by emulsion-gelation followed by supercritical CO2 for wound healing

Beatriz G. Bernardes¹, Clara López-Iglesias², Raquel Costa³⁴⁵, Carlos A. García-Gonzaléz², Ana L. Oliveira¹

¹ Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, PT; ² Universidade de Santiago de Compostela, Department of Pharmacology, Pharmacy and Pharmaceutical Technology, I+D Farma group (GI-1645) and Health Research Institute of Santiago de Compostela (IDIS), E-15782 Santiago de Compostela, ES; ³ Universidade do Porto (i3S), Instituto de Investigação e Inovação em Saúde, Porto, PT; ⁴ Universidade do Porto, Department of Biomedicine, Biochemistry Unit, Faculdade de Medicina, Porto, PT; ⁵ Instituto Politécnico do Porto, Escola Superior de Saúde, Porto, PT

Introduction

Chronic wounds are one of the major therapeutic and healthcare challenges. The wound healing process is divided in three important stages (haemostasis, inflammation and proliferation/maturation) that deploy a series of biochemical reactions which induce the repair of the injury. During wound healing, a fluid (exudate) can be produced as a natural response towards healing. However, its excessive production can be detrimental, once it is an excellent culture medium for bacterial growth, delaying the inflammatory phase. Nowadays, the design and development of biocompatible, biodegradable and adaptable materials that promote the tissue repair, prevent the infection and inflammation and ensure the management of exudate are a constant need for wound management.

Aerogels are nanostructured dry materials with high porosity, large surface and low bulk density [1]. They can provide advanced performance for wound healing due to their high porosity and large surface area, which can be tailored for a fast and directional fluid transfer of the exudate. They can also act as carriers for bioactive compounds and can present an adequate biological performance to provide the required wound physiological environment. Silk fibroin (SF) protein, obtained from Bombyx Mori, is an excellent carrier of bioactive compounds while supporting cell proliferation, being presently used in wound healing and regeneration. In this work, we propose the use supercritical CO₂ technology to develop SF aerogel particles as a controlled release system of adenosine, a protein that is herein proposed for the first time being expected to trigger the healing process of chronic wounds, promoting angiogenesis, reducing inflammation and accelerating wound closure (Figure 1).

Experimental Methods

Silk fibroin extracted from Bombyx mori cocoons was used to prepare SF aerogel particles by emulsion-gelation from SF aqueous solutions at different concentrations (3% and 5% (w/v)) as aqueous phase in water-in-oil emulsions followed by supercritical CO₂ drying (Figure 2A). The oil phase was prepared by using paraffin oil with different percentages of Span 80 surfactant. For the characterization of the SF particles, Fourier Transform Infrared with Attenuated Total Reflectance (FTIR-ATR) spectroscopy were used to investigate the secondary structure formation and conformation. The chemical structure and crystallinity of the produced matrices were analysed by FTIR-ATR and X-Ray Diffraction (XRD). Textural properties were assessed by helium pycnometry and N₂ adsorption-desorption (BET) tests. The drug loading capacity and release of adenosine in SF aerogel will be evaluated. In vitro tests will be performed using human skin cells to access the cell viability and therapeutic effects of the developed systems.

Results and Discussion
SF gel particles were produced with an emulsion-gelation method using different concentrations of SF, stirring velocity (600 and 1100 rpm) and Span 80. Namely, the SF particles with a stirring velocity of 600 rpm were more spherical and homogeneous (Figure 2B). The diameter of the particles (Dv10 and Dv90) was 509 ± 3 µm and 1073 ± 91 µm for 5%SF (w/v) aerogel particles and 368 ± 2 µm and 1075 ± 22 µm for 3%SF (w/v) aerogel particles, considering the average size of all the methodologies adopted.

The obtained aerogels were characterized in terms of textural and chemical properties. Analysis of the surface area of 5%SF (w/v) and 3%SF (w/v) by BET. All aerogels had high surface area being the 5%SF (w/v) solution higher than 3%SF (w/v) solution at the same conditions, 3%Span80 and 600rpm (515 ± 26 m²/g and 458 ± 23 m²/g, respectively). The surface area was lower at 5%SF and 3%SF with 3%Span80 and 1100rpm (147 ± 7 m²/g and 177 ± 9 m²/g, respectively. The surface area of 5%SF (w/v) particles with 10%Span and 600 rpm was 160 ± 8 m²/g. According to ATR-FTIR analysis, it was possible to verify the presence of the main characteristic bands of SF assigned to the presence of β-sheet structure, characterized by strong bands on amide I and II regions (Figure 2C). Also, it was detected the presence of Span80. The non-complete removal of Span80 may explain why the surface area of the SF aerogels with 10%Span is lower. According to XRD analysis, it is possible to verify the existence of a broad peak referring to SF (Figure 2D).

**Conclusion**

Physicochemical and textural characterization of the SF aerogels showed excellent properties and low particle size deviation, suggesting that the method is suitable for the production of particles for wound healing applications. In the future, we intend to load these particles with pharmaceutical drugs relevant for wound healing applications.

**Acknowledgement**

This research was funded by Xunta de Galicia [ED431C 2020/17], MCIUN [RTI2018-094131-A-I00], Agencia Estatal de Investigación [AEI] and FEDER funds. B.B. acknowledges to the COST Action CA18125 “Advanced Engineering and Research of aeroGels for Environment and Life Sciences” (AERoGELS), funded by the European Commission, for the granted Short Term Scientific Mission to perform the aerogels synthesis in the Universidade de Santiago de Compostela. This work was also supported by National Funds from Fundação para a Ciência e a Tecnologia (FCT), through project UID/Multi/50016/2019.
Figure 2
A) Methods used for the development of the SF aerogel particles; B) Influence of stirring velocity on SF gel particles (3%SF and 5%SF with 3% Span80); C) FTIR-ATR analysis; D) XRD analysis: I. 5%SF 600rpm 10% Span80 + ADO; II. 3%SF 600rpm 10% Span80 + ADO; III. 3%SF 600rpm 3% Span80.

References
Gold nanoparticles as a part of a photothermal therapy system

Tânia Ferreira-Gonçalves, Eduardo Costa, João M.P. Coelho, Maria Manuela Gaspar, Lia Ascensão, Pedro Faisca, Isabel V. Figueiredo, Carlos A. García-González, David Ferreira, Hugo Ferreira, Catarina P. Reis

Introduction
Photothermal therapy (PTT) is attracting increased attention for the treatment of superficial localized tumors, relying on the induction of local hyperthermia of tumor cells upon their irradiation with light beams. PTT efficacy depends, however, on the heat generated and, on the depth reached by the light. Some strategies to improve PTT efficacy includes the use of the near infrared (NIR, 650 to 900 nm) radiation to enhance the penetration depth of the light, combined with gold nanoparticles (AuNPs) to enhance the photothermal effect.

Experimental Methods
Core AuNPs were synthesized by a novel method using tetrachloroauric acid and a mixture of reducing agents, and subsequently coated with a combination of hyaluronic and oleic acids, for improving the NPs biocompatibility, biodegradability, and lifetime. This coating also promotes the binding of specific cell receptors of the tumor cells. The particles were physico-chemically characterized, and in vitro and in vivo tests were carried out in breast cancer models to assess their safety and efficacy, when applied alone or combined with NIR irradiation.

Results and Discussion
AuNPs presented a predominant spherical morphology with sizes under 350 nm, polydispersity index lower than 0.4 and enhanced absorbance in the NIR. The particles showed no toxicity in vitro and promising efficacy in vivo when administering the NPs in situ and later irradiating them externally. Histopathological analysis of tumors treated with both AuNPs and laser irradiation showed the presence of necrosis in most of the tumors and no effect or practically absence in healthy surrounding cells, which are very encouraging outcomes.

Conclusion
The results are promising, however, there is still room for improving the system, namely by reducing even more the invasiveness of the treatment through the combined use of aerogels structures. Aerogel’s unique properties make them ideal candidates to minimize the exposure of healthy tissues to laser radiation, acting as light and thermal insulators, as well as to incorporate the nanoparticles into their skeletal structure and thus potentiating a topical application of the particles. For these reasons, some exploratory methods were carried to produce and design aerogels structures for PTT applications.

Acknowledgement
This work was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal, under the References SFRH/BD/147306/2019, UIDB/00645/2020 and UID/DT/04138/2019. Authors are also thankful to FCT/MCTES for the financial support to CESAM (UIDP/50017/2020, UIDB/50017/2020), through national funds. T. F.-G.
acknowledges to the COST Action “Advanced Engineering and Research on aeroGels for Environment and Life Sciences” (AERoGELS, Ref. CA18125), funded by the European Commission, for the granted Short Term Scientific Mission to perform the aerogels synthesis in the Universidade de Santiago de Compostela.

References
IBU/ZIF-8@Graphene Oxide Aerogels as scaffolds for bone tissue regeneration.

Alejandro Borrás, Ana M. López-Periago, Concepción Domingo

ICMAB-CSIC, SFFM, Campus UAB, ES

Introduction
Graphene Oxide (GO) has become a very promising and studied material in all the biologically related fields given its biocompatibility. Furthermore, its high mechanical strength paired with a high elasticity and flexibility and the possibility to be formed into a porous aerogel [1] elevates this material to the position of a highly qualified candidate for 3D scaffolding. In addition, the oxygen functionalities present in the 2D flakes allow for the anchoring of any wanted molecule or structure. In this case we are anchoring ZIF-8. ZIF-8 is also a very well-studied MOF with a high surface area and porosity [2] that has been considered as a drug delivery system for its high loading capacity and low toxicity [3]. In this case, the ZIF-8 has been heavily impregnated with Ibuprofen, a well-known drug that is used worldwide for its anti-inflammatory properties.

Experimental Methods
The fabrication and impregnation of the composite is fully carried out in a one-pot synthesis under supercritical CO₂, which implies the use of a green solvent on top of the cheap and easily accessible other reagents, such as the MOF precursors, the ibuprofen and the GO.

Two approaches are being followed, in the first one, all the reagents (Zn(acac)2, Hmim, GO and Ibuprofen) are dissolved in ethyl acetate inside a glass vial and placed inside a 100 mL autoclave filled with supercritical CO2 at 200 bar and 40 ºC.

On the second approach the composite is formed beforehand following the same procedure as before and is afterwards impregnated with Ibuprofen following a typical CO₂ impregnation.

Results and Discussion
The state of the project is still on its early stages. However, we have obtained 3D aerogels of GO fully covered with ZIF-8 nanocrystals. In addition, we have successfully impregnated ZIF-8 crystals with ibuprofen.

Conclusion
In this work we are focusing on the preparation of a Zeolitic Metal-Organic framework (MOF) ZIF-8@Graphene oxide (GO) hybrid aerogel. This composite is structured as a hierarchical porous system formed by the macroporous GO aerogel and the microporous ZIF-8 MOF. Therefore, the presented composite could function as a dual therapy agent, firstly with the 3D GO scaffold as a growing media for the generation of new cells and secondly with the controlled release of ibuprofen on the desired area through the ZIF-8 nanocrystals.

Acknowledgement
The authors acknowledge the funds provided by the spanish ministry of universities for the projects: SEV-2015-0496, CTQ2017-83632
A. Borrás also acknowledges the funds provided by the FPI grant: BES-2017-081148
GO Aerogel

Macroscopic picture of a GO aerogel

ZIF-8@GO

SEM image where ZIF-8 fully covers graphene oxide flakes

References


Mechanical properties of a conductive porous material produced by biopolymer cryogel as template and its potential application as extracellular matrix in biological cell culture

Ricardo Starbird-Perez1, Roy Zamora-Sequeira2, Laria Fabiola Rodriguez-Quesada1,8, Fernando Alvarado-Hidalgo3,8, Karla Ramirez-Sánchez1, Carlos A. García-González4, Monica Prado-Porras2, Juan Jose Montero-Rodriguez2, Fabian Vasquez-Sancho3, Esteban D. Avendano-Soto3, Giovanni Saenz-Arce5

1 Instituto Tecnologico de Costa Rica, School of Chemistry, Cartago, CR; 2 Instituto Nacional de Aprendizaje, Centro Nacional Especializado en Industria Gráfica y Plástico (CEGRYPLAST), San José, CR; 3 Universidad de Costa Rica, Centro de Investigación en Ciencia e Ingeniería de Materiales (CICIMA), San José, CR; 4 Universidade de Santiago de Compostela, Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, R+D Pharma group (GI-1645), Santiago de Compostela, ES; 5 Universidad Nacional, Departamento de Física, Heredia, CR; 6 Instituto Tecnologico de Costa Rica, Escuela de Ingeniería Electrónica, Cartago, CR; 7 Universidad de Costa Rica, Centro de Investigación en Enfermedades Tropicales (CIET), San José, CR; 8 Instituto Tecnologico de Costa Rica, Master Program in Medical Devices Engineering, Cartago, CR

Introduction
Scaffolds composed of biopolymers as extracellular matrix (ECM) have received significant attention for their potential therapeutic applications. Physical, mechanical and electrical properties of the substrate in cell culture may affect the cellular behaviors like migration, and stem cell differentiation [1,2]. In this work, an electrical conductive interconnected composite was synthesized [3], using starch/κ-carrageenan/poly(vinylalcohol)/poly(vinylalcohol)-g-N-methyl-4(4’-formylstyrlyl)pyridinium methosulfate acetal, and it has been crosslinked by gamma radiation. The biopolymer, κ-carrageenan, was added to the formulation in order to enhance the biocompatibility and the electrical response and the poly(vinylalcohol)-g-N-methyl-4(4’-formylstyrlyl)pyridinium methosulfate acetal has been incorporated to covalently bond the synthetic and the natural polymers. The physicochemical, mechanical and electrical properties of the Poly(3,4-ethylenedioxythiophene) (PEDOT) composite and the templates were characterized. The mechanical and solubility responses of the porous materials at macroscale were tuned by the starch/κ-carrageenan/PVA/PVA-SbQ biopolymers composition and the material properties were evaluated at nanoscale using atomic force microscopy. The electroactive nanostructures were formulated in order to evaluate its potential application as extracellular matrix in biological cell culture.

Experimental Methods
Starch aqueous dispersions with varying concentrations of κ-carrageenan, PVA and PVA-SbQ (0–1.0 wt.%) were prepared in deionized water. The resulting clear and homogeneous solutions were stirred for 20 s and then immediately transferred to cylindrical moulds. The moulds were, once gelified, stored at 4°C during 48 h for retrogradation. Then, the simples were lyophilized for 4 hours to dry them. A sterilization process, using gamma radiation doses of 25 kGy, was applied in order to promote the crosslinking reaction of the PVA-SbQ. The PEDOT was produced by adding 150 mg of the starch/κ-carrageenan/PVA/PVA-SbQ cryogel template to 2.5 mL of iron chloride (III) in ethanol. Subsequently, 2.5 mL of EDOT in 2-propanol (0.5 M) was added to the template. After 24 h of reaction, a homogenous blue color was obtained.

The compressive strength of the aerogel specimens was evaluated with a universal testing machine and a dynamic mechanical analyzer (DMA). The recorded force was divided by the initial length of the specimen to determine the compressive strain at 10% deformation. The conductive cryogel surface topography, adhesion, and tunneling-atomic
force microscopy (TUNA) electrical conductivity were obtained simultaneously atomic force microscope (AFM) operating in peak force tunneling mode. A 1 V DC bias was applied between the sample and the electrically conductive tip as the tip scanned the sample in contact mode.

**Results and Discussion**

Compression performance tests were used to evaluate the mechanical properties of the cryogel templates as a function of the PVA-SBQ presence. The highest value for the elastic modulus was obtained for the St-κCa-PVA-SbQ (3.25±0.56 MPa) and the lowest was for the St-κCA cryogels (1.95±0.60MPa). The elastic behavior is dominated by the elastic deformation of the porous structure and the macropores freely deform by bending into their open space. The polymerization of the PEDOT into the cryogel templates resulted in an enhancement of the mechanical properties. Similar mechanical properties but with higher κ-Ca and PVA-SbQ contents were denoted as structurally stable materials with high mechanical strength. It can be concluded that the mechanical properties were improved by the incorporation of the crosslinking agent.

The conductive porous material was investigated by electrochemical AFM to obtain the surface topography, adhesion, and electrical conductivity images. The topography of St-κCA cryogel showed a rough morphology and the conductivity analysis confirmed that the regions of high conductivity were found regardless of the mechanical properties. In similar studies, the current differences in some places were attributed to the high charge carriers due the doped-PEDOT areas, which tend to be concentrated in specific areas.

**Conclusion**

The conductive porous PEDOT-based composites were prepared through a nanostructured template-assisted processing approach using starch (S)/κ-carrageenan (κ-C)/poly(vinylalcohol) (PVA) and poly(vinylalcohol)-g-N-methyl-4(4'-formylstyryl)pyridinium methosulfate acetal (PVA-SbQ) cryogel as templates. S/κ-C/PVA/PVA-SbQ cryogels presented an attractive combination of mesoporosity and macroporosity. The mechanical and electrical properties at macro and local scale were evaluated proving the suitability of the PVA-SbQ and the κ-carrageenan in the formulation. The unique combination of properties obtained for this conductive nanoporous material is of high relevance in the biomedical field as an electroactive extracellular matrix for cell stimulation and it will be the subject of further studies.

**Acknowledgement**

The authors would like to thank to the Ministerio de Ciencia, Tecnología y Telecomunicaciones (MICITT) Project number FI038B-19, Vicerrectoría de Investigación (VIE), project number 5402-1360-4401, Dirección de Posgrado, CIEMTEC and CIB research Centers from Instituto Tecnológico de Costa Rica. Also to the Vicerrectoría de Investigación, Universidad Nacional, Costa Rica. Work supported by Xunta de Galicia [ED431C 2020/17], MCIUN [RTI2018-094131-A-I00], Agencia Estatal de Investigación [AEI] and FEDER funds. Work carried out in the frame of the COST Action CA18125 “Advanced Engineering and Research of aeroGels for Environment and Life Sciences” (AERoGELS), funded by the European Commission.
Mechanical properties of a conductive porous material produced by biopolymer cryogel as template
Characterization and potential application of the biopolymer cryogel as template of a conductive porous material.

References
Fabrication of chitosan/alginate aerogels with antibacterial properties

Jelena M. Pajnik¹, Ivana Z. Lukic², Jelena K. Dikic¹, Svetolik N. Maksimovic², Stoja L. Milovanovic²

¹ University of Belgrade, Innovation Center of the Faculty of Technology and Metallurgy, Karnegijeva 4, Belgrade, RS; ² University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, Belgrade, RS

Introduction
Natural polysaccharides such as chitosan and alginate are broadly utilized for various biomedical applications due to their biocompatible/biodegradable properties and ability to act as carriers for different drugs enabling their controlled release. Chitosan and alginate are both water-soluble polymers and capable to form hydrogels under defined conditions. Process of elimination of a fluid from a hydrogel, in which liquid phase is replaced with a gas by preserving the gel structure, results in a formation of an aerogel. The type of extraction of the liquid phase i.e. drying of hydrogels defines the synthesis of this innovative porous material. This step can be performed with the application of supercritical CO₂-assisted drying. This study was dedicated not only to investigate application of supercritical CO₂ for preparation of chitosan/alginate aerogels, but also to test supercritical solvent impregnation (SSI) as a technique for loading of obtained aerogels with compounds with proven antimicrobial properties.

Experimental Methods
Chitosan/alginate aerogel preparation was performed in several steps. First, chitosan and alginate 1.5 mass% solutions were prepared by dissolving chitosan and alginate powder in 2% (v/v) acetic acid and water, respectively. Hydrogel was formed by mixing chitosan and alginate solutions at room temperature for 24 h. Then hydrogel was poured in plastic mold and immersed into the 5% (w/v) aqueous solution of CaCl₂ during 30 min. Subsequently, water was gradually replaced with ethanol during several days to form an alcogels. Obtained alcogels were cut into disc shape (diameter ~10 mm) and dried at 45 °C and pressures of 100 bar. Scanning Electron Microscopy (SEM) was used to evaluate textural properties of the aerogels. Obtained aerogels were impregnated with thymol/carvacrol mixture at 35 ⁰C, 30 MPa during 5 and 18 h in a high pressure view cell using green medium, supercritical carbon dioxide. Mass ratio of thymol/carvacrol was set to 1/1. Aerogels before and after the SSI were analyzed by Fourier transform infrared (FTIR) spectroscopy. The antibacterial activity of impregnated aerogels was tested towards Gram-negative bacterium Escherichia coli strain DSM 498 and Gram-positive bacterium Staphylococcus aureus strain ATCC 25923. The disk diffusion method was used for a qualitative assessment of antibacterial activity, which was reported as the presence/absence of the inhibition zone.

Results and Discussion
Porosity of the obtained samples was 86%. Highly porous structure of the chitosan/alginate aerogels was confirmed by scanning electron microscopy. As expected, loading of thymol/carvacrol mixture into aerogels increased with an increase in process time. Namely, amounts of loaded thymol/carvacrol mixture were 31 and 43.7% for time intervals of 5 and 18 h, respectively. Presence of thymol/carvacrol on the surface of the impregnated chitosan/alginate aerogels was confirmed by FTIR analyses. As it can be seen from the Figure 1, thymol/carvacrol impregnated aerogels exhibited strong antibacterial activity towards tested bacteria strains while controlled samples (pristine aerogels) had no antibacterial activity.

Conclusion
Chitosan/alginate aerogels were successfully loaded with high amounts of thymol/carvacrol mixture with the application of SSI. Functionalized material exhibited strong antibacterial effective towards both Gram-negative and Gram-positive bacteria, indicating various potential application.

Acknowledgement
Acknowledgements: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-9/2021-14/200287 and 451-03-9/2021-14/200135). Work was carried out in the frame of the COST-Action "Advanced Engineering of aeroGels for Environment and Life Sciences" (AERoGELS, Ref. CA18125) funded by the European Commission.

Antibacterial activity of impregnated aerogels towards E. coli (a) and S. aureus (b)
Initial number of bacteria (t₀): E. coli = 3.7 • 10⁸; S. aureus = 4.7 • 10⁸ CFU cm⁻³
Introduction
Aerosol administration of therapeutic drugs is the most efficient pathway for lung inflammatory diseases, such as asthma and chronic obstructive pulmonary disease (COPD). Indeed, β2-agonists like salbutamol sulfate (SS) are quick-acting bronchodilator drugs administered as an aerosol formulation. Aerogel microparticles (aerogel/MPs) hold a great potential for this administration route, thanks to their high surface area, low density, high porosity and drug loading efficiency [1]. The light weight of aerogels allow for large particle size with suitable aerodynamic diameters, which improves their flow properties and resistance to phagocytosis by pulmonary macrophages [2]. For this reason, the biological effect of SS-loaded alginate aerogel/MPs produced by inkjet printing followed by supercritical drying [3] were herein evaluated in vitro. The cytocompatibility and the anti-inflammatory effect were evaluated in lung epithelial cells and macrophages, respectively.

Experimental Methods
The SS loading was carried out in three different ways to achieve different drug distributions within the particles: (1) by incorporating SS in the 0.35 wt.% alginate ink at a concentration of 12 wt.% (Aero-SS-i), (2) by dissolving it in the gelation bath of CaCl₂ at a concentration of 0.4 wt.% (Aero-SS-b), and (3) by combining both loading strategies (Aero-SS-ib). After gelation and solvent exchange to ethanol, supercritical drying was performed (120 bar, 40 ºC, 3.5 h) to obtain the aerogels used for the cytocompatibility tests. Unloaded alginate aerogel/MPs, raw alginate and free salbutamol were also tested as controls. Morphology of the aerogels was evaluated using scanning electron microscopy (SEM) and textural properties were tested by nitrogen adsorption-desorption analysis.

The cytocompatibility of the aerogel/MPs was measured in the human pulmonary epithelial A549 and NCI-H460 cell lines, as model cells of the alveolar and lung epithelium, respectively. The cells were incubated with the aerogel/MPs at different concentrations and the viability was determined by the MTS colorimetric assay. The potential release of reactive oxygen species (ROS), which can contribute to exacerbate inflammatory diseases, was also evaluated in A549 cells incubated with the SS-loaded aerogel/MPs or the control samples. Finally, the therapeutic potential of the formulation was tested in an in vitro model of inflammation by using lipopolysaccharide (LPS) pre-treated human monocytic (THP-1) cells. The anti-inflammatory potential of the SS-loaded aerogel/MPs was based on their capacity to reduce the levels of the proinflammatory cytokines MCP-1 and TNF-α, in comparison with cells treated only with LPS, as described before for SS [4].

Results and Discussion
Aerogels were obtained in the form of a white, free-flowing powder. The SEM images showed particles with high sphericity and sizes between 10–25 mm (Fig. 1). The particles presented excellent textural properties, with surface areas in the 200-300 m²/g range and mean pore diameter of ca. 4.0 nm for the three aerogel processing strategies tested.

After incubation of the aerogel/MPs with the pulmonary epithelial cell lines at different concentrations, no significant reduction of the cell viability or increase in ROS levels were detected for the aerogel/MPs or the individual components, even at the highest concentrations tested (500 µg/mL for aerogel/MPs and 15 µg/mL for SS and SS-
loaded aerogel/MPs). Induction of ROS in the lung is one of the mechanisms that can contribute to exacerbate inflammatory diseases such as asthma or COPD, and even contribute to the fibrotic and carcinogenic process [5]. Thus, the aerogel/MPs showed a good cytocompatibility for pulmonary administration to treat inflammatory diseases. All the SS-loaded aerogel/MPs were able to inhibit the release of MCP-1 and TNF-α in LPS-stimulated THP-1 cells, but only Aero-SS-b and Aero-SS-ib reduced significantly both cytokine levels (Fig. 2). In fact, Aero-SS-ib produced the highest cytokine inhibition, even in non-stimulated cells and similar to the free SS. Hence, SS-loaded aerogel/MPs prepared by combining both techniques, incorporation in the ink and dissolution in the gelation bath, could induce a more controlled drug release in the physiological media in comparison with the individual drug loading strategies. The higher drug load in these aerogels was observed in the SEM images with the presence of SS precipitates in their surface (Fig. 1).

**Conclusion**

Alginate aerogel/MPs prepared by inkjet printing and loaded with SS showed a good cytocompatibility and a safe profile for pulmonary administration. All the SS-loaded aerogel/MPs tested were able to reduce the inflammation in an in vitro cell model. Yet, Aero-SS-ib seems to be the best processing candidate for the treatment of lung inflammation because it produced the highest cytokine inhibition. The combination of both drug loading strategies induced the highest therapeutic efficacy in an in vitro inflammation model.

**Acknowledgement**

Work carried out in the framework of the COST-Action "Advanced Engineering of aeroGels for Environment and Life Sciences" (AERoGELS, ref. CA18125) funded by the European Commission. Work supported by Xunta de Galicia [ED431C 2020/17], MCIUN [RTI2018-094131-A-I00], Agencia Estatal de Investigación [AEI] and FEDER funds. L.D.-G. acknowledges Xunta de Galicia for a predoctoral fellowship [ED481A-2018/294].
MCP-1 and TNF-α concentration in LPS pre-treated THP-1 cells after 24 hours of incubation with SS-loaded and unloaded aerogel/MPs.

References
AER InvF-07

Polysaccharide κ-Carrageenan as doping agent in conductive coatings for Electrochemical Controlled Release of Dexamethasone at Therapeutic Doses

Karla Ramírez-Sánchez1, Ricardo Starbird-Perez1,2, Fernando Alvarado-Hidalgo1,3, Aura Ledezma-Espinoza1, Andrés Sánchez-Kopper1, Esteban Avendaño-Soto2,4, Monica Prado5

1 Instituto Tecnológico de Costa Rica, Centro de Investigación y de Servicios Químicos y Microbiológicos (CEQIATEC), School of Chemistry, Cartago, CR; 2 Universidad de Costa Rica, Centro de Investigación en Ciencia e Ingeniería de Materiales (CICIMA), San José, CR; 3 Instituto Tecnológico de Costa Rica, Master Program in Medical Devices Engineering, Cartago, CR; 4 Universidad de Costa Rica, School of Physics, San José, CR; 5 Universidad de Costa Rica, Centro de Investigación en Enfermedades Tropicales (CIET), Faculty of Microbiology, San José, CR

Introduction

Conductive polymers are a new generation of smart materials extensively used in organic bioelectronics and in active controlled release systems. PEDOT, a conductive polymer synthetized from EDOT, has been recently studied for the incorporation of charged drugs during the electropolymerization process. Numerous aerogels from dierent sources have been used as extracellular matrices for cell colonization in implantable devices, many of them are loaded with bioactive agents such as anti-inflammatory molecules (i.e., dexamethasone) to improve the therapeutic efficacy and safety of the drugs, playing important roles in treatment of several chronic diseases, avoid immune responses, and provide a potential stimulation of dierent types of cells.

In this work, a method to induce the loading of dexamethasone into PEDOT films during the polymerization process onto gold electrodes was established. The polysaccharide κ-Carrageenan (κC) was also incorporated to maintain the electrochemical stability and biocompatibility of the PEDOT matrix and the subsequent drug release using electrical stimulation. Therapeutic doses of dexamethasone appropriate for mesenchymal stem cells diferentiation were achieved during the electrical stimulation of the bioelectronic device.

Experimental Methods

κC (0.2% w/v) in deionized water was prepared and sonicated before adding the monomer EDOT and Dx at three different concentrations. The deposition of the dispersion was carried out on the surface of gold bare electrodes through an electropolymerization process. Profilometry analysis were made in order to study the electrode topography. Thus, the arithmetical mean roughness of the surface (Sa) was calculated by using a 2 μm tip radius and a force of 1 mg. Raman spectroscopy analysis was carried out using a confocal µ-Raman microscope with a 532nm excitation laser. The presence of PEDOT and Dx/κC was confirm at the relative wavenumber of 1435 cm⁻¹ and 1625 cm⁻¹, respectively.

Drug release experiments from the modified electrode were studied using cyclic voltammetry sweeps over a period of 300 min at room temperature. A second release event, without electrical stimulation, was performed as negative control to quantify the passive drug release process. Dexamethasone concentration in the extracted samples was determined using a quadrupole time of flight (Q-tof) mass spectrometer coupled with an UPLC system. Quantification of the drug was carried out using Multiple Reaction Monitoring (MRM) acquisition method with the optimized transition of 471.1584 m/z for the precursor ion and 78.9585 m/z for the product ion.

Results and Discussion

The roughness data of the electrodes surfaces before and after drug release did not show significant differences between them. The lack of significative variation during the delivery of dexamethasone may indicate the reliability of
electroactive composite for cell culture studies since no additional mechanism may be seemed due to the topography changes.

Qualitative composition analysis of the coating using confocal µ-Raman spectroscopy during electrical stimulation showed a strong signal in the spectral range of 1421–1442 cm\(^{-1}\), associated with PEDOT presence. Additionally, a relative intense band at 1625 ± 30 cm\(^{-1}\) was detected, corroborating the qualitative existence of Dx and κC in the conductive film.

The electrochemical controlled release studies from PEDOT:κC:Dx showed a characteristic reduction potential signal range at −100 to −400 mV. This signal indicates the release of the drug from the stimulated electrode, which disappeared completely after 160 cycles of electrical stimulation, confirming the complete dexamethasone migration from the conducting composite.

HR-mass spectrometry was used to calculate the accumulative concentration of the released Dx. The amount of drug released on stimulated electrodes using 1 mM and 5 mM initial formulations were 300 nM and 600 nM, respectively. These values are relevant therapeutic dosages for the mesenchymal stem cell cultures differentiation or during anti-inflammatory treatment.

Finally, the surface area of the unstimulated electrode is associated with promoting larger amounts of passive drug release, yet, in our case, the electrode surface and total area are maintained virtually constant. A low diffusion value was identified in the beginning of the process, maybe it depends on the slow penetration of supportive electrolyte into the polymeric film. Nevertheless, after 80 min, the diffusivity of the passive Dx release process is reflects.

**Conclusion**

Therapeutic doses of dexamethasone were successfully delivered using an electroactive controlled system. Incorporation of κ-carrageen as doping agent in the matrix avoided delamination and changes in the film roughness. The formulation and method established along with a proper active electrochemical stimulation profile allowed the delivery of the drug at adequate concentrations to induce differentiation in mesenchymal cell cultures and in anti-inflammatory responses.

**Acknowledgement**

The authors would like to thank Vicerrectoría de Investigación from Instituto Tecnológico de Costa Rica and the Ministerio de Ciencia, Tecnología y Telecomunicaciones de Costa Rica. Part of this work was carried out in the frame of the COST-Action “Advanced Engineering of Aerogels for Environment and Life Sciences” funded by the European Commission.

**References**


4:30 p.m. – 6:00 p.m.

Track08

**AERO S03 | Drug-delivery systems and Nutraceutical/food systems**

**Chairs**

*Catarina Pinto Reis*
Faculdade de Farmácia, Universidade de Lisboa, Lisboa, PT

*Bruno Sarmento*
i3S, Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, PT
AERO S03-KL03

Bioaerogels prepared using the Pressurized Gas eXpanded liquid (PGX) process as delivery systems for hydrophobic bioactives

Feral Temelli¹, Ricardo Couto¹², Bernhard Seifried², Byron Yépez², Paul Moquin²

¹ University of Alberta, Department of Agricultural, Food and Nutritional Science, Edmonton, CA; ² Ceapro Inc, 7824-51 Avenue, Edmonton, CA

Introduction
The global market for functional foods and natural health products is expected to grow by US$ >12 billion within the next decade according to marketing experts. These products are formulated with various bioactives, which are mostly isolated from natural sources. In general, bioactives are sensitive to heat, light and oxygen and can degrade easily. Many bioactives are hydrophobic and some are crystalline in nature, making them poorly bioavailable and challenging to incorporate into aqueous-based products. Such hydrophobic bioactives include coenzyme Q10 (CoQ10), vitamin D (VitD), and vitamin E (VitE). CoQ10 and VitE are antioxidants, associated with reducing the risk of cardiovascular diseases and cancer, while VitD plays an important role in calcium absorption and bone health. CoQ10 and VitD are crystalline powder, but VitE is a viscous liquid. Delivery systems are needed for such bioactives for ease of handling, incorporation into aqueous-based formulations, enhance their stability and maximize their health benefits.

Experimental Methods
Bioaerogels prepared using water-soluble polysaccharides, including oat β-glucan (BG), gum arabic (GA) and sodium alginate (SA) can be utilized as delivery systems for the hydrophobic bioactives to overcome some of the challenges. Conventional methods of aerogel production are slow and expensive as they involve several steps: preparation of hydrogel, conversion to alcogel by lengthy solvent exchange, and drying, preferably by supercritical CO₂ (SC-CO₂) to prevent collapse of the network. Pressurized Gas eXpanded liquid (PGX) Technology is a faster more affordable way to manufacture bioaerogels. The aqueous biopolymer solution is contacted with the PGX fluid (CO₂+Ethanol at 100 bar, 40°C) through a co-axial nozzle to precipitate the biopolymer while removing the water. Then, flushing with CO₂ removes residual ethanol and impurities. Resulting bioaerogel powders have unique morphologies of micro/nano-sized particles as shown in Fig. 1 and large specific surface areas, ranging from 24.1 m²/g for BG [1], to 65 m²/g for GA [2] and 164.5 m²/g for SA [3].

Adsorptive precipitation using SC-CO₂ was employed to load the PGX-processed bioaerogels with the hydrophobic bioactives under different processing conditions followed by fast depressurization. CoQ10 was loaded on BG, GA and SA, while VitD and VitE were loaded on GA and SA. The loaded powders were characterized in detail (loading content, morphology, thermal behavior, crystallinity, release kinetics, stability) in an effort to understand their potential as delivery systems.

Results and Discussion
Helium ion microscopy images demonstrated uniform coating of the bioaerogels by the bioactives. Loading levels varied depending on the biopolymer and the bioactive. CoQ10 loading (w/w) was 3.6% on GA [2], 17.5% on BG [4] and 46.9% on SA [3]. However, CoQ10 loading per unit area was 0.56, 7.2 and 7.5 mg/m² for GA, BG and SA, respectively, demonstrating the higher level of interaction between CoQ10 and the fibrous networks of BG and SA (Fig. 1). A similar trend was observed with the vitamins with SA resulting in higher loading compared to GA (4.4 and 7.1 mg/m² on SA vs 3.2 and 4.8 mg/m² on GA for VitD and VitE, respectively) [5]. The long hydrophobic tail present in the structures of CoQ10 and VitE seems to enhance their interactions and loading on the fibrous network of the negatively charged SA as opposed to the spherical network of GA.
Differential scanning calorimetry and X-ray diffraction analysis demonstrated a reduction in the crystallinity of CoQ10 and VitD after loading on the bioaerogels. Release kinetics in simulated intestinal fluid (SIF) showed a sustained release of the vitamins from GA of up to 20% of the original amount over 4 h but it was limited (up to 5%) from SA, indicating a higher level of interaction. For CoQ10, its sustained release from SA in SIF and simulated gastric fluid (SGF) was at 11.5%, whereas its release from BG and GA was higher in SGF reaching 7.4% and 30.5%, respectively. Considering that the release of pure bioactives was ≤3%, their loading on the bioaerogels resulted in a substantial increase in their availability under simulated digestive tract conditions. In addition, the stability of CoQ10 on BG, GA and SA powders was demonstrated over 1 mo of storage. The aqueous dispersions of CoQ10-loaded BG and GA were quite stable for 6 mo and 1 mo of testing, respectively.

Conclusion
The findings of the studies on adsorptive precipitation of hydrophobic bioactives on the PGX-processed polysaccharide aerogel powders demonstrated the great potential of this technology. The different combinations of bioactives and biopolymers studied provided insight on the development of customized delivery systems for potential targeted applications with anticipated enhanced bioavailability and ease of incorporation into aqueous-based product formulations.

Acknowledgement
The authors gratefully acknowledge the financial support of the Natural Sciences and Engineering Research Council of Canada (NSERC) Collaborative Research and Development (CRD) grant (Project no: CRDPJ 500236-16) and Ceapro Inc.

References
Protective and Functional Coatings of Alginate Aerogel Particles as a Drug Carrier

İşık S. Akgün¹, Enis Demir², Murat İşik², Nadin Ekmekçiyan², Erkan Şenses¹, Can Erkey¹

¹ Koç University, Chemical and Biological Engineering Department, Istanbul, TR; ² Ashland Specialty Ingredients, Istanbul, TR

Introduction
Due to their open pore structures, very high specific surface areas and pore volumes, aerogels in the form of particles are attracting increasing attention for a wide variety of applications such as drug delivery. In general, aerogels provide immediate release due to the adsorption of drugs in the amorphous state and due to the large surface area in contact with the body fluid [1]. Coating the surface of aerogel particles with a thin polymer layer may act as an armor to protect the aerogel matrix and provide prolonged and controlled release of drugs [2]. Coating is an essential component of particle formulations in various industries, particularly in the pharmaceutical industry. However, coating of aerogels based on current technologies in pharmaceutical industry is a challenging task since tablets and aerogels have quite different properties such as very different densities, porosities, and mechanical strength [3].

Experimental Methods
The main target of this study was to successfully coat aerogel particles via keeping their porous structures and understanding drug release mechanism from both coated and uncoated aerogels. Spherical alginate aerogel particles were synthesized by dripping an alginate solution from a nozzle into a CaCl₂ solution followed by solvent exchange and drying with supercritical CO₂ in an Applied Separations Speed SFE. The mean particle size and the mean particle density were 4.20 ± 0.02 mm and 0.05 ± 0.01 g/cm³, respectively. Subsequently, ibuprofen was loaded via supercritical deposition at 40°C and 220 bars. A Wurster fluidized bed was used for both protective and functional coating of alginate aerogel particles. Copovidone based ultrahigh solid film coating system, Aquarius™ Genesis by Ashland Specialty Ingredients, was applied as protective coating around the unloaded aerogel particles whereas methacrylic acid-ethyl acrylate copolymer based Aqueous Control ENA by Ashland Specialty Ingredients was used for functional coating of ibuprofen loaded aerogels. Gastric resistance of functional coated aerogels was tested in 0.1 M HCl. Drug release was measured in phosphate buffer at pH 7.2. Release profiles were determined using HPLC.

Results and Discussion
For protective coating, variations occurring in coating layer thickness (CLT) around alginate aerogel particles and changing coating layer surface morphology with process time, bed temperature, atomizing air pressure and polymer rheology were investigated. Three bed temperatures which were 30°C, 50°C and 70°C and three atomizing air pressures (AP) which were 1.3, 1.5 and 1.7 bars were studied. In all three bed temperatures, aerogel particles were successfully coated. As an example, photographs of uncoated aerogels and coated aerogels with high level of coating uniformity at 50 °C bed temperature and 1.7 bar AP after 30 minutes are given in Figure 1. Coating time for all the runs ranged from 5 minutes to 40 minutes and the CLT ranged from 12.4 ± 4.6 μm to 170.6 ± 43.3 μm. The smoothest coating layer surface and the highest coating efficiency which was 69.2 ± 0.4 % with a linear increase in coating layer thickness were achieved at 50 °C with 1.7 bar. Due to the moderate polymer viscosity and elastic modulus, the lowest contact angle as ~118° was achieved at 50°C which indicating a good droplet spreading on an aerogel surface. An increase in atomizing air pressure from 1.5 bar to 1.7 bar resulted in a smoother coating layer. A high mean coating polymer solution droplet velocity with a narrow droplet size distribution led to a homogeneous spreading and less variance in CLT at 1.7 bar. Coatings at 50°C were very similar in smoothness and morphology to coatings on pharmaceutical dosage forms demonstrating the suitability of Wurster fluidized bed for successful coating of aerogel
Ibuprofen loaded aerogels were also successfully coated for enteric release without damaging the pores of aerogels. SEM images of both outer surface and cross-section of a functional coated ibuprofen loaded aerogel at 50 °C, 3 hours are given in Figure 2. Almost 94% of the ibuprofen was released from uncoated aerogels within 120 min. at a pH of 1.0, whereas ibuprofen release from aerogels coated with methacrylic acid-ethyl acrylate copolymer-based solution was prevented at the same pH with 160 ± 21 µm CLT. The change of the release medium from acid to PBS buffer at a pH of 7.2 resulting in a release of all the loaded ibuprofen in less than 10 min.

**Conclusion**

Highly porous alginate aerogel particles which has very high potential in pharmaceutical industry as drug carriers were successfully coated with a copovidone and methacrylic acid-ethyl acrylate copolymer-based solutions, separately. Based on the results presented, bed temperature was a more important parameter for CLT whereas both AP and bed temperature affected coating layer surface morphology and coating polymer rheology. Moreover, enteric coating of ibuprofen loaded aerogels improve pH specific release of ibuprofen which is a poorly water-soluble drug.

---

**Figure 1**

Alginate aerogel particles. (a) before the coating (b) after 30 minutes coating with pink polymer solution in a Wurster fluidized bed at 50 °C bed temperature and 1.7 bar AP. (c) SEM image of cross-section of a coated aerogel particle. (d) Light microscopy image of cross-section of a coated aerogel particle.

**Figure 2.**

Functional coated and drug loaded alginate aerogel particles. (a) outer surface (b) cross section.

**References**


Whey protein aerogel particles as templates to produce oleogels with tailored digestibility

Sonia Calligaris, Stella Plazzotta, Marilisa Alongi, Lara Manzocco

University of Udine, Department of Agricultural, Food, Environmental and Animal Sciences, Udine, IT

Introduction

Oleogels are defined as gels in which the continuous liquid oil phase is immobilized into a supramolecular network of molecules known as oleogelators. A lot of research efforts has been dedicated to developing oleogels intended as fat substitutes in foods to create healthier foods with reduced content of saturated/trans fatty acids. Today, oleogels seem to be promising also for the delivery of bioactive lipophilic molecules and as modulators of the gastrointestinal lipolysis, opening novel opportunities not only in foods but also for pharmaceutical and cosmetic applications. Oleogels can be obtained by exploiting the network-forming ability of lipophilic molecules upon self-assembly in oil or by generating scaffolds made of hydrophilic components able to entrap oil. Recently, aerogels made of hydrophilic food polymers, such as carbohydrates and proteins, have been suggested as potential templates for oleogel preparation, by simple absorption of the oil in the aerogel dried network. Up to now, limited information is available on these novel oleogels, especially considering their gastrointestinal behavior. Based on these considerations, the aim of the present study was to explore the possibility to prepare oleogels from whey protein (WP) aerogels by using different oils and evaluate the digestibility of the obtained systems.

Experimental Methods

To this aim, protein aerogel particles were prepared by supercritical-CO$_2$ drying (SCD) of heat treated and ground WP hydrogels (20% w/w, pH=5.7). The particles were then dispersed into edible oils (castor, cod liver, corn, extra virgin olive, flaxseed, MCT, peanut and sunflower oil) and collected by centrifugation. The obtained oleogels were analyzed for oil content, microstructure, and rheological properties. Some selected oleogels were then subjected to in vitro digestion, conducted according to the INFOGEST protocol.

Results and Discussion

Optical microscopy showed that aerogel particles formed a homogeneous network in all the tested oils, probably due to hydrophilic inter-particle interactions, so that the oil was efficaciously embedded both in the pores of the particles and in the interparticle spaces, as also confirmed by confocal microscopy. Solid-like materials containing up to 80% oil and with mechanical properties comparable to those of conventional fats were obtained. Interestingly, the oil type did not affect the oleogel structure. The only exception was observed for castor oil, whose high polarity probably hindered particle networking. The digestion trials showed that, differently from native WP, which were completely digested in the stomach, circa 50% of the WP aerogel particles remained undigested after gastric digestion, being then fully digested in the intestinal phase. At the same time, the lipolysis degree of the oil contained in the oleogel was significantly different as compared to that of the bulk oil. It can be hypothesised that the protein network embedding the oil was able to modify the lipase action at the interface.

Conclusion

This work demonstrates that WP aerogel particles could be regarded as versatile oleogel templates allowing the structuring of many edible oils into solid-like materials. The developed structure could have peculiar delivering capacity of health-promoting lipophilic components not only in foods but also in human body, modulating their release.

Acknowledgement

Work carried out in the framework of the COST Action "Advanced Engineering and Research on aeroGels for Environment and Life Sciences" (AERoGELS, ref. CA18125) funded by the European Commission.

Page 641 of 2028
References


AERO S03-03

Potential of whey protein aerogels as delivery systems in food: structural characterization and physical stability

Stella Plazzotta, Sonia Calligaris, Lara Manzocco

University of Udine, Department of Agricultural, Food, Environmental and Animal Sciences, Udine, IT

Introduction
During the last years, a lot of research has been focused on natural biopolymer-based aerogels, which can fulfill the requirements for food applications. Being based on food-grade polysaccharides and proteins, these aerogels are claimed to provide new opportunities for food applications, including edible delivery systems for bioactive and labile components. In both cases, the stability of the loaded molecule might be increased not only during food processing and storage, but also during digestion, allowing for a controlled release after intake. The aerogel loading may be easily performed by immersing the aerogel into a liquid, which permeates into the aerogel and saturates the internal volume. The embedded liquid may interact with the aerogel polymer chains and either reinforce or weaken their architecture. Structural integrity upon liquid loading strongly affects the feasibility of exploiting aerogels as delivery systems in foods, since influencing the effective entrapment and release of the loaded components during food processing, storage, and in vivo delivery. Both composition and process can be adjusted to get aerogels showing specific structural response to contact with hydrophilic or lipophilic liquids. In this regard, protein aerogels appear promising in the light of their compatibility with food components. As well known, the drying technique strongly affects the aerogel micro-structure by steering pore size and distribution, and thus its loading and liquid retention capability. While supercritical-CO₂ drying (SCD) generally preserves the original network, leading to homogeneous pores with low dimension, freeze-drying (FD) commonly results in macropores and cracks upon sublimation.

Experimental Methods
Based on these considerations, the aim of this study was to investigate the potential of whey protein (WP) aerogels as delivery materials in foods. To this aim, WP monolithic hydrogels (20% w/w, pH 5.7) were dried by FD or SCD. The obtained aerogels were firstly characterized for their structural features (density, volume contraction, firmness, SEM microstructure), adsorption isotherm and glass transition temperature. Subsequently, the FD and SCD aerogels were used as templates for absorption of water and oil by immersion.

Results and Discussion
Heat treatment of 20% WP solutions near their isoelectric point resulted in white monolithic hydrogels, whose microstructure consisted in the association of spherical hydrated protein particles, called microgels. The hydrogels were subjected to FD and SCD. SCD resulted in denser aerogels (d=0.29 g/cm³), which underwent a greater volume contraction (ΔV=39%) as compared to the FD samples (d=0.22 g/cm³; ΔV=15%). Accordingly, the SEM microstructure of the two samples appeared different. The FD aerogels showed an open protein matrix with large pores (2-5 µm), in which the microgel particles appeared fused together. A more compact and homogeneous matrix was observed in the SCD samples, which showed lower-dimension pores (< 1 µm) and a particulate network in which the structure and organisation of the original microgel particles were maintained. Despite these differences, both aerogels showed a type III isotherm upon water vapour absorption, indicating a limited capacity of interacting with water molecules, and similar glass transition at 161 °C. An ERH of circa 80% was identified as the maximum humidity value below which both samples maintained their porosity at 20 °C.

A slower uptake of both water and oil was observed in the SCD aerogel as compared to the FC one. Water absorption caused destructuring of both aerogel samples. By contrast, the aerogels retained their integrity upon oil-loading.
generating materials containing 75% (w/w) oil, independently on the drying technique applied for their preparation. However, the SCD sample presented higher firmness (F=29.5 N) and oil holding capacity (OHC=96.3%) than the FD one (F=18.5 N, OHC=44.7%).

Conclusion
The results acquired in this work demonstrate the potential of WP aerogels to be used as innovative delivery systems in foods, and the possibility to steer their properties by changing the drying technique. Based on their fast dissolution in water, FD aerogels would be optimal candidates for rapid release of loaded compounds; by contrast, the SCD aerogels should be selected for the delivery and controlled release of oil and lipophilic compounds.

Acknowledgement
Work carried out in the framework of the COST Action "Advanced Engineering and Research on aeroGels for Environment and Life Sciences" (AERoGELS, ref. CA18125) funded by the European Commission.

References
Ultrasonic Detection of Aerogel coupons for Biomedical Applications

Firouzeh Sabri

UofM, Physics and Materials Science, Memphis, US

Introduction
Ultrasound imaging is a non-invasive rapid diagnostic technique with no known biological risks to tissue or to the patient. We have previously shown that polyurea crosslinked silica aerogels can be easily identified in vivo and are distinguishable from the surrounding bone and tissue scanned at 13MHz[1]. The aerogel implants demonstrated hyperechoic behavior and significant posterior shadowing. The impedance mismatch contributes to a clear outline of the aerogel coupon and visibility of structural details is expected to be frequency dependent and was not explored in our earlier study. In this study we expand the library of ultrasonic properties available for aerogels and investigate other types of aerogels, beyond silica-based aerogels and as a function of frequency.

Experimental Methods
Six different types of aerogels were evaluated. Coupons of a similar size were extracted from each aerogel type and scanned under different conditions. An Edan U50 Prime ultrasound unit with a linear probe array was used for this study. Aerogels were scanned systematically from 8Mhz upto 13.5 MHz. Image analysis and processing was performed by means of NIH open-source ImageJ software. Attenuation coefficient was calculated using linear regression of pixel intensity from the acquired images.

Results and Discussion
Submuscular and subcutaneous positioning of aerogel coupons investigated as a function of frequency showed unique characteristics influenced by the porosity and the Young's modulus of each aerogel type.

Conclusion
Diagnostic ultrasound techniques can be readily used to detect Aerogels both in vivo and in vitro. The unique structure of aerogels allows for a clear distinction from surrounding tissue and depends on acoustic impedance and attenuation coefficient. Some frequency dependence in the range of 8-13.4 MHz was also observed. Further studies in other frequency ranges will be performed in the next phase of our study.

Acknowledgement
The University of Memphis Faculty Research Grants.

References
[1] https://doi.org/10.1371/journal.pone.0066348
6:00 p.m. – 6:30 p.m.

Track08

**AERO Closing | Closing session**

**Chairs**

Carlos A. García-González  
University of Santiago de Compostela, Pharmacology, Pharmacy and Pharmaceutical Technology Dept., Santiago de Compostela, ES  
Fernando Monteiro  
University of Porto, Porto, PT

**AERO Closing-01**

**Closing session**

Carlos A. García-González¹, Fernando J. Monteiro²

¹ University of Santiago de Compostela, Pharmacology, Pharmacy and Pharmaceutical Technology Dept., Santiago de Compostela, ES; ² i3S - Instituto de Investigação e Inovação em Saúde; INEB - Instituto de Engenharia Biomédica, Faculdade de Engenharia da Universidade do Porto, Porto, PT
<table>
<thead>
<tr>
<th>Time</th>
<th>Track 01</th>
<th>Track 02</th>
<th>Track 03</th>
<th>Track 04</th>
<th>Track 05</th>
<th>Track 06</th>
<th>Track 07</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:30</td>
<td>GW AW01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>George Winter Award 2021</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:30</td>
<td>KG AW02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Klaas de Groot Award 2021</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:00</td>
<td>K06</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biomimetic Nanomaterials and Bioinspiring Devices</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:00</td>
<td>K07</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biomaterials for Cardiac Tissue Engineering and Regenerative Medicine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:30</td>
<td>K08</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mechanomodulatory Biomaterials and its Impact on Cell Behaviour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Program Wednesday, 8 September, 2021
GW AW02-01
Development of fiber-reinforced composites for dental and medical reconstructive biomaterial

Pekka Vallittu

University of Turku, Biomaterials Science / Institute of Dentistry, Turku, FI

Development of dental and medical biomaterials has been limited to use only isotropic bulk materials until recently, when first clinically applicable fiber-reinforced composites (FRC) have become to the clinical use in late 1990s. Present applications of FRC can be found in all disciplines of clinical dentistry and in applications of bone reconstructive surgery. All of these applications are having fibers of glasses in the biostable resin-based matrix of FRC. Orientation of fibers in the polymer matrix may vary from unidirectional to bidirectional weaves and random fiber oriented mats. On the other hand, fibers can also vary in terms of length. Continuous and discontinuous (short) fibers are both utilized in clinical dentistry nowadays. Other properties of FRC in relation to the direction of fibers, like optical properties and bonding properties are also having their implications in dentistry, where transmission of polymerization light is a desired property. Due to bone like biomechanical properties and high toughness, FRCs have found their implications in bone surgery too: FRC with bioactive glass is already in clinical use as bone growth inducing implant for large bone defects in cranium. Biological mechanism of action of the implant is based ion dissolution from the bioactive glass which influence the cells differentiation to osteogenic cells and the simultaneous physicochemical modification of the FRC surface. Lecture will show clinical examples of the use of glass fibers in prosthetic applications, and give an insight to bioactive surgical FRC implants.
10:15 a.m. – 11:00 a.m.

Track01

**KdG AW02 | Klaas de Groot Award 2021**

**Chair**  
Mário A. Barbosa  
University of Porto, Porto, PT

**KdG AW02-01**

**Klaas de Groot Award: Some reflections on scientific progeny**

**Clemens A. van Blitterswijk**  
*Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, NL*

We are all products of our past. This is no different for scientists as for the species seen in Darwin’s tree of life. There is a difference still, a species is moulded over time by a combination of chance and the influence of its environment, on which it, usually, has little influence. A scientist, though, is a conscious being that should at least be partially able to select its own environment and possibly even its scientific ancestry, thus controlling its own development.

As the Klaas de Groot award is on mentorship and guidance I thought that, as its recipient, rather than highlighting some of our group’s research, it might be more interesting to reflect on how our scientific predecessors may and should, even today, still affect our careers and scientific choices. I will try to do this by mixing some personal anecdotes with historical examples and a few biological metaphors on scientific organizations.

Perhaps, these reflections may be of some use to younger scientists to make the right choices early in their career and not make the mistakes of their predecessors. For those for which this advice comes too late (my generation) it will at least recall some memories on the inspirational impact of Klaas de Groot on the early generation of biomaterial scientists.
11:30 a.m. – 1:00 p.m.

Track01

K06 | Biomimetic Nanomaterials and Biosensing Devices

Chairs
Nicholas Dunne  
Dublin City University, IE
Cristina C. Ribeiro  
INEB/i3S, INEB, Porto, PT
Ioannis Tsamesidis (YSF)  
Aristotle University of Thessaloniki, Dentistry, Thessaloniki, GR
Plastic antibodies for (bio)sensing

M. Goreti F. Sales

University of Coimbra, Chemical Engineering Department of the Faculty of Sciences and Technology, Coimbra, PT

(Bio)sensors have evolved in recent years and generally meet point-of-care (POC) requirements. They are compact analytical devices that can be built from low-cost materials in a suitable size and in a portable manner as required for POC applications. Electrochemical biosensors are at the forefront of such developments, easily recognisable from blood glucose monitoring strips. Optical biosensors are also popular devices, implemented in the population mainly through pregnancy tests and the rapid antigen/antibody tests in COVID-19 management.

In terms of development, biosensors require a biorecognition element that selectively binds to the target biomolecule. Various compounds can be used for this purpose, with antibodies playing the main role. Their operation is effective and similar to an ELISA test, but with a different transduction mode. However, all procedures for the production of natural antibodies in the laboratory are still very complex and expensive. They require highly skilled personnel, special handling and storage conditions, and complex facilities/equipment that often require the use of animals [1]. Moreover, handling antibodies in the commercial solutions is a difficult task. These biological materials are unstable and change their functionality due to slight variations in temperature, pH or solution composition. Antibodies may also become unusable once bound to the antigen, as this binding cannot be reversed without altering the 3D antibody assembly.

In contrast, plastic antibodies are synthetic “analogues” capable of selectively targeting a specific analyte, offering greater stability and lower cost than natural antibodies, as well as the possibility of scaling [2]. Such interaction between the antibody and its target analyte can even occur in direct contact with complex samples, avoiding sample pretreatment and opening doors to POC applications. The most successful synthetic route for obtaining plastic antibodies uses molecular imprinting technology [3], in which a polymeric material facilitates antigen-antibody interactions through simulated Fab regions. Briefly, an imprinted polymer is made by growing a rigid polymeric network (of individual monomers and crosslinkers to precisely tailor a molecular shape in a 3D imprinted site) around a target molecule; the imprinted sites are generated as the template emerges from the polymer matrix and theoretically matches the size and shape of the target.

Polymer antibodies have already been used in biosensors for a variety of biomolecules, such as various proteins that signal cancer or cardiovascular disease or extracellular vesicles, among others [4,5]. Several examples of these specific applications will be presented, coupled to the processes involved in their production. Interestingly, plastic antibodies also open up for a wide range of other applications in tissue engineering.

Acknowledgement

The author gratefully acknowledges funding from the European Commission through the project MindGAP (FET-Open/H2020/GA829040).

References


Nanodiamonds magnetometry for a real-time detection of free radical in sperm cells

Aldona Mzyk1,2, Claudia Reyes-San-Martin1, Thamir Hamoh1, Yue Zhang1, Runrun Li1, Alina Sigaeva1, Jakub Kawalko3, Romana Schirhagl1

1 University Medical Center Groningen/University of Groningen, Department of Biomedical Engineering, Groningen, NL; 2 Institute of Metallurgy and Materials Science, Polish Academy of Sciences, Krakow, PL; 3 AGH University of Science and Technology, Academic Centre for Materials and Nanotechnology, Krakow, PL

Introduction
The free radical theory of infertility is derived from the more general free radical theory of ageing. It states that free radicals are the reason why sperms become dysfunctional. Some free radicals are also needed to maintain crucial functions in sperms including sperm capacitation, the acrosome reaction, and sperm-oocyte fusion. However, despite their undeniable importance we know relatively little about where free radicals are generated exactly, which ones play a role or if we can alter their generation. Unfortunately, free radicals are short lived and thus very difficult to detect with the state of the art methods. As a result, detecting radicals is despite the enormous potential barely used as clinical marker.

Experimental Methods
We have used commercial 70nm oxygen terminated FNDs (Adámas Nano, Raleigh, NC, USA). Boar sperm commercially available from Varkens KI Nederland BV was separated using Ficoll gradient, and selected motile sperm cells were plated on fibronectin or hyaluronic acid coated dishes. Sperm cells were treated with FNDs suspension in Human Tubal Fluid (HTF) for capacitated state and modified-HTF (HTF medium without bicarbonate, calcium salts and serum) for uncapacitated state. Biocompatibility tests like MTT assay, DCFDA and membrane integrity assay was performed. Confocal and scanning electron microscopy were performed on fixed cells to characterize the location of FNDs on sperm cells. We have applied diamond magnetometry (the fluorescent nanodiamonds are used as sensors) to visualize free radical generation in real-time in single cells with nanoscale resolution, which is not possible with other methods. We have used our home-built magnetometers to perform a specific type of diamond magnetometry called relaxometry. These measurements reveal a signal, which is equivalent to T1 from conventional MRI but from nanometer sized voxels around the diamond particles (rather than millimeter to micrometer as in conventional MRI). T1 is a measure for spin noise and (after calibration) allows quantifying free radical generation.

Results and Discussion
In this studies we have developed novel approach to detect free radical in sperm cells. To prove our concept, we first tested the biocompatibility of FNDs and the effect of them on sperm cells metabolic activity, reactive oxygen species formation and membrane permeability. The results of this set of experiments indicated no significant difference compared to the control group for FNDs in both capacitated and uncapsacitated sperm cells. Confocal and scanning microscopy images have shown that FNDs attached preferentially to the head of sperm cells. The fluorescent nanodiamonds have been used in the relaxometry measurements to detect real-time changes of free radicals level in the sperm transition between the uncapacitated and capacitated stage. We have been tracking capacitation progress for two hours using one selected nanodiamond and observed gradual shortening of relaxation time. Decrease of T1 values corresponds to an increase in free radical concentration near the nanodiamond sensor, which was expected for the capacitation process. In the further experiments we have investigated how chemical stressors and antioxidants influence the free radicals generation by the sperms on a different maturation stages.

Page 653 of 2028
Conclusion
We have shown for the first time that fluorescent nanodiamonds can be used for innocuous selective labelling and localized, real-time measurements of free radical in single sperm cells using diamond magnetometry. Our research lay the foundation for further work with human clinical samples.

Acknowledgement
This research was funded by the ERC starting grant ERC-2016-StG-714289.

Figure 1. Free radicals detection in sperm with fluorescent nanodiamonds. Images of nanodiamonds (red spots) interacting with uncapacitated and capacitated sperm cells are shown in (A). (B) and (C) depict the results of free radical measurements before and after capacitation.
K06-03

A lab-on-a-chip approach for lung cancer assessment: Size-based enrichment of circulating tumour cells (CTCs) combined with circulating cell-free DNA (cfDNA) methylation analysis

Angela Carvalho¹²⁴, Gabriela Ferreira¹², Duarte Seixas¹²³, Vera Constâncio³⁴, Fernando J. Monteiro¹²⁵, Carmen Jerónimo³⁴⁶

¹ INEB-Instituto de Engenharia Biomédica, Universidade do Porto, Porto, PT; ² i3S-Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, PT; ³ Cancer Biology and Epigenetics Group, IPO Porto Research Center (GEBC CI-IPOP), Portuguese Oncology Institute of Porto (IPO Porto), Porto, PT; ⁴ Porto Comprehensive Cancer Center (P.CCC), Porto, PT; ⁵ Faculdade de Engenharia, Departamento de Engenharia Metalúrgica e Materiais, Universidade do Porto, Porto, PT; ⁶ Department of Pathology and Molecular Immunology, Institute of Biomedical Sciences Abel Salazar, University of Porto (ICBAS-UP), Porto, PT

Introduction

Lung cancer (LCa) remains the leading cause of cancer-related mortality, worldwide, with late diagnosis and limited therapeutic approaches still constraining patient’s outcome [1,2]. Liquid biopsies have been introduced as a minimally invasive way to retrieve multiple cancer biomarkers for tumor characterization and microfluidic devices have shown promising results regarding biomarkers isolation efficiency and specificity [3]. In this work, we proposed a microfluidic chip for size-based isolation of circulating tumour cells (CTCs) from plasma samples of LCa with downstream circulating cell-free DNA (cfDNA) methylation analysis.

Experimental Methods

The microfluidics layer was developed in polydimethylsiloxane (PDMS) via soft-lithography and bonded to coverglass by plasma cleaning, creating the final chip. A total of 22 LCa plasma samples (2mL) were processed through the chip and CTCs were isolated through size in chambers with sequential rows of micropatterns with varying interspacing, followed by cfDNA extraction from the processed plasma. CTCs were identified in-chip as DAPI+/EpCAM+/CD45− in non-small cell lung cancer patients (NSCLC) and DAPI+/CD133+/CD45− for small cell lung cancer (SCLC) samples. A collection of samples was further stained for Vimentin (VIM). Promoter methylation levels of ADCY4me, MIR129-2me and HOXA11me were assessed in ctDNA by multiplex quantitative methylation-specific PCR.

Results and Discussion

CTCs were detected in a total of 18 samples. CTC count increased according to disease progression, with a higher average count in late-stage samples (3.7 ±2.0) compared to a lower (1.3±1.8) in early-stage patients. A total of 8 DAPI+/EpCAM+/CD45− negative cells were identified, highlighting the limitations associated with EpCAM identification of CTCs in LCa.

Results regarding cfDNA extraction and quantification revealed that samples processed in-chip showed a tendency for higher DNA concentration than the respective unprocessed samples. A trend was also observed for higher cfDNA concentration in late-stage samples as well as higher number of methylated genes. HOXA11me displayed the highest individual LC detection.

In the 4 samples with no CTCs detection, at least one gene promoters’ methylation was identified in all early-stage samples (n=3) and cfDNA methylation of all 4 genes was observed in the late-stage sample (n=1).

Conclusion

The designed chip showed efficacy in CTCs isolation from a minute sample volume (2 mL), with increased detection in late-stage samples and higher cfDNA concentration levels and number of methylated genes. Plasma processing
through the microfluidic device allowed for a combinatorial analysis of both cancer biomarkers, showing a promising approach for LCa diagnosis and assessment.

Acknowledgement

This work was supported by FEDER funds through COMPETE (POCI-01-0145-FEDER-030831) and by Portuguese funds through Fundação para a Ciência e a Tecnologia (PTDC/BTM-TEC/30831/2017) in the framework of project TRIMARKCHIP. PCCC Beacon projects-2021 (Carmen Jeronimo & Fernando J. Monteiro).

References


Electrochemical behaviour of the nanotubular TiAlV surface in the presence of bacteria

Jaroslav Fojt, Eva Pruchova, Vojech Hybasek, Jitrenka Jiru, Ludek Joska

University of Chemistry and Technology in Prague, Department of Metals and Corrosion Engineering, Prague, CZ

Introduction
Biofilm-related infections (BRI) are an overwhelming issue for health care systems in Europe and worldwide. Common examples of biofilm-related infections include implant-related infections, like those that may follow joint replacement, osteosynthesis, mechanical heart valves, breast implants, indwelling catheters, ventriculo-peritoneal shunts etc. BRIs are characterized by chronic behaviour or frequent recurrences, antibiotic resistance, complex and prolonged treatment, poor prognosis, high social and economical costs and difficult diagnosis. In vitro experiments showed that young biofilm could be easily eliminated by antibiotic treatment compared to the matured biofilm. Therefore, early and aggressive antibiotic treatments are recommended for biofilm infections. However, early diagnosis of BRI is currently difficult and most of the clinical biofilm infections are actually matured biofilms that are usually difficult to eradicate with antibiotic treatment. The ability to acquire real-time measurements of environmental parameters of interest including within the implant surface in situ would significantly enhance the information available to medical professionals to detect and effect early inflammation. The titania nanotubes have a big ratio of the real/geometric area and thus with a relatively small dimension of the sensor provide a large active surface. This surface could provide changes in the electrochemical response, which reflects changes caused by bacteria presence.

Experimental Methods
The nanotubular surface of Ti-6Al-4V alloy was prepared by anodic oxidation in fluorides containing electrolyte and covered by several bacteria strains: E. Coli, S. Aureus and P. Aeruginosa. The sample with bacteria were cultivated in agar with nutrient and electrochemical impedance spectra, open circuit potential and polarisation resistance were measured throughout 48 hours exposure.

Results and Discussion
The presence of bacteria was detected during up to 24 hours. The open circuit potential decreases reflecting the oxygen consumption by bacteria. The electrochemical impedance spectra showed several frequency windows, which reflects processes on the surface. The first one was at high frequencies and corresponds to the fast reactions such as nitrate metabolism and also reflecting surface coverage by bacteria. The slow reactions were detected at middle-low frequencies. One of the possible reaction candidates is iron ions oxidation/reduction. The impedance spectroscopy showed increasing sensitivity to used bacteria strain E. Coli, P. Aeruginosa and S. Aureus, respectively.

Conclusion
The results indicate that nanostructured Ti-6-Al-4V surface should be a possible candidate for bacteria detection sensors.

Acknowledgement
The work was supported by the Ministry of Health of the Czech Republic, grant nr. NU20-06-00424.
Figure 1.
Normalised impedance response of the nanostructured surface covered by bacteria (left) and surface with E. coli after 50 hours cultivation.
Development of *in vitro* flow model to test antimicrobial coatings for blood-contacting materials

Juliane Valtin$^{1,3}$, Stephan Behrens$^2$, Manfred Maitz$^1$, Lars D. Renner$^1$, Frank Sonntag$^2$, Carsten Werner$^{1,3}$

$^1$Leibniz Institute of Polymer Research Dresden, Institute of Biofunctional Polymer Materials, Dresden, DE; $^2$Fraunhofer Institute for Material and Beam Technology IWS, Micro- and Biosystems Engineering, Dresden, DE; $^3$TU Dresden, Center for Regenerative Therapies Dresden, Dresden, DE

**Introduction**

Serious medical consequences and cost-intensive management of catheter-associated blood stream infections have led to an increased interest in antimicrobial coatings. Many candidate materials show promising performance in standard microbiological assays, but fail in clinical trials [1]. This circumstance highlights the need for more realistic *in vitro* models to test antimicrobial coatings in a relevant physiological environment. The realistic emulation of shear forces is of particular interest as bacterial adhesion and proliferation *in vivo* are strongly dependent on the hydrodynamic flow conditions.

**Experimental Methods**

*Flow model setup:* Our microfluidic model consists of two main components: the pumping chip connected to a controlling unit regulating the speed of the blood flow (previously described in [2]), and the microchannel chip for the detection of bacterial adhesion and proliferation (Figure 1). The microchannel chip is made of three layers: (1) the upper layer with Luer-Lock connections for in- and outlet (polycarbonate), (2) the middle layer which determines the shape of the channels (3M™ Adhesive Transfer Tape 94714 LE), and (3) the bottom layer with customizable surface material. Most experiments were performed with a bottom layer made of thermoplastic polyurethane.

*Incubation conditions:* Human whole blood, anticoagulated with 5 µM hirudin, was stored in a blood bag reservoir, slowly drawn through the microfluidic system and collected in small caps after passing the pumping and microchannel chip. The collected blood samples were analyzed for inflammation (e.g. complement component 5a) and hemostasis parameters (e.g. prothrombin fragment F1+2, platelet factor 4). Bacterial adhesion was detected via fluorescence microscopy through the microchannel chip. All experiments have been performed at 37°C and 5% CO$_2$ incubation. Pumping speed of the flow model can be adjusted in the range of 0.09 and 13.5 µL/s corresponding to wall shear stresses from 0.07 to 10.5 dyne/cm$^2$ (channel height of 300 µm).

*Antimicrobial surfaces:* The microchannel surfaces were coated with PEGylated copolymers which are further equipped with antimicrobial components (e.g. hexetidine).

*Bacteria:* Gram-positive bacterial species *Staphylococcus aureus* (RN4220) and Gram-negative bacterial species *Escherichia coli* (MG1655) were used for bacterial colonization. Both strains are labeled with green fluorescent protein. A bacterial solution (OD$_{600}$ = 0.1) was applied to microchannels before blood incubation for 1 hour at 37°C.

**Results and Discussion**

Initially, the flow model was optimized to reduce inflammation and hemostasis of pumped blood cells. By broadening in- and outlets, adjusting channel height and optimizing bonding procedure for Luer-Lock ports, hemocompatibility was improved significantly and is comparable to our well established quasi-static whole blood screening chamber [3].

Using the novel flow model, the antimicrobial efficiency of copolymer coatings was shown. Pre-tests with lysogeny broth media displayed a reduction of bacterial surface coverage up to 50 % for both bacterial strains. Whole blood...
experiments revealed lower inflammation response due to the reduced bacterial adhesion and proliferation on the surface. Fluorescence microscopy images of the microfluidic chip after incubation confirmed this observation.

**Conclusion**

The reported results suggest that our developed flow model allows evaluation of antimicrobial coatings under physiological conditions. By adjusting the wall shear stresses, the model can be adapted for defined test conditions.

**Acknowledgement**

Financial support from the Federal Ministry of Education and Research (Project RESPONSE - Partnerschaft für Innovation in der Implantattechnologie) is gratefully acknowledged.

---

**Figure 1**

Microchannel design with three parallel channels (A), layer construction of microchannel chip with Luer-Lock ports (B)

**References**


A Long-Range Sensing Mechanism of Cell Sensing of Substrate Nanotopography

Julien Gautrot, Stefania Di Cio

Queen Mary, University of London, School of Engineering and Materials Science, London, GB

Introduction
The mechanical properties and nanotopography of the extracellular matrix have an important impact on cell phenotype. Such physical cues have been shown to regulate cell adhesion and spreading, cell motility, proliferation and differentiation in a wide range of cells, stem cells and in cancer. However, detailed mechanisms underlying mechanical and nanotopography sensing remain unclear. We developed nanoscale engineered extra-cellular matrices to study such mechanisms. In particular, we show that focal adhesions, typically regarded as essential mechanosensors, are not primary sensors of the nano-scale geometry of the ECM and that the dynamics of the microscale acto-myosin network acts instead as a global sensor of the ECM nanoscale geometry.

Experimental Methods
We used nanopatterned polymer brushes to control the nanoscale geometry of the ECM and cell adhesions. We characterised cell and focal adhesion morphology via confocal microscopy. We studied actin dynamics via live imaging and study the dynamics of regulators of actin assembly and disassembly. We use engineered protein expression, siRNA and inhibitors to explore the imapct of focal adhesion formation, actin contractility and dynamics on nanotopography/nanogeometry sensing.

Results and Discussion
Here, we use nanotopographical patterns of polymer brushes generated via electrospun nanofibre lithography (ENL) to investigate the mechanisms of nanotopography sensing by cells. We observe the dysregulation of actin dynamics, resulting in the surprising formation of actin foci. This alteration of actin organisation is regulated by myosin contractility but independent of adapter proteins such as vinculin. This process is highly dependent on differential integrin expression as β3 integrin expressing cells, more sensitive to nanopattern dimensions than β1 integrin cells, also display increased perturbation of actin assembly and actin foci formation. We propose that, in β3 integrin expressing cells, contractility results in the destabilisation of nanopatterned actin networks, collapsing into foci and sequestering regulators of actin dynamics such as cofilin that orchestrate disassembly.

Conclusion
In contrast to the sensing of substrate mechanics and ECM ligand density, which are directly orchestrated by focal adhesion assembly, we propose that nanotopography sensing is regulated by a long-range sensing mechanism, remote from focal adhesions and mediated by the actin architecture.

Acknowledgement
Funding from the ERC (ProLiCell, 772462) is gratefully acknowledged.
Nanoscale sensing
Long range mechanisms for the sensing of ECM nanoscale topography

References
11:30 a.m. – 1:00 p.m.

Track02

**S09 | Role of Biomaterial Properties on Cell-Scaffold Interplay**

**Chairs**  
**Valeria Chiono**  
Politecnico di Torino, Department of Mechanical and Aerospace Engineering, Turin, IT  
**Alice Zoso**  
Politecnico di Torino, Dept of Mechanical and Aerospace Engineering, Torino, IT  
**Clara M. Guarch Perez (YSF)**  
Amsterdam UMC, University of Amsterdam, 1Department of Medical Microbiology and Infection Prevention, Amsterdam institute for Infection and Immunity, Amsterdam UMC, Amsterdam, NL

*Tissue engineering aims at the reproduction of cell-biomaterial interactions sensed by cells in vivo. The symposium will highlight recent evidences on the influence of biophysical and biochemical cues of biomaterials in mediating cell phenotype or response to treatment. Such knowledge is fundamental for tissue regeneration and modelling.*

*The keynote speaker will introduce the subject of the symposium, by highlighting how biomaterials properties may affect the mechano-chemical processes occurring at cell-biomaterials interface. The selected abstracts will cover innovative approaches in studying biomaterial impact on cell fate and behaviour, covering different biomaterials composition and architectures and different cell types.*
S09-KL01

Mechanochemical processes at the cell/material interface

Manuel Salmeron-Sanchez

University of Glasgow, Centre for the Cellular Microenvironment, Glasgow, GB

The physical properties of the extracellular matrix (ECM) and the use of growth factors are powerful tools to control cell behaviour, including fundamental processes such as cell migration and (stem) cell differentiation. Integrins are mechanotransducers that feel and respond towards the viscoelastic properties of the ECM. Growth factors are important molecules that trigger signalling cascades that control e.g. osteogenesis and vascularisation. The field has moved from soluble administration/release of growth factor from biomaterials to solid-phase presentation of growth factors, recapitulating aspects of the ECM. We have developed material systems that allow simultaneous stimulation of integrins and growth factors receptors. We have engineered polymers and 3D hydrogels that unfold and assemble proteins to allow exposure of the integrin and growth factor binding regions. For example, we show the use of BMP-2 in synergy with α5β1 integrins to promote osteogenesis and regeneration of critical-sized defects and VEGF to promote vascularisation. Using laminin-based hydrogels we have demonstrated the combined effect with NGF to trigger neural processes.

In parallel, we have also engineered dynamic systems that allow control over temporal release of adhesion molecules and growth factors. Unconventionally, we have used non-pathogenic bacteria in co-culture with stem cells that have been engineered to control release of a fibronectin fragment and BMP-2 in a dynamic way. We present bacteria-based materials in which symbiotic bacteria/mammalian cell interactions occur and their use for stem cell engineering. We have first genetically modified L. lactis to produce a fibronectin fragment (FNIII7-10) that allows integrin binding and cell adhesion to the bacteria biofilm and then stimulation of osteogenesis by inducing the expression of BMP-2 in a dose-controlled manner.

Acknowledgement
Support from the EPSRC Programme grant (EP/P001114/1) is acknowledged.

References
Force-FAK Signaling Coupling at Individual Focal Adhesions Coordinates Mechanosensing and Microtissue Repair

Marc A. Fernandez-Yague1,7,8, Dennis W. Zhou1,7,8, Elijah N. Holland1,7,8, Jeroen Eyckmans4, Christopher S. Chen4,5, Jianping Fu3, David D. Schlaepfer2, Andrés J. García1,7,8

1 Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA, Atlanta, US; 2 Moores Cancer Center, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Diego, La Jolla, CA 92093, USA, San Diego, US; 3 Department of Mechanical Engineering, Department of Biomedical Engineering, Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI 48109, USA, Ann Arbor, US; 4 Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA, Boston, US; 5 Department of Biomedical Engineering, Boston University, Boston, MA 02215, USA, Boston, US; 6 School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA, Atlanta, US; 7 Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA, Atlanta, US; 8 Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332, USA, Atlanta, US

Introduction

How adhesive forces are transduced and integrated into biochemical signals at focal adhesions (FAs) is poorly understood. Using cells adhering to deformable micropillar arrays, we demonstrate that traction force and FAK localization, as well as traction force and Y397-FAK phosphorylation, are linearly coupled at individual FAs on stiff, but not soft, substrates. Similarly, FAK phosphorylation increases linearly with external forces applied to FAs using magnetic beads. This mechanosignaling coupling requires actomyosin contractility, talin-FAK binding, and full-length vinculin that binds talin and actin. Using an in vitro 3D biomimetic wound healing model, we show that force-FAK signaling coupling coordinates cell migration and tissue-scale forces to promote microtissue repair. A simple kinetic binding model of talin-FAK interactions under force can recapitulate the experimental observations. This study provides insights on how talin and vinculin convert forces into FAK signaling events regulating cell migration and tissue repair.

Experimental Methods

Cells: eGFP-WT vinculin, eGFP-VH vinculin, eGFP-A50l-vinculin, and vinculin-null MEFs were generated. WT GFP-FAK, E1015A GFP-FAK, K454R GFP-FAK (kinase-dead), Y397F GFP-FAK, and FAK-null MEFs were derived by transducing FAK−/− MEFs with lentiviral constructs for FAK variants.

mPADs analyses: Microfabricated post array detectors (mPADs) device silicon masters were fabricated using PDMS replica molding.

Western blotting: Equal amounts of protein (10 μg) were boiled (95° C) in Laemmli sample buffer for 5 min, separated by SDS-PAGE. Membranes were incubated with antibodies overnight at 4°C and imaged with a LI-COR Odyssey Imager (LI-COR Biosciences).

Magnetic bead experiments: Fibronectin was coupled to tosyl-activated paramagnetic Dynabeads M-450 (At 6 mm distance, the force on a single bead was 30 pN or 10 pN

Microtissue fabrication and experiments: Cells (1.2 x 10⁶) were suspended in 1.75 mg mL⁻¹ neutralized rat tail collagen type I (Ibidi) and seeded in the device.

Results and Discussion

By analyzing individual FAs in cells adhering to deformable micropillar arrays, we demonstrate that traction force and FAK localization and traction force and Y397-FAK phosphorylation are linearly coupled at individual FAs on stiff, but not soft, substrates. Importantly, disruption of talin binding to FAK using the E1015A FAK mutant eliminates force-
FAK localization and force-Y397-FAK phosphorylation. We further demonstrate that force-FAK coupling regulates two important cellular responses: (i) signaling in response to applied external forces using magnetic beads, and (ii) coordinated cell migration and tissue-scale forces driving microtissue repair using an *in vitro* 3D biomimetic wound healing model of fibrous tissue. Consistent with the results on micro post arrays, mutation of talin-binding site in FAK significantly disrupts these two cellular processes. Using a conventional wound scratch assay, Lawson et al. reported reduced wound closure for FAK E1015A-expressing cells compared to wild-type FAK-expressing cells, and no differences between FAK E1015A-expressing cells and FAK-null controls [1]. In contrast, fibroblasts migrating in 3D collagen matrices do not display a lamellipodium, and migration is driven by mechanosensitive proteins that rely on actomyosin contractility [2]. We used a biomimetic 3D *in vitro* model of fibrous tissue repair with cells dispersed within a collagen construct in which wound healing involves coordinated tissue-scale forces, matrix assembly, and cell migration to restore 3D tissue architecture. In contrast to the reduced cell migration reported by Lawson et al., we observed increased cell speed, straightness index, persistence time, and diffusivity for FAK E1015A-expressing cells compared to wild-type FAK-expressing cells. Furthermore, we observed higher contractile forces in microtissues containing wild-type FAK-expressing cells compared to microtissues seeded with FAK E1015A-expressing cells. Finally, we present a simple kinetic binding model of talin-FAK binding interactions under a force that recapitulates the experimental observations. Our model proposes force-dependent structural changes in talin that regulate its interaction with FAK. Although talin at FAs is stretched by forces [3,4] and stretching modulates vinculin binding [5] to our knowledge, there is no direct experimental evidence showing that FAK binding to talin requires a mechanical stretch.

**Conclusion**

These experimental and modeling analyses provide insights into how talin, FAK, and vinculin convert forces into early signaling events regulating mechanotransduction. This conceptual framework is relevant to adhesive force-signaling coupling at migratory cell fronts, force-regulated morphogenesis, and stem cell lineage commitment in response to matrix stiffness. Furthermore, this fundamental understanding of mechanosignaling can ultimately be exploited to design cell-biomaterial interactions.

**Acknowledgement**

We acknowledge support from the National Institutes of Health Award No. R01 EB024322 (A.J.G.), R01 CA102310 and R01 CA247562 (D.D.S.), MSCA-H2020 898737 (M.F.Y) and R21 EB028491 (J.E.), and the NSF Science and Technology Center for Engineering Mechanobiology (C.S.C.).
Force-FAK localization and force-pY397-FAK are linearly coupled at individual FAs.

a, Schematic of individual FA traction force measurement for a cell using mPADs. Fluorescence images showing (b) mPAD posts, (c) tFAK, (d) pY397-FAK, and (e) eGFP-vinculin for cell. f-i, Heatmaps for (f,h) tFAK (g,i) pY397-FAK staining at FAs. j, Force (mean ± SD) tFAK integrated intensity (mean ± SD). m, Force (mean ± SD) tFAK integrated intensity (mean ± SD) at FAs on 14 kPa mPADs. n, Force (mean ± SD) tFAK integrated intensity (mean ± SD) at FAs on 14 kPa mPADs. o, Force vs tFAK intensity at individual FAs on 14 kPa. p, Force vs tFAK intensity at individual FAs on 5 kPa. q, Force vs pY397-FAK intensity at individual FAs on 5 kPa. r, Force vs tFAK intensity at individual FAs on 14 kPa.

FAK-talin binding regulates wound closure of 3D microtissues.

a, Schematic of microtissue assembly and wound closure assay. b, Wound area (top) and log-normal curve fit for wound area normalized to initial area (bottom) as a function of time for individual microtissues. c, Amplitude (mean ± SD) of normalized wound area profile. d, Closure rate (mean ± SD) of normalized wound area profile. e, Net force difference (mean ± SD) b-f. Microtissue width contraction (mean ± SD) g, pY397-FAK integrated intensity (mean ± SD) h, Brightfield and fluorescence i, Intensity heatmaps (DNA, tFAK, pY397-FAK, F-Actin, IgG control) for microtissues.

References
The mechanobiology of (de)myelination - a novel tissue engineered model to dissect the impact of ECM mechanical properties alterations on oligodendrocyte differentiation

Eva D. Carvalho¹,², Miguel R.G. Morais¹, Helena Ferreira¹,³, Hendrik Hubbe⁴, Marco Araújo¹, Eduardo Mendes⁴, Cristina C. Barrias¹,³, Ana P. Pêgo¹,²,³

¹ i3S|INEB - Instituto de Investigação e Inovação em Saúde | Instituto de Engenharia Biomédica, Porto, PT; ² FEUP - Faculdade de Engenharia da Universidade do Porto, Porto, PT; ³ ICBAS - Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, Porto, PT; ⁴ TU Delft - Delft University of Technology, Chemical Engineering Department, Delft, NL

Introduction
With over 2.5 million people affected worldwide, multiple sclerosis (MS) represents a serious health, economic and social burden with no long-term suitable treatment. Remyelination failure in MS is a longstanding problem that remains to be solved. The formation of a glial scar containing reactive astrocytes overexpressing altered extracellular matrix (ECM) proteins contributes to a complete alteration of the microenvironment surrounding these cells. We hypothesize that oligodendrocytes (OLs) respond to these dynamic biomechanical changes altering their differentiation capacity, and in turn the remyelination is compromised. By tuning glial scar-mechanosensing mediated pathways one expects to be able to promote remyelination.

Here we propose a novel fully three-dimensional tissue-engineered model to study the impact of mechanical properties changes of the ECM on OL differentiation. The platform consists of an array of polydimethylsiloxane (PDMS) microstructures with biologically relevant dimensions and low stiffness designed to act as surrogate axons, embed within a modified alginate (ALG) matrix to fully recreate the 3D environment of the ECM. PDMS possesses advantageous characteristics as optical transparency that allows the user to follow live myelination, biocompatibility, and easiness of production. ALG was chosen as the polymer to mimic the extracellular milieu due to its biocompatible, non-toxic and tunable matrix mechanical properties.

Experimental Methods
PDMS microstructures were fabricated through replica moulding by mixing PDMS base and curing agent in a weight ratio of 10:1. PDMS stiffness was varied by adding to the PDMS mixture a low viscosity PDMS.

ALG hydrogels were produced by combining modified ALG formulations containing the cell adhesive peptide RGD (GGGGRGDSP) or the matrix metalloproteinase sensitive peptide PVGLIG (GGYGPVG↓LIGGK). Primary rat oligodendrocyte precursor cells (OPCs) growth and differentiation were optimized by varying the alginate content (0.5–1.5 wt/v%), PVGLIG (130–400 µM) and RGD concentration (40–200 µM) and polymer chain oxidation status.

Results and Discussion
Axon surrogates were produced using a low viscosity PDMS, corresponding to an advance in the creation of structures with a great aspect ratio and reduced stiffness. OPCs not only adhere and differentiate but also generate spread myelin sheaths when cultured on the PDMS microstructures. Interestingly automated image analysis (using open-source image analysis software – Fiji, Ilastik and Cell Profiler) suggested that softer PDMS structures (Young’s modulus of 1250 kPa vs 364 kPa) promoted higher expression levels of OL differentiation markers.

Alginate hydrogels were non-toxic to OPCs and differentiation status was favoured in matrices with high PVGLIG and oxidation content (Figure 1). The impact of the mechanical properties’ changes on OL differentiation was assessed by culturing OPCs in increased alginate content hydrogels (1%, 2% and 3% wt/v). OL metabolic activity and differentiation (assessed by the expression of the myelin basic protein, MBP) was favoured in softer matrices.
(shear modulus, $G^* \sim 100$ Pa) in comparison with low expression of MBP and decreased cell volumes for $G^*$ around 350 Pa and 1300 Pa. Additionally, impaired OL differentiation was verified in hydrogels with similar stiffness values but with increased stress-relaxation times, which indicates an enhanced cellular behaviour in matrices with augmented capability of dissipating cell-induced forces.

To recreate a glial scar in vitro, primary rat astrocytes were also embedded within the ALG matrices and found to extend long processes, with increased cellular complexity for high PVGLIG matrices (Figure 1). Activation with LPS/IFN-γ lead to an increase of Lcn2 and Col4 expression, strongly indicating that astrocytes acquire an astrogliosis-like phenotype.

**Conclusion**

In this work we have established an in vitro platform that can function as a toolbox to dissect (de)myelination processes occurring under differential mechanical conditions. OLs respond to alginate matrices mechanical properties alterations (stiffness and stress-relaxation properties) by changing their differentiation pattern. By combining the culture of OLs and activated astrocytes within the ALG hydrogels we are expecting to understand the crosstalk between these cells and investigate the effects of matrix alterations provoked by astrogliosis processes on OL differentiation.

**Acknowledgement**

The authors acknowledge the funding from projects UTAPEXPL/NTec/0057/2017 (FCT - UT Austin Portugal Program) and GRANT13074566 (Air Force Defense Research Sciences Program, USA). E. D. Carvalho acknowledges FCT from her Ph.D. fellowship (grant SFRH/BD/140363/2018).
Influence of culture substrates on morphology and function of pulmonary alveolar cells in vitro

Chiara Emma Campiglio1, Marina Figliuzzi2, Sara Silvani2, Matteo Tironi2, Sara Conti2, Federica Boschetti3, Andrea Remuzzi1

1 University of Bergamo, Department of Management, Information and Production Engineering, Dalmine, IT; 2 Istituto di Ricerche Farmacologiche Mario Negri-IRCCS, Department of Biomedical Engineering, Bergamo, IT; 3 Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering, Milan, IT

Introduction

Cell’s microenvironment has been shown to exert influence on cell behavior. In particular, matrix stiffness and cell interaction strongly impacts cell morphology and function. The alveolar epithelium is a dynamic tissue normally undergoing a slow, but constant renewal [1]. It is predominantly composed of alveolar Type I (ATI) and Type II (ATII) epithelial cells. ATII pneumocytes basically secrete the surfactant proteins implied in the regulation of alveolar surface tension during gas exchange, in alveolar fluid balance and in host defense. However, isolated ATII pneumocytes cultured in vitro lose their specific features and acquire ATI epithelial cell characteristics, stopping surfactant production [2]. Continuous cell lines are an alternative to primary ATII epithelial cell cultures. A representative cell system used to investigate ATII epithelial cells is the A549 lung epithelial adenocarcinoma cell line. A549 cells are usually cultured in conventional two-dimensional (2D) systems. Under such conditions, A549 cells flatten, lose differentiated Type II cell morphologic characteristic, and stop synthesis and secretion of biochemical markers of surfactant.

The purpose of this study was to analyze the influence of different culture substrates on phenotype and functional properties of A549 cells in order to understand in which in vitro culture condition these cells are able to acquire and maintain ATI or ATII phenotypes.

Experimental Methods

Two different substrates were used for A549 cell culture: a rigid surface of cell culture treated Thermanox™ Coverslips, and a polydimethylsiloxane (PDMS) artificial elastic membrane (Elastosil® Film). Firstly, the two substrates were characterized by a tensile testing and, to provide a more suitable substrate for cell adhesion, they were coated with fibronectin. For cell culture experiments, A549 cells were seeded at concentration of 1 x 10⁴ cells/cm² on Thermanox™ Coverslips and Elastosil® Film, and maintained in culture for 72 h. Metabolic activity was quantified by resazurin assay, while the morphological characterization was performed by optical and scanning electron microscopy (SEM). In order to characterize A549 cell functional changes, an immunofluorescence analysis was performed for YAP-1 and surfactant protein C expression, focal adhesion and actin filaments organization.

Results and Discussion

A549 cells were successfully seeded onto Thermanox™ Coverslips and Elastosil® Film, substrates with elastic modulus of 1,350 ±290 and 0.85 ±0.01 MPa, respectively (Figure 1A). The resazurin assay showed that there are no statistically significant differences in the metabolic activity measured on cells seeded on different substrates (Figure 1B). However, the two substrates influenced cell morphology and the actin cytoskeleton organization. The morphology and distribution of A549 cells on Thermanox™ Coverslips and Elastosil® Film substrates were examined by SEM analysis. We observed that cells grown on Thermanox™ Coverslips remained flat, expressing a low number of microvilli while cells cultured on Elastosil® Film maintained the typical ATII cell morphology, with rounded shape and large number of microvilli on cell surface (Figure 1C-D). The different distribution of F-actin filaments was also associated with a different expression of focal adhesions identified in A549 cells cultured on Thermanox and Elastosil.
substrates. The acquired sections (z-axis) confirmed a different profile in cell organization: cells remained round-shaped on Elastosil® Films (19 μm thickness), while appeared flat in a thinner layer on Theranox™ Coverslips (9 μm thickness) (Figure 2). Further, the Yes-associated protein (YAP) and its transcriptional coactivator PDZ-binding motif (TAZ) were translocated to the nucleus in A549 cells cultured on the more rigid polyester substrate, yet it remained mostly cytosolic in cells on soft PDMS substrate. Immunofluorescence staining for surfactant-C revealing a high expression of surfactant-C in cells cultured on Elastosil® Film, but not in cells cultured on Theranox™ Coverslips. Therefore, A549 cells grown onto soft Elastosil® Film exhibited morphology and functionality that suggest retention of alveolar epithelial Type II phenotype, while A549 cells grown onto more rigid substrates acquired an alveolar Type I phenotype.

Conclusion
The presented results suggest that different substrates induced important changes in cell morphology and cytoskeleton organization. A stiffer substrate led to nuclear YAP/TAZ formation in lung epithelial cells and to a reduction of surfactant C expression, inducing cells to acquire ATI phenotype, while soft substrate induced to retain an ATII phenotype. The results from this study bring us a step closer to understand how cell substrate and, in particular, substrates stiffness influence morphology and function of alveolar epithelial cells.

References
The Visco-Elasticity of 2D Protein Networks Regulates Stem Cell Expansion at Liquid-Liquid Interfaces

Julien Gautrot, Dexu Kong, Lihui Peng

Queen Mary, University, School of Engineering and Materials Science, London, GB

Introduction
The mechanical behaviour of the extracellular matrix has an important impact on cell phenotype. Despite the importance of mechanotransduction in regulating a wide range of phenotypes, we recently reported the surprising observation that cells (keratinocytes and mesenchymal stem cells) can adhere, spread and proliferate at the surface of liquids\(^1\)-\(^3\). This observation is particularly surprising as the reinforcement of cell adhesion is thought to require a solid elastic or viscoelastic substrate that can resist cell-mediated contractile forces. Our work has evidenced the formation of protein nanosheets, self-assembled at the liquid-liquid interface, displaying strong mechanical properties that can provide a sufficient mechanical scaffold to promote cell adhesion and expansion. We showed that this is sufficient to regulate stem cell phenotype. However, the parameters controlling the self-assembly and the mechanical properties of protein nanosheets remain poorly understood. In this work we investigate the assembly of polymers and proteins at liquid-liquid interfaces, and the impact of pro-surfactants with a wide range of chemistries. We identify structural features that control the visco-elastic properties of the resulting nanosheets and regulate associated cell phenotype.

Experimental Methods
Assembly at liquid-liquid interfaces is studied using interfacial rheology. Protein nanosheets are characterised by scanning electron microscopy, atomic force microscopy and X-ray photoelectron spectroscopy. Cell adhesion and phenotype was characterised by fluorescence microscopy and qPCR.

Results and Discussion
In this work, we show the importance of parameters such as pH and concentration on protein self-assembly and the impact it has on interfacial mechanics. Importantly, we demonstrate the impact that pro-surfactant-protein interactions play on regulating the assembly and the interfacial mechanical properties of the corresponding interfaces. In addition, we show how these parameters regulate interfacial viscoelasticity over a wide range, and ultimately regulate cell adhesion and proliferation. Finally, we demonstrate the proof-of-concept of using such liquid substrates, in the form of emulsions, for stem cell culture in 3D bioreactors, and their simple recovery by centrifugation.

Conclusion
Overall, our results suggest that nanoscale mechanical properties of biomaterials may dominate over bulk physical properties. This concept has important implications for the design of biomaterials in the field of regenerative medicine and allow the rational design of liquid substrates for tissue engineering.

Acknowledgement
Funding from the Leverhulme Trust (RPG-2017-229, Grant 69241), the ERC (ProLiCell, 772462) and the China Scholarship Council (201708060335) is gratefully acknowledged.
Biomicrodroplet
MSCs culture on microdroplets stabilised by protein nanosheets

References
Light-responsive cell culture platform combines mechanical topography with protein patterns to guide cell alignment

Mari Isomäki¹, Chiara Fedele¹, Elina Mäntylä², Soile Nymark², Teemu O. Ihalainen², Arri Priimagi¹

¹ Tampere University, Faculty of Engineering and Natural Sciences, Tampere, FI; ² Tampere University, BioMediTech and Faculty of Medicine and Health Technology, Tampere, FI

Introduction
Reproducing interaction between cells, and between cells and extracellular matrix (ECM), remains a challenge when developing biomimetic materials. In vivo, cells are surrounded by a constantly changing microenvironment, which regulates many cell functions such as differentiation, migration and cell death.¹ Without the natural microenvironment, some of the biological cell functions can be lost or altered, thus there is a need for developing novel materials for improved in vitro models.² Smart stimuli-responsive materials are an emerging group of materials, which can react to external stimuli. Light is an interesting energy source for stimuli-responsive materials, as it can be localized over a substrate, it is non-invasive, and its properties can be finely tuned. One of the primary molecules used for photosensitive materials are azobenzenes. The molecular process behind the photoinduced motions in azobenzene-based systems is the isomerization of the azobenzene molecule between the thermodynamically stable trans isomer and metastable cis isomer. Azobenzenes can be incorporated into glass-forming materials, which can be surface patterned via light-induced mass migration to form sinusoidal surface relief gratings (SRGs).³ The aim of this research is to combine the dynamic nature of living tissue with in vitro cell culturing. Here we have developed a dynamic platform, which enables the control of cell alignment and migration in a non-invasive way.

Experimental Methods
Disperse Red 1-glass (DR1g) was spin coated on clean glass coverslips to obtain a 500 nm-thick layer. Then, a solution of Sylgard 184 was diluted in n-hexane and spun over DR1g to form DR1g-PDMS bilayer. Samples were photopatterned using laser interference lithography (488 nm light), and the formation and erasure of SRGs on the bilayer was studied with diffraction measurements and with atomic force microscopy (AFM). Microcontact printing (μCP) technique was used to print FITC-conjugated gelatin and collagen I on flat and surface patterned bilayer surface. Cell alignment on the bilayer was studied with Madin-Darby canine kidney type II epithelial cells (MDCK II), which were seeded on top of surface and protein patterned samples and cultured for 72h. Microcontact-printed samples were passivated with Pluronic F-127. The alignment of MDCK II cells was analyzed with confocal microscopy and image analysis.

Results and Discussion
The results show that SRGs were efficiently produced on DR1g-PDMS bilayer. Compared to plain DR1g film, the SRG formation and erasure on the bilayer was slower and the dynamics depended on the PDMS film stiffness. MDCK II cells cultured on DR1g-PDMS bilayer with SRGs showed elongated morphology after 24h from cell seeding (Figure 1a), as confirmed by image analysis conducted on focal adhesions. The focal adhesions were oriented in the same direction as SRGs, whereas on flat areas they had a random orientation. 72h post-seeding focal adhesions were still oriented along the SRGs, thus the SRG topography provides strong guidance for the cells. These results are consistent with our previous studies.⁴ DR1g-PDMS bilayer was also chemically patterned with proteins, to assess the chemical functionalization of the platform.⁵ Protein patterns with uniform and micropatterned areas were achieved. MDCK II cells were seeded on top of the protein patterned DR1g-PDMS bilayer and their attachment and alignment on the pattern was studied (Figure 1b). Cells adhered well on the protein patterned areas and aligned according to the pattern (24h post-seeding), until cells spread to the passivated areas (72h post-seeding). To study
the different effect of topographical and chemical cues over cell migration, MDCK II cells were also seeded on bilayer with combined surface topography and protein pattern. Cells attached to areas with the protein pattern, however, after 24h from cell seeding the cells aligned along the topography rather than the protein pattern. This indicates that the surface pattern is dominating the cell orientation over the protein pattern in longer cell culturing times, probably due to protein adsorption from cell medium and passivation reduced effect. The results showed that the DR1g-PDMS bilayer can be surface patterned dynamically and further modified by protein patterning, which enables the tunability of the platform for different applications.

Conclusion
Azobenzene-containing DR1g was chosen as light-responsive material to study collective cell migration on purely topographical versus purely chemical cues. The presence of a biocompatible PDMS coating did not inhibit the SRG formation and erasure but altered the dynamics. Based on the results, the light patterned DR1g-PDMS bilayer can guide cell alignment and, interestingly, the topographical signal was able to drive cell migration for longer culture times. The possibility to control material properties non-invasively and remotely with light will open new ways to design next-generation smart materials for biomedical research.

![Figure 1](attachment:image)

**References**


Clickable microgels with hydrophobic topographical domains and in situ tunable matrix properties as dynamic cellular microenvironments

Mariana I. Neves¹,²,³, Ana L. Torres¹,², Lorenzo Moroni⁴,⁵, Cristina C. Barrias¹,²,⁶

¹ Universidade do Porto, i3S - Instituto de Investigação e Inovação em Saúde, Porto, PT; ² Universidade do Porto, INEB - Instituto de Engenharia Biomédica, Porto, PT; ³ Universidade do Porto, FEUP - Faculdade de Engenharia, Porto, PT; ⁴ Maastricht University, MERLN - Institute for Technology-Inspired Regenerative Medicine, Maastricht, NL; ⁵ Università del Salento, CNR NANOTEC - Institute of Nanotechnology, Lecce, IT; ⁶ Universidade do Porto, ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Porto, PT

Introduction

The extracellular matrix (ECM) is a key component of a cell microenvironment, regulating many cellular processes through biochemical and mechanical signaling. Hydrogel systems have been widely used to build artificial cellular niches, which should allow mimicking both the structure and the dynamic properties of the native ECM. Strain-promoted azide-alkyne cycloaddition (SPAAC) is a biorthogonal conjugation strategy, involving a specific reaction between strained cyclic ring-containing alkynes and azides, which proceeds under mild, copper-free, biocompatible conditions. For this reason, SPAAC has been widely used for biomolecules and living cells labelling [1]. While in polymer chemistry SPAAC emerged as an attractive strategy for (bio)functionalization and/or network formation [2,3], here, we further exploited the ability of pendant cyclooctyne groups to undergo physical crosslinking via hydrophobic interactions. By taking advantage of this feature, we formulated dual-crosslinked alginate microgels that present hydrophobic microdomains and can be functionalized with azido-functionalized compounds, in situ and in the presence of cells, in a time-controlled fashion. This microgel system provides topographical cues to entrapped cells and may sequester molecules with hydrophobic moieties, while allowing on-demand dynamic switch of matrix properties.

Experimental Methods

Ultra-pure alginate was functionalized with a cyclooctyne (BCN-amine) by carbodiimide chemistry, and the modification degrees of alkyn-agarates (ALK) were assessed by ¹H NMR. ALK hydrophobicity and hydrophobic-driven self-assembly potential were characterized by contact angle measurements, hydrophobic probes (ANS and Coomassie), scanning electron microscopy and viscometry. ALK microgels (acellular and cell-laden) were prepared by ionic crosslinking with Ca²⁺ using a coaxial airflow droplet extrusion device [4]. SPAAC conjugation with azide-functionalized compounds was performed at 37°C, in pre-gel solutions (0.9% w/v NaCl) or pre-formed microgels (culture medium). Grafting kinetics and efficiency were estimated using fluorescent azido-tags. Clickable microgels laden with mesenchymal stem cells (MSC) were analyzed throughout culture for cell number, viability (live/dead assay) and metabolic activity (resazurin assay).

Results and Discussion

ALK derivatives with varying modification degrees were successfully produced. Expectably, as the content of grafted hydrophobic octyne moieties increased, ALK derivatives become less hydrophilic, showing decreased wettability, stronger interactions with hydrophobic probes and increased viscosity in aqueous solutions. Also, at higher modification degrees, ALK derivatives become less water soluble and showed ability to spontaneously establish concentration-dependent physical cross-linkages between polymer chains, via pendant cyclooctyne groups, forming filamentous structures. By taking advantage of this property, and further promoting secondary ionic crosslinking, we were able to produce dual-crosslinked ALK microgels with structured hydrophobic microdomains. These not only add topographical features to the otherwise smooth bulk hydrogel, but also provide binding regions for sequestering...
compounds with hydrophobic sites, such as proteins, as verified using an extrinsic hydrophobic fluorescent probe. The ability to multi-functionalize the clickable ALK microgels, in situ and on-demand, was confirmed by performing consecutive SPAAC conjugations with sequentially added azido-functionalized florescent tags of different colors. The kinetic profiles demonstrated that the reactions proceeded rapidly (less than 30 min) and efficiently, under physiological conditions (i.e., in culture medium at 37ºC). The live/dead and resazurin assays performed after SPAAC reaction on MSC-laden ALK microgels showed that cells remained viable and metabolically active throughout 14 days of culture, confirming the cytocompatibility and specificity of the strategy. In situ SPAAC conjugation with fluorescent azido-tags could be successfully performed at different time points, proving that a fraction of octyne groups remained reactive, enabling temporal control of the hydrogel matrix throughout the culture.

**Conclusion**

By taking advantage of the intrinsic hydrophobicity of cyclooctyne groups, which can be conjugated with azido-conjugated compounds via SPAAC, we successfully formulated dual crosslinked microgels with topographical hydrophobic domains that can be (bio)functionalized, on-demand, in the presence of cells.

**Acknowledgement**


**References**


11:30 a.m. – 1:00 p.m.

Track03

S10 | Advances in Functionalization and Fabrication of Gelatin Hydrogels for Biomedical Applications

Chairs
- Silvia Faré
  Politecnico di Milano, Dept Chemistry, Materials and Chemical Engineering, Milan, IT
- Nicola Contessi Negrini
  Imperial College London, London, GB
- Susanne Heid (YSF)
  Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, DE

The presentation will provide an overview of pros and cons associated with crosslinking chemistries, both from a processing (laser- versus extrusion-based 3D-printing and electrospinning) and an application perspective. Furthermore, the use of crosslinkable gelatin in vivo will be reported along with the trajectory towards Phase I clinical trials.

The Keynote Speaker will discuss advanced chemistries and fabrication technologies to prepare gelatin scaffolds. The other four speakers will be selected to present specific chemistries and/or fabrication technologies to regenerate different tissues, aimed at clinical applications or advanced in vitro models, to capture the interest of a wide audience.
S10-KL01

Crosslinkable gelatins - From Innovative Chemistry and Valorization of Bioinks towards Human Trials: Utopia or Reality?

Lana Van Damme$^{1,2}$, Jasper Van Hoorick$^1$, Phillip Blondeel$^2$, Peter Dubruel$^1$, Aleksandr Ovsianikov$^3$, Sandra Van Vlierberghe$^{1,4}$

$^1$ Ghent University, Polymer Chemistry & Biomaterials Group, Ghent, BE; $^2$ Ghent University Hospital, Department of Plastic & Reconstructive Surgery, Ghent, BE; $^3$ Technische Universität Wien (TU Wien), 3D Printing and Biofabrication Research Group, Vienna, AT; $^4$ Vrije Universiteit Brussel, Brussels Photonics, Brussels, BE

Biofabrication is a specific area within the field of tissue engineering which takes advantage of rapid manufacturing (RM) techniques to generate 3D structures which mimic the natural extracellular matrix (ECM). A popular material in this respect is gelatin, as it is a cost-effective collagen derivative, which is the major constituent of the natural ECM. The material is characterized by an upper critical solution temperature making the material soluble at physiological conditions. To tackle this problem, covalent crosslinking is realized by functionalizing gelatin with (photo-)crosslinkable functionalities. In this respect, methacrylamide-functionalized gelatin (gel-MA/gel-MOD) currently is the gold standard. However, gel-MA poses some important limitations with regard to network homogeneity and processing capabilities exploiting laser-based additive manufacturing (AM). As a result, we have developed mono- and bifunctional (patented) crosslinkable gelatin derivatives which offer distinct advantages (e.g. solubility at room temperature) for various AM techniques. The hydrogels support processing with digital light projection, multiphoton lithography (2PP) and extrusion-based additive manufacturing. Furthermore, they are suited to encapsulate a.o. adipose tissue-derived stem cells and to support differentiation into different lineages (e.g. adipogenic and osteogenic). The gelatin derivatives offer a unique opportunity to serve a plethora of tissue engineering (TE) applications (including ocular, adipose, bone, etc.) and some gelatins are currently being valorized by a spin-off company as bioink constituents. In addition to their in vitro biocompatibility assessment, we also performed in vivo animal trials (mice) which indicated that the implanted constructs actively stimulate vascularization. Currently, the regulatory trajectory towards first in-human trials of an injectable crosslinked bifunctional gelatin to serve adipose tissue engineering applications is ongoing.

Acknowledgement

The authors would like to acknowledge Jos Olijve (Rousselot) for providing the raw materials and to provide financial support. In addition, Prof. Blondeel and Prof. Van Vlierberge would like to thank Research Foundation Flanders (FWO) for providing financial support to research project 3S039319.
Gelatin-based constructs obtained via ZPP
Example of the improved ZPP performance of the different thiol-ene formulations as opposed to the conventional gel-MOD (all structures written at 100 mm s\(^{-1}\) and 100 mW laser power using 0.68 molar of P2CK).
Comparison between chain growth- and step growth-crosslinked gelatin for adipose tissue engineering

References


Modulation of gelatin hydrogel properties by tuned bioorthogonal crosslinking

Nicola Contessi Negrini¹, Ana Angelova Volponi², Paul T. Sharpe², Adam D. Celiz¹

¹ Imperial College London, Department of Bioengineering, London, GB; ² King’s College London, Centre for Craniofacial and Regenerative Biology, Faculty of Dentistry, Oral & Craniofacial Sciences, London, GB

Introduction
Gelatin hydrogels are promising biomaterials to engineer biomimetic extracellular matrix for tissue engineering and in vitro modelling. However, the development of a cytocompatible crosslinking strategy to stabilize these hydrogels at physiological temperature and simultaneously control physico-mechanical properties to guide a desired cell response is still challenging [1]. Here, we prepare gelatin hydrogels using bioorthogonal click chemistry and demonstrate that the hydrogel properties can be tuned by fixing their concentration and modulating their crosslinking to guide in vitro cell response.

Experimental Methods
Gelatin hydrogels were prepared by bioorthogonal crosslinking reaction (Fig.1, left) between tetrazine (Tz) and norbornene (Nb) [2]. Tz- and Nb-gelatin derivatives (Gel_Tz and Gel_Nb) were synthesised with different degrees of modification (DOM) by decorating gelatin either with Tz or Nb, respectively, using EDC/NHS coupling reaction. ¹H NMR was performed to measure the DOM of gelatin derivatives. Gelatin hydrogels at fixed 8% w/v concentration were prepared by mixing Gel_Tz and Gel_Nb obtained with different DOM (i.e., low, medium, and high), at different Gel_Tz:Gel_Nb ratios R (i.e., 0.5, 1, and 2). The evolution of the rheological properties (storage modulus G' and time required for crosslinking t_cross) of the hydrogels during crosslinking were recorded by a rheometer (Anton Paar MCR302). The swelling of crosslinked hydrogels was investigated by measuring their weight variation after immersion in PBS at 37 °C up to 2 weeks. The compressive mechanical properties of swollen hydrogels were tested by Instron 5900R. Human Dental Pulp Stem Cells (hDPSC) were added to the gelatin derivatives solutions and cell-laden hydrogels were prepared (1·10⁶ cells ml⁻¹). After 1 day of culture, a live/dead staining was performed to evaluate cell viability and distribution in the 3D hydrogels. Cell metabolic activity was measured over 2 weeks of in vitro culture by AlamarBlue™ assay. Cell morphology was evaluated after 14 days by F-Actin/nuclei staining.

Results and Discussion
Gel_Tz and Gel_Nb were successfully synthesised with different controlled DOM (i.e., 5, 10, and 15%). After mixing the gelatin derivatives, an increase in G' was observed with time, confirming the ongoing crosslinking reaction between Tz and Nb to achieve a formed hydrogel (G’>G”). Hydrogels prepared using higher DOM and equimolar Tz:Nb ratio (i.e., R=1) were characterized by a quicker crosslinking reaction and higher G’ values at plateau (t_cross=10-20 min, G’=1000-1500 Pa), compared to hydrogels prepared with lower DOM and defect of Tz or Nb (i.e., R=0.5 and R=2, respectively; t_cross=60-70 min, G’=300-600 Pa). Crosslinked hydrogels were stable in PBS at 37 °C for 2 weeks, and higher weight variation was measured for lower DOM hydrogel and hydrogels prepared with defect of Tz or Nb (i.e., R=0.5 and 2). Compressive mechanical properties of swollen hydrogels were tuned both by varying the DOM and the R ratio (Fig.1, right). Lower Young Modulus E was measured for hydrogels with low DOM and R0.5 and R2 (E<1 kPa), while higher mechanical properties characterized hydrogels with high DOM and R1 (E>1 kPa). Viable hDPSC (viability>85%) distributed in the 3D structure of the hydrogels (Fig.2, left) were observed in all the hydrogel formulations, proving the cytocompatibility of the developed hydrogels. Cell morphology after 14 days of culture heavily depended on the mechanical properties of the hydrogels (Fig.2, right). Elongated cells were observed for hydrogels with lower mechanical properties, while less elongated cells were observed in hydrogels with higher mechanical properties.
mechanical properties, proving the importance of modulating the hydrogel properties to guide a desired cell response [3]. Cell metabolic activity increased over time in all the hydrogel formulations; elongated cells showed higher metabolic activity values compared to less elongated cells. Hydrogels prepared with excess Nb or Tz were successfully adhered and remained in contact over culture, potentially allowing for future use as compartmentalized cell co-culture platforms.

**Conclusion**

Cytocompatible gelatin hydrogels with tuneable properties were successfully developed by varying the DOM and Tz:Nb ratio of the hydrogels. All hydrogel formulations can be used to culture viable and metabolically active cells. Tuning the properties of the hydrogels allowed successful modulation of cells response.

**Acknowledgement**

Rousselot Biomedical®, Confidence in Collaboration in Advanced Therapy, UKRI Future Leader Fellowship.

**References**

[1] Campiglio, CE, Contessi Negrini, N, Fare, S, Draghi, L, Cross-Linking Strategies for Electrospun Gelatin Scaffolds, Polymers, 2019, 12, 2476


Electroconductive and injectable hydrogels based on gelatin and PEDOT:PSS for nervous tissue regeneration

Franco Furlani\(^1\), Margherita Montanari\(^1\), Nicola Sangiorgi\(^1\), Emanuela Saracino\(^2\), Elisabetta Campodoni\(^1\), Alessandra Sanson\(^1\), Valentina Benfenati\(^2\), Anna Tampieri\(^1\), Silvia Panseri\(^1\), Monica Sandri\(^1\)

\(^1\) Institute of Science and Technology for Ceramics (ISTEC-CNR), National Research Council of Italy, Faenza, IT; \(^2\) Institute of Organic Synthesis and Photoreactivity (ISOF-CNR), National Research Council of Italy, Bologna, IT

Introduction
Hydrogels are hydrated networks able to mimic the natural Extra Cellular Matrix (ECM) and are widely used for different applications, including biomedicine and soft electronics. [1] Nervous tissue is an excitable system, with poor self-healing ability when damaged by trauma and diseases. Recently, great attention has been focused on developing electroconductive hydrogels to support recovery of the degenerated nervous tissues, [2],[3] by enhancing cell adhesion, cell growth and formation of functional neuronal networks in the scaffold. [4],[5] Among neural cells, glial cells, called astrocytes, play a critical role in nervous system recovery and in the response of the damage tissue to the implanted scaffold. In the present work, an electroconductive scaffold is prepared and characterized and its biocompatibility with different cell types including astrocytes are evaluated.

Experimental Methods
Hydrogels were fabricated by using three different components, namely \(i\) gelatin, a biomimetic polymer, \(ii\) genipin, a crosslinking agent and \(iii\) different amounts of poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT/PSS), an electroconductive polymer. Rheological analyses were performed to investigate the gelation time and mechanical properties at the end of the gelation. Mechanical properties were also investigated with compression tests using Dynamic Mechanical Analysis (DMA). Electroconductive properties of resulting hydrogels were assessed by Electrochemical Impedance Spectroscopy. Furthermore, swelling and degradation after hydrogels incubation in media able to mimic physiological conditions of temperature, pH and osmolarity were considered. Finally, hydrogels biocompatibility was investigated by using different cell types, including primary rat astrocytes. Alamar blue assays was used to this aim.

Results and Discussion
Genipin was able to promote homogenous gelation of the resulting composite networks. Gelation time of the system without the conductive polymer was close to three hours, whereas in the presence of PEDOT/PSS it was reduced down to 30 minutes. Hydrogels fabricated in the presence of PEDOT/PSS displayed an increase of the shear and elastic modulus but resulted to be less resistant to deformation. This behavior was attributed to the ability of conductive polymer to partially prevent networks bending and stretching. All hydrogels displayed an excellent stability. Additionally, the presence of PEDOT/PSS enhanced the electroconductivity of resulting hydrogels. Hydrogels resulted to be biocompatible towards different cell lines, including primary rat astrocytes.

Conclusion
The presence and the amount of PEDOT/PSS resulted to finely tune some pivotal physical-chemical features – including gelation time, mechanical performance, electroconductive properties, swelling and degradation – of resulting hydrogels. These electroconductive hydrogels can be proposed as electrically conductive interfaces with human tissues, e.g. as neural probes and skin electrodes. Resulting hydrogels can be also potentially proposed as \textit{in-vivo} injectable biomaterials for regeneration of electroconductive tissues, e.g. muscular and neural tissues. Furthermore, this system is promising for development of 3D bio-printed electroconductive biomaterials for regenerative medicine.
Acknowledgement

This study was supported by the grant POR-FESR financed from Regione Emilia-Romagna Mat2Rep, Biomateriali multifunzionali per l’autoriparazione di tessuti e organi (with European Fund for Regional Development. PG/2018/626605).

References


Cell proliferation and morphology of NIH/3T3 cells are influenced by stress-relaxation behavior which can be modified by pre-crosslinking in alginate-gelatin-based hydrogels

Jonas Hazur1, Nadine Grummel2, Dirk W. Schubert3, Ben Fabry2, Aldo R. Boccaccini1

1 University Erlangen-Nürnberg, Institute for Biomaterials, Erlangen, DE; 2 University Erlangen-Nürnberg, Department of Physics, Erlangen, DE; 3 University Erlangen-Nürnberg, Institute for Polymer Materials, Erlangen, DE

Introduction

Synthetic and natural hydrogels are widely used as extracellular matrices in biofabrication, 3D cell culture and tissue engineering. Their physical and chemical properties sensitively influence the behavior of the embedded cells, and it is therefore of considerable interest to understand how these hydrogel properties must be optimized to achieve desired cell functionality. In an ideal matrix, attaching cells are able to establish cell-material interactions, e.g. via integrin-binding and are able to elongate, proliferate and migrate. Alginate hydrogels show good biocompatibility, but do not offer binding-sites for cellular attachment. In this study, we investigated the influence of a priorly established pre-crosslinking technique on the mechanical behavior of alginate-gelatin-based hydrogels and the resulting changes of cell morphology, proliferation and migration of embedded NIH/3T3 cells.

Experimental Methods

The materials used in this project were alginate-gelatin-based hydrogels. On the one hand, native alginate (Alg) and on the other hand oxidized alginate (ADA) was mixed with gelatin (GEL) in the same ratio. After that, the hydrogels were crosslinked with adjusted Ca\(^{2+}\) concentrations, as well as 5 % microbial Transglutaminase (mTG). In order to characterize the mechanical properties, uniaxial compression tests of cylindrical samples were performed in order to determine the Young’s moduli. Furthermore, samples were compressed to 5 % strain and the stress-relaxation was measured over a time period of 10 min. For the biological characterization of NIH/3T3 cells, their morphology was evaluated with Fiji ImageJ and Clickpoints Software and roundness values were compared. Apart from that, migration distance and motility of the cells was investigated with a custom Clickpoint-based Python script. Lastly, the viability of cells was determined with a formazan salt (WST-8) assay, which indicates high cellular activity by a color change upon bioreduction.

Results and Discussion

First of all, we were able to prepare 3D hydrogel matrices with similar Young’s moduli, while substituting ADA with alginate, by adjusting the Ca\(^{2+}\)-crosslinking concentration. With these samples we could show that by the substitution of ADA with alginate, the stress relaxation behavior is altered. The native alginate polymer has an increased molecular weight compared to its oxidized form. Our hypothesis is, that this reduction of the molecular weight leads to the slower stress-relaxation behavior as observed. Furthermore, a significant effect on the cell behavior of NIH/3T3 cells could be observed. Embedded in ADA-GEL samples, cells proliferated a lot more. Accompanied by that, distinctly more elongated cells were observed in these hydrogel matrices.

Secondly, our previously described pre-crosslinking technique [1] was investigated thoroughly within this study. From a mechanical point of view, we observed significantly faster stress relaxation behavior within pre-crosslinked hydrogels. This lead to significantly enhanced cell proliferation, increasingly stretched morphology of the cells, as well as an increased fraction of motile cells in ADA-GEL hydrogels.

Conclusion
In this study, we could emphasize the importance of mechanical stress-relaxation behavior for 3D cell culture *in vitro*. The positive effects of stress-relaxation on a variety of critical cell characteristics, such as morphology, proliferation and migration behavior could be observed. Furthermore, the pre-crosslinking of ADA-GEL-based hydrogels did enhance these positive effects, by rendering the matrix with a faster stress-relaxation behavior. Our hypothesis is that due to the pre-crosslinking, micro-gel particles form within the matrix, which are rather stiff and consist of high alginate content. These micro-gel particles are thought to be surrounded by a rather soft, gelatin rich matrix. In the latter, cells can elongate, migrate and proliferate easily, while the stronger crosslinked parts are impenetrable, but stabilize the structure before being post-crosslinked, which can be advantageous for 3D biofabrication approaches.

**Acknowledgement**

Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Projektnummer 326998133 – TRR 225 (subproject A01).

**References**

Chitosan/gelatin/k-carrageenan sponge-like scaffolds for bone tissue engineering

Konstantinos Loukelis¹, Danai Papadogianni¹, Maria Chatzinikolaidou¹²

¹ University of Crete, Department of Materials Science and Technology, Heraklion, GR; ² FORTH, IESL, Heraklion, GR

Introduction

Since there is an increasingly high demand for optimal biomaterials for a variety of different bioengineering applications, researchers around the globe are constantly trying to fabricate new biofunctional scaffolds that can facilitate and support cell growth and tissue formation. Two of the most prominent biomaterials are chitosan and gelatin, whose chemistry allows for extensive biochemical interactions and also present excellent cell adhesion and cytocompatibility capabilities [1]. Kappa carrageenan is another natural biomaterial, mainly used in the food industry as preservative, which possesses two biologically important chemical groups per subunit, one hydroxyl and one negatively charged sulfur trioxide group. These two groups contribute to its great biological response, when combined with other biomaterials [2,3]. Taking this into consideration, we currently aim to produce kappa carrageenan/chitosan/gelatin scaffolds, reinforced with potassium chloride and covalently crosslinked with glutaraldehyde, in order to subsequently assess their physico-chemical and biological properties. This idea is based on the fact that a polyelectrolyte complex (PEC) can be formed between the negatively charged sulfur trioxide group of kappa carrageenan and the positively charged amino group of chitosan, leading to a sponge-like formation. Moreover, potassium chloride is being used to negate the sulfur trioxide groups that have not participated in the polyelectrolyte complex, allowing for the transition of the non-reacted kappa carrageenan chains from coil to helix formation, endowing the scaffolds with a more robust structure.

Experimental Methods

Four different scaffolds compositions have been prepared: (i) chitosan/gelatin/kappa carrageenan, (ii) chitosan/gelatin/kappa carrageenan enriched with potassium chloride, (iii) chitosan/kappa carrageenan, and (iv) chitosan/gelatin (as control). For all the scaffolds containing kappa carrageenan, three different concentrations have been used, 1% w/v, 0.75% w/v and 0.5% w/v, while keeping chitosan/gelatin concentration the same. Chitosan/gelatin blend was prepared following an established protocol [1]. 2.5% w/v chitosan solution was prepared in 0.25 M acetic acid for 1 h at 50 °C and 5% w/v gelatin solution was prepared in deionized water for 20 min at 50 °C as well. Then, the two solutions were mixed in a volume ratio of 2:1 chitosan:gelatin. Another chitosan solution was prepared without gelatin, in order to be used for the construction of kappa carrageenan/chitosan scaffolds. The same procedure was followed for the preparation of the kappa carrageenan/chitosan/gelatin scaffolds enriched with potassium chloride, with the only difference being that 0.2 M potassium chloride was added in each kappa carrageenan solution prior to the mixing with the chitosan/gelatin blend. The final solutions, independently of their composition, were allowed to mix for 4 h at 60 °C under stirring. Finally, 10 ml of the mixture were transferred into a 15 ml falcon and after addition of 50 μl of 0.25% v/v glutaraldehyde, 800 μl of the blend were cast onto the wells of a 24 well-plate and allowed to freeze-dry for 24 h. Mechanical analysis of the materials was conducted by employing a CellScale UniVert unit (tension, compression and bending testing), while the measurement of swelling ratio, degradation, pore size distribution and FTIR characterization of the different scaffold types were performed. The pore size and morphology has been examined by means of scanning electron microscopy. Swelling ratio and degradation rate at 37 °C in PBS were addressed. Cell viability and proliferation assessment has been performed by means of the PrestoBlue cell viability assay using MC3T3-E1 pre-osteoblastic cells.
Results and Discussion
The scaffolds enriched with potassium chloride demonstrated the highest Young modulus while the non-enriched 1% kappa carrageenan/chitosan/gelatin had comparable Young modulus to that of chitosan/gelatin (control). Kappa carrageenan/chitosan combinations presented the lowest values. All materials had high swelling ratios above 2500% and degradation times with a mass loss of 30% after 21 days. In addition, all kappa carrageenan-containing materials depict big pores above 120 μm, while chitosan/gelatin scaffolds presented lower values. FTIR analysis indicated that the polyelectrolyte formation has occurred and crosslinking with glutaraldehyde occurred for covalent binding. Cell viability and proliferation increased up to 14 days in culture, validating the excellent biocompatibility of all the scaffold compositions. Ongoing osteogenic differentiation assays including the ALP activity, calcium mineralization and collagen secretion are in progress.

Conclusion
The kappa carrageenan/chitosan/gelatin scaffolds demonstrated high mechanical properties, high swelling ratio, 30% mass loss after 3 weeks, and an excellent biocompatibility, rendering them promising candidates for bone tissue engineering.

Acknowledgement
The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the “First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant” project number HFRI-FM17-1999.

References
Assessment of collagen-based bioactive scaffolds with human osteoblast and osteoclast indirect co-culture systems

Giorgia Montalbano\(^1\), Giorgia Borciani\(^1,\)\(^2\), Priscila Melo\(^1\), Sonia Fiorilli\(^1\), Gabriela Ciapetti\(^2\), Chiara Vitale-Brovarone\(^1\)

\(^1\) POLITECNICO DI TORINO, DEPARTMENT OF APPLIED SCIENCE AND TECHNOLOGY (DISAT), TORINO, IT; \(^2\) ISTITUTO ORTOPEDICO RIZZOLI, Biomedical Science and Technologies Lab, IRCCS, BOLOGNA, IT

Introduction
In the last decades, bone tissue engineering aimed at developing smart bioactive biomaterials and constructs able to direct cell activity and to provide alternative strategies to the standard pharmacological and surgical approaches \([1]\). Especially in the case of diseases such as osteoporosis, biomimetic three-dimensional (3D) scaffolds, reproducing the chemical composition and morphological features of native bone tissue, can provide key stimuli to support a suitable balance between osteoblast (OB) and osteoclast (OC) activities. More recently, cell co-culture systems have been demonstrated to be a useful tool for the pre-screening of innovative biomaterials and scaffolds for bone regeneration, enabling the simulation of the natural cell crosstalk mechanisms, and reproducing a more effective in vitro bone model \([2]\).

In this study, 3D scaffolds were produced by extrusion printing of a bioactive hybrid formulation combining type I collagen, strontium containing mesoporous bioactive glasses (Sr-MBG) and rod-like hydroxyapatite (HA) nanoparticles. Subsequently, the ability of the resulting constructs to promote cell adhesion, proliferation and differentiation was assessed by means of human co-culture of OBs and OCs.

Experimental Methods
A hybrid bioactive biomaterial ink was obtained by combining Sr-MBGs and rod-like HA nanoparticles with a 1.5 wt% collagen solution. 3D printed scaffolds were fabricated by using a commercial 3D Bioprinter (BIOX, Cellink) equipped with a temperature controlled pneumatic extrusion printhead and exploiting the freeform reversible embedding of suspended hydrogels (FRESH) method \([3]\). After incubation at 37 °C, the resulting scaffolds were chemically crosslinked with 0.5 wt% genipin solution in 70% ethanol (GEN/EtOH). After the physico-chemical and morphological assessment, the scaffolds were cultured in presence of human OBs derived from trabecular bone samples and human peripheral blood mononuclear cells (PBMCs) isolated from fresh buffy coat as OC precursors. The indirect co-culture was set seeding OBs onto the scaffold surface, while PBMCs were placed onto the transwell device, to be cultured in a medium free of osteogenic inducers, up to 21 days. Cell viability was detected by means of Alamar Blue and Live/Dead assays, while cell adhesion and morphology were investigated by Scanning Electron Microscopy (SEM). Alkaline Phosphatase, Alizarin Red S and von Kossa stain were used to investigate OB maturation, while Tartrate-resistant acid phosphatase, Phalloidin-FITC and Hoechst 33258 staining enabled the detection of PBMC differentiation towards pre-OCs.

Results and Discussion
3D printed bioactive composite scaffolds were successfully obtained by processing a hybrid formulation combining type I collagen, Sr-MBGs and HA nanoparticles. The resulting constructs (10x10x1 mm\(^3\)) were characterized by a mesh-like pattern and 1 mm pores. The subsequent chemical crosslinking with GEN/EtOH led to the increase of the overall material stiffness and stability. The indirect co-culture system using human OBs and PBMCs demonstrated the ability of the designed scaffolds to promote cell adhesion and high proliferation rates up to 21 days, while by colorimetric and fluorescent assays the differentiation of cells between 7 and 14 days after seeding, without the addition of osteogenic inducers in the culture medium, was highlighted.
Conclusion
Bioactive 3D printed scaffolds were successfully designed by processing a novel biomaterial ink combining type I collagen, Sr-MBGs and HA nanoparticles with the aim to mimic the native features of human bone tissue. Human OB/OC co-culture system proved the ability of the designed scaffolds to support cell adhesion, proliferation, and differentiation by paracrine activity in complete medium free of osteogenic inducers, up to 21 days.

Acknowledgement
This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation program (grant agreement No 681798-BOOST).

Live/Dead Assay on OBs (left) and nuclei ad actin stains on PBMCs (right) after 14 days of co-culture

References
Making it stick: A novel approach to bind and present physiological amounts of BMP-2 via collagen-based substrates

Stylianos O. Sarrigiannidis, Oana Dobre, Aleixandre Rodrigo-Navarro, Matthew Dalby, Manuel Salmeron-Sanchez

University of Glasgow, GLASGOW, GB

Introduction
The challenge in utilizing growth factors in tissue engineering is to keep them active and stable within the material for a prolonged period [1]. Collagen type I, a material often used in tissue engineering, does not have a high affinity and binding capacity to growth factors. Researchers have been incorporating growth factors such as Bone Morphogenic Protein 2 (BMP2) into collagen sponges for bone regeneration for several years [2], a system that is FDA approved (INFUSE®). However, very high, carcinogenic concentrations of BMP2 are required for this procedure and BMP2 swiftly leaks out of material [3]. The aim of this study is to incorporate BMP-2 into collagen substrates that can be presented locally to the biological environment. We have genetically engineered bacteria which express new recombinant peptide fragments that combine collagen binding domains (CBD) of fibronectin (FN) or bacterial collagenases (COLG, COLH) and the growth factor binding domains (GFBD) of fibronectin [4]. The fragments can be isolated and incorporated stably into a collagen substrate allowing it to sequester growth factors (e.g. BMP2) and promote osteogenesis.

Experimental Methods
The custom fragments (FN-CBD + GFBD, COLG-CBD + GFBD and COLH-CBD + GFBD) were expressed in BL21Star E. Coli bacteria utilizing a pet100 vector. Bacteria growth conditions were optimized, and the expressed protein was purified using a nickel column. Fragment attachment to collagen was verified by immunostaining and HisTag ELISA in multiple collagen hydrogel/scaffold systems (2D – on top of collagen-PEG gels, 2.5D – within collagen sponge and 3D – incorporated into collagen-PEG gel). Growth factor absorption - release experiments were conducted by incorporating Alexaflor488 labelled BMP-2 into each hydrogel/scaffold system at different concentrations and then placing it in PBS for up to 7 days. Aliquots of the supernatant were removed and analyzed at different time points. Human Mesenchymal Stem Cells (hMSCs) were cultured and seeded on the collagen hydrogel systems to conduct cell viability, morphology and differentiation experiments.

Results and Discussion
The fragments successfully bind to collagen substrates and allow for the sustained release of BMP-2 from 2D, 2.5D (fig 1) and 3D collagen hydrogel systems. The COLG-CBD+ GFBD peptide showed the highest affinity for collagen and naturally retained more BMP2 compared to the other groups (figure 1). Osteogenic differentiation experiments showed that the collagen substrates (2D, 2.5D and 3D) treated with the peptide and BMP-2 show upregulation of osteogenic markers. The translocation of Runx-2 to the nuclei of hMSC after 7 days cultured in a 2.5D collagen systems can be seen in fig. 2 (1μg/mL of BMP2 was used for the BMP2 conditions). When the nuclear intensity was quantified the COLG-CBD+GFBD+BMP2 showed the highest translocation of Runx-2 to the nucleus. This is consistent with other osteogenic differentiation experiments such as upregulation of osteopontin (OPN) in 2D after 21 days and von Kossa studies after 28 days.

Conclusion
This system is an innovative method of absorbing and retaining physiological amounts of BMP-2 to promote osteogenic differentiation of hMSCs, which can be expanded to other GFs in the future for other tissue regeneration.
An in vivo experiment (critical defect murine model) will be conducted this summer to further showcase the validity of this system.

Acknowledgement

This project is supported by Medical Research Scotland and Collagen Solution Ltd. Special thanks to the CeMi Lab.

Figure 1
BMP-2 (1μg/mL) absorption and release from 2.5D collagen scaffold treated with different collagen binding peptides with growth factor sequestering capabilities.

Figure 2
Runx-2 translocation into the nucleus of MSCs (7d culture) - Runx-2 (red), F-actin (green), Dapi (blue). Note: 1μg/mL of BMP2 was used.

References

11:30 a.m. – 1:00 p.m.

Track04

S11 | Nanostructure-based Biomaterials: Design and Biological Interactions

Chairs

Lino S. Ferreira
University of Coimbra, Coimbra, PT

Ana Marina Ferreira Duarte
Newcastle University, School of Engineering, Newcastle upon Tyne, GB

Madison J. Ainsworth (YSF)
University Medical Center Utrecht, Orthopedics, Utrecht, NL

The program will provide new insights on the most advanced nanosystems for precision medicine, with a particular focus on cancer treatment and RNA-based therapies. The symposium will also describe advanced approaches for the in vitro testing of drugs, based on 3D in vitro disease models.
S11-KL01

Nucleic acid delivery systems for RNA therapy and genome editing

Daniel G. Anderson

Institute for Medical Engineering and Science, Cambridge, US

RNA nano formulations have received much attention for their role in bringing the covid pandemic under control. However, vaccines are only a small part of the broad potential use of RNA in human therapeutics. Here we describe our work developing nanoformulations for RNA therapy and genome editing. Libraries of degradable polymers and lipid-like materials have been synthesized, formulated and screened for their ability to delivery RNA payloads inside of cells. These nanoformulations facilitate in vivo delivery to a range of tissues, and can enable targeted gene suppression with siRNA, gene expression with mRNA, or even permanent genetic editing using the CRISPR/Cas9 system. We will describe the development and use of this technology, and describe their potential as therapies for a range of different diseases.
S11-KL02

Leveraging polymer biomaterials to improve the design of nanomedicines

Clara Mattu, Giulia Brachi, Gianluca Ciardelli

Politecnico di Torino, DIMEAS, Torino, IT

Nanomedicines, i.e., nano-encapsulated drugs, have demonstrated great potential in the treatment of several hard-to-tackle diseases, including cancer. When properly designed, nanomedicines are able to negotiate with several biological barriers, enhancing the delivery of the encapsulated drugs at the target location. [1]

Among the design parameters, the choice of the constituent material for nanomedicines plays a fundamental role, as it dictates the encapsulation efficiency, the release kinetics, and the interactions with the biological milieu. [2,3]

This presentation will discuss the role of the materials in the design of drug delivery systems, including nanomedicines for combinatorial drug delivery and for the localized treatment of site-specific pathologies. [3-5]

Acknowledgement

European Union’s Horizon 2020 research and innovation programme - Marie Sklodowska-Curie grant agreement No 658665.

References

S11-03

Nucleic acid-based nanostructures functionalized with neurotrophin-mimetic aptamer aiming at neuronal targeting

Ana S. Martins1,2, Sara D. Reis1, Ruxandra Baboi1,3, João Cortinhas1, Marco M. Domingues4, Nuno C. Santos4, Sofia D. Santos1, Ana P. Pêgo1, Pedro M. Moreno1,5

1 i3S/INEB - Instituto de Investigação e Inovação em Saúde / Instituto de Engenharia Biomédica, Universidade do Porto, Porto, PT; 2 FEUP - Faculdade de Engenharia da Universidade do Porto, Porto, PT; 3 FCUP - Faculdade de Ciências / ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, PT; 4 Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, PT; 5 FEUP / ICBAS, Universidade do Porto, Porto, PT

Introduction

The impact of neurological disorders and the lack of efficient therapies drive an urgent need for development of new treatments. Nucleic acid (NA)-based drugs (siRNA, miRNA, aptamers, antisense-oligonucleotides) can be seen with immense therapeutic potential, however, there is still a need for an effective brain delivery and a specific neuronal targeting [1], [2]. DNA-based nanostructures (DNA-NS) can be promising NA drugs carriers for brain and neuronal delivery, application for which DNA-NS potential is still largely unexplored.

Using principles of DNA nanotechnology for seamless bottom-up self-assembly, we are engineering a novel multifunctional DNA-NS integrating different NA drugs and functionalized with multiple ligands, aiming at three outcomes: an effective brain delivery, a specific neuronal targeting and a neuroprotective therapeutic effect.

In the present work, we have functionalized the DNA-NS with a neurotrophin-mimetic aptamer conferring neuronal cell targeting. Additionally, we are exploring if the aptamer-functionalized DNA-NS (DNA-NS-Apt) is activating the cognate neurotrophin receptor.

Experimental Methods

DNA-NS are produced by bottom-up self-assembly of four single-strand oligonucleotides and characterized by gel electrophoresis, dynamic light scattering (DLS) and atomic force microscopy (AFM). The functionalization with aptamers is done through direct hybridization of DNA-NS anchors with a reverse complementary DNA sequence included in the aptamer (Fig. 1). The DNA-NS-Apt was characterized by gel electrophoresis and DLS.

DNA-NS-Apt biological functionality was evaluated in terms of neuronal targeting capability and specific neurotrophin receptor activation in neuronal and non-neuronal cell lines (Fig. 2), as well as in primary cortical neuronal cells. Neuronal targeting has been assessed by confocal microscopy and flow cytometry, whereas receptor activation has been evaluated by Western blotting (WB).

Results and Discussion

DNA-NS can be produced with high uniformity, with an assembly yield of 88.3 % ± 2.1 %, according to gel quantifications. A mean hydrodynamic diameter of 19.37 nm ± 0.32 nm was measured by DLS (z-average, ± SD) and height of approximately 5 nm was detected by AFM. Being intrinsically modular, DNA-NS allow spatially and stoichiometrically precise functionalization with ligands, as verified with DNA-NS-Apt, by PAGE and DLS. A mean hydrodynamic diameter of 43.33 nm ± 0.35 nm was measured by DLS.

DNA-NS-Apt showed an enhanced cell binding in neuronal cell lines expressing the specific neurotrophin receptor, in comparison with the DNA-NS and the DNA-NS functionalized with a scrambled aptamer (DNA-NS-scrApt). Accordingly, low cell binding was observed when using non-neuronal cells (not expressing the neurotrophin receptor) (Fig. 2). These results reveal the neuronal targeting potential of DNA-NS-Apt. Internalization routes will be investigated to analyse the DNA-NS-Apt intracellular fate.
Preliminary evaluations of neurotrophin receptor phosphorylation (receptor activation) show a tendency for a low level of activation (partial activation) by DNA-NS-Apt, in comparison with the control DNA-NS-scrApt.

**Conclusion**

In summary, these are the first demonstrations of DNA-based nanostructures capable of neuronal cell targeting, through aptamer functionalization. Work is on-going regarding further multi-functionalization with other NA drugs and ligands. The proposed system will be further characterized both structurally and biologically, aiming to translate it into a unique and efficient nanotechnology to promote brain delivery, neuronal targeting and, ultimately, neuroprotection.

**Acknowledgement**

This work was supported by Fundação para a Ciência e Tecnologia (PTDC/NAN/MAT/30898/2017 project and PhD fellowship SFRH/BD/137075/2018) and Projects NORTE 01 0145 FEDER 0000008, PPBI POCI 01 0145 FEDER 022122 and NORTE 01 0145 FEDER 0000012 (N2020).

---

**Fig. 1**  
Representative sketch of DNA-NS functionalized with 3 aptamers (Apt).

**Fig. 2**  
Cell binding assay of DNA-NS. The cell binding activity of DNA-NS, DNA-NS-Apt, and DNA-NS-scrApt was evaluated in neuronal and non-neuronal cell lines by flow cytometry. Results from 2 independent experiments.

**References**


S11-04

Engineering nanoparticles for targeted delivery of RNA-based therapies

Vitor Francisco¹, Josephine Blersch¹, Catarina Rebelo¹, Artur F. Rodrigues¹, Hugo Fernandes¹,², Lino Ferreira¹,²

¹ Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, PT; ² Faculty of Medicine, University of Coimbra, Coimbra, PT

Introduction
RNA-based therapies are highly selective and affect in many ways the biology that cannot be achieved by small molecules. Non-viral vectors such as nanoparticles are very promising formulations for the delivery of RNA-based therapies but they suffer from limited success in cell targeting, cell internalization and endolysosomal escape. To this end, it is crucial to develop formulations that enable their delivery to a target cell, with potential off-target effects and simultaneously increase their efficacy in the intracellular delivery. The goal of the current work is to obtain biocompatible light-activatable nanoparticles (NPs), allowing precise control of the timing and spatial release of the RNA molecules, in order to accelerate the translation of these therapies.

Experimental Methods
By Michael-type addition chemistry we produce polymers with chemical diversity. Then, the polymers were conjugated with light sensitive molecules to increase their hydrophobicity as well as confer light responsiveness properties. The polymers obtained were precipitated in water to form NPs and then complexed with siRNA and miRNA or directly added to mRNA to form polyplexes. The NP library was characterized for size, zeta potential, light disassembly, siRNA complexation, cellular internalization and gene knockdown activity.

Results and Discussion
Herein, we synthesized a nanoparticle library with more than 250 formulations, based in two different principles with a variety of physico-chemical properties and responsiveness to UV light. We evaluated (i) formulations synthesized with light responsive group in different positions of the polymers structure and (ii) complexation of different RNA molecules, siRNA, miRNA and mRNA (Figure 1). Next, we have performed high-throughput screenings in reporter cells to identify formulations that were rapidly taken up by cells and deliver efficiently RNA molecules (more effectively than the commercial transfection agent lipofectamine RNAiMAX). We have selected candidates for subsequent studies regarding their specificity to skin cells (some NPs were more internalized by a specific type of cell than other), endolysosomal escape and functional studies before and after light activation. This study indicates that the compositional elements of the formulations, other than zeta potential of the NPs, RNA complexation ability, trigger responsiveness, cellular uptake, play a key role in determining an efficient RNA transfection vector. Next, we have confirmed the advantages of one of the candidate formulations in a wound healing animal model, for the delivery of a skin regenerative miRNA identified recently by us.

Conclusion
Using the appropriate cell model allied with high content analysis we can rapidly monitor several parameters, such as cellular uptake, cytotoxicity, intracellular trafficking and RNA delivery efficacy. This strategy allows precise delivery of RNA-based therapies and thus provides an effective design approach to address critical issues in non-viral gene delivery.

Acknowledgement
The authors would like to thank the financial support of ERA Chair project (ERA@UC, ref:669088) through EU Horizon 2020 program, the POCI-01-0145-FEDER-016390 (acronym: CANCEL STEM), POCI-01-0145-FEDER-029414 (acronym: LightBRARY) and UID/NEU/04539/2019 projects through Compete 2020 and FCT programs.

Figure 1. Schematic representation of the polymer design strategies for the complexation of RNA molecules. These steps include: (i) monomer selection, (ii) polymer synthesis, (iii) RNA molecules complexation, (iv) efficacy of RNA therapeutic delivery with light trigger and (v) cell targeting.

References
High-throughput production of polyethylene glycol-tyramine microcapsules for non-invasive delivery of immunoprotected beta cells

Nuno Araújo-Gomes¹, Barbara Zoetebier Liszka¹, Bas van Loo¹, Suzanne Nijhuis¹, Alexandra M. Smink², Bart de Haan², Paul de Vos², Marcel Karperien¹, Jeroen Leijten¹

¹ University of Twente, Developmental Bioengineering, Enschede, NL; ² University of Groningen, Section of Immuoendocrinology, Department of Pathology and Medical Biology, University Medical Center, Groningen, NL

Introduction
Type 1 diabetes is an auto-immune disease that results in the destruction of destroys the insulin-producing beta cells of the pancreas. Transplantation of pancreatic islets or beta cells is anticipated as a high potential curative solution for patients suffering from Type 1 diabetes. However, to implanted cells currently require immunosuppressants to evade the host's immune response to the implant, which causes adverse effect such as an increase to susceptibility of collateral and opportunistic infections [1]. To this end, several immunoprotective biomaterial strategies have been developed to protect engrafted beta cells by acting as e.g. semi-permeable microcapsules [2], which prevent the diffusion of molecules larger than 150 kDa (i.e. antibodies) while allowing for diffusion of small sized molecules, such as insulin and glucose. However, clinical translation of these immunoprotective microcapsules is currently challenged by the low-throughput production rate of the currently explored platforms. Here, we describe the development of a novel non-degradable immunoprotective material that allows for continuous high-throughput microfluidic production of beta cell containing microgels.

Experimental Methods
8-arm enzymatically crosslinkable 20 kDa polyethylene glycol–tyramine (PEG-TA) conjugates were synthesized in a two-step reaction: 1) ester activation and 2) amidation. PEG-TA conjugates (2.5%, 5%, and 10% w/v) were used to fabricate microcapsules up to a diameter of 200 µm with a shell thickness of 20 µm. Microfluidic droplet generation was optimized for production of hollow PEG-TA microgels using a delayed outside-in crosslinking strategy based on horseradish peroxidase and hydrogen peroxide diffusion [3]. Microcapsule permeability assessment was performed by incubation with fluorescently labeled immunoglobulin (IgG) and bovine serum albumin (BSA). Cytocompatibility, proliferation, cyto-immunity permselectivity and insulin release profiles were assessed in vitro using MIN6B1, a pancreatic beta cell model and THP1, a monocytic cell line. In vivo analysis was carried out using a C57BL/6 mouse model by: 1) implanting hollow microgels in to confirm the low immunogenicity of the microgels and 2) implanting MIN6B1 laden gels subcutaneously to determine the survival and function of the microencapsulated β-cells.

Results and Discussion
Monodisperse PEG-TA microgels of 150-200 µm diameter containing up were produced on the PMMA on-chip platform at a rate of ~45 Hz allowing for the continuous microencapsulation of ~50 beta cells/min. Microgel permselectivity assessment confirmed that in microgels composed of 10% PEG-TA, molecules of >150kDa could not diffuse into the microcapsules offering immunoprotectivity for at least 30 days (Fig.1a). Moreover, microgels still were permeable to smaller molecules like (BSA), guaranteeing nutrient, hormone, and growth factor diffusion. Immunological assessment revealed that microgel contact with undifferentiated THP1 cells elicited low-to-no immune cell activation and cell attachment to the microgels. Interestingly, multiplexed ELISA of whole blood exposed to microgels demonstrated an inverse dose-dependent response of several pro-inflammatory cytokines with the immunoprotective formulation of 10% PEG-TA thus inducing the lowest inflammatory reaction. Post-encapsulation, beta cells remained viable and glucose responsive, with no loss-of-function. Upon reaching confluency, microgel withstood the growing internal mechanical pressures and remained intact, which allowed the microgels to act as a
proliferation-restrictive barrier yielding non-proliferating monodisperse microencapsulated β-cell spheroids (Fig.1c). In vivo analysis showed that the implantation of hollow microgels on mice elicited the formation of minor fibrotic structures surrounding the material, most likely due to the geometric profile of the microparticles. The implantation of cell-laden gels revealed that the encapsulated cells formed microaggregates that remained viable and glucose responsive after 7 days (explantation day), confirming the immunoprotectivity and semi-permeable character conferred by the PEG-TA microgel (Fig.2).

Conclusion
High-throughput production of beta cell containing PEG-TA hollow microgels represents a promising and innovative approach for the production and delivery of immunoprotected glucose responsive non-autologous beta cells for the treatment of type 1 diabetes.

Acknowledgement
Financial support was received from the European Research Council (ERC, Starting Grant, #759425) and JDRF award 2-SRA-2018-684-S-B.

PEG-TA microgels are immunoprotective and act as a restrictive barrier to cell proliferation. Fig.1 - a) Confocal images of hollow and cell-laden 10% PEG20-TA microgels incubated with FITC- BSA and IgG; b) Actin stained beta-cell aggregate development within the gel (red dashed line); c) EDU staining and quantification of the proliferative cells within the PEG-TA microgel at 2, 7, 14 and 30 days. *Indicates significance with p < 0.05
PEG-TA microgels remain viable and glucose responsive 7 days post-implantation.

Fig. 2. - a) Live/Dead staining of an explanted MIN6B1 cell-laden microgel - the cell aggregates maintain viability after explantation; b) Insulin release profile and stimulation index of the explanted microgels - beta-cell aggregates maintain responsiveness post-implantation.

References
[2] B. Kupikowska-Stoba, D., Lewińska, 'Polymer microcapsules and microbeads as cell carriers for in vivo biomedical applications', Biomaterials Science 2020, 8, 1536-1574
11:30 a.m. – 1:00 p.m.

Track05

ESB-SIBB S02 | ESB-Iberian Society for Biomechanics and Biomaterials (SIBB) Joint Session II: Biomaterials for cancer models and treatment

Chairs

Diego Velasco Bayón
University Carlos III of Madrid (UC3M), Bioengineering and Aerospace Engineering, Leganés (Madrid), ES

João Mano
University of Aveiro, PT

Victoria Leiro (YSF)
i3S - Instituto de Investigação e Inovação em Saúde da Universidade do Porto, INEB - Instituto de Engenharia Biomédica, Porto, PT

Biomaterials for cancer models and treatment Cancer is the leading cause of death worldwide, and the Portuguese and Spanish biomaterials scientific community is clearly trying to fight it. This symposium highlights the role of biomaterials for use as cancer models and for use as nanoparticles for targeted therapies, exploring a wide range of applications, including brain, gastric, lung, bone, pancreatic and breast cancers.
Selective targeting of cancer stem cells by hyaluronic acid-coated nanoparticles based on naproxen

Eva Espinosa-Cano1,2, Miguel Huerta-Madroñal1, Patricia Camara-Sanchez2,3,4, Joaquin Seras-Franzoso4, Simo Swartz Jr2,4, Ibane Abasolo2,3,4, Julio San Roman1,2, Maria Rosa Aguilar1,2

1 Consejo Superior de Investigaciones Científicas, CSIC, Institute of Polymer Science and Technology, Madrid, ES; 2 Networking Biomedical Research Center in Bioengineering, Biomaterials and Nanomedicine, CIBER-BBN, Madrid, ES; 3 Universidad Autónoma de Barcelona, Functional validation and preclinical research, Barcelona, ES; 4 Vall d’Hebron Institut de Recerca, Drug delivery and targeting CIBBIM-Nanomedicine, Barcelona, ES

Introduction

Cancer Stem Cells (CSC) are a small cell subpopulation within the tumour, with high tumorigenic potential and the ability to self-renew. They are associated with the acquisition of drug resistance and the development of early, aggressive metastatic relapses. In breast cancer, major CSC markers are CD44+/CD24- phenotype and high aldehyde dehydrogenase 1 (ALDH1).

Chronic inflammation has long been linked to carcinogenesis and play a key role in the different stages of tumour development. Non-steroidal anti-inflammatory drugs (NSAIDs), and in particular naproxen (NAP), have shown to be effective in the treatment of breast cancer [1]. NAP anti-cancer properties have been attributed to the reduction of prostaglandin E2 levels (the major product of the activation cascade of both cyclooxygenase isoforms (COX1/COX2)) and COX-independent pathways, being both mechanisms associated to CSC maintenance. However, repeated-administration and efficacy of free forms of NAP are hampered by the drug's high hydrophobicity and dose and time-dependent gastrointestinal side effects.

The aim of this work is the development of NAP-based polymeric nanoparticles (NP) with higher activity and lower side effects than the free NAP. NP were coated with hyaluronic acid (HA) in order to actively target CD44 receptors, overexpressed in breast CSC.

Experimental Methods

NAP-based cationic NP were prepared by nanoprecipitation of poly(HNAP-co-VI)(71:29), as recently described [2]. HA-coating was performed by electrostatic interaction of NAP-based NPs and HA at pH 4.0. Synthesis conditions were optimized and the influence of pH and ionic strength on hydrodynamic properties as well as stability were assessed.

Particle size distribution and zeta potential were determined by DLS and LDE, respectively; Morphology was assessed by cryo-TEM. Esterase-mediated NAP release kinetics was determined by UV spectrophotometry during 14 days.

Haemocompatibility parameters (aPTT, PT and TT) were determined. NP uptake was studied to evaluate CD44-mediated active targeting of CSC and non-CSC populations. For this assay, an in vitro CSC fluorescent model of MCF-7 cell line was used [3]. Cell viability and IC50 of MCF-7 (CD44+) and HepG2 (CD44-) cancer cells in the presence of the NP were evaluated by MTT assay. NP cytotoxicity was also evaluated in cells from the mammary tumor microenvironment (RAW264.7, HUVEC and MCF-7) using AlamarBlue assay. ELISA kits were used to study COX-dependent markers (PGE2 and VEGF) and apoptosis markers (p53 levels) in NP-treated MCF-7. Wound healing assay was performed to evaluate NP capacity to inhibit MCF-7 migration.

Results and Discussion

Page 705 of 2028
HA was physically adsorbed on the surface of NAP-based NPs by electrostatic interaction as confirmed by an inversion in the sign of surface charge and a significant increase in NPs diameter. HA-coated NPs were spherical as observed by cryo-TEM.

HA-coated NP were stable at physiological pH as HA-coating kept the surface charge constant. However, a significant increase in mean size was observed as the pH increased. HA-coating acted as a barrier layer providing a better control in NAP-release. HA-coated NPs caused a lower haemolysis of red blood cells if compared with uncoated NAP-based NPs.

CD44-HA interaction plays a key role in the internalization of the NP, as CSC presented a significantly higher endocytosis rate of coated-NPs than the differentiated breast cancer cells. Uncoated-NAP NPs did not reduce MCF-7 or HepG2 cells viability below 90% at any of the tested concentrations, while HA-coated NPs were cytotoxic at concentrations of polymer above 0.1 µg/mL for MCF-7 or above 1 µg/mL for HepG2. HA-coated NPs are 10 times less effective in cells with low CD44 expression (HepG2) when compared to CD44-overexpressing cells (MCF-7).

HA-coated NPs did not reduce cell viability of macrophages RAW264.7 at doses below 50 µg/mL, however, these NPs affected HUVEC cell viability (66 ± 6% for 50 µg/mL) which is highly desirable in an anti-tumour treatment.

Non or little effect was observed on PGE2 or VEGF, respectively. A significant dose-dependent accumulation of p53 was observed for HA-coated NPs at 12.5 and 25 µg/mL. Cell migration was also inhibited by HA-coated NPs at 12.5 and 25 µg/mL.

**Conclusion**

NAP-based cationic NP were successfully coated by HA that provided superior pH stability, a better control on esterase-mediated NAP release and a better haemocompatibility, than uncoated-NAP NPs. HA-coated NPs are better and faster internalised in CSC than in non-CSC as a direct consequence of the CD44 targeting. This feature coupled to the anti-inflammatory activity from NAP makes HA-coating of poly(HNAP-co-VI)(79:21)-based NPs a potent strategy for targeting CSC subpopulation within breast tumors by either directly attacking CSC or preventing their occurrence derived in response to a pro-inflammatory state. The system also allowed to reduce the dose of NAP needed to achieve pro-apoptotic and anti-migratory activity against luminal breast cancer cells.

**Acknowledgement**

Authors would like to thank the Spanish Ministry of Science, Innovation and Universities (MAT2017-84277-R), ISCIII (PI18_00871 co-founded by ERDF) and CIBER-BBN (EXPLORE) for the financial support of this project. E. Espinosa-Cano thanks FPU15/06109. The kind support by Alvaro Gonzalez-Gomez, Rosana Ramirez and Rafael Nunez is greatly appreciated.

**References**


Zwitterionic polyphosphazene nanoparticles for the genetic modulation of the glioblastoma niche

Carla Garcia-Mázás1, Elia Bozzato2, Veronique Preat2, Noemi Csaba1, Marcos Garcia-Fuentes1

1 Universidad de Santiago de Compostela, CiMUS Research Center, Santiago de Compostela, ES; 2 Université Catholique de Louvain, Louvain Drug Research Institute, Brussels, BE

Introduction
Glioblastoma cells of high malignancy are protected and supported by their tumoral niche. It is now understood that disruption of this tumoral niche is critical to generate long-lasting and effective antitumoral responses in combinations with standard chemotherapy [1]. Some Bone Morphogenetic Proteins (BMPs) are known to disrupt glioblastoma malignant cells in clinically relevant models [2-4]. In this work we show that BMPs can be delivered in genetic form, as plasmid DNA (pDNA), when associated to suitable nanocarriers. Nanocarriers for pDNA delivery based on polyphosphazene combinations have shown high transfection/toxicity ratio and the capacity to deliver gene therapies in vivo [5]. In this work, we designed new nanocarriers with further transfection capacity based on this technology and tested them in advanced glioblastoma treatments.

Experimental Methods
Polyphosphazenes were synthesized based on a thiol-ene reaction previously reported by us [5]. Four cationic and one anionic polyphosphazene were synthesized. The polymers were characterized by 31P and 1H NMR. Polymeric nanoparticles (NPs) were prepared by ionic complexation of cationic polyphosphazenes with plasmid DNA, with or without the anionic polyphosphazene. The NPs were characterized for particle size, zeta potential, morphology and plasmid association. Their toxicity and transfection efficiency were evaluated in vitro in 2D and 3D U87MG cultures. Therapeutic activity was evaluated in vitro in U87MG neurosphere models. In vivo efficacy was tested in U87MG ectopic models using NMRI nude mice. The animals were treated with the nanoparticles loaded with U87MG either alone, combined with temozolamide, or with a battery of controls. Antitumoral activity was analyzed by measuring tumor size, survival and the expression of malignancy markers in the tumor by qRT-PCR.

Results and Discussion
We obtained several NP prototypes based on the use of cationic polymers alone or associated with the anionic polymer, with a size around 100 nm and positive charge. Nanocomplexes were able to associate effectively the polynucleotides. The in vitro and in vivo toxicity assays showed that the anionic polymer reduces the toxicity of all cationic nanoparticles. Studies performed in vitro showed that an aliphatic cationic polyphosphazene derivative had the best transfection/toxicity ratio, particularly when combined with the anionic polymer. Indeed, this optimized NPs prototype showed 3-fold higher transfection than the experiment benchmark (Lipofectamine 2000). The optimized NPs formulation was used to deliver a BMP encoding plasmid, and this gene therapy was capable of suppressing tumor formation in a neurosphere assay. The NPs combined with temozolamide were capable of inhibiting tumor growth in an in vivo study (Figure), resulting on statistically significant effect as compared with any other groups. Indeed, this antitumoral activity resulted in >50% survival by the end of the study, whereas all other groups had minimal or no animal survival. The study indicated that the presence of BMP in the tumor normalizes the expression of efflux pump MRD, which can be induced by the presence of temozolamide. This normalization of MRD expression results in more effective antitumoral effects for this therapeutic combination.

Conclusion
Optimized nanoparticles based on polyphosphazenes for their use as gene delivery carriers have been synthesized and characterized. These nanoparticles have an excellent delivery/toxicity profile. When loaded with a BMP-encoding
plasmid, these nanoparticles result in synergistic and highly efficient glioblastoma treatments in combination with temozolamide.

Acknowledgement
This work was financed by Ministerio de Economía y Competitividad (MAT2017-84361-R, Feder Funds). CGM acknowledges a predoctoral grant from Education, Culture and Sport Ministry (Grant number FPU16/03836).

Figure 1
(A) Tumor volume after treatment (red arrows) for different combinations of blanz nanoparticles (NPs), BMP4 loaded NPs, and temozolamide (Tz). (B) Representative images of tumors for the indicated experimental groups.

References
Targeting CD44v6-expressing gastric cancer cells with half-antibody conjugated nanoparticles

Bianca N. Lourenço¹²³, Rúben F. Pereira¹²⁴, Cristina C. Barrias¹²⁴, Claudia Fischbach⁵⁶, Carla Oliveira¹⁷, Pedro L. Granja¹²

¹³S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, PT; ² INEB - Instituto Nacional de Engenharia Biomédica, Universidade do Porto, Porto, PT; ³ FEUP - Faculdade de Engenharia da Universidade do Porto, Porto, PT; ⁴ ICBAS - Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, Porto, PT; ⁵ Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University, Ithaca, US; ⁶ Kavli Institute at Cornell for Nanoscale Science, Cornell University, Ithaca, US; ⁷ IPATIMUP - Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, PT

Introduction

Gastric cancer (GC) remains a major cause of death worldwide mainly due to late detection in advanced stage. Hence, there is an unmet clinical need for the development of reliable, non-invasive screening methods for early detection and treatment of GC. Cancer-targeting nanoparticles have attracted considerable attention as theranostic tools. Nevertheless, very few de novo expressed molecules specific of GC cells have been identified and, therefore, the development of GC-targeted strategies is only in the beginning. Recently, we demonstrated that CD44v6 is not only de novo expressed in GC¹, but also promotes GC malignancy², suggesting that it may enclose a key marker for the early diagnosis and treatment of GC. Although antibody-targeted NPs have been commonly explored given the innate high-binding affinity and specificity of antibodies, their clinical translation has been hampered by the uncontrolled and random conjugation of antibodies to nanoparticles, resulting in loss of binding specificity³. Here, we designed a modular nanoscale system that specifically targets CD44v6-expressing GC cells by conjugating in a site-specific manner a novel engineered CD44v6 half-antibody fragment to model polystyrene nanoparticles (PNPs) via an efficient bioorthogonal thiol-Michael addition click chemistry⁴.

Experimental Methods

Anti-human CD44v6 antibody was incubated with different concentrations of tris(2-carboxyethyl)phosphine (TCEP) for 3 h and then separated by SDS-PAGE. Binding affinity of generated CD44v6 half-antibody fragments to GC cells was analyzed by flow cytometry using an isogenic human CD44v6-expressing GC cell line, established and reported by our group², ⁵, and its parental cells, lacking CD44 expression, as control. After antibody reduction, 200 nm carboxylated Texas Red-labeled PNPs were functionalized with a heterobifunctional maleimide crosslinker that allowed their ‘click’ conjugation to the native reactive thiol groups of the novel chemically reduced CD44v6 half-antibody fragment by bioorthogonal thiol-Michael addition click reaction. CD44v6 half-antibody conjugated PNPs were characterized by dynamic light scattering and zeta-potential measurements, and their targeting ability to CD44v6-expressing GC cells was evaluated by flow cytometry and further confirmed by immunofluorescence.

Results and Discussion

CD44v6 half-antibody fragments with intact antigen-binding sites to CD44v6-expressing cells and reactive thiol groups were obtained by preferential reduction of disulfide bonds bridging the two half chains of the anti-human CD44v6 antibody in 1 mM of TCEP. Using this optimized antibody reduction condition, our results showed the successful site-specific conjugation of modified-PNPs maleimide groups to half-antibody thiol groups as shown by the increase of particle size and surface charge compared to control conditions. This bioorthogonal click strategy overcomes major pitfalls of current approaches exclusively based on carboxyl-amine coupling of full antibodies, providing superior control over site-oriented ligand conjugation, essential to obtain cell-specific targeting. Notably, our results confirmed the specific targeting ability of the newly engineered CD44v6 half-antibody conjugated PNPs.
that exhibited a significant increased binding to CD44v6-expressing GC cells (1.65-fold) compared with their counterparts, highlighting the receptor-mediated targeting potential of these nanoparticles.

Conclusion
Collectively, by exploring the native reactivity of half-antibody fragments and the bioorthogonal nature of click reactions, our findings not only open new avenues on the use of CD44v6 half-antibody fragments as new targeting ligands, but also highlight the broad significance of our nanoscale system that stands as a promising and clinically relevant tool for the early diagnosis and therapy of GC. Additionally, the rational design of this modular nanoscale system may be explored for the development of several other nanotechnology-based disease-targeted approaches.

Acknowledgement
This work was supported by Norte Portugal Regional Operational Programme (NORTE2020) under the Portugal 2020 Partnership Agreement through the European Regional Development Fund, projects NORTE-01-0145-FEDER-000012, NORTE-07-0124-FEDER-000029 through COMPETE 2020-Operational Programme for Competitiveness and Internationalization (POCI) Portugal 2020 and Portuguese Foundation for Science and Technology (FCT) in the framework of the projects POCI-01-0145-FEDER-007274, POCI-01-0145-FEDER-016390, PTDC/CTM-NAN/120958/2010, and FCT-PhD-Fellowship to BNL (SFRH/BD/87400/2012). The authors acknowledge the support of the i3S Scientific Platforms (PPBI-POCI-01-0145-FEDER-022122).

References
ESB-SIBB S02-04

Targeting Fatty Acid Synthase in Sensitive and Resistant Lung Adenocarcinoma Models Cultured on Polycaprolactone Electrospun Scaffolds

Emma Polonio-Alcalá¹², Santiago Ruiz-Martínez², Marta Planas³, Lidia Feliu³, Teresa Puig², Joaquim Ciurana¹

¹ Product, Process and Production Engineering Research Group (GREP), Department of Mechanical Engineering and Industrial Construction, University of Girona, Girona, ES; ² New Therapeutic Targets Laboratory (TargetsLab) - Oncology Unit, Department of Medical Sciences, University of Girona, Girona, ES; ³ Laboratori d’Innovació en Processos i Productes de Síntesi Orgànica (LIPPSO), Department of Chemistry, University of Girona, Girona, ES

Introduction

Lung cancer is the first leading cause of cancer-related mortality in men and women. More than 85% of lung cancer patients correspond to non-small cell lung cancer (NSCLC) subtype, of which around 40% are adenocarcinomas. Epidermal growth factor receptor (EGFR) activating mutations are the most common genetic alterations in lung adenocarcinoma. Different EGFR tyrosine kinase inhibitors (EGFR-TKI) have been developed during the past few years, such as gefitinib or osimertinib. Nevertheless, approximately 70% of patients are diagnosed with locally advanced or metastatic disease when the treatment is not curative.

In physiological conditions, cells are enfolded by the extracellular matrix (ECM), a fibrous network that plays a key role in some cellular processes, for instance morphogenesis, differentiation and homeostasis. Three-dimensional (3D) cell culture has burst into oncology field providing structures that serve as a physical support for cancer cells, allowing interactions between cell-cell and cell-matrix. Electrospinning is a technique used to manufacture nanofiber scaffolds with a diameter fiber size close to ECM. Polycaprolactone (PCL) is a suitable synthetic polymer for cell culture purposes due to its biocompatibility, biodegradable, viscoelastic properties and its low cost. Interestingly, using PCL electrospun scaffolds to culture EGFRm lung adenocarcinoma sensitive and resistant to EGFR-TKIs models, we have demonstrated an enrichment of cancer stem-like cells, a tumor subpopulation related to drug resistance, relapse and metastasis.

Fatty acid synthase (FASN) is an enzyme responsible for the synthesis de novo of long-chain fatty acids. The overexpression and hyperactivation of this enzyme have been related to aggressiveness and poor prognosis. We have previously shown that FASN inhibition had cytotoxic effects in sensitive and EGFR-TKIs resistant cell models. Additionally, the FASN inhibitors (-)-epigallocatechin-3-gallate (EGCG) and its derivative G28 synergistically interact with gefitinib or osimertinib in EGFR-TKI resistant EGFRm lung adenocarcinoma models.

The purpose of this study is to determine the role of FASN and its related signaling pathways and compare the effect of FASN inhibitors on cell proliferation of sensitive and EGFR-TKI resistant lung adenocarcinoma models cultured in monolayer and on 15% PCL electrospun scaffolds.

Experimental Methods

PCL was dissolved in acetone at 15% (weight/volume) concentration at 60°C under agitation. Scaffolds were manufactured through the Spraybase® electrosprinning machine using a stainless steel 18G needle. Through the Syringe Pump Pro software, 5 mL of solution were ejected at a flow 6 mL/h and voltage 7kV. mRNA and protein expression of FASN and its related signaling pathways were analyzed through RT-qPCR and Western blot. The cytotoxic effect of EGCG and G28 on EGFRm lung adenocarcinoma cells cultured on electrospun scaffolds were tested by MTT assay.

Results and Discussion

Page 711 of 2028
Sensitive and resistant to EGFR-TKIs lung adenocarcinoma cells cultured in 3D showed an overexpression of FASN mRNA and protein levels compared to monolayer culture. An activation of the related signaling pathways EGFR/STAT3, PI3K/AKT, and MAPK was also observed in 3D cultured cells. Additionally, EGCG and G28 treatments had a similar cytotoxic effect in cells cultured in monolayer and in 15% PCL electrospun scaffolds.

**Conclusion**
The results exhibited in this study using 15% PCL electrospun scaffolds support FASN inhibition as an alternative therapy for sensitive and resistant EGFRm lung adenocarcinoma.

**Acknowledgement**
Authors thank R. Rosell and M. A. Molina from laboratory of Oncology Pangaea (Barcelona, Spain) for kindly provided cell models.

**References**
**ESB-SIBB S02-05**

**Stratified 3D Microtumors as Organotypic Testing Platforms for Screening Pancreatic Cancer Therapies**

*Maria V. Monteiro, Vítor M. Gaspar, Luís F. Mendes, Iola F. Duarte, João F. Mano*

*Aveiro University, CICECO-Aveiro Institute of Materials, Aveiro, PT*

**Introduction**

Pancreatic ductal adenocarcinoma (PDAC) is a highly deadly and complex neoplasia with a very low 5-year patient survival. The poor prognosis of this malignancy is related with its unique bioarchitecture in which an abundant juxtatumoral fibrotic stroma shields cancer cells mass hampering anti-cancer therapeutics delivery.\(^1\) A growing evidence has suggested that cancer associated fibroblasts (CAFs), generally localized in periductal/periacinar regions close to the tumor mass, are master players in orchestrating such active desmoplastic tumor microenvironment.\(^2\) To date, few 3D in vitro tumor models have been able to fully recapitulate such PDAC tumor features, resulting in poorly predictive preclinical drug screening data of PDAC candidate therapies. Aiming to recapitulate key disease hallmarks, in this work we bioengineered truly organotypic and physiomimetic 3D microtumor models that recapitulate PDAC unique cytoarchitecture, as well as biomolecular and desmoplastic characteristics. The capacity of such 3D stratified microenvironment spheroid models (STAMS) to emulate various tumor hallmarks including the native spatial stratification of cancer-stromal cells, de novo ECM deposition and secretion of key molecular biomarkers and drug resistance were evaluated.

**Experimental Methods**

3D STAMS were established by using the Human Pancreatic cancer (PANC-1) cell line (ATCC® CRL-1469™) and Human Immortalized Pancreatic CAF-Stellate Cells. STAMS platforms are comprised by a cancer cell-rich core enveloped by stromal CAFs. This cytoarchitecture was established through a two-step strategy. In the first stage, a 3D spheroid core comprising PANC-1 cells was maturated for 6 days under low-adhesion conditions. Afterwards, human pancreatic stellate CAFs were and allowed to attach to 3D PDAC spheroids establishing the stratified model. The cell viability, necrotic regions formation and cellular tracking were all evaluated along time. Moreover, glycosaminoglycans (GAG) and total collagen levels were also analyzed to evaluate de novo ECM deposition. The quantification of soluble biomolecular markers secreted by 3D PDAC microtumor models was performed by ELISA. 3D PDAC models were then used as testing platforms to evaluate different anti-cancer therapeutics (Gemcitabine, Olaparib and Irinotecan). PANC-1 mono-culture 3D spheroids and Random spheroids in which cancer cells and CAFs were randomly distributed within the microtissue were used as controls.

**Results and Discussion**

The engineered 3D STAMS model showed to emulate PDAC key biomarkers and cytoarchitectural hallmarks, namely its cancer-stroma stratification and tumor microenvironment composition, as well as the activated fibrotic stroma that promoted an increased ECM deposition. Interestingly, STAMS reproduced gemcitabine resistance as generally observed in the clinical setting for this standard-of-care therapy, confirming the influence of TME composition and drug sensitivity, emphasizing the importance of mimicking the cytoarchitecture cellular organization of this malignancy in an *in vitro* setting.

**Conclusion**

Overall, 3D STAMS platforms represent a valuable testing tool for evaluating the complex interactions between stromal and pancreatic cancer cells, as well as to accurately perform preclinical drug discovery and screening. The ease of assembly, reproducibility and low-cost of these platforms also highlights their potential inclusion in high-content, high-throughput screening platforms.

Page 713 of 2028
Acknowledgement
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. This work was also supported by the Programa Operacional Competitividade e Internacionalização (POCI), in the component FEDER, and by national funds (OE) through FCT/MCTES, in the scope of project PANGEIA (PTDC/BTM-SAL/30503/2017). Vítor Gaspar acknowledges funding in the form of a Junior Researcher Contract under the scope of the project PANGEIA. Maria Monteiro acknowledges an individual PhD fellowship from the FCT (2020.07692.BD).

The authors declare that a patent application (National Institute of Industrial Property - INPI - 116856) has been submitted for this model with M.V.M, V.M.G and J.F.M stated as inventors.

Figure 1
Schematics of 3D STAMS platform components and use as living tumor models for in vitro drug screening of candidate therapeutics.

References

Calcium-Deficient Hydroxyapatite Microspheres as Antitumoral Carriers for Bone Cancer

Cédric Labay\textsuperscript{1,2,3}, Linh Johansson\textsuperscript{1,2}, Maria P. Ginebra\textsuperscript{1,2,3}, Cristina Canal\textsuperscript{1,2,3}

\textsuperscript{1} Universitat Politècnica de Catalunya, Biomaterials, Biomechanics and Tissue Engineering Group (BBT), Dpt. of Materials Science and Engineering (CEM), Barcelona, ES; \textsuperscript{2} Barcelona Research Center in Multiscale Science and Engineering, Barcelona, ES; \textsuperscript{3} Research Centre for Biomedical Engineering (CREB), Barcelona, ES

Introduction

To tune the resorption rate of calcium phosphate bone grafts used for bone regeneration, research on calcium phosphate cements (CPC) evolved from the manufacturing of dense macroporous blocks to the synthesis of micro-sized particles [1] or thin filaments for 3D-printed scaffolds. The excellent biocompatibility of CPC-based biomaterials, together with their porosity has fostered interest on enhancing their functionality by including cargos such as stem cells, growth factors or active molecules [2,3]. The development of microporous CPC microspheres with controlled size, topography and porosity allowed important advances these last years, regarding the incorporation of biological moieties or drugs and their subsequent controlled release [4,5]. The novelty of this work is to investigate CPC microspheres as carriers for antitumoral drugs, by loading doxorubicin (DOX) into the microspheres and assessing the \textit{in vitro} biological effects on Sarcoma Osteogenic SaOs-2 cell line. By conferring such added therapeutic value to the proven bone regenerative properties of the CPC biomaterial, the designed DOX-loaded microspheres aim to the refill of a diseased site after a bone tumor removal surgery, in order to enhance bone regeneration while preventing the recurrence of cancer cells.

Experimental Methods

In this work, Calcium-Deficient HydroxyApatite (CDHA) microspheres were synthesized by emulsion from α-TCP coarse powder. Characterization of the CDHA microspheres was performed using: i. Scanning Electron Microscopy (SEM) to analyze the shape and the topography of the microspheres, ii. Dynamic Light Scattering (DLS) to evaluate the size distribution of the microspheres and iii. Mercury Intrusion Porosimetry (MIP) and N\textsubscript{2} Adsorption with BJH to determine the porosity and the specific surface area, respectively.

After selecting the microspheres with diameter size ranged from 100 µm to 150 µm by sieving, the microspheres were loaded with an auto-fluorescent antitumoral molecule by soaking them into a 0.4 M DOX aqueous solution for 4 h. Loading kinetics of DOX into the microspheres was plotted from the monitoring of the absorbance of the loading solution. Penetration of the DOX into the CDHA microspheres was assessed by confocal microscopy (CLSM). Subsequent release experiments from the DOX-loaded CDHA microspheres were performed to PBS release medium to evaluate the release kinetics and the final percentage of DOX released. \textit{In vitro} experiments of the DOX-loaded microspheres were performed with Sarcoma Osteogenic (SaOs-2) and human Mesenchymal Stem Cells (hMSC) to study the cell viability and the selectivity of the loaded microspheres at 24 and 72 h on a cancer and a healthy bone cell line.

Results and Discussion

CDHA microspheres with a size range 100 to 700 µm were synthesized, consisting of needle-like calcium-deficient hydroxyapatite crystals (Figure 1a). We observed that drug loading efficiency was microsphere size-dependent, with the highest efficiency being around 60% for CDHA microspheres with the smallest diameter size range (100 – 150 µm). This was associated with a higher specific surface area of the smaller carriers. Assessment with CLSM of the cross-section of a DOX-loaded microsphere revealed that DOX is adsorbed within the first 15-20 µm of the microspheres (Figure 1b). This was related to the release pattern from the DOX-loaded microspheres, showing a
burst release of the antitumoral with 90% of the final [DOX] released in 1 hour. In views of decreasing the release rate, plasma polymerization with polycaprolactone (PCL) was investigated on the surface of the DOX-loaded CDHA microspheres. In vitro experiments revealed cell viabilities down to 20% with SaOs-2 after 72 h in presence of DOX-loaded microspheres, either uncoated or PCL-coated, while hMSC presents a maintained cell viability around 80% in the same cell culture conditions.

**Conclusion**
The loading of doxorubicin into the CDHA microspheres from an aqueous solution is size dependent. The antitumoral agent mainly adsorbs onto the surface of the microspheres which promotes a fast subsequent release of doxorubicin. Evidence of selectivity of the DOX-loaded CDHA microspheres on osteosarcoma cells (SaOs-2) was revealed by in vitro cell cultures, when compared the cytotoxic effects with those observed on human mesenchymal stem cells (hMSC).

**Acknowledgement**
This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (Grant agreement Nº 714793). Authors belong to the SGR2017 1165.

**References**
ESB-SIBB S02-07

Theranostic hydroxyapatite nanoparticles with intrinsic anti-cancer activity against triple negative breast cancer

Tiago P. Ribeiro, Fernando J. Monteiro, Marta S. Laranjeira

1 University of Porto, INEB-Instituo Nacional de Engenharia Biomédica, Porto, PT; 2 University of Porto, Institute for Research and Innovation in Health (i3S), Porto, PT; 3 University of Porto, FEUP-Faculty of Engineering, Porto, PT

Introduction
Theranostic nanometric systems are compact devices with both therapeutic and diagnostic capabilities. Moreover, materials with intrinsic anti-cancer activity are revealing great promise as co-adjuvants of common cancer therapies such as chemotherapy and radiotherapy [1]. For instance, one material with an unexpected intrinsic anti-cancer activity is hydroxyapatite. This calcium phosphate is a highly biocompatible and biodegradable material, mainly used as a synthetic bone substitute due to its similarity with natural bone apatite [2]. Yet, several studies reported that hydroxyapatite nanoparticles are selectively toxic against certain types of cancer cells, meaning that healthy cells are not affected. Moreover, the unique crystal structure of hydroxyapatite enables it to be modified through ion doping, which creates additional features for biomedical applications, such as magnetism and antimicrobial activity [3-5].

Experimental Methods
In this study, hydroxyapatite and iron doped hydroxyapatite nanoparticles were synthetized, through a wet-chemical precipitation method, characterized and their influence on triple negative breast cancer cells was evaluated. Physicochemical characterization was done with TEM for size and morphology determination, XRD and FTIR for crystallinity, phase purity and chemical composition evaluation and SQUID for the study of the magnetic properties. In vitro toxicity towards MDA-MB-468 human breast cancer cells was determined through metabolic analysis, live-dead fluorescence assay and ultrastructure analysis. Contrast potential for bioimaging was determined in a clinical 3T MRI equipment.

Results and Discussion
The physicochemical characterization of the produced particles, through the wet chemical precipitation method, revealed that both where, in fact, hydroxyapatite with a hexagonal crystalline conformation. Iron incorporation, by calcium substitution, was confirmed through XRD, where a less crystalline structure with a smaller crystallite size was obtained. This occurs because iron ions introduce defects into the crystal structure and size decreases because iron has a smaller atomic radius than calcium. Quantitatively, the produced particles had about 10% iron ions, which provided the material with a saturation magnetization of 0.5 emu/g and a superparamagnetic behaviour. These magnetic properties were further explored, and when the nanoparticles were exposed to an MRI magnetic field, they were able to produce significant contrast, meaning that they could be used as contrast agents for cancer diagnosis. Finally, regarding the intrinsic anti-cancer activity, it was confirmed that both non-doped and iron doped hydroxyapatite nanoparticles were toxic towards triple negative breast cancer cells in a concentration dependent manner, with the iron doped ones being more effective. Through an ultrastructure analysis, the cells were mainly suffering from mitochondrial stress and cell membrane deregulation. These led to the conclusion that the calcium excess was working as a mitochondria disruptor, while iron ions were probably contributing to the formation of reactive oxygen species through the Fenton reaction. The latter being supported by some test conditions where the production of lipid droplets was detected, which are indicators of oxidative stress.

Conclusion
In conclusion, these findings regarding iron doped hydroxyapatite nanoparticles, serve as a foundation for future development of cancer theranostic devices.

Acknowledgement

The authors acknowledge project Biotherapies (NORTE-01-0145-FEDER-000012), supported by Norte Portugal Regional Operational Program (NORTE2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). This work was also financed by FEDER - Fundo Europeu de Desenvolvimento Regional funds through the COMPETE 2020 Operational Programme for Competitiveness and Internationalization (POCI), PORTUGAL 2020, in the framework of the project “Institute for Research and Innovation in Health Sciences” (POCI-01-0145-FEDER-00727). The authors also acknowledge the support of the i3S Scientific Platforms, Advanced Light Microscopy (ALM) and Histology and Electron Microscopy (HEMS), members of the national infrastructure PPBI - Portuguese Platform of Bioimaging (PPBI-POCI-01-0145-FEDER-022122) and the Biointerfaces and Nanotechnology (FCT-UID/BIM/ 04293/2019). Additionally, the authors acknowledge the collaboration of João A. M. Santos and Pedro Conde from the Medical Physics, Radiobiology and Radiation Protection Group, IPO Porto Research Center (CI-IPOP), Portuguese Oncology Institute of Porto (IPO Porto), Porto, Portugal, during MRI image acquisition.

References

Rapid Assembly of Fibrillar dECM-Hyaluronic Acid Hydrogels for 3D Breast Cancer In Vitro Modelling

Luís P. Ferreira, Carole Jorge, Vitor M. Gaspar, João F. Mano

University of Aveiro, CICECO - COMPASS, Aveiro, PT

Introduction
Currently available 3D in vitro breast cancer models focus mostly on the independent recapitulation of either cell population interactions or singular ECM-specific moieties. The influence of tumor microenvironment cellular and extracellular populations is critically inferred and yet mischaracterized in preclinical validation models resulting in a poor correlation between in vitro analysis and clinical trials data [1]. Alternatively, high-throughput compatible models seeking to provide a holistic representation of the tumor microenvironment interplay between cancer cells and their surrounding ECM are underdeveloped [2]. Within this context, decellularized scaffolds provide a new pathway to recapitulate major tumor hallmark characteristics in complex cell-representative coculture models capable of integrating ECM-specific stimuli and bioactive components [3]. Therefore, providing a more biomimetic window into the process of tumor modeling and cell-cell interaction analysis. Seeking to overcome these disadvantages, herein we developed fibrillar dECM/hyaluronic acid conjugate hydrogels for establishing organotypic 3D breast cancer-stromal models which recapitulate the cellular and matrix landscape of the human tumor. These surrogate biomimetic matrices were combined with cancer and stromal cells and rapidly processed into spherically structured 3D tumor models using superhydrophobic surfaces.

Experimental Methods
To this end, ECM derived from porcine adipose mammary tissues was decellularized [2], characterized and homogenized into fibrillar micro-fragments suitable for inclusion in the process of microtumor assembly. High molecular weight hyaluronic acid was modified with methacrylic moieties in accordance with previously established methods [4]. Breast cancer cells (MDA-MB-231) and Breast Cancer Associated Fibroblasts (BCAFs) were directly cultured with conjugated dECM microfibrillar fragments within photocrosslinked hyaluronic acid hydrogels (dECM/HA-MA) assembled in super-hydrophobic surfaces [5]. Obtained microgels ability to recapitulate key solid tumor hallmarks was evaluated through characterization of morphology, viability, and necrotic core formation analysis.

Results and Discussion
The optimized dECM/HA-MA spherically structured 3D microgels supported cancer-stromal cells and 3D spheroids viability, as well as served as unique platforms for evaluating such co-cultures interactions and in vitro maturation along time. The conjugation of HA hydrogels with dECM microfragments better recapitulated the diverse landscape of mammary ECM. Serving as a platform in which tumor-targeting combinatorial therapeutical compounds can be tested.

Conclusion
Overall, the addition of dECM fragments, preserving ECM fibrillar architecture and mammary specific components, within a secondary matrix of HA-MA enabled the development of dECM-enriched scaffolds in which co-culture interactions could be evaluated in a Tumor-ECMs mimetic context capable of partially portraying the ECMs influence in cancer cell behavior.

Acknowledgement
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER
under the PT2020 Partnership Agreement. This work was also supported by the Programa Operacional Competitividade e Internacionalização (POCI), in the component FEDER, and by national funds (OE) through FCT/MCTES, in the scope of the project PANGEIA (PTDC/BTM-SAL/30503/2017). Vítor Gaspar acknowledges funding in the form of a Junior Researcher Contract under the scope of the project PANGEIA. The authors acknowledge the financial support by the Portuguese Foundation for Science and Technology (FCT) through an individual Doctoral Grant (SFRH/BD/141718/2018, Luís Ferreira).

References

11:30 a.m. – 1:00 p.m.
Track06

ESB-BMSJ S | ESB-Biomaterials Science Journal (BMSJ) Joint Session: Biomaterials Science Journal Lectureship Symposium

Chairs
Pamela Habibovic
Univ. Maastricht, NL
Maria Southall
Royal Society of Chemistry, Publishing, Cambridge, GB
May A. Tayyem (YSF)
Queen's University Belfast, School of Pharmacy, Belfast, GB

Biomaterials Science is very happy to be the official journal of the European Society of Biomaterials and sponsor a session at ESB 2021.

Bimaterials Science is published on a not-for-profit basis by the Royal Society of Chemistry and publishes high impact work focused on exploring the design, function, and interactions of biomaterials. Its scope encompasses new concepts in biomaterials design, studies into the interaction of biomaterials with the body, and the use of materials to answer fundamental biological questions. The journal is led by Editor-in-Chief Jianjun Cheng, alongside an international Editorial Board including expert Associate Editors.

This session features presentation of the 2021 Biomaterials Science lectureship to Nasim Annabi (UCLA). We are happy to have Khuloud Al-Jamal as second keynote speaker in the session.
ESB-Biomaterials Science-KL01

Multifunctional adhesive biomaterials for medical applications

Nasim Annabi

University of California, Department of Chemical and Biomolecular Engineering, UCLA, Los Angeles, US

Tissue engineering is an interdisciplinary field incorporating concepts from engineering, biological sciences, and medicine with the goal of engineering biological substitutes to maintain, restore and promote normal tissue function. Polymer-based biomaterials have played an important role in the development of tissue constructs that mimic the structures and physical properties of the native tissues. However, there are still many challenges in micro-engineering biomaterials with tunable physical and biological properties for the development of fully functional tissue constructs. The combination of advanced biomaterials with micro- and nanoscale technologies have been shown to hold great potential to address the current challenges in tissue engineering. Our research focuses on developing biomimetic elastin-based biomaterials and nanocomposite hydrogels, with controlled architecture, and physical and biological properties, utilizing recombinant proteins. These advanced biomaterials are integrated with different micro- and nanofabrication technologies to engineer biomimetic tissue constructs for engineering soft and elastic tissues. Furthermore, we have developed new chemistries to improve the adhesion of these biomaterials to the tissue surfaces and use them as multi-functional bioadhesives for sealing and repair of soft tissues such lung, heart, skin, and cornea. In addition, our group has designed different nano delivery planforms which can be incorporated in these bioadhesives for gene and drug delivery applications. In this presentation, I will outline our recent work on the development of adhesive and elastic hydrogels for tissue engineering along with their clinical applications as tissue adhesives and surgical sealants.
Track to understand or understand to track extracellular vesicles?

Khuloud Al-Jamal

King’s College London, School of Cancer and Pharmaceutical Sciences, London, GB

The ability to track extracellular vesicles (EVs) in vivo without influencing their biodistribution is a key requirement for their successful development as drug delivery vehicles and therapeutic agents[1]. Here, we evaluated the effect of five different optical and nuclear tracers on the in vivo biodistribution of EVs. Exp293F EVs were labelled using either a non-covalent fluorescent dye DiR[2], or covalent modification with 111Indium-DTPA[3], or bioengineered with fluorescent (mCherry) or bioluminescent (Firefly and NanoLuc luciferase) proteins fused to the EV marker, CD63. To focus specifically on the effect of the tracer, we compared EVs derived from the same cell source, and administered systemically by the same route and at equal dose into tumour-bearing BALB/c mice. 111Indium and DiR were the most sensitive tracers for in vivo imaging of EVs, providing the most accurate quantification of vesicle biodistribution by ex vivo imaging of organs and analysis of tissue lysates. Specifically, NanoLuc fused to CD63 altered EV distribution resulting in high accumulation in the lungs, demonstrating that genetic modification of EVs for tracking purposes may compromise their physiological biodistribution. Blood kinetic analysis revealed that EVs are rapidly cleared from the circulation with a half-life below 10 min. Our study demonstrates that radioactivity is the most accurate EV tracking approach for a complete quantitative biodistribution study including pharmacokinetic profiling. In conclusion, we provide a comprehensive comparison of fluorescent, bioluminescent, and radioactivity approaches, including dual-labelling of EVs, to enable accurate spatiotemporal resolution of EV trafficking in mice, an essential step in developing EV therapeutics.

References
“BeeInspired”: Drug load patches for wound healing

Katia R. Amaral, Lúcia F. Santos, Ana S. Silva, João F. Mano

CICECO - Aveiro Institute of Materials, University of Aveiro, Aveiro, PT

Introduction

Transdermal drug delivery patches are used to improve wound healing. Still, the limited amount of drugs that these patches can carry leads to a frequent patch replacement which can cause reinjury of the wound, thus resulting in pain and delayed wound healing.1,2 Inspired by this limitation and stimulated by the ability of honeybees in transporting large quantities of pollen particles in between their legs’ hairs, we propose the development of an algae-derived micropatterned patch composed of spaced micropillars with high aspect ratio able to transport high quantities of drug particles. After satisfactory results using polydimethylsiloxane 3, methacrylated laminarin is now used to microfabricate the patch, granting anti-inflammatory properties and a moist environment for a faster healing, while improving the delivery of poorly soluble drugs such as ciprofloxacin, used as an antibiotic.

Experimental Methods

Firstly, methacrylated laminarin micropatterned patch (MLMP) was prepared by a soft lithography technique and mechanical characterization was performed. Then, PCL microparticles were produced by an emulsion technique in order to investigate the entrapment efficiency of MLMP by gently pressing the particles onto the MLMP. Herein, we also demonstrated that microparticles could be immobilized in the developed MLMP. For this purpose, ciprofloxacin-encapsulated alginate microparticles (CLM) were produced by an electrospray technique. MLMP entrapping either ciprofloxacin powder (CP) or CLM were investigated. Free MLMP and non-loaded alginate microparticles were used as controls for the different assays. In vitro release studies of the drug were made at pH 7.4 (physiological) and pH 5.5 (wound). Moreover, the cells biocompatibility of the mentioned constructs was evaluated using human-dermal fibroblast. After incubation for 3, 7 and 14 days, analysis of cells viability, mitochondrial quantification and cell proliferation were performed. Finally, growth inhibition of MLMP and the different constructs was investigated using E. coli (gram negative) and S. aureus (gram positive) cultures.

Results and Discussion

We herein report a novel biomimetic structure with spaced micropillars able to physically support the entrapment of drug particles, as proven by the successful entrapment of PCL microparticles in the MLMP. Sustained and controlled releases were evidenced for both constructs (MLMP + CP and MLMP + CLM) at both pHs, with an initial burst release of 10 % of CP from the MLMP in the first hour. Suggested by this initial burst release followed by a more controlled and sustained release evidenced for the CP-MLMP system at both pHs, we hypothesis that MLMP had a probable superficial adsorption of CP, as usually seen in the marketed patches. This event demonstrated that our MLMP could be applied either in wound healing situations or other events that require topical transdermal delivery of a drug, since an initial burst provides immediate relief followed by prolonged release to promote gradual healing. Furthermore, bacterial assays showed that MLMP containing CP manifested remarkable antimicrobial activity against both strains. Interesting, free MLMP and MLMP with non-loaded alginate microparticles shown antibacterial activity against E. coli, but the same result was not evidenced for S. aureus. As reported in literature, the laminarin present antibacterial activity against gram-negative bacterium, corroborating the results of MLMP against E.coli.4 Finally, cellular assays were performed attesting the biocompatibility of our MLMP (free of drug).

Conclusion

The biocompatibility of our MLMP (free of drug) together with an enhanced antimicrobial activity and with the ability to fix high dosages of CP or CP in drug vehicles (alginate microparticles), make these natural-derived hierarchal...
optimized patches valuable systems for clinical applications. Additionally, since the particles’ entrapment can be easily accomplished by simple contact of the patch with any powdered product, these constructs could be a valuable solution for the treatment of diseases that require high drug concentrations, enabling less-frequent dosing and thus significantly improving patient compliance. We also hypothesize that the applicability of these systems could be extended from wound healing applications to other high-value uses, such as the biomedical, biotechnological, environmental and industrial fields, where fixing high content of microparticles could be an added-value.

Acknowledgement

The authors acknowledge the financial support by the European Research Council grant agreement ERC-2014-ADG-669858 for project “ATLAS”. The work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID /CTM /50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement.

Fabrication and characterization of methacrylated laminarin micropatterned patch (MLMP).

A) Schematic illustration of the fabrication of MLMP by soft lithography; B) Photograph of photocrosslinked MLMP; C) Two-dimensional and D) three-dimensional fluorescence microscopy visualization of MLMP. Scale bar is 200 μm; E) Representative compressive stress-strain curve for MLMP; F) Mechanical performance of the developed MLMP – Young’s modulus, Ultimate Compressive Strength and Maximum Strain.
Microparticles with 80μm diameter, in vitro release assays and antimicrobial activity MLMP.
A) Photograph of PCL microparticles entrapped in MLMP; B) Fluorescence microscopy of Nile Red-PCL microparticles entrapped in MLMP. Scale bar is 100μm. C) Fluorescence microscopy of MLMP with non-loaded alginate microparticles. Scale bar is 200 μm; D) In vitro cumulative release of ciprofloxacin from MLMP; E) Graphical representation of the inhibition zone (mm) of the different constructs; F) Disposition of the different combinations on plate; G) Antibiograms to Gram-positive S.aureus and H) Gram-negative E.coli.

References
[2] 10.4155/tde.10.16
Multifunctional lipid-based magnetic nanovectors: Combining chemotherapy and magnetic hyperthermia to treat glioblastoma multiforme

Carlotta Pucci, Daniele De Pasquale, Gianni Ciofani

Istituto Italiano di Tecnologia, Smart Bio-Interfaces, Pontedera, IT

Introduction
Glioblastoma multiforme (GBM) is the deadliest brain tumor, with a median survival rate of only 12-15 months and a 5-year survival of around 5%. Difficulties in treating GBM arise from its diffuse nature and genetic variability that makes conventional therapies extremely inefficient. Moreover, most of the drugs cannot easily penetrate the blood-brain barrier (BBB), and have poor selectivity. For this reason, research is focusing on finding more efficient treatments.

In this work, we propose a multimodal approach based on the synergic action between chemotherapy and magnetic hyperthermia. To achieve this, lipid nanovectors (LMNVs) were loaded with a drug (chosen among a library of drugs tested on different glioblastoma cell lines) and superparamagnetic iron oxide nanoparticles (SPIONs), inducing lysosomal membrane permeabilization (LMP) upon interaction with an alternated magnetic field (AMF). Thanks to the functionalization with peptides (angiopep-2) or cancer cell membrane extracts (homotypic targeting), nanovectors can accumulate in tumor tissues, and more efficiently cross the BBB.

Experimental Methods
LMNVs were synthetized by hot ultrasonication, and subsequently functionalized with either angiopep-2 or GBM cell membrane extracts. The physicochemical properties of the nanovectors were studied by DLS, TEM, TGA, whereas the successful functionalization was demonstrated by fluorescence spectroscopy and SDS-PAGE. Drug loading and release was measured by HPLC. The cytotoxicity of the nanovectors was studied in vitro by WST-1 assay and immunocytochemistry, with or without alternated magnetic field (AMF) stimulation. The preferential uptake of the nanovectors by GBM cells was assessed in static and dynamic conditions, and compared with the uptake by healthy cells lines (human astrocytes, endothelial cells, and neuronal-like cells). The intracellular fate of Ang-LMNVs was studied by confocal microscopy.

Results and Discussion
Thanks to functionalization, we were able to selectively target GBM cells, both in static and dynamic conditions, while the uptake by healthy cells was, in comparison, negligible. On the other hand, bare LMNVs uptake was lower and aspecific. Functionalized nanovectors were also found to be more efficient in crossing a fluidic BBB model as compared to bare LMNVs. LMNVs were internalized in lysosomes. Upon interaction with AFM, the SPIONs-mediated heat generation induced destabilization of the lysosomes membrane (LMP effect) and triggered the leakage of lysosomal enzymes, such as cathepsin B, in the cytosol with consequent cell death. Alongside, the drug encapsulated in the nanovectors was able to induce an increase in p53 expression, leading to cell apoptosis. The toxic effect of the drugs increased when the drug was loaded in the nanovectors as compared to the free form, probably due to a higher cellular uptake mediated by the nanovectors. Moreover, the release of the drug was found to be higher at acidic pH conditions, similar to those found in cancer cells and in acidic organelles, such as lysosomes. Therefore, AMF-induced LMP might foster the release of nutlin-3a trapped within lysosomes, increasing its efficiency. The combined effects of chemotherapy and magnetic stimulation, induced a significant reduction of GMB cells viability (<
1\%), and, even after 3 days from the end of the stimulation, no further cell proliferation was detected, proving the high efficiency of the approach.

Conclusion
Thanks to the synergic effect between chemotherapy and magnetic hyperthermia, LMNVs efficiently activates several apoptotic pathways in GMB cells, hindering cell proliferation even after the chronic AMF stimulation protocol. Moreover, the improved BBB crossing abilities and targeting efficiency of functionalized LMNVs make this system a potential candidate for the treatment of GBM.

Acknowledgement
The research leading to these results has received funding from AIRC under IG 2020 - ID. 24454 project – P.I. Ciofani Gianni.
Particle-coated hydrogels for adhesion to internal tissues

**Maïlie Roquart**¹², Estelle Palierse¹, Nicolas Golse³, Eric Vibert³, Mathieu Manassero⁴⁵, Sophie Norvez¹, Laurent Corté¹²

¹ ESPCI Paris, PSL University, Chimie Moléculaire, Macromoléculaire et Matériaux, Paris, FR; ² Mines ParisTech, PSL University, Centre des Matériaux, Evry, FR; ³ Hôpital Paul Brousse, Centre Hépato-biliaire, Villejuif, FR; ⁴ Ecole Nationale Vétérinaire de Maisons-Alfort, Service de Chirurgie, Maisons-Alfort, FR; ⁵ CNRS, Biologie, Bioingénierie et Bioimagerie Ostéo-articulaires, Paris, FR

**Introduction**

Improving the fixation of hydrogels to the soft and wet surface of internal organs could create opportunities for the development of innovative surgical approaches and implantable technologies. Adhesion by particle bridging appears as an interesting solution to that respect [1]. In this approach, particles are deposited at the tissue-hydrogel interface and adhesion is created by the adsorption of the macromolecules composing the hydrogels and the tissues to the surface of these particles, which can then act as connectors. In vivo experiments on rat models showed that nanoparticles can be used to fix polymer membranes to tissues, onto a resected liver surface and a beating heart [2]. More recently, in vivo experiments on pig models have shown that in the presence of blood, the use of procoagulant silica nanoparticle coatings can further enhance this hydrogel-tissue adhesion by triggering the formation of an interfacial blood clot [3]. In addition to these specific particle bridging mechanisms, intrinsic viscoelastic properties of hydrogels also play an important role in their adhesion to soft internal tissues through the dissipation of mechanical energy [4]. Taking these considerations into account, we investigate here how the adhesion on internal tissues depend on the properties of hydrogel substrates and particle coatings in order to design membranes able to adhere by simple contact and withstand surgical and physiological stresses.

**Experimental Methods**

**Materials:** PEG-based hydrogel membranes of 0.5-2 mm thickness were fabricated using formulations based on photoactivated thiol-acrylate chemistry [5]. Powders of aggregated silica nanoparticles having a 30 nm diameter (Ludox TM-50®) were prepared from aqueous solutions by a series of steps comprising dialysis, drying, grinding and sieving.

**Liver tissue sourcing:** Pig livers were purchased from butcher’s shop or harvested from surgery school (Fer-à-Moulin, AP-HP). They were stored at 4°C and used from 3h to 76h after harvesting.

**Adhesion measurements:** Peeling experiments were performed on a tensile test apparatus (AllAround, Zwick) equipped with a 10 N force cell. For each measurement, a 1cm-wide hydrogel stripe, coated or not, was placed onto either a model glass surface or the surface of porcine liver capsule. Contact was applied for given times and pressures. Peeling was then performed at constant angle (90°) and speed (1 mm/s).

**Surface characterization:** The surface of coated membranes was characterized by SEM on a FEI Nova NanoSEM 450 apparatus. Specimens were sputter coated with a 2 to 4 nm layer of gold/palladium and then observed with a secondary electron TLD detector and a backscattered electron detector (CBS) which gives a chemical contrast image. This analysis was completed with an energy-selective X-ray microanalysis system (EDS) to trace the presence of elements on the surface that are representative of the particles (Si), of the gel (S) and of biological compounds (N).

**Results and Discussion**

To explore these adhesion mechanisms and design clinically relevant adhesives, we synthesized resorbable PEG hydrogel membranes having adjustable topologies and cross-linking densities. The viscous dissipation within these
gels could be greatly modulated. For instance, adhesion energy on model glass surfaces varied from 20 to 250 J.m$^{-2}$ by varying the thiol-acrylate ratio (Figure 1a). Coatings of silica nanoparticle aggregates were deposited on those membranes by a simple brush spreading process. Their adhesive performances and the underlying microscopic mechanisms were characterized by ex vivo peeling tests on the capsule of porcine livers.

Figure 1b shows typical peeling curves after a 5 min contact on the liver capsule for highly dissipative membranes with and without silica particle coating. For the uncoated membranes, a normalized peeling force of about 4 N.m$^{-1}$ is obtained and peeling is accompanied by a small deformation of the liver tissue (Figure 1c). For the coated ones, the peeling force can be 3 to 4 times higher with a noticeably larger deformation of the liver (Figure 1d). SEM observations (Figure 1e) and chemical analysis indicate that silica aggregates remain on the hydrogel surfaces after peeling and are coated with biological compounds.

**Conclusion**

Through ex vivo experiments on porcine liver surfaces, we showed that the adhesion of PEG hydrogels is strongly enhanced by the deposition of silica nanoparticle coatings. Using intrinsically dissipative hydrogels, adhesion energies up to 15 J/m$^2$ were obtained for contact times and pressures that are relevant for surgical practice. These results justify the implementation of on-going in vivo tests to evaluate the robustness of these adhesives in surgical conditions.

**Acknowledgement**

Financial support from the French National Research Agency (ANR-18-CE19-0022-04 NanoBioTape) is acknowledged. We thank Maria Simoes (Mines ParisTech) for technical support on SEM experiments.

**References**


11:30 a.m. – 1:00 p.m.

Track07

N12 | Biomaterials for Cardiovascular Applications and Angiogenesis

Chairs

Didier Letourneur
Université de Paris, INSERM U1148, LVTS, Paris, FR

Chiara Rinoldi
Institute of Fundamental Technological Research Polish Academy of Sciences, Department of Biosystems and Soft Matter, Warsaw, PL

Batur Ercan (YSF)
Middle East Technical University, Ankara, TR
Modelling early stage cardiac fibrosis through “bioartificial” scaffolds: an in vitro platform for preclinical validation of new cardiac regenerative therapies

Mattia Spedicati¹,², Gerardina Ruocco¹,², Irene Carmagnola¹,², Alice Zoso¹,², Valeria Chiono¹,²

¹ Politecnico di Torino, Department of Mechanical and Aerospace Engineering, and POLITO BioMedLab, Turin, IT; ² Interuniversity Center for the promotion of the 3Rs principles in teaching and research, Centro 3R, Politecnico di Torino Unit, Turin, IT

Introduction
Myocardial infarction, the leading cause of mortality and morbidity worldwide, provokes the loss of billions of cardiomyocytes and the deep remodelling of cardiac extracellular matrix (ECM) with the progressive formation of a stiff and dysfunctional fibrotic tissue, rich in crosslinked collagens. Post-infarct microenvironment is mainly populated by activated cardiac fibroblasts called myofibroblasts (MyoFs) [1]. Moreover, cardiac fibrotic tissue is characterised by high variability in terms of composition, size, stiffness and structure, depending on location and time from heart attack. Currently, there is no effective treatment to reverse cardiac fibrosis, therefore heart transplantation remains the only available clinical approach. Following the 3Rs principle (Reduction, Replacement, Refinement), in vitro models of human cardiac post-infarct tissue could improve preclinical validation of new therapies aimed at heart regeneration.

The aim of this work was to engineer in vitro models of early-stage human cardiac fibrotic tissue, exploiting 2D and 3D “bioartificial” synthetic/natural polymer scaffolds.

Experimental Methods
Randomly oriented 2D nanofibrous polycaprolactone (PCL; Mw: 43.000 Da) scaffolds were fabricated by electrospinning (Linari Engineering), with small and interconnected porosities, closely mimicking the ECM morphological features. 3D square-meshed PCL scaffolds with different interconnected pore sizes (150 µm; 350 µm) were fabricated through melt-extrusion additive manufacturing (Rokit Invivo). The three scaffold types reproduce the structure of early stage cardiac fibrotic tissue with different severity.

To mimic cardiac ECM chemical composition, gelatin (G; Type A) was grafted on scaffold surfaces using mussel-inspired pre-coating [2]. Physicochemical characterisations were performed after each functionalization step. G grafting efficacy was confirmed by QCM-D, ATR-FTIR, and static contact angle analyses that was also used to assess coating stability upon 1 week incubation in phosphate buffered saline (PBS) at 37°C. Adult human cardiac fibroblasts from healthy human ventricle (HCFs) were cultured on 2D and 3D scaffolds for up to 3 weeks.

Results and Discussion
2D electrospun scaffolds showed homogeneous morphology with no process defects (Figure 1 a). 3D scaffolds, with both 150 µm and 350 µm pore sizes showed well defined and homogeneous porosity (Figure 1 b-c). Regarding the biomimetic coating, QCM-D and ATR-FTIR analyses confirmed G successful deposition. The static contact angle measurements showed higher wettability for G modified scaffolds, which was maintained upon 1 week incubation in phosphate buffered saline (PBS) demonstrating coating stability.

Biological validation showed bioartificial scaffolds capability to sustain HCFs adhesion and spreading. Phalloidin staining analysis highlighted cell coverage on all scaffolds and control samples after 3 weeks culture time (Figure 2). Interestingly, HCFs activation, triggered by intrinsic scaffold stiffness, was demonstrated by the increase (compared to control) in α-smooth muscle actinin (α-SMA) and Discoidin Domain-containing Receptor 2 (DDR2) expression. HCFs on control samples did not express α-SMA and DDR2, as evaluated by immunostaining, while HCFs cultured...
on 2D and 3D functionalized scaffolds expressed both fibrotic markers. The secretion of typical cardiac ECM proteins (Fibronectin, Laminin, Tenascin and Collagens) was confirmed by immunofluorescence analysis.

**Conclusion**

This work proposes such 2D and 3D engineered “bioartificial” tissues as platforms for *in vitro* modelling of early stage cardiac fibrotic tissue with different thickness and severity depending on scaffold architecture. Results will be beneficial for the preclinical investigation of new therapies and the study of different degrees of cardiac fibrosis.

**Acknowledgement**

This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme grant agreement No-772168.

---

**References**


Elastin-like Recombinamer and Collagen Bi-Layered Construct as a Model of the External Elastic Lamina and Tunica Adventitia of the Vascular Wall

Miguel González-Pérez, Dimitria B. Camasão, Diego Mantovani, Matilde Alonso, José C. Rodríguez-Cabello

Laval University, Laboratory for Biomaterials and Bioengineering, Quebec City, CA; University of Valladolid, BIOFORGE, Valladolid, ES

Introduction
The development of bioinspired models of the vascular wall presents a special interest in the biomedical field. Whether to serve as a model for studying common vascular diseases or for becoming the future solution for evaluating the activity of drugs and bioactive molecules in vitro [1]. Based on previous promising approaches employing collagen as scaffold for vessel walls [2] and the second main principal component present on these tissues, namely elastin, a bi-layered model of the external elastic lamina (EEL) and tunica adventitia of the vascular wall was designed. Collagen type I was isolated directly from rat tail tendon and embedded with fibroblasts (FBs) to reproduce the tunica adventitia, whereas the elastin replicating the EEL was recombinantly produced in Escherichia coli bacteria. This family of polypeptides, also known as elastin-like recombinamers (ELRs), were designed containing cell-adhesive, protease-sensitive sequences and click reactive groups to constitute the EEL matrix. Mechanical and histological analysis were performed to evaluate the cell-remodeling activity and the approximation of the designed model to the native EEL and tunica adventitia physiology under in vitro maturation for two weeks.

Experimental Methods
A biocasting technique comprising the use of a multiwell plate containing a cylindrical mandrel in the center was developed. Evolution in the mechanical response of the model, namely elastic modulus, tensile strength and strain at break, was investigated by circumferential tensile stress-relaxation test. Whereas histology allowed to evaluate the distribution of cells, collagen and ELR matrix morphology, and production of α-smooth muscle actin (α-SMA), focal adhesion kinase (FAK), and acid polysaccharides at 3, 7 and 14 days.

Results and Discussion
The obtained data identified the elastic and more viscous character conferred by the ELR and collagen matrix, respectively. Maturation in vitro revealed the ability of the RGD cell-adhesive epitopes to deviate the HDFn polarization from the surface of the model, in contact with the culture medium, towards the ELR/collagen interface instead. Histology showed the presence of α-SMA, FAK, and acid polysaccharides indicating the active cell remodeling activity and differentiation of the embedded FBs into myofibroblasts, thus suggesting their active participation in the acquisition of a physiological-like morphology and environment.

Conclusion
A biocasting technique for fabricating a bi-layered model of the EEL and tunica adventitia from FBs, collagen and bioactive click-crosslinkable ELRs was designed. The developed model positively resembled the native morphology and composition while confirmed the ability of the moldable collagen matrix and the encoded bioactive sequences in the ELR counterpart to direct the behavior of FBs cells.

Acknowledgement
The authors are grateful for funding from the Spanish Government (MAT2016-78903-R, RTI2018-096320-B-C22, FPU15-00448 and EST18/00068), the Junta de Castilla y León (VA317P18), the Interreg V A España Portugal POCTEP (0624_2IQBIONEURO_6_E), the Centro en Red de Medicina Regenerativa y Terapia Celular de Castilla Page 734 of 2028
y León, the Natural Sciences and Engineering Research Council of Canada (NSERC), the NSERC Create Program in Regenerative Medicine, the Canadian Foundation for the Innovation and the Fonds de Recherche du Québec (Nature et Technologies, and Santé).

References


A textile approach of tissue-engineered vascular graft: implantation, conservation and sterilization

Diane Potart, Maude Gluais, Nicolas Da Silva, Yoann Torres, Nicolas L'Heureux

Université de Bordeaux, Science de la Vie et de la Santé (SVS), Bordeaux, FR

Introduction
To meet the clinical need for small vascular graft, we propose a new “human textile” approach creating a completely biological Tissue-Engineered Vascular Graft (TEVG). Vessels were produced by weaving into a tube yarn of Cell-Assembled extracellular Matrix (CAM) synthetized in vitro by human skin fibroblasts. Our TEVG clinical use as an off-the-shelf product requires in vivo validation, flexible production, final product storage and sterility assurance. Therefore, the objectives of this study are to 1) assess the human TEBV functionality in an immunodeficient rat model, 2) find the best conservation method maintaining CAM mechanical properties and matrix structure, and 3) find the best terminal sterilization method to simplify TEBVs production while preserving its biomechanical and biological properties.

Experimental Methods
Well-organized human CAM sheets were produced in vitro after maintaining human skin fibroblasts in culture during two months with 20% bovine serum and 0.5 mM ascorbate. CAM sheets were cut into 2mm wide ribbons and woven into small tubes (8 mm long, 1.6 mm inner diameter). Collars were especially created at each end allowing TEVGs suture with native vessels. Those TEVGs were implanted in abdominal aortas of immunodeficient rats by a microvascular surgeon and their functionality and remodeling will be tested up to 12 months.

CAM sheet were cut into 5 mm wide ribbons that were conserved in conditions compatible with clinical use (-80°C, -20°C, 4°C (dry or wet) and Room Temperature (RT) (dry or wet)). After 1 year, ribbons were mechanically tested and compared to control (-80°C, 1 night of conservation).

Our CAM was sterilized using gamma irradiation (dry or wet, with high or low dose rates), electron beam irradiation, ethylene oxide (EtO), or supercritical carbon dioxide (scCO2). Sterilized 5 mm wide ribbons (16 cm long, n=8/condition/time) were implanted subcutaneously in immunodeficient rats (4 ribbons/rat, 54 rats) and tensile tests were performed before implantation and after 2, 4, 20, and 40 weeks.

Subcutaneous explants were fixed and embedded in paraffin, and will be stained (H&E, Masson’s Trichrome) or processed for immunofluorescence (including antibodies against collagen-I and M1/M2 immunostaining).

Sterilized sheets are currently tested using DSC (Differential Scanning Calorimetry) and FTIR (Fourier Transform InfraRed spectroscopy). Endothelial cells proliferation on sterilized CAM sheets is also evaluated.

Results and Discussion
Preliminary surgeries in 1 mm diameter abdominal aortas of nude rats demonstrated graft implantability and the absence of transmural leakage. To this day, we are following 12 successful grafts and we already reached 6 months of survival.

After 1 year of conservation, ribbons showed no loss of strength, and RT dry condition even lead to a force increase (+26% / control). This could be due to a sample rehydration incapacity. This hypothesis is supported by the decrease in section of these samples (-60% / control) and requires further evaluation. We also noticed that wet conditions (4°C and RT) showed lower max strain (-10% / control).

Before implantation, the ribbons strength of the gamma-wet group was significantly lower than control (sterile production group) by 34%, while the EtO group was stronger (+20% / control). After five months, the gamma-wet...
group remained the weakest, while scCO2 group seemed to be stronger than other conditions. 10 months results are under evaluation. Histological and physicochemical evaluation with DSC and FTIR are expected soon, as well as endothelialization results.

**Conclusion**

Our next step will be TEBV long-term implantations to assess its remodeling. We are expecting a graft spontaneous endothelialization by the host, avoiding its thrombosis.

We confirmed that our CAM can be easily conserved without losing mechanical properties up to 1 year, and that sterilization methods influence CAM mechanical properties. ScCO2 appears to be a promising solution. Histological and physicochemical analyses are expected to complete these results, highlighting the possibility of having a “off-the-shelf” product.

**Acknowledgement**

This work was supported by the "European Research Council" (ERC). We thank Marie Sarrazin, Sylvie Rey from "BioTis", Alexandra Gaubert from "ChemBioPharm", Julien Izotte, and Lea Mora Charrot from the Animal facility for their technical support.

**References**


Functionalization of biodegradable Zn-based alloys with poly(ε-caprolactone) and bioactive peptides to control degradation and enhance endothelialization

Claudia García Mintegui, Victor Chausse, Cédric Labay, Carles Mas Moruno, José Luis Cortina, Marta Pegueroles

Technical University of Catalonia, Barcelona, ES

Introduction

Zinc alloys arise as potential candidates for bioresorbable stents due to their moderate degradation rate in comparison with Mg-based and Fe-based alloys [1]. However, the control of the degradation behavior and the optimization of endothelial cell response remains an unmet challenge. This study explores different strategies to coat Zn-based alloys with poly(ε-caprolactone) (PCL) to control degradation. Subsequently, PCL-coated surfaces will be functionalized with bioactive synthetic peptides in order to accelerate surface endothelialization.

Experimental Methods

Zn, Zn-0.5Mg, and Zn-2Ag discs (Goodfellow) were ground with SiC abrasive paper up to grit 4000. PCL coatings were prepared as follows: (a) PCL nanocoating was developed by ε-caprolactone polymerization via cold plasma-polymerization process; (b) PCL (Mw: 70,000-90,000 g/mol) was dissolved in chloroform in 5.0 wt.% and spin-coated at 5,000 rpm onto samples surfaces during 30 s. Later, coated surfaces were functionalized with RGD, REDV linear and RGD+REDV dual peptides, synthesized in solid-phase, and covalently attached to the samples surfaces using EDC/NHS activation and coupling. Surface physicochemical characterization was performed by X-ray photoelectron spectroscopy (XPS) and a dual beam focused ion beam/field emission scanning electron microscope (FIB/FESEM). Electrochemical behavior in DMEM solution (37 ± 1 °C) was evaluated by potentiodynamic tests (ASTM G5-14) and electrochemical impedance spectroscopy (EIS) at 1, 2, and 3 days of immersion. Peptides immobilization to the surface was characterized by fluorescence microscopy and XPS. In vitro biological response was evaluated after 6 h of adhesion of human umbilical vein endothelial cells (HUVECs) and posterior live/dead and DAPI/Alexa Fluor 546 phalloidin staining.

Results and Discussion

FIB/FESEM determined a uniform PCL nanolayer of ~ 20 nm after the plasma-polymerization process (Fig. 1). The spin-coated samples presented a homogeneous PCL microlayer of ~ 2 μm. Potentiodynamic tests of spin-coated samples exhibited a noteworthy decrease in current density values suggesting that PCL is acting as a corrosion barrier. EIS supported that the corrosion resistance was provided by the PCL coating. No significant differences in corrosion were noticed for PCL-nanocoated samples with respect to untreated samples, as expected due to the nanothickness of the coating. PCL-coated Zn alloy surfaces were successfully biofunctionalized as observed by fluorescent confocal microscopy and N 1s XPS spectra. HUVEC adhesion studies indicated a good cell attachment and aggregation for Zn and PCL-coated surfaces (Fig. 2 a, c). However, changes in HUVEC adhesion morphology were observed for PCL-coated surfaces, with higher cell spreading that can foster endothelialization (Fig 2 d). REDV, RGD and the mixture of both peptides have been shown to accelerate endothelial cell adhesion, proliferation and migration [2]. However, the adhesion of RGD and REDV mixture was not correctly controlled. The covalent immobilization of RGD+REDV platform onto PCL-coated surfaces was correctly achieved, and allows to control the conformation of the peptides and the disposal to the cells. Finally, the combination of antifouling PCL micro-coating with pro-endothelialization peptidic platform showed a better biological performance.
Conclusion

PCL coating onto Zn-Alloys was successfully achieved by two different techniques, controlling the degradation rate through the thickness of the PCL layer. The electrochemical results suggest that the degradation behavior strongly depend on the thickness of the layer. In this work, the combination of antifouling PCL-microcoating with pro-endothelialization multipetidic RGD+REDV platforms enhanced surface endothelialization being a possible solution to overcome the reported poor *in vitro* cell biocompatibility of Zn alloys.

Acknowledgement

Financial support was received from Spanish Government, MINECO/FEDER (RTI2018-098075-B-C21 and MAT2017-83905-R) and the Government of Catalonia (AGAUR 2017 SGR 1165 and FI scholarship for C.G.M.)

References


Extrusion of nanocomposite poly-l-lactic acid for stent application

Lison Rocher, Gary Menary, Alex Lennon

Queen's university, School of Aerospace and Engineering, Belfast, GB

Introduction
Drug eluting stents are the current gold standard to treat coronary artery disease but they remain in the body and may cause late stage complications after a couple of years [1]. To overcome this issue, bioresorbable vascular stents (BVS) can fully degrade in the body within three years after the vessel healing. However current limitations of BVS (like their lower mechanical properties, microstructural heterogeneities and lack of radiopacity) have to be improved before their use in clinical practice [2,3]. To manufacture BVS, small tubes are extruded and then biaxially stretched before laser-cutting to a stent design and crimping onto a balloon.

To address the issues related to BVS, tungsten disulphide (WS₂) inorganic nanotubes (INT) were investigated to reinforce poly-l-lactic acid (PLLA) and increase its radiopacity. The aim of this study was to produce and characterise PLLA/WS₂ tubes loaded with 0.5wt% and 3wt% of nanoparticles to create a suitable preform for the stretch blow moulding process.

Experimental Methods
Medical grade PLLA (Corbion, Netherlands) was reinforced with INT-WS₂ (Holon Institute of Technology, Israel). Prior to extrusion, the polymer was ground into powder and mixed with the nanoparticles. The mix was dried for 4h at 120°C to avoid hydrolytic degradation. Neat PLLA tubes were produced following the same process. The tubular extrusion was performed at 230°C with a twin-screw extruder to ensure good mixing followed by quenching in a water bath.

The dispersion of the nanotubes was studied using scanning electron microscopy (SEM) and high-resolution x-ray micro-computed tomography (μCT). Gel permeation chromatography (GPC) was used to evaluate the effect of processing on the PLLA molecular weight (Mw). Thermal and morphological properties were determined using differential scanning calorimetry (DSC) at a rate of 10°C/min. Uniaxial tensile testing was performed to evaluate the tubes mechanical properties.

Results and Discussion
Extruded tubes produced have 1.90 ± 0.03 mm outside diameter and 0.59 ± 0.02 mm wall thickness along with good concentricity measured by optical microscopy.

GPC analysis indicated about 12% decrease of Mw for both the neat PLLA and 0.5wt% samples during drying and extrusion and about 34% for the sample loaded with 3wt% of nanotubes. This suggests that excessive loading of nanotubes can be detrimental to PLLA molecular chains during the extrusion process.

No agglomerate was observed from SEM images and the nanotubes looked well dispersed in PLLA (Figure 1a). Comparison of nanotubes length before and after processing showed a significant shortening due to the high shear induced in the extruder (from 4.05 ± 1.7μm to 2.2 ± 0.8μm). The nanotubes radiodensity and the high resolution of the instrument allow the evaluation of dispersion with μCT. The inclusion analysis indicated the presence of small agglomerates and a higher nanotube concentration toward the inner wall of the preform (Figure 1b), which was not detected with SEM due to highly localised scanning.

Mechanical properties results indicated similar ranges in term of Young’s modulus and elongation at break for all samples but the ultimate tensile strength for the 3wt% sample was decreased by ~8% compared to the neat PLLA tube (Figure 2a). These results can be due to the nanotubes shortening during extrusion, the decrease of Mw at 3wt%, or the state of dispersion, which could be improved during the expansion process.
The DSC results showed that the nanotubes have a strong nucleation effect on PLLA and impact its crystallization temperatures ($T_{cc}$ and $T_c$). No changes in glass transition and melting temperatures were observed (Figure 2b). Enthalpy measurements revealed a low level of crystallinity ($X_c < 13\%$) for all samples, indicating efficient quenching post-extrusion.

**Conclusion**

PLLA/WS$_2$ was investigated as a new material to improve BVS. Neat PLLA and PLLA/WS$_2$ tubes with appropriate dimensions were produced by extrusion. GPC measurements indicate that an excessive loading of nanotubes decreases PLLA $M_w$, which can explain the small drop of tensile strength observed. The SEM and μCT results present complementary information suggesting that dispersion should be improved during expansion process. DSC data reveal a strong nucleation effect from the nanotubes. While no mechanical improvement was found in extruded tube preforms, further work will explore the impact of the nanotubes on the biaxially stretched PLLA microstructure that can be expected in stretch-blow-moulded tubes.

**Acknowledgement**

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 813869. This abstract reflects only the author’s view and the REA is not responsible for any use that may be made of the information it contains. The authors would like to thank Boston Scientific Ltd. for their support and collaboration.

**References**


The Influence of Sterilization on PLLA for Cardiovascular Stent Applications

Emily Morra, Alex Lennon, Fraser Buchanan

Queen's University Belfast, School of Mechanical and Aerospace Engineering, Belfast, GB

Introduction
Sterilization of any medical device is a critical prerequisite for successful clinical application and represents the final processing step prior to device use in vivo. Sterilization is the process by which a product is sterilized within its final barrier system, thus ensuring a device meets required sterility assurance levels. Biodegradable polymers such as poly (l-lactic acid) (PLLA), used as a key component of many biodegradable stent designs, present challenges for sterilization in that they are temperature sensitive and undergo hydrolytic degradation. The most common techniques used in sterilization of biodegradable polymers are ethylene oxide gas (EtO) or radiation-based techniques such as gamma or electron beam (e-beam) at a dose range of 15-25 kGy. In the sterilization industry alternative techniques such as vaporized hydrogen peroxide (VHP) are also being explored to broaden available sterilization modalities. The development of new techniques corresponds with a push from environmental agencies to decrease reliance on EtO, which is a flammable and carcinogenic gas [1]. Each of these techniques has aspects which can affect biodegradable stent properties, and characterizing response to sterilization is critical to determining a device’s ability to meet functional requirements throughout its lifetime.

Experimental Methods
Medical grade PLLA (Purasorb PL38, Corbion, Gorinchem) was processed using compression moulding into 1 mm thick sheets which were then annealed and laser cut into test specimens. Samples were treated with one of three sterilization techniques at a range of doses: e-beam (5, 15, 25, 40, or 60 kGy), VHP (2, 4, 6, or 8 pulses), or EtO (typical product cycle). These doses were chosen to reflect the range a device would be exposed to during sterilization as well as lower and more extreme values. Sterilization work was carried out by STERIS (Tullamore, Ireland). Sterilized samples were characterised using a variety of techniques to assess the influence of sterilization on initial material properties. This included uniaxial tensile testing and dynamic mechanical analysis (DMA) for mechanical properties, differential scanning calorimetry (DSC) for thermal properties and crystallinity, and gel permeation chromatography (GPC) for molecular weight.

Results and Discussion
E-beam sterilization was found to influence initial material properties and the magnitude of this effect corresponded to increasing dose. E-beam caused a decrease in ultimate tensile strength and percent strain at break, which was especially evident at the selected extreme doses of 40 and 60 kGy. Samples treated with VHP did not experience the same corresponding decrease in ultimate tensile strength and percent strain at break, across doses used for sterilization as well as the more extreme dose. Similar to VHP, the EtO cycle chosen did not cause a change in either property. Young's modulus determined by tensile testing remained relatively unaffected by sterilization across all doses and treatments and this finding corresponded to the storage moduli calculated using DMA. The observed mechanical behavior was supported by GPC results, where E-beam caused a decrease in molecular weight corresponding to dose received while VHP and EtO did not cause a significant decrease in molecular weight. The dominant effect of e-beam sterilization on biodegradable polymers is degradation through chain scission and this was evident in the experimental results. While the doses used for terminal sterilization do not reach the extremes purposely selected for this experiment, some level of degradation must be considered when determining device lifetime. The heat and humidity required for EtO sterilization can also induce degradation, but this can be mitigated...
by using an optimized EtO cycle design. Similar to an EtO cycle, VHP sterilization is performed under vacuum to maximise vapour penetration. Although not as stable as EtO in vapour form, VHP efficacy can be optimised by controlling concentration, exposure time, and delivery mechanisms. VHP sterilization does not generate toxic residuals, unlike with EtO gas. VHP also has a lower temperature processing window and largely reduced cycle time compared to EtO. This reduces device exposure time to temperature, humidity, and vacuum, which in turn reduces the likeliness of degradation occurring.

**Conclusion**

VHP represents a novel sterilization technique which has received industry and regulatory attention, however its suitability for bioresorbable polymers has not been extensively characterised. The stability observed in samples treated across the range of VHP doses indicate that it could be a viable alternative sterilization modality for use with PLLA.

**Acknowledgement**

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 813869. The authors would like to thank Boston Scientific Ltd. for their support and collaboration, and STERIS for providing sterilization advice and facilities.

**References**

From *in vivo* to *in vitro*: Perfusable multiscale vascularized tissues

Prasanna Padmanaban, Jiena Zhang, Jeroen Rouwkema

University of Twente, Biomechanical Engineering Department / Faculty of Engineering Technology / Technical Medical Centre, Enschede, NL

**Introduction**

Knowledge about the flow field of the blood in surrounding tissues and the red blood cells within capillaries is important for a physical understanding of vascular networks remodeling and organization [1-2]. Keeping this in mind, our aim is to study and tune the vascular organization within both artificial eggshell systems and engineered perfusable muscle tissues using external flows.

**Experimental Methods**

Both artificial eggshell systems and muscle tissue perfusion chambers were replica molded using PDMS. Red blood cells velocities and vascular network properties such as vessel diameter, length, sprouting etc. have been characterized using in-house assembled microscopy.

**Results and Discussion**

We conduct an experimental study on the flow field, both within the endothelialized perfusable microchannels in muscle tissues and the microchannel placed on the top of vascularized membrane within artificial eggshell systems (see the Figure Multiscale vascularized tissues) to understand the influence of fluid flow on vascular network organization. We achieved spatiotemporal control of fluid perfusion and vascular cells within both the systems. Our experimental data are complemented by numerical simulations.

**Conclusion**

Coupling fluid flow parameters to the organization of vascular networks leads to a deeper understanding regarding the structural information and mechanical environment of these developing vascular networks, which is an important component in controlling vascular organization in engineered tissues.

**Acknowledgement**

The authors acknowledge the financial support from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (724469).

References


N12-08

Vascularization and high-resolution imaging of 3D polysaccharide-based scaffolds as model of liver sinusoid

Alessandra Dellaquila1,2, Didier Letourneur1, Teresa Simon-Yarza1

1 Université de Paris, INSERM U1148, LVTS, Paris, FR; 2 University of Bielefeld, Biomolecular Photonics, Department of Physics, Bielefeld, DE; 3 Elvesys Microfluidics Innovation Center, Paris, FR

Introduction

Liver capillaries, known as sinusoids, play a fundamental role in hepatic pathophysiology, such as filtering of molecules from the blood, clearing of wastes and managing the vascular immune response [1]. Despite the recent advancements in tissue engineering, the development of 3D in vitro models of the hepatic sinusoid is still hampered by the following drawbacks: (i) fabricating scaffolds with physical properties that mimic the in vivo microenvironment, (ii) engineering a functional vascular network within the scaffold and (iii) in-depth imaging characterization of 3D vascularized constructs.

To address these limitations, we developed vascularized polysaccharide scaffolds: here, we present their fabrication and characterization for optimal vasculature formation. Imaging by conventional and high-resolution microscopy was performed for in-depth characterization of the constructs.

Experimental Methods

The scaffolds were fabricated by freeze-drying from a solution of pullulan and dextran, known to be biocompatible polysaccharides [2]. The tubular channels within the porous matrix were obtained by subtractive molding technique: briefly, the polymeric mixture was casted onto a custom-made mold containing suture monofilaments (50 µm in diameter), that were removed after the hydrogel cross-linking [3]. The empty channels were selectively coated with collagen type I for cellular adhesion prior to freeze-drying. To enhance the imaging of channels by confocal microscopy, the porous gels were engineered by optimizing the fabrication and freeze-drying process. The scaffolds physical properties were evaluated by porosity and swelling tests and mechanical characterization was performed by nanoindentation. Fourier-transform infrared spectroscopy (FT-IR), elemental analysis and second-harmonic generation (SHG) were used to assess the presence of the collagen coating.

The scaffolds were subsequently seeded in vitro with human umbilical vein endothelial cells (HUVECs) as a model of liver vasculature. Different cellular densities (ranging from 1 · 106 to 10 · 106 cells/mL) and seeding strategies were investigated and their impact on scaffold vascularization was evaluated. Endothelium formation over time (up to 21 days) was followed by combining confocal and multiphoton microscopy (MPM) imaging. Morphological and functional endothelium studies (staining for actin, CD31 and VE-Cadherin) were performed by either confocal, MPM or light sheet microscopy (Figure 1).

Results and Discussion

The results of physical and mechanical studies of the collagen coated scaffolds showed a porosity of about 40% and Young’s modulus below 12 kPa, indicating the capability of the constructs to mimic soft tissues [4]. FT-IR, elemental analysis and SHG imaging confirmed the successful selective coating of the channels with collagen (Figure 2a). Imaging by confocal microscopy was enhanced thanks to the optimized scaffold fabrication while the use of MPM and light sheet techniques enabled a faster and deeper imaging of the channels within the 3D matrices with reduced photodamage. Under optimal seeding conditions, a complete endothelium was obtained after 4 days of culture and the HUVECs started forming sprouts within the porous gel at day 7 (Figure 2b), showing preliminary vascular angiogenesis. The expression of adhesion molecules such as CD31/PECAM1 confirmed the formation of functional vessels (Figure 2c).

Page 745 of 2028
Conclusion
This study reports the fabrication and characterization of vascularized porous polysaccharide scaffolds and their in-depth imaging. Current studies are focusing on the integration of other hepatic cell types and on dynamic studies of the endothelium under flow to build a perfusable vascularized liver model.

Acknowledgement
This project received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie Grant Agreement No. 766181.

References
Bioengineering Self-Organizing Microvasculature via Spatiotemporally Controlled VEGF$_{165}$ Bioavailability: An aptamer-based approach

Deepti Rana, Vasileios Trikalitis, Vincent Rangel, Jeroen Rouwkema

University of Twente, Department of Biomechanical Engineering, Technical Medical Centre, Enschede, NL

Introduction
Achieving hierarchically organized vasculature within an artificially developed tissue analogue is critical for its successful implantation. In native ECM, spatiotemporally controlled multiple growth factor’s bioavailability guides the vascular morphogenesis. While previous strategies have shown to achieve stable release rates via protein immobilization or coupling within polymer matrices (via linker proteins or peptides), they often fail to recapitulate the dynamic, multifunctional behaviour of the ECM. Therefore, we harnessed the unique properties of DNA based aptamers that are affinity ligands designed to recognize proteins with high affinity and specificity, for controlling growth factor bioavailability within 3D polymer matrix that in-turn could guide the developing self-organizing microvascular networks. Herein, we aim to develop vascular endothelial growth factor (VEGF$_{165}$) specific aptamer-functionalized platform that could spatiotemporally control VEGF bioavailability (retention & release) and study its effect for guiding self-organizing microvascular networks by employing bio fabrication techniques such as 3D bioprinting & photo-patterning.

Experimental Methods
The aptamer-functionalized hydrogel was prepared via visible light induced photo-polymerization of gelatin methacryloyl (GelMA) and acrydite-functionalized aptamers having DNA sequence specific for VEGF$_{165}$. For bioprinting, two bio-inks were prepared (i) with aptamer-functionalized hydrogel & (ii) GelMA + fluorescent blue microbeads (control); followed by mixing with human umbilical vein endothelial cells (HUVECs) and mesenchymal stem cells (MSCs). The construct were 3D bioprinted as lines of aptamer-functionalized bio-ink next to control GelMA lines, making an interface. Subsequently, photo-crosslinked and loaded with VEGF$_{165}$ for 1 hr. It was expected that the aptamer regions would be able to sequester VEGF$_{165}$ from the culture medium, compared to plain GelMA. To understand this effect on complex shapes and designs, photo-patterning technique with similar hydrogel formulations were used. All of these samples were studied for triggered VEGF release efficiency via complementary sequences (CS) addition (using ELISA assay) and its effect on microvascular network formation in 3D (cell viability & immunostainings).

Results and Discussion
The results obtained from ELISA experiments confirmed triggered release of VEGF$_{165}$ from the aptamer-functionalized hydrogels in response to CS addition. Without CS addition, these hydrogels showed zero leakage of VEGF$_{165}$ for 10 days. Furthermore, the bioprinted and photo-patterned aptamer-functionalized regions revealed high cellular viability and ability to guide microvascular network formation (by HUVECs and MSCs) confined within these regions and minimal invasion to GelMA regions, even after 10 days of culture (see Figure). The differences in microvascular organization was observed in the samples with triggered VEGF release on day 5, compared to control. These observations altogether confirmed the ability of bioprinted and patterned aptamer functionalized hydrogels in controlling self-organizing microvascular networks within 3D microenvironment.

Conclusion
The present study confirms the potential of aptamer-functionalized platform for guiding self-organizing microvascular networks within 3D microenvironment by spatiotemporally controlling VEGF$_{165}$ bioavailability.

Acknowledgement
This work is supported by an ERC Consolidator Grant under grant agreement no 724469.

Guided self-organizing microvascular network formation by controlling VEGF bioavailability. Self-organizing microvascular network formation confined within the VEGF_{165}-loaded 3D bioprinted aptamer regions after culturing for 10 days. Even though same cell concentrations and culture conditions were used for both regions, the localized aptamer-bound-VEGF_{165} molecules showed enhanced network formation confined within aptamer regions. The presence of blue fluorescent beads marks GelMA region. The image is a maximum projection of confocal z-stack and scale bar is 100 microns.

References
1:15 p.m. – 1:45 p.m.

Track01

**YSF NC | YSF National Chapter Meeting**

*This meeting can only be attended by the YSF NCs and the YSF Board.*
1:15 p.m. – 1:45 p.m.

Track02

Social Activity 5 | Yoga

*Prof. Filipa Ribeiro will give 30min yoga class at lunch time.*

**Filipa Ribeiro**

YOGA SHALA MATOSINHOS, Matosinhos, Portugal
3:45 p.m. – 5:15 p.m.

Track01

K07 | Biomaterials for Cardiac Tissue Engineering and Regenerative Medicine

Chairs
Jeroen Leijten
University of Twente, Enschede, NL
Manuel Salmeron Sanchez
University of Glasgow, GB
Shivesh Anand (YSF)
Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, NL
Optimizing synergy between materials and cell microenvironments using functional polyesters

Milica Radisic

University of Toronto, IBME, Toronto, CA

Material based technologies play an integral role in many medical treatment strategies, including joint replacements, vascular stents, drug delivery methods, contact lenses and medical sutures. The application of successful technologies continues to grow, with an estimated market size of $130 billion USD by 2020. Although successes are notable, a number of biomaterial-based devices are limited in application by non-specific material properties to tissue type and undesired inflammation at the device interface. In application, polyester materials have traditionally been an effective polymer strategy, with successes in tissue engineering scaffolds and a variety of biomedical devices. Leveraging advantageous polyester degradability and well understood chemical synthesis, these materials serve as a strong functional platform to further optimize material-cell microenvironments. In this talk, I will describe the development of polyesters that (a) exhibit controllable mechanical properties to mimic the elastic properties of cardiac tissue extracellular matrix (ECM) and (b) incorporate a biomimetic moiety of mammalian immunity to recapitulate mechanistic inflammation regulation at the material surface.

Development of engineered cardiac tissue requires material scaffolds that match the elastic behavior of cardiac ECM. Using polycondensation techniques to incorporate unsaturated carbonyl groups into elastic polyester material gels, we generated materials that can be molded into intricate designs with secondary crosslinking to generate constructs with highly tunable elasticity. By adapting material synthesis conditions (i.e. monomer ratios, reaction time), elastic mechanical behavior was highly tunable across a range of soft biomaterial applications. Material applicability was highlighted in a number of micro-designed elastomeric scaffold supports for engineered tissue, both for in vitro organ-on-a-chip and in vivo cardiac patch technologies, wherein the two-step synthesis process allowed for micro molding into intricate shapes prior to gelation.

With biomaterial application in vivo, inflammation at the biomaterial surface limits device application. To tackle this, we looked to itaconate, a powerful small molecule metabolite of innate immunity that has recently emerged as a regulator of innate immune inflammation. This molecule has been previously limited therapeutically by short circulation times. To circumvent this, itaconate was incorporated directly into material backbones to impart biomimetic small molecule modulation, with quantified hydrolytic degradation release from polyester chains. Harnessing hydrolytic degradation release from polyester backbones, itaconate polymers resulted in the mechanism specific immunoregulatory properties on macrophage polarization in vitro. In a functional assay, the polymer-released itaconate inhibited bacterial growth on acetate. Translation to an in vivo model of biomaterial associated inflammation, intraperitoneal injection of ITA polymers demonstrated a rapid resolution of inflammation in comparison to a control polymer silicone, demonstrating the value of sustained biomimetic presentation of itaconate. Using scalable polyester synthesis techniques, we have demonstrated the power of instructive biomaterial technologies as functional modulators of the cell microenvironment.
Piezoelectric Patches Improve Cardiac Electrical Proficiency After Myocardial Ischemic Injury

**Luis M. Monteiro**¹, Pedro J. Gouveia²,³, Susana Rosa¹, Francisco Vasques-Nóvoa⁴,⁵,⁶, Ifigeneia Bardi⁷, Rita Gomes¹,⁵,⁶, Simão C. Santos¹, Leonardo Ricotti⁸, Rui Cerqueira⁶, Adelino Leite-Moreira⁶, Filippo Perbellini¹⁰,⁷, Cesare Terraciano⁷, Perpétua Pinto-do-Ó⁴,⁵,⁸, Lino S. Ferreira¹,¹¹, Diana S. Nascimento⁴,⁵,⁸

¹ University of Coimbra, Center for Neuroscience and Cell Biology (CNC), Coimbra, PT; ² Royal College of Surgeons in Ireland (RCSI), Department of Anatomy & Regenerative Medicine, Dublin, IE; ³ Royal College of Surgeons in Ireland (RCSI), Advanced Materials and BioEngineering Research (AMBER) Centre, Dublin, IE; ⁴ University of Porto, i3S - Instituto de Investigação e Inovação em Saúde, Porto, PT; ⁵ University of Porto, INEB - Instituto Nacional de Engenharia Biomédica, Porto, PT; ⁶ Faculty of Medicine of the University of Porto, Departamento de Fisiologia e Cirurgia Cardiotórica, Porto, PT; ⁷ Imperial College London, National Heart & Lung Institute, London, GB; ⁸ University of Porto, ICBAS - Instituto de Ciências Biomédicas de Abel Salazar, Porto, PT; ⁹ Scuola Superiore Sant'Anna, The BioRobotics Institute, Pontedera, IT; ¹⁰ Hannover Medical School, Institute of Molecular and Translational Therapeutic Strategies (IMTTS), Hanover, DE; ¹¹ University of Coimbra, Faculty of Medicine, Coimbra, PT

**Introduction**

Ischemic heart diseases (e.g. myocardial infarction (MI)) are the leading cause of death worldwide. Novel approaches have been focusing on restoring cardiac function by targeting cardiac repair, however in vivo studies promoting both contractile and electrical functional recovery are scarce.

Piezoelectric materials acquire electric charges upon mechanical deformation. Since the heart exhibits cyclic movements, implantation of these materials on the myocardium holds great potential for designing biomaterials with self-sustained electrical stimulation, harnessing the contractile proficiency of healthy regions of the ventricle to stimulate damaged ones.

Although a therapeutic tissue engineering strategy with piezoelectric materials is yet to be tested in vivo, these materials have already been tested on hearts in the context of recharging the battery of electronic pacemakers [1]. Herein, the therapeutic potential of biomaterial-based films with piezoelectric properties, similar to scaffolds previously produced by us [2], was evaluated, as cell-free patches, on viable or MI heart tissue on both ex vivo and in vivo rodent models.

**Experimental Methods**

Flexible patches with piezoelectric properties (Piezo patches) were obtained by electrospinning polyvinylidene fluoride-trifluoroethylene aligned nanofibers on polycaprolactone (PCL) nanofilms, obtained by spin coating. As an inert control, PCL patches were produced, containing PCL nanofibers instead. The patches were characterized mechanically through tensile tests.

These materials were applied to ex vivo cardiac tissue on Langerdoff-perfused working healthy and MI rat hearts and on healthy rat cardiac slices. The effects of the patches on the former were assessed by electrocardiography (ECG) before and after patch application, on both healthy and MI hearts; while the effect on cardiac slices were characterized by contractility measurements.

To evaluate the effect of the patches on an in vivo disease model, they were implanted in the hearts of an in vivo murine model of MI. One month after the induction of the MI and subsequent patch implantation, mice were evaluated functionally by echocardiography and surface ECGs. Their hearts were harvested and processed for histology.

Histological sections were used to morphometric analysis (e.g. infarct region area, left ventricle (LV) dilation,
cardiomyocyte (CM) cross-sectional area) and to perform Laser Capture Microdissection of LV regions near or far from the implanted Patches, which were collected and their RNA extracted and sequenced by RNASeq for transcriptomic analysis.

**Results and Discussion**

Piezo and PCL patches exhibit an elastic modulus of 7.89 and 11.16 MPa respectively, in the same range as other cardiac patches (e.g. 6.7 MPa) [3].

None of the patches cause relevant changes in ECG parameters on Langendorff-perfused rat hearts (neither healthy nor MI). This shows that the Piezo patches have no negative effect on the electrophysiological and mechanical characteristics of Langendorff-perfused working hearts, in contrast with other studies [4].

When applied in healthy viable rat cardiac slices, Piezo patches cause an increased contractility force amplitude, when compared with the PCL patches, although without statistical significance. This suggests that the Piezo patch might be generating sufficient local electrical stimulation that induces an improvement on the contraction efficiency of the cardiac slices.

Echocardiographic evaluations performed on MI mice, one month after the implantation, showed a tendency for improvement in the Piezo group, suggesting an improved systolic function and decreased LV remodeling. Moreover, LV mass was increased in the PCL group, suggesting a compensatory hypertrophic response to the MI. Importantly, the Piezo group exhibited significantly improved electrical integrity, as observed by statistical significant differences on several ECG parameters.

Although no differences were observed in histological parameters (e.g. infarct size, LV dilation), transcriptomic analysis of LV tissue microdissected from regions near or far from either Piezo or PCL patches, showed that LV far from the PCL patch showed an upregulation of several genes associated with extracellular matrix remodeling, when compared with Piezo group, in the same tissue region. Curiously, the cross-sectional area of the CMs on that same region was statistically significantly increased in the PCL group, which aligns with the observed increase in LV mass.

**Conclusion**

*Ex vivo* experiments with perfused hearts and cardiac slices showed that the Piezo patch doesn’t negatively affect tissue function in a short time-frame and slightly improves contractility. In a longer time-frame (one month), the Piezo patch causes an improvement in an MI mouse model, mainly on electrical integrity. This study shows for the first time the possibility to harness the contractile motion of the heart tissue to further electrically stimulate the same tissue for improvement of function, viability and remodeling.

**Acknowledgement**

This work was funded by FEDER through the Program COMPETE and by Portuguese fund through FCT in context of the projects PTDC/BTM-SAL/29229/2017 and POCI-01-0145-FEDER-016385, as well as the European projects RESETageing (ref. 952266) and ERAatUC (ref. 669088), and the project POCI-01-0145-FEDER-030985. LM wishes to thank FCT for a BD fellowship (SFRH/BD/129991/2017).

**References**


Predictive and experimental study of intelligent nanoparticles for controlling cardiac ECM after myocardial infarction

Giovanni Barcaro¹, Susanna Monti², Antonio Rizzo¹, Nicoletta Barbani³, Anthea Villano¹, Francesca Sergi¹, Chiara Bulgheresi¹, Rachele Rosso⁴, Daniela Rossin⁴, Claudia Giachino⁴, Caterina Cristallini¹

¹ National Research Council (CNR), IPCF, Pisa, IT; ² National Research Council (CNR), ICCOM, Pisa, IT; ³ University of Pisa, Department of Civil and Industrial Engineering, Pisa, IT; ⁴ University of Turin, Department of Clinical and Biological Sciences, Turin, IT

Introduction
Recent advancements in nanotechnology have the potential to revolutionize both preventive and therapeutic approaches for treating cardiovascular disease [1]. Nanoengineering approaches could be exploited to develop scaffolds recapitulating the complex signals necessary for cardiac regeneration. Recently, cardiac scaffolds were modified with nanoparticles (NPs) able to recognize a specific ECM metalloproteinase (MMP) with the aim to prevent left ventricular (LV) dysfunction after myocardial infarction (MI) [2]. It is known that an increased LV myocardial MMP activity and selective upregulation of MMPs in heart failure occur. The control of MMP activation and expression in the failing human LV myocardium represents a significant therapeutic target for heart disease. Our idea was to exploit the molecular imprinting polymers (MIP) in order to restore the correct MMPs/TIMPs balance. In this work, we have explored more in depth the potential of this technique through a wide scenario of evaluations from the computational study to the synthesis and physico-chemical, dimensional and biological characterization.

Experimental Methods
Methacrylic acid (MAA), poly(ethylene glycol) ethyl ether methacrylate ((PEG)EEMA) as monomers and trimethylolpropane methacrylate (TRIM) as cross-linker were used. Human MMP-9 was used as template. MD simulations were performed by using the AMBER16 software employing the General Amber Force Field (GAFF) for MAA and (PEG) EEMA species and the ff14SB Force Field for the MMP-9 protein. The structure of isolated MMP-9 protein was extracted from PDB database. MIP NPs were synthesized using precipitation radical polymerisation of MAA and (PEG)EEMA with monomer ratio 75/25 (selected from the computational analysis) with a low coverage of the protein. FT-IR Chemical Imaging was used to evaluate the chemical map of pure MMP-9 and NP aggregates before and after template extraction. DLS analysis was carried out on different NP aqueous dispersions. SEM analysis was performed on both MIP NPs and control NPs. HPLC analysis was performed to evaluate rebinding capacity and selectivity of MIP NPs towards MMP-9. Viability assay was carried out on H9c2 cardiomyoblasts incubated with increasing MIP NPs concentrations at 24, 48 and 72 hrs through Propidium Iodide Flow Cytometry analysis.

Results and Discussion
The evolution of the interaction between MMP-9 and MIP NPs as a function of monomer concentration was evaluated by a new and original computational protocol. A stronger water/protein interaction than the monomers/protein one was observed, although, in the case of low coverage, this effect was reduced especially for (PEG)EEMA monomers that resulted more strongly anchored to the protein surface. FT-IR Chemical Imaging allowed to identify a diagnostic band for MMP-9 and detect this band both before and after template extraction from MIP NPs. An interesting similarity between a partial coverage where the enzyme structure is exposed respect to monomer shell and chemical map showing a surface presence of enzyme on NPs was observed. An high percentage of MMP-9 entrapped into NPs was evaluated by HPLC confirming the strong interaction protein/NPs and an elevated enzyme extraction. SEM analysis showed an average value of sphere dimension only slightly higher for MIP NPs than control NPs. This result
was confirmed by DLS analysis indicating that surface sites can take place allowing the removal of MMP-9 after imprinting. HPLC analysis showed a rebinding capacity of MIP NPs towards the enzyme confirming the presence of highly-specific recognition zones in agreement with the computational study. MIP NPs exhibited also selectivity towards MMP-9 respect to an analogue enzyme (MMP-2). Finally, no cytotoxic activity of MIP NPs was observed on H9c2 cells: the viability values were close to 100% independently of dose and incubation time.

**Conclusion**

Molecular imprinting was proposed for the production of nanoparticles capable of modulating enzymes of the MMP family for restoring the correct MMPs regulation in the cardiac microenvironment. This study aims to underline the importance of a comprehensive study, using a large and synergetic set of analyses and methodologies, to better explore and imitate the complex interactions between engineered nanosystems and native tissue for progress in myocardial regeneration.

**Acknowledgement**

This work was supported by transnational EU project INCIPIT M-ERA.NET 2 call 2016, MIUR FIRST
Rebinding MMP-9: MIP vs CP
MMP-9 rebinding assay of MIP and control NPs (CP).

References
Extracellular vesicles as IncRNA-H19 carriers to the cardiovascular system

Andreia Vilaça¹,²,³, Marida Sansonetti⁵, Hugo Fernandes⁴,¹, Leon de Windt², Lino Ferreira⁴,¹

¹ University of Coimbra, Centro de Neurociências e Biologia Celular, Coimbra, PT; ² Maastricht University, Department of Molecular Genetics, Maastricht, NL; ³ Maastricht University, CARIM School for Cardiovascular Diseases - Faculty of Health, Medicine and Life Sciences, Maastricht, NL; ⁴ University of Coimbra, Faculty of Medicine, Coimbra, PT; ⁵ Hannover Medical School, Institute of Molecular and Translational Therapeutic Strategies (IMTTS), Hannover, DE

Introduction

Long non-coding RNAs (lncRNAs) have increasingly been reported as major epigenetic regulators of gene expression within the cardiovascular system, and are therefore very attractive as drug candidates or targets [1]. They are >200nt long and can fold into complex 3D structures, a feature usually required to exert their biological function. These characteristics complicate chemical RNA synthesis as well as intact and functional RNA delivery. Extracellular vesicles (EVs) have been shown to naturally contain lncRNAs [2] and to successfully deliver these to the cardiovascular system [3], mediating tissue regeneration [4]. Moreover, previous studies have shown that lncRNAs can be loaded in EVs following donor cell transfection and transferred to recipient cells [5]. However, whether different splice variants of a given lncRNA can be located within EVs and how recipient cells uptake them remains elusive. Thus, in this work, we aimed at identifying different splice variants of a given IncRNA inside EVs and their uptake by major cardiac cell types.

Experimental Methods

This work focused on the lncRNA-H19, known to participate in several biological processes involved in cardiac repair upon delivery by EVs. We overexpressed the mouse H19 gene in human mesenchymal stem cells (WJ-MSC) for 48h, after which we collected the EVs and parental cells. Expression of the IncRNA-H19 splice variants (variants 201, 202, 203, 204, 205 and 207, i.e. 6 out of 7 known splice variants) in the EVs or the parental cells was assessed by RT-qPCR. Subsequently, both PKH67-labeled native EVs or unlabeled H19-EVs were used to treat cultures of neonatal mouse cardiomyocytes or fibroblasts and mouse aortic endothelial cells (MAECs) for 4h to evaluate the uptake of EVs by each cell type through confocal microscopy and the expression of the different splice variants by RT-qPCR. To confirm the functional transfer of lncRNA-H19 from the EVs, we treated MAECs with H19-EVs or vector-EVs and assessed migration (via wound closure assay) and angiogenesis (via tube formation assay) profiles for 12h. Finally, we evaluated the EV tropism on rat cardiac slices by incubating them with PKH67-labeled EVs for 4h or 24h and evaluating the PKH67 internalization in cardiomyocytes and endothelial cells through immunohistochemistry.

Results and Discussion

Our results showed that H19-transfected WJ-MSCs express all analyzed splice variants of IncRNA-H19 and that a similar expression profile was seen in the EVs collected from these cells at the same timepoint. We further demonstrate that EVs are uptaken by the major three cardiac cell types and that all analyzed splice variants, with the exception of H19-207, increase following H19-EV treatment in all cell types. Moreover, we show that transfection of MAECs with H19-EVs significantly (p<0.05) accelerated the angiogenic response of these cells, resulting in a >10% increase in the total tube length and in a >20% increase total number of junctions, compared to control. In the wound closure assay, H19 did not seem to improve basal EV function, since both H19-EVs and vector-EVs elicited a strong enough response to match that of the positive control (bFGF). In this case, all conditions tested significantly (p<0.05)
decreased wound area, relative to untreated MAECs. Finally, analysis of EV tropism in cardiac slices indicates that the EVs are quickly internalized and processed by cardiomyocytes and endothelial cells. We further noted that EVs accumulate preferentially in the cells layering the surface of the cardiac slice but they are also found in deeper cell layers, albeit at lower amounts.

**Conclusion**

Collectively, our results indicate that different splice variants can be loaded into EVs. These are readily uptaken by different cardiac cell types inducing relevant biological functions. This study encourages the use of EVs for cardiac delivery of IncRNAs and paves the way for personalized splice variant delivery, particularly interesting in the context of disease.

**Acknowledgement**

This work was funded by the FCT PhD Studentship (SFRH/BD/119187/201650), FCT Project “Exo-Heart”: POCI-01-0145-FEDER-029919, co-promotion Portugal2020 project “LABEL-radio-marcação de vesiculas extracelulares para aplicações em diagnostico e terapêuticas” (Ref: 49268), European projects ERAatUC (ref. 669088) and RESETageing (ref: 952266).

**References**


Magneto-responsive Core-shell Microbeads for Engineering Peristalsis and Alveolar Breathing In-vitro

Ludovica Cacopardo, Nicole Guazzelli, Arti Ahluwalia

University of Pisa, Pisa, IT

Introduction
Physiologically relevant in-vitro models need to reproduce both structural and mechanical features of the in-vivo environment. 3D scaffolds and cell spheroids are emerging as powerful tools to replicate three-dimensional structures. While, bioreactors are employed to apply uniaxial or biaxial stretching to cell monolayers. However, despite the complexity of these systems, to date, the replication of multidirectional (radial) three-dimensional (out of plane) stretching is a challenge [1, 2].

Here, we present a new bioprinting strategy to obtain core-shell structures, able to replicate the structure and motility of the intestinal and alveolar barrier thanks to magneto-responsive materials (Figure 1). In the case of the alveoli the air-containing beads will be embedded in a magneto-responsive septum-like gel able to mimic the in-vivo deformation mechanism. The core-shell structures were characterised as a function of different extrusion velocities, while deformation was evaluated as on the base of different magnet combinations and magnetite nanoparticle (NP) concentrations.

Experimental Methods
A core-shell microbead (COSMIC) generator was designed and fabricated using commercial coaxial needles (16-26 and 19-26 Gauge) connected with two syringes, which are actuated with two stepper motors. The extrusion velocity is controlled via computer thanks to a graphical user interface. The core-shell structures were characterised using 1 and 2% w/v alginate in the shell and i) 1 - 2 % w/v FITC-alginate (1:100 w/w, Creative PEGWorks, USA), ii) air or iii) 0.1% and 1% w/v pluronic-127 solution in the core. FITC-alginate was used for imaging purposes only, i.e. it does not affect bead extrusion.

A 0.1 M calcium chloride (CaCl₂) solution was placed under the needle to allow alginate crosslinking. Spheres dimension was quantified in function of different relative core/shell extrusion velocities (10, 20, 40 µL/s) using brightfields and fluorescence images (Olympus, Japan) and the Image-j software.

Cell viability tests were performed using Caco-2 cells with an encapsulation density of 1M/mL. All reagents were purchased from Sigma-Aldrich (Milano, Italy), unless differently specified.

Results and Discussion
The obtained diameters range from about 700 to 1700 µm for the core with a shell thickness ranging from about 90 to 500 µm. In the beads with a solid FITC-alginate core, the best results in terms of distinction between core and shell were obtained for shell extrusion velocity higher than core velocity. For instance, Figure 2a show a bead obtained using the following velocities: core at 20 µL/s and shell at 40 µL/s (c20s40). Differently, for the air core, the best results were obtained at for core extrusion velocity higher than shell velocity (Figure 2b: c40s10). In the case of the liquid core, good results were obtained for all the velocities combinations. However, core-shell distition was more defined in the case of higher pluronic concentrations.
The best deformation performance was obtained with a combination of the 10 and 20 mm magnets with strain levels around 7%. No significant differences were observed as a function of the NP concentration. Indeed, despite the higher magnetic responsivity, increasing NP concentration may result in less compliant structures.

Finally, cell testing confirmed the cytocompatibility of the encapsulation procedure, showing that our strategy is suitable to obtain cell-laden core-shell structures.

**Conclusion**

In conclusion, the main advantage of our strategy is the combination of structure and 3D stretching, which, to the best of our knowledge, has not been implemented in-vitro. Thus, COSMIC represents a step further towards the definition of physiologically relevant in-vitro models, which improve the translation between research and clinical applications and also have the potential to reduce animal tests as required by EU directives.

The fabrication and characterisation of the septum-like gel and the design of an automated stimulation system based on electromagnets are on-going. Further developments foresee the evaluation of the 3D structure and of the stretching effects on cell morphology, organization and polarization.

**Figure 1:**
Schematic illustration of the core-shell microbead generator and structures

**Figure 2:**
a) $c_{20s40}$ 2% alginate core-shell structures; b) $c_{18s40}$ air core - 2% alginate shell bead; c) $c_{20s40}$ 1% pluronic core - 2% alginate shell bead.

**References**

[1] Sakalem et al., 2018, 'New methodologies for old problems: Tridimensional gastrointestinal organoids and guts-on-a-chip', *Journal of Coloproctology*, 38, 90-93


Temporal changes in the secretome of microgel-entrapped vascular cells during in vitro priming improve their angiogenic potential

Ana L. Torres1,2, Sílvia J. Bidarra1,2, Daniela P. Vasconcelos1,2, Judite N. Barbosa1,2,3, Eduardo A. Silva4, Diana S. Nascimento1,2,3, Cristina C. Barrias1,2,3

1 Universidade do Porto, i3S – Instituto de Inovação e Investigação em Saúde, Porto, PT; 2 Universidade do Porto, INEB – Instituto de Engenharia Biomédica, Porto, PT; 3 Universidade do Porto, ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Porto, PT; 4 University of California - Davis, Department of Biomedical Engineering, Davis, US

Introduction
Strategies to promote tissue revascularization are central for tissue engineering and regenerative medicine. We previously reported the design of microgels that guided the assembly of entrapped outgrowth endothelial cells (OEC) and mesenchymal stem cells (MSC) into primitive vascular beds [1]. This system presented high angiogenic/vasculogenic potential upon transplantation in mice, standing out as an attractive strategy for minimally invasive therapeutic vascularization [2]. Along in vitro culture, cohesive vascularized microtissues were formed, with capillary-like networks enveloped in endogenous ECM. While dynamic cellular alterations associated with in vitro vascular morphogenesis are still elusive, they are known to include changes of the cells secretome that will affect their therapeutic potential. Notably, most clinical trials for cardiovascular cell therapies focused on the possible benefits of paracrine modulators, rather than on cells ability to directly participate in neo-tissue formation. While several studies have reported the paracrine effects of MSC and endothelial cells [3,4], the secretory activity of cocultured cells remains poorly studied. Herein, we evaluated how dynamic changes of the secretome of microgel-entrapped vascular cells during culture affect their in vitro/in vivo angiogenic and inflammatory potential.

Experimental Methods
OEC and MSC were entrapped in RGD-alginate microgels as described [1] and cultured for 14 days. Conditioned media (CM) from in vitro cultured microgels were collected at days 1 and 14 for analysis. The levels of matrix metalloproteinases (MMP) were quantified by gelatin zymography, and migration/invasive potential was probed by microgel entrapment in fibrin gel. Angiogenesis-related proteins were probed by multiplex analysis. The in vivo angiogenic potential of CM was analyzed using chick embryos chorioallantoic membranes (CAM) assay. Cytokine composition and in vivo inflammatory profile of primed-microgels CM were probed by multiplex-cytokine analysis and an air-pouch mouse model, respectively. To evaluate response to pro-inflammatory conditions, cell-laden microgels were pre-conditioned with CM from lipopolysaccharide (LPS)-activated macrophages. Then, microgels were incubated for 24h in basal medium and CM were collected for multiplex analysis.

Results and Discussion
During in vitro maturation (from day 0 to day 14), cells in microgels remodeled the original alginate hydrogel to create their own niche, forming ECM-rich microtissues permeated by endothelial capillary-like networks. Maturation enhanced MMP2 and MMP9 secretion, which may help implanted cells to proteolytically invade host tissues and undergo tubulogenesis in the external milieu, as suggested by the fibrin assay. The release of multiple pro-angiogenic factors with proven ability to promote EC survival/function and blood vessel formation was also enhanced, suggesting that in vitro maturation translated into a more pro-angiogenic secretome, which was confirmed in vivo by the CAM assay. Multiplex analysis of cytokines and the air-pouch model were used to investigate changes in the in vitro/in vivo inflammatory profile of cells secretome, but no significant differences were observed for non-primed (day 1) vs. primed cells (day 14). In a clinical setting, microgels should be locally delivered into injured tissues, where the pro-
inflammatory milieu may induce further alterations of entrapped cells secretome. Significantly, upon pre-conditioning with CM from LPS-activated macrophages, cells in matured microgels (day 14) were clearly more responsive than the control (day 1). Overall, they adopted a more pro-angiogenic profile, with increased or de novo expression of several angiogenesis-related proteins. Also, they showed de novo secretion or up-regulation of different cytokines, which may reflect on their immunomodulatory potential.

**Conclusion**
The secretome of microgel-entrapped vascular cells dynamically changes along in vitro culture, as cells undergo morphogenesis into primitive vascular beds. Globally, the secretome of matured microgels presented improved angiogenic potential and altered immunomodulatory properties, which may partially explain their better angiogenic/vasculogenic potential upon transplantation.

**Acknowledgement**
ERDF funds (COMPETE2020, POCI) and Portuguese funds (FCT) for ANGIONICHE project (POCI-01-0145-FEDER-028744, PTDC/BTMMAT/28744/2017), FCT for fellowship SFRH/BD/94306/2013 and contract IF/00296/2015 and BiotechHealth PhD Programme.

**References**
Ultra-High Throughput Production of Human Cardiac Organoids using In-Air Microfluidics generated Hollow Core-Shell Microgels

Bas van Loo1, Nuno Araújo-Gomes1, Simone ten Den2, Vincent de Jong1, Marcel Karperien1, Robert Passier2, Tom Kamperman1, Jeroen Leijten1

1 University of Twente, Developmental BioEngineering, Enschede, NL; 2 University of Twente, Applied Stem Cell Technology, Enschede, NL

Introduction
Pluripotent stem cells-derived organoids hold great potential for tissue engineering and drug testing purposes. However, organoid production is characterized by batch-process techniques that associate with artisan, labor-intensive, and low throughput production processes.1–3 Microfluidic technology offers solutions for improved production of cellular aggregates in terms of quality and production rate, which can be achieved via microfluidic encapsulation of cells in hollow core-shell microgels.4 We therefore hypothesized that microfluidic droplet generation could also be leveraged to realize high throughput production of pluripotent stem cell-derived organoids. Advantageously, we recently invented In-Air microfluidics, which allows the ultra-high throughput microfluidic production of micromaterials in an off-chip manner without the use of oils and surfactants.5 We here report that hollow core-shell microgels generated with In-Air microfluidics allowed for the production of embryoid bodies, which autonomously transformed in cavitated organoids that committed subsequently to the mesodermal and myocardial lineage.

Experimental Methods
We developed a novel In-Air microfluidics setup for the production of hollow core-shell microgels, which consisted of a three nozzle design (Figure 1a). The first microjet was composed of a solution containing 10% dextran and 0.05 M CaCl2 and was actuated with a piezoelectric actuator (5 Vpp, 5 kHz) (figure 1b). The second microjet contained a solution of 0.2% alginate and 10% (v/v) ethanol (EtOH) which encapsulated the droplets of the first microjet. The third microjet contained a solution of 0.2 M CaCl2 and 55% (v/v) EtOH which encapsulated the droplet containing the solutions of the first and second microjet. The resulting layered droplets were subsequently captured in a 0.1 M CaCl2 collection bath. This process allowed for the inside-out and outside-in crosslinking of the alginate shell, resulting in the monodisperse production of shape-stable hollow core-shell microgels with ~200 µm diameter and ~10 µm shell thickness (Figure 2a) at flow rates of over 3 ml/min. Human embryonic stem cells genetically modified with a dual-fluorescent reporter (i.e., MESP1/NKX2.5 and NKX2.5/ α-Actinin) were encapsulated at 0.5–10×106 cells/ml (Figure 2b). Cell-laden microgels were collected in a surplus of collection medium, which guaranteed that the EtOH concentration within the core consistently remained <1%. Embryoid body formation cells was achieved by culturing in E8 stem cell medium, and functional myocardiac organoid formation was achieved using BPEL differentiation medium. Embryoid bodies and cardiac organoids were analyzed using bright field microscopy and various modes of fluorescent confocal microscopy.

Results and Discussion
To allow for shape-stable hollow core-shell microgel formation, material concentrations and flow rates were optimized. Using different nozzle sizes (50/100/150 µm), monodisperse hollow core-shell microgels of various sizes (i.e., 80-300 µm in diameter) could be produced. By adjusting the frequency of the piezoelectric actuator, the number of cores per particle could be controlled (i.e., 1, 2, or 3 cores per particle). Moreover, by using the alginate shell as a scaffold material, it was possible to produce photocrosslinkable PEGDA hollow core-shell microgels, as well as enzymatic crosslinkable tyramine-conjugated dextran hollow core-shell microgels. In-air microfluidic encapsulation
of various human pluripotent stem cells (i.e., embryonic stem cells) associated with viabilities of >95% immediately upon encapsulation. Within 24 hours, the microencapsulated cells aggregated into embryoid bodies, which continued to be metabolically active and proliferate for at least 14 days of culture. Remarkably, the microencapsulated embryoid bodies cavitated and formed organoids in a fully self-instructed and autonomous manner (Figure 2c-e) while remaining positive for Sox2 and Oct3/4 (Figure 2f-h). Upon differentiation, the human embryoid bodies initially underwent mesoderm transition within four days of culture (Figure 2i) and then committed to the myocardial lineage. After 7 days of culture, cardiomyocytes were positive for NKX2.5 (Figure 2j), and showed positive for α-Actinin markers after 14 days (Figure 2k), resulting in cardiac organoids that displayed self-induced synchronized cardiac contractions.

**Conclusion**

Hollow core-shell microgels generated with In-Air microfluidics allowed for the production of human embryoid bodies that autonomously transformed in cavitated organoids, which could be triggered to become functional cardiac organoids. We anticipate that the facile, high-throughput, and clean nature of our platform can facilitate upscaled investigations for developmental studies, cell therapies, and drug screens.

**Acknowledgement**

The authors acknowledge funding from Dutch Arthritis Foundation (#17-1-405) and European Research Council (ERC, Starting Grant, #759425).
Encapsulation of Human Pluripotent Stem Cells for Embryoid Body and Cardiac Organoid Formation.

(a) In-Air Microfluidics allowed for hollow core-shell microgel formation. (b) Human Pluripotent Stem Cell encapsulation (c) resulted in Embryoid Body formation (d) with high viability. (e, f) Confocal microscopy revealed cavity formation in the Embryoid Bodies. (g,h) Embryoid Bodies remained pluripotent as shown by Sox2 and Oct3/4 staining. (i) Upon myocardial differentiation, cells showed positive for mesoderm transition by MESP1 fluorescent reporter after 4 days, (j) and positive for differentiation towards myocardiocytes by NKX2.5 after 7 days, (k) and α-Actinin after 14 days.

References

3:45 p.m. – 5:15 p.m.

Track02

**K08 | Mechanomodulatory Biomaterials and its Impact on Cell Behaviour**

Chairs
- **Cristina C. Barrias**
i3S-Institute for Investigation and Innovation in Health, Porto, PT
- **David Mooney**
Harvard University, School of Engineering and Applied Sciences, Cambridge, US
- **Maria Gabriella G. Fois (YSF)**
Maastricht University, MERLN, Maastricht, NL
K08-KL01

Extracellular matrix viscoelasticity and its impact on cells

Ovijit Chaudhuri

Stanford University, Department of Mechanical Engineering, Stanford, US

The extracellular matrix (ECM) is a complex assembly of structural proteins that provides physical support and biochemical signaling to cells in tissues. Over the last two decades, studies have revealed the important role that ECM elasticity plays in regulating a variety of biological processes in cells, including stem cell differentiation and cancer progression. However, tissues and ECM are often viscoelastic, displaying stress relaxation over time in response to a deformation, and viscoplastic, exhibiting irreversible deformations in response to mechanical stress. We have been investigating the impact of ECM viscoelasticity on cells. Our approach involves the use engineered biomaterials for 3D culture, in which the mechanical properties can be independently modulated. In this talk, I will discuss our recent findings on how matrix viscoelasticity regulates the migration stem cells and cancer cells, and breast cancer progression.

Acknowledgement
We acknowledge support from a National Institutes of Health National Cancer Institute grant (R37 CA214136) and a National Science Foundation CAREER award (CMMI 1846367). 
A 3D Bioprinted Breast Cancer-Adipose Tissue Model Utilizing Adipose-Derived Stromal Cell Spheroids

Hannes Horder1, Mar Guaza Lasheras1, Nadine Grummel2, Ali Nadernezhad3, Johannes Herbig3, Süleyman Ergün4, Jörg Teßmar3, Jürgen Groll3, Ben Fabry2, Petra Bauer-Kreisel1, Torsten Blunk1

1 University of Würzburg, Department of Trauma, Hand, Plastic and Reconstructive Surgery, Würzburg, DE; 2 Friedrich-Alexander University Erlangen-Nürnberg, Department of Physics, Erlangen, DE; 3 University of Würzburg, Chair for Functional Materials in Medicine and Dentistry, Bavarian Polymer Institute, Würzburg, DE; 4 University of Würzburg, Department of Medicine, Institute of Anatomy and Cell Biology, Würzburg, DE

Introduction
The advancement of biofabrication techniques, including 3D bioprinting, has enabled the development of more representative and physiologically relevant disease models, such as in cancer research. In breast cancer, various stages of tumor development and progression are promoted by adipose tissue, an important part of the tumor microenvironment. Therefore, in this study, we employed human adipose-derived stromal cell (ASC) spheroids as building blocks for adipose microtissues to establish a 3D-printed breast cancer model in which crucial aspects of the interplay between adipocytes and breast cancer cells can be recapitulated.

Experimental Methods
Cell culture; 3D cell culture; preparation of hyaluronic acid-based hydrogels; analysis of spheroid sedimentation behavior; determination of shear viscosity; 3D bioprinting; adipogenic differentiation; quantification of intracellular triglyceride and DNA content; co-culture; qRT-PCR; viability assay; detection of secreted proteins; (immuno-)histochemistry

Results and Discussion
First, we focused on the generation of ASC spheroids on a large-scale for use in an extrusion-based bioprinting setup. A system based on agarose molds was applied to obtain 256 spheroids per well, i.e., a total of 3072 spheroids per 12-well plate within 48 h in a highly controlled manner with a regular round shape and reproducible size. Spheroids composed of 2500 cells (diameter of 228 µm ± 22 µm) were then used to optimize the printing process in terms of homogeneous spheroid distribution and spheroid viability. Spheroids were dispersed in a solution of thiol-modified hyaluronic acid (HA–SH), which was UV-crosslinked to stable hydrogels with allyl-modified poly(glycidol) (P(AGE-co-G)). The addition of 1 wt.% unmodified high molecular weight HA (hmHA) increased bioink viscosity improving printability and, at the same time, decreased spheroid sedimentation during printing and UV-crosslinking, resulting in a distinctly more homogeneous spheroid distribution in the final constructs. Analysis of the influence of different printing process parameters on the viability of ASC spheroids revealed nozzles with an inner diameter of at least 330 µm and a printing pressure of 1 bar to be most suitable for spheroid printing, as shown by viability assays. To assess whether the printing process impairs the adipogenic differentiation capacity of the spheroids, adipogenesis in printed and cast constructs was compared. Histological and quantitative detection of accumulated triglycerides after 21 days revealed substantial adipogenic differentiation, compared to non-induced controls. These findings were corroborated by strongly elevated expression levels of adipogenic marker genes such as PPARγ, C/EBPa and FABP4 in differentiated constructs. Furthermore, extracellular matrix (ECM) deposition of induced versus non-induced spheroids was immunohistochemically evaluated. The development of a tissue-specific ECM is considered an important feature in 3D tissue constructs, since, in addition to providing physical support, the ECM actively contributes to the behavior of the cells and the complex interaction with their environment. Characterization of adipogenically differentiated spheroids showed an ECM composition resembling adipose tissue, with highly elevated...
expression of laminin and collagen IV, two main components of the adipocyte basement membrane, as well as enhanced collagen I and collagen VI expression, along with a marked reduction of fibronectin, as compared to non-induced controls. In all experiments, no significant differences of the differentiation capacity between printed and cast constructs were observed. Subsequently, these established adipose microtissues were implemented in a printed 3D breast cancer model as a proof-of-concept study. A tumor compartment consisting of an invasive tumor cell line (MDA-MB-231) was printed on top of the adipose tissue construct using the same HA-based hydrogel as bioink. The resulting co-constructs were then cultured for a further nine days. Histological and quantitative assessment of lipid storage revealed a considerable reduction of lipid content in the adipose microtissues upon co-culture with MDA-MB-231 cells, as compared to the monoculture control. Immunohistochemical analysis of major ECM components and quantitative assessment of the respective staining demonstrated a distinct ECM remodeling with increased collagen I, VI and fibronectin expression in the co-cultured adipose microtissues.

**Conclusion**

Taken together, our printed co-culture model allowed us to investigate characteristic cancer cell-induced alterations in the adipocyte phenotype also observed in vivo, i.e., reduction of lipid content and remodeling of the adipose ECM. In future studies, the use of advanced bioinks that support tumor cell migration as well as the inclusion of other cell types may contribute to further improve the biofabricated 3D model and elucidate the complex cell-cell and cell–matrix crosstalk within the tumor microenvironment.

**Acknowledgement**

This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation), Project number 326998133, TRR 225 (subprojects A02, B02, B04, C02).
K08-03

Tethering cells via enzymatic oxidative crosslinking enables mechanotransduction in non-cell-adhesive materials

Tom Kamperman1,2, Sieger Henke1, João F. Crispim1, Niels G. Willemen1, Pieter J. Dijkstra1, Wooje Lee1, Herman L. Offerhaus3, Martin Neubauer4, Alexandra M. Smink5, Paul de Vos5, Bart de Haan5, Marcel Karperien1, Su R. Shin2, Jeroen Leijten1

1 University of Twente, Developmental BioEngineering, Enschede, NL; 2 Brigham and Women’s Hospital, Harvard Medical School, Division of Engineering in Medicine, Boston, US; 3 University of Twente, Optical Sciences, Enschede, NL; 4 University of Bayreuth, Physical Chemistry II, Bayreuth, DE; 5 University of Groningen, University Medical Center Groningen, Pathology and Medical Biology, Groningen, NL

Introduction
The interaction between cells and materials is a determining factor for the efficacy of cell-based products and therapies. Cell-matrix interactions are essential to transduce biomechanical cues that control cellular functions including migration, proliferation, apoptosis, metabolism, and differentiation.[1] Engineered living tissues often emulate these interactions by endowing biomaterials with constitutively active bioligands such as RGD-type moieties, which challenges control over cell fate and associates with a strong inflammatory response upon implantation.[2] Here, we report on a method to tether intrinsically non-cell-adhesive biomaterials to cells via discrete inducible on-cell crosslinking (DOCKING). This method crosslinks tyramine-functionalized macromolecules to tyrosine residues that are naturally present in extracellular protein domains via an enzyme-mediated oxidative reaction. In short, DOCKING is a bio-inspired and cytocompatible cell-material tethering method that enables the formation of intrinsically non-cell-adhesive yet mechano-instructive biomaterials eliciting a minimal inflammatory response, which primes it as a promising new strategy to control interactions between cells and materials.

Experimental Methods
To visualize DOCKING, mesenchymal stem cells (MSCs) and 3T3 cells were labeled with tyramide-AF647 by incubation with 3 U/ml horseradish peroxidase (HRP) and 0.3 mg/ml H2O2 in PBS. MSCs were individually tethered within 3D Dex-TA microneches using a previously reported microfluidic encapsulation system.[3] Hydrogels' stiffness was determined by nano-indentation with an atomic force microscopy (JPK NanoWizard) combined with inverted optical microscopy (Zeiss Axio Observer Z1). Viability and metabolic activity of cells was analyzed by staining with 2 µM calcein AM (live), 4 µM EthD-1 (dead), and 0.5 mg/ml MTT (metabolically active) in PBS and visualization using brightfield and fluorescence microscopy (EVOS FL). Adipogenic differentiation was analyzed using Oil-Red-O staining. Osteogenic differentiation was analyzed using Alizarin Red S staining. For transcriptome analysis, MSCs from three different human bone marrow donors were tethered and cultured in soft and stiff Dex-TA microgels for sixteen hours, then cultured for eight hours in bipotential differentiation medium, and subsequently lysed and sequenced. To identify cellular protein targets of DOCKING, biotin-tyramide was tethered to MSCs, isolated from cell lysates using a pull-down assay, and analyzed using liquid chromatography combined with mass spectrometry (LC-MS). Dex-TA gel disks were formed by mixing 100 mg/ml Dex-TA, 3 U/ml HRP, and 0.3 mg/ml H2O2 in PBS. Dex-TA-RGD hydrogels were formed by adding 2.2 mM RGD-Tyr to the hydrogel premix. To compare host response, hydrogel disks were subcutaneously implanted in C57BL/6 mice for four weeks.

Results and Discussion
Here, we report on the discrete inducible on-cell crosslinking (DOCKING), of non-cell-adhesive biomaterials onto cells via oxidative crosslinking of phenolic moieties. Specifically, we demonstrated that tyramine-functionalized dextran (Dex-TA) could be enzymatically crosslinked with tyrosine-rich extracellular fibronectin, thereby forming
pericellularly tethered hydrogel microniches. The intrinsically bio-inert and non-cell-adhesive Dex-TA hydrogel elicited a reduced inflammatory response upon implantation in mice as compared to its RGD-modified cell-adhesive counterpart. Importantly, enzyme-mediated oxidative crosslinking enabled the controlled mechanotransduction from non-cell-adhesive Dex-TA hydrogel to encapsulated cells via cell tethering. As a proof-of-concept, we leveraged an advanced microfluidic system to tether individual mesenchymal stem cells (MSCs) within micrometer-sized hydrogel matrices (i.e., microgels) using DOCKING to control MSC function and fate at single cell resolution. Through timed modulation of microgel stiffness, we could program the lineage commitment of microencapsulated stem cells, thereby indicating DOCKING-mediated mechanotransduction in a bioligand-free material. Proteome and transcriptome analyses revealed that DOCKING predominantly targets proteins associated with the cellular meta-adhesome, and that 3D on-cell-tethered biomaterial mechanics can induce early onset cell-fate-programming mechanotransduction pathways independent of cell size changes.

Conclusion
In summary, DOCKING provides a unique method to discretely tether cells to molecules or materials, including those that are otherwise non-cell-adhesive. This novel method opens up numerous opportunities in the biomedical field that, for example, benefit from mitigating the chronic inflammation associated with conventional bioligand-functionalized materials as well as providing novel strategies to mechanically program and study (stem) cells in a 3D, temporally controlled, and single-cell-resolution manner.

Acknowledgement
TK acknowledges funding from a Rubicon award by the NWO (019.183EN.017). JL and MK acknowledge funding from the Dutch Arthritis Foundation (#12-2-411 to JL and MK, and #LLP-25 to MK). JL acknowledges funding from an Innovative Research Incentives Scheme Vidi award (17522) and a NWO-Groot consortium grant (SCI-MAP; OCENW.GROOT.2019.079) from the Dutch Research Council (NWO), and an ERC Starting Grant (#759425) from the European Research Council. SRS acknowledges funding from the National Institutes of Health (R01AR074234) and the Gillian Reny Stepping Strong Center for Trauma Innovation at Brigham and Women’s Hospital.
Figure 2. DOCKING enables engineering of mechanically instructive stem cell niches. (a-d) Oil-Red-O staining (ORO; a), hyperspectral coherent anti-Stokes Raman scattering (CARS; b), Alizarin Red staining (AR; c) and hyperspectral spontaneous Raman (d) confirmed the adipogenic and osteogenic differentiation of MSCs tethered within soft and stiff Dex-TA hydrogel microniches, respectively. (e,f) Quantification of the per-cell adipogenic (e) and osteogenic (f) differentiation as a function of microniche stiffness and culture medium. GM: growth medium, DM: differentiation medium, ****: p < 0.0001 (Mann-Whitney), white scalebars: 50 µm, black scalebars: 10 µm.

References
A biomimetic alveolar in vitro model to study lung cancer

Chiara Tonda-Turo, Michela Licciardello, Gianluca Ciardelli

Politecnico di Torino, Department of Mechanical and Aerospace Engineering, POLITOBIOMED LAB, Torino, IT

Introduction
Lung cancer, also known as lung carcinoma, is a malignant tumour characterized by uncontrolled cell growth in the specific tissue of respiratory system [1]. The lack of new therapies to treat lung carcinoma requires experimental models that can reproduce the behaviour of healthy and pathological tissues [2] to be applied in the study of pathology development. Here, we describe the implementation of a novel technological biomimetic platform to mimic both the multicellular composition of the alveolus and its vascular network, as well as the composition and structure of extracellular matrix. Our aim is to design and fabricate new structures, composed by biomaterials, that resemble the alveolar wall structure.

Experimental Methods
The basement membrane layer morphology was reproduced through electrospinning of a gelatin/polycaprolactone (GL/PCL) solution. Nanofibrous GL/PCL membranes were obtained after optimizing the electrospinning process and solution parameters. HULEC-5a cells (human lung microvascular endothelium cells) and A549 cells (human alveolar basal epithelial cells) were cultured on one side of the GL/PCL membrane respectively, to recreate the alveolar barrier. On the other hand, the connective tissue layer was replicated through bioprinting (ROKIT InVivo, Rokit, Seul) of collagen type I hydrogel loading human lung fibroblasts (MRC-5 cell line). Bovine collagen powder was dispersed in 0.5 M acid acetic solution, then the acid pH of solution was neutralized by adding 1M NaOH to induce the sol-gel transition. Rheological characterization was carried out to evaluate the viscoelastic properties of the material. MRC-5 cells were loaded into the hydrogel prior to gelification and their viability was monitored. Cellularized hydrogels were then printed on the GL/PCL membranes and the printing parameters were optimized in order to obtain a stable gel structure. The cellularized 3D structure was cultured both in static and dynamic (Livebox2 bioreactor, IVtech srl) conditions.

Results and Discussion
Nanofibers morphology was characterized by scanning electron microscopy (SEM) and the GL/PCL mats obtained by electrospinning process appear to be characterized by randomly oriented defect-free nanofibers (fig. 1A). Cyclical traction tests were performed to evaluate the effect of the physiological breathing motion (10% of strain at 0.2 Hz) on membranes mechanical properties demonstrating good fatigue resistance during the 40 tested cycles. In vitro cells tests confirmed the adhesion and proliferation of HULEC-5a and A549 cells on the membranes and each cell density was optimized to achieve a complete coverage of the mats to recreate a biomimetic alveolar barrier. The printing of collagen hydrogels was achieved extruding the sol-phase material from the syringe (kept at 4°C to avoid the collagen gelation) onto the platform at 37°C. Cellularized collagen hydrogels were obtained showing a high survival of MRC-5 encapsulated (fig. 1B). Finally, a complex 3D model of the multilayered architecture of the physiological alveolar structure was obtained by adding the cellularized hydrogel between the endothelium and the epithelium layers. Obtained results suggest that the realized alveolar in vitro model can be applied as a biomimetic environment recapitulating the alveolar structure to study pathology progression and to test novel treatments for lung cancer. Furthermore, the developed structures were integrated into a microfluidic system in which the physiological microenvironment could be reproduced with higher fidelity thanks to dynamic and three-dimensional cell culture conditions.

Conclusion
A biomimetic in vitro alveolar unit model was implemented mimicking the alveolar wall structure, physiologically characterised by an epithelium layer and a connective layer rich in capillaries. The developed model can be upgraded by culturing cancer derived cells to study pathology progression and predict drug efficacy.

Acknowledgement
This project has been partially financed from Regione Piemonte under the Digital tEchnology For Lung Cancer Treatment (DEFLeCT) project.

Fig. 1.
A) Scanning electron microscope (SEM) image of GL/PCL nanofibers.
B) Representative image of MRC-5 incapsulated into a collagen hydrogel after 7 days (blue: DAPI staining).

References
Tissue engineered hybrid scaffold with relevant stiffness and extracellular matrix (ECM) as long-term in vitro model for low-grade serous ovarian carcinoma (LGSOC)

Elly De Vlieghere\textsuperscript{1,2}, Eva Blondeel\textsuperscript{1,2}, Nathan Carpentier\textsuperscript{4}, Sebastian Riemann\textsuperscript{3}, Wim Ceelen\textsuperscript{3,2}, Sandra Van Vlierberghe\textsuperscript{4}, Olivier De Wever\textsuperscript{1,2}

\textsuperscript{1} Ghent University, Department of Human Structure and Repair - Laboratory of Experimental Cancer Research, Gent, BE; \textsuperscript{2} Ghent University, Cancer Research Institute Ghent (CRIG), Gent, BE; \textsuperscript{3} Ghent University, Department of Human Structure and Repair - Laboratory of Experimental Surgery, Gent, BE; \textsuperscript{4} Ghent University, Polymer Chemistry and Biomaterials Group, Centre of Macromolecular Chemistry, Gent, BE

Introduction
Low-grade serous ovarian carcinomas (LGSOC) are known as a silent killer as they have formed peritoneal metastasis before they are diagnosed. Currently, LGSOC patients receive the same treatment as high-grade (HG)SOC, despite poor treatment responsiveness. To provide LGSOC patients with an adequate therapy, clinically relevant models recapitulating the tumor environment are needed.

Experimental Methods
Hybrid scaffold: The scaffolds are produced by extrusion-based 3D-printing AUP\textsuperscript{[1]} with the 3D Bioplotter (SysEng Bioscaffolder, Hünxe, Germany) to have similar dimensions in swollen state as a peritoneal metastasis nodule. The scaffolds are cylinder-shaped with 5mm diameter, 4mm height, 500µm pore size and 300µm strut diameter. Plasma treatment improves interaction with a methacyrylamide-modified gelatin (2w/v% GelMA solution containing 2 mol% Irgacure 2959 relative to the amount of methacrylamide moieties.) coating and enables cell interaction. The scaffolds are seeded with a mixture of cancer cells and cancer associated fibroblasts (CAFs) and type I collagen gel (2mg/ml).\textsuperscript{[2]} In vitro cultures were monitored by phase-contrast microscopy and bio-luminescent imaging (BLI).

Results and Discussion
AUP scaffolds with 3 different PEG backbones (4, 8 and 10 kDa) were printed. Printing conditions were optimized so each scaffold has the same dimensions (Ø and h 5 mm, 125 mm\textsuperscript{3}, pores +/- 500 µm). Stiffness was measured by indentation, the lower linear modulus was used to compare animal and human tissue with the printed scaffolds. 8 and 10 kDa scaffolds have a similar stiffness as normal tissue while 4 kDa scaffolds mimic the tumor stiffness (fig 1A-B).

LGSOC scaffolds were evaluated as long term in vitro culture model for cancer combined with CAFs. With BLI (fig 2A) we evaluated the viability by bioluminescent imaging (BLI) of the cancer cells independent of the CAFs while being in co-culture. Long term (1 month) follow-up shows that LGSOC cells responded to trametinib, however low activity remains (fig 2B). These cells have the possibility to become resistant to treatment.

Conclusion
Hybrid scaffolds mimic the tumor and its environment on a cellular and biophysical level. The 3D co-cultures allows long-term therapy evaluation, on cancer cells and CAFs. In addition, this long-term evaluation allows to investigate the role of the tumor environment on therapy resistance.

Acknowledgement
Elly De Vlieghere is supported by FWO as a post-doc fellow (12Y8119N) and CRIG YIPOC grant
Figure 1: Relevant Stiffness
A) Stiffness of different biological samples determined by indentation. B) Stiffness comparison between different scaffold types and biological samples

Figure 2: Long term follow-up
BLI images (A) and quantification (B) of LGSOC scaffolds in vitro treated by 1nM trametinib

References
[2] De Jaeghere - De Vlieghere et al., 2018, Biomaterials 2018
**Aligned melt electrowritten fiber tracts and PTEN dysfunction reveal an alternating morphology mechanism for migrating brain tumor cells**

**Annalena Wieland**, Pamela L. Strissel, Hannah Schorle, Ezgi Bakirci, Paul D. Dalton, Dieter Janzen, Markus Eckstein, Carmen Villmann, Reiner Strick

*University Hospital Erlangen, Department of OB/GYN, Laboratory of Molecular Medicine, Erlangen, DE; University Hospital Wuerzburg, Department of Funktional Materials in Medicine and Dentistry, Wuerzburg, DE; University of Oregon, Knight Campus for Accelerating Scientific Impact, Eugene, US; University Hospital Wuerzburg, Institute of Clinical Neurobiology, Wuerzburg, DE; University Hospital Erlangen, Institute of Pathology, Erlangen, DE*

**Introduction**

Cell migration and invasion are hallmarks of development and cancer. For the ability of cells to migrate, dynamic and spatially regulated changes of the cytoskeleton, cell-cell adhesion and cell-extracellular matrix (ECM) interaction must occur. Brain tumor cells sense ECM structures as migratory cues, where they become polarized by reorganizing the actin cytoskeleton to facilitate both a protrusive leading and a contractile trailing edge. They are attracted to white matter tracts, formed by axons organized into aligned bundles, but also within the perivascular spaces of blood vessels, where they migrate and metastasize to surrounding brain regions. One explanation for the brain tumor cell movement is the high mechanical rigidity of axons and blood vessels. Glioblastoma multiforme (GBM) arise from glial cells in the central nervous system and represent the most common and aggressive human brain tumors. Malignant progression results in an extensive invasion throughout the brain. GBM tumors show an impassive response to current therapeutic approaches predicting a poor survival for patients. Thus, it is crucial to increase our understanding of tumor behavior in the microenvironment to improve therapeutic strategies by establishing new tumor models. Synthetic biomaterials, which are commonly employed in tissue engineering, are a valuable tool for modeling 3D tumor growth, migration and invasion. Melt electrowriting (MEW) allows to directly write or print complex multi-scaled architectural structures made of biodegradable substrates, such as poly(ε)-caprolactone (PCL). Therefore, aligned MEW-fiber constructs are capable of recapitulating topographical aspects of the in vivo environment, providing important material cues for 3D tumor cell modelling. A key player involved in regulating migration and invasion is the Phosphatase and Tensin Homolog (PTEN) protein. PTEN is a tumor-suppressor antagonizing the oncogenic Phosphoinositide 3-kinase (PI3K) pathway. PTEN loss of function results in constitutively active PI3K signaling and induces cell proliferation, as well as migration. PTEN loss of function mutations, are one of the most common genetic alterations in GBM and are directly associated with a malignant transformation. Interestingly, PTEN was also frequently found mutated in brain metastases descending from breast cancers.

**Experimental Methods**

Various designs of PCL scaffolds were 3D printed using MEW. PCL-scaffolds were functionalized with laminin to mimic axons and blood vessels to study GBM and breast tumor cell migration. Using live cell imaging we investigated scaffold cell attraction, adhesion, migration speed and cell movement along PCL-scaffold tracts. Scanning electron microscopy (SEM) and immunocytochemistry were performed to visualize the 3D cellular morphology on scaffolds. Additionally, we identified the genes involved in the tumor cell migration using RNA-sequencing and quantitative real-time polymerase chain reaction (qRT-PCR).

**Results and Discussion**

In this investigation we employed different 3D MEW printed PCL-scaffolds to mimic in vivo-like migratory cues of the brain to study the migration of GBM and breast tumor cells. We found similar biological behaviors in terms of...
scaffold attraction, adhesion, 3D cell shape, migration speed, and cell movement along PCL- scaffold tracts. We determined PTEN as one of the main factors for cell adhesion and migration, which we verified with different cell lines and PTEN genotypes. In addition, using RNA-sequencing of 2D and 3D - PCL GBM cells, we uncovered RHOB GTPase up-regulated in 3D-PCL migration. Inhibitory studies confirmed the role of RHOB in cell migration. Furthermore, we describe a unique PTEN-dependent "alternating morphology" - mechanism shared by migrating GBM and breast cancer cells in live cell imaging, where cells change from a flat to a round appearance with a boosted acceleration rate.

**Conclusion**

Taken together, our data helps to understand, which physical attributes are required for how GBM and breast cancer cells migrate and disseminate within the brain. We present a MEW-based 3D model to study the migratory behavior of brain tumors as well as brain metastases. Furthermore, we unraveled that PTEN and RHOB play a central role in tumor cell migration, which could be of particular interest for the development of new therapeutic approaches to slow down brain tumor progression.

**Acknowledgement**

This work was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation): Project number 326998133, TRR 225 (sub-project B01).

---

**References**

K08-07

Exfoliated black phosphorus influences healthy and cancer bone cell behaviors

Maria Grazia Raucci1, Ines Fasolino1, Maria Caporali2, Alessandra Soriente1, Manuel Serrano Ruiz2, Maurizio Peruzzini2, Luigi Ambrosio1

1 National Research Council, Institute of Polymers, Composites and Biomaterials (IPCB-CNR), Naples, IT; 2 National Research Council, Institute of Chemistry of Organometallic Compounds (ICCOM-CNR), Sesto Fiorentino, IT

Introduction
Currently, osteosarcoma is the most common bone cancer which mainly affects young people. Surgical resection of tumor followed by chemotherapy for micro-metastasis inhibition constitutes the current standard procedure. However, chemotherapy treatment uses pharmacological agents with the effect of blocking cell proliferation, without any distinction between healthy and cancer cells. In recent years, several studies have focused the attention on Photodynamic Therapy (PDT) or Photothermal treatment (PTT) as minimally invasive therapeutic procedure that can apply a selective cytotoxic activity toward cancer cells [1]. In this context, we propose the use of 2D photothermal transducing agent based on few-layer black phosphorous (2D BP) as an alternative tool for osteosarcoma treatment and report how 2D BP can inhibit cancer cell proliferation and simultaneously to stimulate newly forming bone tissue generation after osteosarcoma resection without PTT. In particular, we have developed 2D and 3D in vitro models to evaluate the efficacy of 2D BP on healthy (HOb) and cancer (Saos-2) cells and we also propose an in vitro co-culture model to study the effect of 2D BP to prevent cancer development in an inflammatory microenvironment.

Experimental Methods
2D BP was obtained by liquid exfoliation process of BP microcrystals. The purity was ascertained by Inductively coupled plasma mass spectrometry (ICP-MS) analysis, the morphological characterization was carried out by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Biological studies were performed by using 2D BP as coating (in vitro 2D model) and solution (in vitro 3D model). In vitro 2D model was used to investigate the selective effect of 2D BP on human healthy (HOb) and cancer cells (Saos-2), with and without PTT, by analyzing cell proliferation, oxidative stress (i.e. Reactive oxygen species- ROS, nitrites levels) and osteogenic differentiation (ALP activity) at day 3 of cell culture. The cell morphology was evaluated by SEM, confocal microscopy (CLSM) and hematoxylin-eosin staining. The effect of 2D BP on inflammatory response through pro and anti-inflammatory cytokine investigations on co-culture model consisting of Saos-2 and HOb, was also investigated. In vitro 3D model was developed by using Matrigel® as support of cell growth. 2D BP solution was injected at different concentrations (5–25–75 µg/mL) in 3D structure. The cell proliferation was evaluated through Alamar blue assay and Ki-67 marker at day3.

Results and Discussion
Our data pointed out that 2D BP induces healthy cell proliferation and inhibit cancer cell proliferation with and without PTT treatment. This behavior was explained by the involvement of 2D BP in the oxidative stress process by different production of nitrites and ROS in both cell lines. Indeed, without PTT treatment, 2D BP coating reduces the ROS and nitrites production in HOb cells suggesting antioxidant properties, meanwhile an opposite effect for Saos-2 was observed (Fig.1A). Furthermore, morphological investigations demonstrated how 2D BP affected cell vitality and morphology. HOb cells showed a good morphology and spreading in the presence of 2D BP, while Saos-2 appeared to lose their characteristic morphology as suffering cells (Fig.1B). Furthermore, it was also demonstrated that 2D BP allows to induce cell proliferation and osteogenic differentiation in basal medium without external growth factors in
HOb by the expression of ALP, an early marker of osteogenesis. Meanwhile, completely metabolic inhibition was observed for Saos-2 cells. The experiments performed on in vitro co-culture model of cancer-related inflammation demonstrated that the anti-proliferative effect of 2D BP is associated to its anti-inflammatory activity. In vitro 3D model demonstrated that 2D BP solution is able to inhibit in higher percentage the tumor cell proliferation (Saos-2) already at 25 µg/mL (Fig.2). This data was confirmed by the inhibition of Ki-67 expression in 3D structures treated by 2D BP 25 µg/mL.

Conclusion
The overall results demonstrated the selective effect of 2D BP on healthy (HOb) and cancer (Saos-2) cells and its property to induce osteogenic differentiation in healthy cells. For the first time, it was demonstrated that 2D BP is able to inhibit in selectively way the proliferation of cancer cells without the use of any chemotherapeutic agents and PTT therapy. These selective effects prompt the application of 2D BP as a highly promising candidate for bone biomedical applications.

Acknowledgement
This study was supported through funds provided by Progetto MIUR PRIN2017 – ACTION, Grant N. 2017SZ5WZB. The authors also thank Mrs. Cristina Del Barone of LAMEST laboratory for SEM investigations, Mariarosaria Bonetti for lab technical support & data elaboration and Dr. Roberta Marzella for support to project management.

Figure 1
Figure 1: A - Effect of 2D BP on Reactive Oxygen Species production (a) and nitrites level (b) from Saos-2 cells, without photothermal treatment. B - Confocal images of HOb and Saos-2 cells without (i), (iii) and with (ii), (iv) 2D BP exposure, respectively.
Figure 2
Effect of 2D BP at different concentrations (5 – 25 –75 µg/mL) on Saos-2 proliferation (2D and 3D in vitro models) without photothermal treatment.

References
3:45 p.m. – 5:15 p.m.

Track03

S12 | Biocompatibility Testing According to ISO 10993: What are the Changes and What can the Biomaterials Community Learn from it?

Chairs
Marc Bohner
RMS Foundation, CH
Nicola Döbelin
RMS Foundation, Bettlach, CH
James Quinn (YSF)
Queen’s University Belfast, School of Pharmacy, Belfast, GB

Learning Objectives:
Learn the basics of ISO 10993
Understand the importance of literature studies
Learn the basics of a tox assessment (occurring prior to any cell culture test)
Learn from practical examples how to approach biocompatibility testing

The main document used commercially for biocompatibility testing is the ISO 10993 standard.

The recent changes of this standard have introduced what could be called a „pharma touch“ since biocompatibility assessment starts with a thorough analysis of potential leachables and their related toxicity. Systematic literature studies are also highly involved.
ISO 10993 is a series of international standards that provides an extensive framework for biocompatibility assessment of medical devices. Currently the series comprises 22 standards, technical specifications and reports, and guidances addressing all aspects of biocompatibility testing including risk assessment, selection of tests and materials, performance of chemical characterizations, in-vitro and in-vivo tests, as well as evaluation and reporting of test results. While cell culture and animal tests constitute an important pillar in biocompatibility testing, it is a common misconception that the standard series requires such tests to be performed under all circumstances. In fact, it puts a strong emphasis on minimizing the costs and the use of animals for biocompatibility testing. Instead, it advocates the use of existing data whenever possible. For example, instead of conducting a cell culture experiment to assess the cytotoxicity of a product, it may be more efficient and economic to determine the leachable and extractable components of the device, and to assess the cytotoxicity of these components based on data available in literature and databases. Many of the materials used in medical devices today have been extensively tested and a wide range of toxicological data is already publicly available. Hence for many products the cost of in vitro and in vivo testing can be drastically reduced without compromising the safety of the product. This presentation gives an overview of the approaches for biocompatibility assessment of medical devices put forward by the ISO 10993 series.
S12-02

A comparative study of the hemocompatibility of polymeric and lipidic nanobiomaterials by linked cytokine flow cytometry analysis and cytotoxicity

Valeria Perugini¹, Cheryl Collins¹, Ruth Schmid², Yrr Morch², Isabelle Texier-Nogues³, Martin Brodde⁴, Matteo Santin¹

¹ University of Brighton, Centre for Regenerative Medicine and Devices, Brighton, GB; ² SINTEF, Biotechnology and Nanomedicine, Trondheim, NO; ³ CEA, Leti, Paris, FR; ⁴ OxProtect, Munster, DE

Introduction
Clinically-efficacious drug delivery systems can be obtained by the combination of nanobiomaterials (NBMs) carriers of different composition with specific drugs. Current regulatory guidelines advise the use of methods optimised for the testing of either biomaterials for medical devices or medicines, but they do not ensure the reliable assessment of the safety of more complex NBMs. Therefore, there is a need to develop a Regulatory Science Framework able to establish in a reliable manner the risk/benefit profile of NBMs by the evaluation of their biological responses. In particular, the systemic administration of NBMs requires an accurate study of their hemocompatibility including the overall haemotoxicity and the activation of inflammatory cells (i.e. monocytes/macrophages and granulocytes) and thrombogenicity (i.e. platelet activation). Biomarkers of inflammation include the secretion of cytokines that can be caused either by the activation of the monocytes/macrophages or as an indirect effect of the NBM's toxicity that disrupt cell integrity. LipImage™ 815 (CEA-Leti, France) are lipid nanoparticles containing a fluorescent dye. They have been tested in pre-clinical studies to target tumour cells and could therefore be used for cancer diagnosis. Poly(2-ethyl-butyl cyanoacrylate) (PEBCA) nanoparticles (SINTEF, Norway) have been loaded with the cytotoxic drug cabazitaxel (CBZ) and used in pre-clinical studies to target tumours as a promising treatment for breast cancer.

In this work, the effect of PEBCA and LipImage™ 815 on whole human blood samples is studied by a combination of protein assay, LDH released activities and flow cytometry analysis of released cytokines as a rapid hemocompatibility test for NBMs.

Experimental Methods
Whole heparinised blood was collected from 6 healthy human donors and spiked with NBMs. NBMs were tested for their cytotoxicity (LDH assay) and total protein concentration (Bredford's method) at different concentrations; LipImage™ 815 at 9, 18, 47, 94, and 940 μg/ml. PEBCA ± CBZ at 1, 2, 5, 10, 100 and 1000 μg/ml, 37°C, static conditions, 24h. Human Th1/Th2 cytokine standards (BD™ Cytometric Bead Array, CBA) were reconstituted in assay diluent and incubated for 15 minutes at room temperature (0 – 2500 pg/mL). After incubation, 6 human Th1/Th2 cytokine capture beads were treated with 50 μL PE-conjugated detection antibodies before being mixed with 10 μL plasma/standards diluted in 40 μL of serum enhancement. Two NMB concentrations were considered below and at the ascertained hemocytotoxicity threshold, 25 and 100 μg/ml. Both samples and standards were then incubated for 3 hours. After 3 wash steps with 1 mL of wash buffer, samples and standards were assessed by BD Accuri C6 flow cytometer and results analysed using a specific BD analysis software. Lysing Solution (Beckman Coulter) was added to the LDH positive controls by incubation for 10 min. All cytokine release data were analysed in relation to the cytotoxicity threshold as determined by LDH assay and total protein concentration and statistically analysed by t-test.

Results and Discussion
The results show that neither PEBCA nor Lipimage were toxic below 100 μg/ml. A significant release of LDH was detected from concentrations of 100 μg/ml and above. This corresponded to a significant reduction of the protein...
concentration in the blood supernatant. Protein concentration was gradually reduced by contact with both types of NBMs to 50% upon incubation with NBMs' concentrations of 100 μg/ml and above suggesting the occurrence of protein adsorption of onto their surfaces that may trigger the activation of inflammatory cells. The release of pro-inflammatory cytokines was detected at similar levels at concentration both below (25 μg/ml) and above (100 μg/ml) the ascertained haemotoxicity threshold showing that the combined assay could discriminate between an inflammation caused by cell activation from that originating from a burst release of the cells due to their loss of integrity. Expected individual variations were observed across donors, while the presence of a cytotoxic drug payload appeared to reduce the release of cytokines supporting the interpretation of the data that the release was not caused by a damage of the cell membrane.

**Conclusion**

The present work demonstrates that the combined protein, LDH and cytokine assay of whole human blood incubated with polymeric and lipidic drug nanocarrier has the potential to be a rapid and very informative method to assess the haemocompatibility of NBMs with/without drug payload which can be adopted by regulatory frameworks aiming to assess the risk/benefit profile of NBMs.

**Acknowledgement**

This work was supported by the EU Horizon 2020 project REFINE, Grant Number 761104.

![Figure 1. Cytokine release in whole human blood following incubation with NBMs](image)

The release of cytokines relevant to a pro-inflammatory pathway (standard) induced by PEBC and Lipimage815 NBMs was tested on the whole blood of two donors below and at the ascertained haemocytotoxicity threshold.

**References**

Tiger 17 and pexiganan peptides anchored onto electrospun polyvinyl alcohol/cellulose acetate mats improve the surface antimicrobial action and hemocompatibility

Marta A. Teixeira\textsuperscript{1}, Shafagh D. Tohidi\textsuperscript{2}, M. Teresa P. Amorim\textsuperscript{1}, Joana C. Antunes\textsuperscript{1}, Helena P. Felgueiras\textsuperscript{1}

\textsuperscript{1} University of Minho, Department of Textile Engineering/Centre for Textile Science and Technology (2C2T), Guimarães, PT; \textsuperscript{2} University of Minho, Department of Mechanical Engineering/Digital transformation colab (DTX), Guimarães, PT

Introduction

The incidence of chronic wounds (CW) is growing at an accelerated rate every year. CW are generally characterized by a poor healing. However, wound dressings functionalized with active biomolecules may encourage and instigate healing in CW, playing an active role in wound closure \cite{1}. The introduction of antimicrobial peptides (AMPs) has been considered very promising as an alternative to antibiotics, mainly for their broad spectrum of antimicrobial activity, quick action, and low tendency to induce resistance. Tiger 17 has demonstrated important effects on different phases of wound healing and recruiting macrophages, promoting the proliferation/migration of keratinocytes/fibroblasts to the wound bed \cite{2}. Pexiganan is recognized for its wide spectrum of antimicrobial activity, acting directly on the anionic phospholipids of the bacterial cell membrane, turning the development of resistance less likely. Also, this AMP has reached phase III of clinical trials aiming at evaluating its wound healing properties towards diabetic foot infections \cite{3}. Electrospinning allows the production of polymeric nanomeshes with an intricate and complex network that can be functionalized with these active biomolecules to address specific CW demands via simple, reproducible and cost-effective approaches \cite{4}. Various synthetic and natural polymers have been used in the production of polymeric nanofibrous mats via electrospinning. Poly(vinyl alcohol) (PVA) and cellulose acetate (CA) are polymers well established for their biocompatibility and biodegradability. In blends, PVA provides excellent electrospinability and mechanical stability, while CA confers cellular viability \cite{5}. The present work reports the antimicrobial effect and hemocompatibility of Tiger17 and pexiganan peptides when immobilized onto PVA/CA crosslinked mats.

Experimental Methods

PVA and CA based meshes were prepared through electrospinning at the ratios 100/0, 90/10 and 80/20 %v/v (at 10 wt\% in a solvent system of 75/25 %v/v of acetic acid/distilled water. Optimal conditions for PVA/CA processing were established at 25 kV, feeding rate of 0.8 mL/h and distance between needle and collector of 18 cm. Crosslinking was performed using vapor of glutaraldehyde (GA) at 60°C during 7h. The blending homogeneity of the polymers and the efficiency of the crosslinking process were examined, together with the mats morphology, mechanical stability, permeability and thermal properties using Fourier-transformed infrared spectroscopy (FTIR), scanning electron microscopy, air and water vapor permeability testing equipment, thermogravimetry and a dynamometer. Tiger 17 and pexiganan were anchored to the mats by physisorption and using poly(ethylene glycol)-spacers as a binding agent, respectively. Their presence on the mats was confirmed using sulfosuccinimidyl-4-o-(4,4- dimethoxytrityl) butyrate. The antimicrobial action was followed against Staphylococcus aureus (\textit{S. aureus}) and Pseudomonas aeruginosa (\textit{P. aeruginosa}), the most prevalent bacteria found in CW. Clotting time was established visually by the loss of movement of re-calcified plasma, following the Lee-White method. Platelets’ adhesion and activation were mapped using brightfield microscopy.

Results and Discussion

Page 788 of 2028
PVA/CA nanofibrous mats were successfully produced via electrospinning, with a porous structure made of continuous nanofibers. Electrospun mats were crosslinked with GA vapor at 60°C by promoting the formation of intermolecular acetal bridges between the -OH in PVA and CA and the difunctional aldehyde molecule of GA, as confirmed by the presence of a FTIR peak at 1143 cm\(^{-1}\), attributed to the -O-C-O vibration of the acetal group. After crosslinking, the average diameters of 100/0, 90/10 and 80/20 PVA/CA nanofibers increased from 223, 217 and 194 nm to 200, 238 and 278 nm, respectively. The permeability index values, even on crosslinked mats, registered values above of 82%, being considered quite appropriated for wound dressing applications. With the introduction of CA in the mats, a slight increase in the ultimate tensile strength and on the thermal stability of electrospun mats was detected, being most important after crosslinking. Mats were successfully modified with Tiger 17 and pexiganan. They were found effective against the two bacteria, particularly pexiganan. Tiger 17 was seen to effect more significantly on the clotting time and platelets activation, enhancing them and, therefore, contributing to a potentially quicker wound closure.

**Conclusion**

Overall, preliminary data revealed the potential of the PVA/CA mats modified with the peptides Tiger 17 and pexiganan for applications in CW healing.

**Acknowledgement**

This research received funding from the Portuguese Foundation for Science and Technology (FCT) under the scope of the projects PTDC/CTM-TEX/28074/2017 (POCI-01-0145-FEDER-028074) and UID/CTM/00264/2021. MAT also acknowledges FCT for PhD grant with the reference SFRH/BD/148930/2019.

**References**

Self-assembled fibrinogen nanofibers promote fibroblast adhesion and prevent *E. coli* infiltration

Arundhati Joshi¹, Naiana Suter¹, Stephani Stamboroski¹², Dorothea Brueggemann¹

¹ University of Bremen, Institute for Biophysics, Bremen, DE; ² Fraunhofer Institute for Manufacturing Technology and Advanced Materials, Bremen, DE

Introduction

Fibrinogen has gained enormous attention in tissue engineering since it plays a central role in blood coagulation and is thought to contribute to the early stages of antimicrobial host defense. Specifically, nanofibrous fibrinogen scaffolds hold great promise for applications in personalized regenerative medicine as they mimic the nanoarchitecture of native blood clots and the extracellular matrix. Although manifold factors, such as hydrophobic surfaces, organic solvents or acidic pH, are already known to induce fibrillogenesis of fibrinogen, the fundamental mechanisms of fibrinogen fiber assembly still remain to be elucidated [1]. In contrast to the aforementioned factors, we recently introduced salt-induced self-assembly as a new approach to prepare nanofibrous fibrinogen scaffolds under physiological conditions [2,3]. Here, we present our findings on the interaction of these scaffolds with fibroblasts and *E. coli* bacteria.

Experimental Methods

The self-assembled fibrinogen nanofiber scaffold morphology was characterized with scanning electron microscopy (SEM) and atomic force microscopy (AFM). To evaluate the potential of self-assembled fibrinogen nanofibers for future wound healing applications, we used NIH 3T3 fibroblasts and *E. coli* bacterial cultures as model systems [4]. Adhesion, growth, morphology as well as migration of fibroblasts on the fibrinogen nanofiber scaffolds was analysed using confocal microscopy, SEM and live cell tracking. Interaction of *E. coli* bacteria with fibrinogen nanofibers was studied with image intensity analysis of light microscopy images and spectrophotometric measurements of bacterial cell lysates.

Results and Discussion

Self-assembly with phosphate buffered saline (PBS) solution yielded very dense nanofiber networks that resembled the structure of native fibrin clots, whereas potassium-based buffers yielded sparse and less defined fiber assemblies [2]. Nanofibrous fibrinogen scaffolds assembled in PBS displayed an average thickness of 3 µm and fiber diameters between 100 and 300 nm (see Fig. 1A). Crosslinking with formaldehyde vapour was found to preserve the fiber topography in an aqueous environment (see Fig. 1B) [3].

The nanofibrous scaffolds promoted the adhesion and growth of 3T3 fibroblasts for up to 72 h [4]. Smaller fibroblasts with many short filopodia, less pronounced actin cytoskeleton and elevated cell bodies were observed on nanofibrous fibrinogen (see Fig. 1C), which was in contrast to planar fibrinogen scaffolds produced in the absence of salt. Higher migration velocities of fibroblasts were observed on the nanofibers in comparison to planar fibrinogen. *E. coli* bacteria failed to migrate through the interconnected pores of fibrinogen nanofibers. In particular, the barrier formed by the fibrinogen nanofiber scaffolds significantly reduced bacterial growth in comparison to controls in the absence of fibrinogen scaffolds, mainly due to lack of access to the nutrient-rich agar underneath. Furthermore, SEM analysis revealed that the cell walls of *E. coli* bacteria on fibrinogen nanofibers were considerably damaged (see Fig. 1D).

Conclusion

In conclusion, self-assembled fibrinogen nanofibers formed interconnected porous networks that exhibited very good biocompatibility by supporting cell adhesion and preventing bacterial infiltration. These findings highlight their great
potential as future protein scaffolds for regenerative medicine and provide a good basis for further cell culture studies with other cell types involved in wound repair, such as keratinocytes and blood platelets.

**Acknowledgement**

Emmy Noether program of the German Research Council under grant number BR 5043/1-1

---

**Characterization and biocompatibility analysis of self-assembled fibrinogen nanofiber scaffolds**

(A) SEM image of fibrinogen nanofibers prepared by salt-induced self-assembly showing porous architecture; inset shows contrasting smooth morphology of planar fibrinogen prepared without salt [1] (B) AFM analysis of cross-linked fibrinogen scaffolds revealed that the fibrous morphology was preserved upon rehydration in water [3]. (C) SEM image of 3T3 fibroblasts on fibrinogen nanofibers at cultivation time of 36 h [4]. (D) SEM image of bacterial colonies growing on nanofibrous fibrinogen scaffoldshowing holes on the bacterial cell surfaces, indicating a disruption of the cell walls [4].

**References**


S12-05

From *in vitro* evolution of new bioactive amorphous calcium ortho/pyrophosphate materials in various acellular/cellular media to *in vivo* animal study

Maximilien Desbord¹, Jérémy Soulié¹, Robin Siadous², Rebecca Landon³, Alice Leroux², Christian Rey¹, Joelle Amedée², Fani Anagnostou³, Christèle Combes¹

¹ Université de Toulouse, Toulouse INP, CIRIMAT - ENSIACET, Toulouse, FR; ² Université de Bordeaux, BioTis Inserm 1026, Bordeaux, FR; ³ Université Paris Diderot, B3OA, Faculté de Médecine Paris Diderot Site Villemin, Paris, FR

Introduction

Amorphous calcium phosphate materials have attracted attention for bone substitution due to their metastability that improves their (bio)chemical reactivity and the subsequent release of active ions and/or dissolution/reprecipitation reactions leading to apatite formation at their surface. Two families of phosphate-based amorphous materials are mainly developed: the phosphate-based glasses that are generally melt-derived and low temperature amorphous calcium phosphates [1, 2]. Recently, we obtained by soft chemistry a new family of mixed calcium orthophosphate/pyrophosphate amorphous materials with structural properties close to those of materials mentioned above [3,4]. It is known that pyrophosphate ions can be hydrolyzed into orthophosphate ones at acidic pHs, at moderate temperature and/or by specific enzymes, alkaline phosphatase (ALP), naturally present *in vivo*, thus contributing to bone neoformation [5]. We aimed at studying for the first time: i) the physico-chemical evolution *in vitro* of such materials including different proportions of pyrophosphate in acellular aqueous media with or without enzymes in a view to control their resorption and biological properties; ii) their *in vitro* cytocompatibility and *in vivo* tissue response in small animal.

Experimental Methods

Mixed calcium ortho- and pyrophosphate amorphous materials of three different compositions (different ortho/(ortho+pyro) ratios, i.e. high, medium and low orthophosphate content) were synthesized by soft chemistry [4]. The evolution tests were carried out at 37°C for 15 days in different aqueous media (simulated body fluid (SBF) solution, Tris buffer with or without ALP) with a solid/ liquid ratio of 1.5 g/L. Materials and media recovered after different evolution times were fully characterized separately by XRD, Raman spectroscopy, SEM and chemical titrations. *In vitro* cytotoxicity and cytocompatibility testings were performed on hBMSCs. Then the most promising material composition was implanted as granules *in vivo* in a critical defect model (rat calvaria) and compared with Activioss™, a 45S5 commercial bioglass.

Results and Discussion

The three amorphous material compositions tested have shown great stability but have all evolved differently in correlation with their initial composition (initial ortho/(ortho+pyro) ratio). The material including the higher pyrophosphate content was the more stable and tended to remain amorphous, while the composition comprising the highest level of orthophosphate evolved into a nanocrystalline apatite analogous to the bone mineral in almost each medium tested. The evolution in the presence of ALP enzymes demonstrated their major role in the evolution of these materials *in vitro*: at day 6 material mass loss and the ortho/(ortho+pyro) ratio in the material were enhanced and directly correlated to the initial proportion of pyrophosphate in the material. The cell study showed that the materials are not cytotoxic and that they are able to activate differentiation of hMSCs and mineralization. Preliminary *in vivo* study testified for higher new bone tissue formed around the material granules than for the commercial bioglass.

Page 792 of 2028
Conclusion
All together these results demonstrated for the first time the *in vitro* and *in vivo* behavior of this new family of amorphous calcium phosphates which thus appeared as promising tunable bioactive bone substitute materials with a resorption and osseo-integration that could be directly controlled by their composition.

Acknowledgement
The authors thank the Agence Nationale de la Recherche (PyVerres project n°ANR-16-CE19-0013) for supporting this research work and the Noraker company (Villeurbanne, France) for providing the Activioss™ granules as a reference material for the *in vivo* study.

References
Resveratrol-Loaded Nanostructured Lipid Carriers: Evaluation of Antioxidant Properties on ARSACS Patient Fibroblasts

Ozlem Sen¹, Melis Emanet¹,², Stefano Doccini³, Filippo M. Santorelli³, Gianni Ciofani¹

¹ Istituto Italiano di Tecnologia, Smart Bio-Interfaces, Pisa, IT; ² Sabanci University, Sabanci University Nanotechnology Research and Application Center (SUNUM), Istanbul, TR; ³ IRCCS Fondazione Stella Maris, Molecular Medicine for Neurodegenerative and Neuro-Muscular Diseases Unit, Pisa, IT

Introduction
Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a rare early-onset neurodegenerative disease, which is caused by mutations in SACS gene that encodes the sacsin protein. The sacsin protein localizes to mitochondria, and it is crucial in neurons for normal mitochondrial localization and morphology [1,2]. Although the exact role of this protein is unknown, it has been suggested that it plays roles in mitochondrial function [3]; on the other side, the decrement of the mitochondrial function is well-known to be related to increased reactive oxygen species (ROS) production [4]. Within this context, we hypothesized the use of resveratrol-loaded nanostructured lipid carriers (Res-NLCs) might decrease the ROS level and inflammation in ARSACS patient cells, owing to the antioxidant activity of resveratrol.

Experimental Methods
NLCs are designed by modification of solid lipid nanoparticles, including solid and liquid lipid phases and prepared by hot homogenization. The colloidal stability of NLCs was monitored using dynamic light scattering and zeta potential measurements. The Res release was assessed in PBS at three different time points (4, 24 and 72 h) and different pH values (pH 4.5, 7.4 and 9.0) and analyzed using a high-performance liquid chromatography (HPLC). The antioxidant capacity of Res-NLCs was measured using a Total Antioxidant Capacity Assay Kit. The cell proliferation of Res-NLCs was assessed on healthy and ARSACS patient fibroblasts using PicoGreen assay. The ROS production was tested via CellROX™ Green Reagent. To analyze the cytokine (interleukin 6, IL-6, and interleukin-8, IL-8) release, ELISA kits were used, and data were normalized according to the total proteins of each sample. Transcription of oxidative stress and mitochondria genes was investigated with quantitative real-time PCR (qRT-PCR). Proteomic analysis was carried out to show regulation of genes on patient fibroblasts upon Res-NLCs treatment.

Results and Discussion
In our study, glyceryl dibehenate and oleic acid were used as solid and liquid lipids, respectively, and resveratrol was added to the formulation to obtain Res-NLCs. Schematic representation and TEM image of Res-NLCs are shown in Figure 1. The stability studies show that the formulations remain stable at 4 °C in long-term storage (up to 2 months). Resveratrol release at pH 7.4 was found 25.2 ± 0.4% and 31.6 ± 3.3% after 24 and 72 h, respectively. The antioxidant ability of Res-NLCs was confirmed using a total antioxidant capacity kit, and it was shown to be concentration-dependent. According to the cell proliferation results, Res-NLCs did not show any toxicity in healthy and patient cells after 24 and 72 h of incubation, demonstrating their biocompatibility. The concentration used for further studies was determined as 140 µg/mL of Res-NLCs (corresponding to 5 µM of resveratrol). Effects on ROS production in tert-butyl-hydroperoxide (TBH)-treated healthy and patient cells, and on inflammatory cytokine release by lipopolysaccharide (LPS)-stimulated cultures were assessed with promising results. The human oxidative stress and mitochondria genes were further evaluated using qRT-PCR array and proteomic analysis in patient fibroblasts upon Res-NLCs treatment.

Conclusion
Taken together, obtained results show that Res-NLCs are a suitable tool in decreasing ROS production and inflammation in ARSACS patient cells.

Acknowledgement
This research was supported by the Italian Ministry of Health (grant no. RF-2016-02361610).

Figure 1. A) Schematic representation and B) representative TEM image of Res-NLCs.

References
Impact of silicone exposure after leakage or rupture of breast implants: histology and molecular analyses

Isabelle Brigaud¹, Tatiana Petithory¹, Carole Arnold¹, Franck Monnien², Isabelle Pluvy², Florelle Gindraux², Marie-Paule Algros², Karine Anselme¹

¹ Institute of Materials Science of Mulhouse, Mulhouse, FR; ² University Hospital of Besançon, Besançon, FR

Introduction
Rupture of the shell of breast implant exposes host tissue to silicone. The more often silicone leakage is confined to the capsular space (intracapsular rupture) and is clinically asymptomatic, indirectly supporting the concept of innocuity of this material. However, accumulating evidences cast doubt on this consensus (1,2). In this study, we addressed the question of breast implant silicone toxicity in vivo. We compared the tissue reaction and gene expression in capsular tissues facing ruptured or intact implants presenting 2 types of topography: OC (open cavity) and SOC (semi-opened cavity).

Experimental Methods
Immune response triggered by silicone exposure was evaluated both at the tissue and at molecular levels. Silicone-driven tissue modifications were observed from hematoxilin-eosin or hematoxilin-eosin-safran stained capsule sections. In parallel, we collected 37 capsular tissues facing textured implants, either intact (n=21) or associated to silent intra-capsular rupture (n=16) and established the molecular profile of key genes representative of immune response (Il8, Il16, Il17, Tgfβ, Mmp9, Timp1) and linked to pathological conditions including BIA-ALCL (Il13)(3). For that, collected tissues were immediately stored in RNAlater solution (Invitrogen, Carlsbad, Calif.) and preserved at -20 °C until further use. Tissue-containing tubes were then placed in a tissue homogenizer (Precellys Evolution, Bertin Instruments Tustin, USA) where tissues were mechanically crushed 30” to liquid at 8500 rpm. Lysates were then centrifuged 2’ at full speed at 4 °C. Recovered clear supernatants were used for subsequent RNA extraction. RNA extraction, RNA quality and integrity check-points together with RT-qPCR steps were carried out as described in (4). Pectoral muscle from breast reduction served as an outgroup calibration sample to calculate relative gene quantifications for all the capsule samples.

Results and Discussion
The contact with silicone led to chronic inflammation characterized by foreign body reaction, granuloma formation, and different forms of tissues metaplasia (synovial, palisade tissue), which were 1) found isolated or mixed in the same tissue and 2) modulated in terms of severity based on amount and duration to silicone exposure. At the molecular level, despite being mute clinically, the exposure to silicone triggered latent inflammation (Il8, Il13, Il16, Il17, Tgfβ) and extracellular matrix remodeling (Mmp9, Timp1). IL16, IL17 and TGFβ gene expressions were found significantly up-regulated in capsules facing ruptured OC-patterned implants compared to intact OC-patterned implants (p < 0.05). Only IL13 gene expression was found similar in tissues located in the vicinity of OC implants, regardless whether implants are intact or ruptured. For any of the 5 inflammatory genes, related gene expression was found similar in tissues facing SOC-patterned implants independently of the implant integrity (intact or ruptured). When we compared the OC- versus SOC-patterned implants, IL8, IL13, IL16, IL17 and TGFβ gene expressions were all found significantly up-regulated. IL13 and IL16 gene expressions were found significantly higher in capsules facing SOC-ruptured implants compared to those facing OC-ruptured implants while gene expression levels for IL8, IL17 and TGFβ were comparable. Both MMP9 and Timp1 gene expressions were found significantly up-regulated in capsules facing ruptured OC-patterned implants compared to intact OC-patterned implants. Up-regulation for MMP9 in tissues facing OC-ruptured implants compared to intact OC-patterned implants.
implants compared to those facing OC-intact implants was noticeably dramatic (~15 folds more in average). If MMP9 gene expression levels were found similar in tissues facing SOC-patterned implants independently of the implant integrity (intact or ruptured), we found that Timp1 was in average ~3 folds more expressed in tissues located in the vicinity of SOC-ruptured implants compared to those in the vicinity of intact SOC implants. Finally, MMP9 gene expression was found significantly higher in capsules facing OC-ruptured implants compared to those facing SOC-ruptured implants while gene expression levels for Timp1 was comparable in those conditions. In general, ECM-related gene expression levels and related variation mirrored expression pattern of the inflammatory genes in view of the different conditions.

Independently, implant topography and silicone exposure were confirmed as pro-inflammatory factors. Combination of these two parameters resulted in a maximal immune response.

Conclusion

Altogether, this study clearly ascertained that host body confronted to silicone develops latent immune response which might predispose to the development of various medical conditions.

We thus encourage surgeons to redefine ruptures, even silent, as urgent condition requiring immediate implant resection.

Acknowledgement

The authors acknowledge Dr Nathalie Bricout (Private Hospital St Germain, St Germain-en-Laye, France) for capsular tissue samples and Ms Anne-Charlotte Heba for her contribution in transcriptomic analyses.
3:45 p.m. – 5:15 p.m.

Track04

S13 | Biomaterials Informatics and Data-Driven Biomaterials

Chairs
Osnat Hakimi
Universitat Internacional de Catalunya, Faculty of Medicine and Health Sciences, Barcelona, ES
Maria-Pau Ginebra
Technical University of Catalonia, ES
Hongyi Chen (YSF)
University College London, Mechanical Engineering Department, London, GB

The aims of this symposium are three folds:

1. To share recent progress in biomaterials big-data curation, sharing, analysis and exploitation
2. To highlight urgent needs of the domain in terms of data organization, open tools and repositories
3. To initiate and foster a community of biomaterials scientists committed to advancing data-driven biomaterials research
Biomaterial surface topography induces cell signaling events that control cell phenotype: how does it work and how can we use it?

Jan de Boer

Eindhoven University of Technology, Eindhoven, NL

Abstract
I will present our latest work on investigating and controlling the interaction between cells and biomaterials through design of surface topography. For instance, we looked into how tenocytes, the cells that build tendon and ligament tissues, interact with their extracellular matrix and how this impacts the formation focal adhesion complexes and F-actin. Transcriptomics analysis of topography exposed tenocytes and molecular biological research shows an interesting relay of the topographical information from the extracellular matrix into the tenocyte phenotype. Inspired by this, we used our high throughput screening platform, the TopoChip, to investigate the relationship between the topographical design parameters and tenocyte phenotype. We use quantitative high content imaging and machine learning algorithms to characterize the response of the cells to the thousands of different surfaces. Using machine learning algorithms, we can describe the relation between surface topography and cell response. If time allows, I will also talk about other cell types and how their phenotype depends on the surface topography, such as macrophages and hepatocytes. The focus of my seminar will be on our effort to digitize life at the interface of biomaterials and cells through parameterization of biomaterial properties, –omics based approaches to analyse cell response and computational science to understand and design bio-active biomaterials.

Biography
Jan de Boer is chair of the BioInterface Science lab at the Institute for Complex Molecular Systems and the department of Biomedical Engineering at Eindhoven University of Technology. His research is focused on understanding and implementing molecular biology in the field of tissue engineering, regenerative medicine and medical devices interface biology. His research is characterized by a holistic approach to both discovery and application, aiming at combining high throughput technologies such as the TopoChip platform, computational modeling and experimental cell biology to streamline the wealth of biological knowledge to clinical applications.
DEBBIE1.0: Biocompatibility data mining from MEDLINE into an open access database

Osnat Hakimi1, Javier Corvi2, Austin McKitrick3, Jose M. Fernandez2, Carla Fuenteslopez4, Clarence Lepine5, Salvador Capella-Gutierrez2, Josep-Lluis Gelpi6, Maria-Pau Ginebra7

Introduction

The majority of biocompatibility data gathered during biomaterials and medical implants research is published in the form of scientific articles. As a result, most biocompatibility information is presented in free text or graphic figures, and is therefore ‘unstructured’, making it a challenging input for computational processing. In the absence of structured biocompatibility data, tasks such as comparing the performance of similar polymers or selecting a biomaterial for a specific medical application relies on manual review of research articles - a daunting manual task, given the volume of work in the field [1].

Emerging computational tools, such as machine-learning-based systems, are becoming essential for coping with this information overload. Among these, text mining systems present an attractive option for automated extraction of information from text documents into structured data sets [2].

Here, we present the first automated system for biocompatibility information extraction from MEDLINE research abstracts. The system is a text mining pipeline, which periodically retrieves abstracts from PubMed and identifies studies where scaffolds or implants have been tested in a biological environment, such as cell culture, tissues, and animal models or human clinical studies. Thereafter, the pipeline identifies concepts of interest in the abstract text using the Biomaterials Annotator, a tool for biomaterials text annotation. These concepts of interest, along with the abstract text and some meta-data are then deposited in DEBBIE, the Database of Experimental Biomaterials and their Biological Effect.

Experimental Methods

The DEBBIE pipeline has four main components (see Figure 1):

1. Data retrieval from Medline using the python Entrez module.
2. Relevance classification using a machine learning algorithm
3. Concept recognition using the Biomaterials Annotator, our semantic annotation tool (github.com/ProjectDebbie/Biomaterials_annotator)
4. Database deposition in a MongoDB database. The database data model is based on the previously published DEB ontology[4].

DEBBIE pipeline has been specified in Nextflow language[3], and its workflow manager was used for orchestration and automated execution of the pipeline. To facilitate the workflow maintainability, extension, adaptation and installation, the DEBBIE sub-components were deployed using software containers (docker). The database and REST API are hosted at the Barcelona Supercomputing Canter’s StarLife server.

So far, the database contents and user interface were tested by 16 scientists, who provided feedback through a questionnaire and a focus group.

Results and Discussion

An experimental version of the DEBBIE database (DEBBIE1.0) is already running, alive, and open for users to test. Users can access the database search page (debbie.bsc.es/search/) and submit an open, single query (for example:
Thereafter, users receive a quick summary (see Figure 2), including the total number of abstracts mentioning the term of interest in the collection, and a line chart showing the number of abstracts per year. Also in the quick summary, there is a bar chart of the top associated terms, namely the concepts most frequently associated with the term of interest. Because the system relies on the Biomaterials Annotator, all identified terms belong to pre-designated relevant categories.

Below the quick summary, users can select and explore categories, providing different aspects related to biomaterials and their biocompatibility. For example, users can select categories such as ‘Associated Biological Process’ (eg. osteogenic differentiation, angiogenesis), ‘Adverse Effects’ (eg. cytotoxicity, inflammation) and ‘Medical Application, Disease or condition’ (example concepts: wound healing, implantation). Each term is an interactive link to the relevant abstracts containing concepts of interest within the search term collection.

The content of DEBBIE can also be accessed programmatically through the RESTful API located at debbie.bsc.es/search/rest. The documentation page for the database provides instructions on how it can be used under the section ‘quickstart’.

While DEBBIE is already open for exploration, the annotation pipeline, the database and web components are still evolving. Ongoing work includes improving the accuracy of the annotations, implementing a multinomial classifier for better relevance filtration and adjusting the user interface and search results display in accordance with users feedback.

Conclusion

We present the first automatically curated biomaterials database, specifically developed for the organization of biocompatibility data. Whilst we are improving the accuracy and performance of the database, feedback and visibility in the biomaterials community is fundamental to the usefulness of this resource.

In addition to the database, all the components, linguistic resources and code developed in the project are openly available through the project’s GitHub page (github.com/ProjectDebbie).

Acknowledgement

This project has received funding from the European Union Horizon 2020 programme under the Marie Skodowska-Curie grant agreement DEBBIE, project number: 751277. O. H. is funded through a Bosch-Aymerich fellowship. J-M.F, S.C-G and J-L.P are partly supported by INB Grant (PT17/0009/0001 - ISCIII-SGEGI / ERDF). M-P. G. acknowledges the ICREA Academia Award from Generalitat de Catalunya. J.C. is partly supported by eTRANSAFE (received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 777365 and support from the European Union’s Horizon 2020 research and innovation programme and EFPIA).
In addition to the text mining pipeline, an interactive user interface is being developed. The user interface enables the submission of keyword queries to the DEBBIE API, which depends on PyMongo (pymongo.readthedocs.io) for database searches and exploration. Python Flask (flask.palletsprojects.com) has been used to structure the application programming interface and web server, implementing search interfaces. Web interface was developed in HTML, CSS, and JQuery JavaScript library, delegating on the Bootstrap library to make the page user-friendly and responsive.

References

Dissolution and biocompatibility of combinatorially sputtered SiCrNbN coatings

Estefanía Echeverri¹, Charlotte Skjöldebrand¹, Gry Hulsart Billström², Cecilia Persson¹

¹ Uppsala University, Materials Science and Engineering, Uppsala, SE; ² Uppsala University, Medicinal Chemistry, Uppsala, SE

Introduction
Hip and knee joint replacements have been some of the most successful surgeries for the treatment of patients with chronic pain due to arthritis. A current challenge is however the younger and more active patients, which demands longer-lasting devices that will withstand several decades of cyclic loading. Corrosion and wear products are herein a concern since they can cause localized inflammation leading to periprosthetic bone loss and potentially implant loosening, necessitating revision surgery [1]. One approach to overcome the long-term issues is to develop materials more resistant to wear and corrosion, as well as materials giving less of an inflammatory response, e.g. by depositing a ceramic coating which acts as a barrier to the release of metal ions from the substrate as well as improving the wear resistance. Silicon nitride is a promising candidate because of its low wear rates and the possibility to limit the adverse effects of wear debris due to its slow dissolution in aqueous solutions [2]. This study aimed to investigate the dissolution and biocompatibility of SiCrNbN coatings deposited on cobalt chromium (CoCr) substrates. We hypothesized that the ceramic coating will reduce metal ion release compared to uncoated CoCr without affecting its biocompatibility.

Experimental Methods
The SiCrNbN coatings were deposited on CoCr disc substrates by reactive sputtering in an in-house built equipment, allowing for combinatorial processes, using Si, Cr and Nb solid targets. Nitrogen was supplied as a reactive gas. To improve the adhesion of the coating a CrN interlayer was deposited. The coatings were characterized in 9 points using x-ray photoelectron spectroscopy (XPS), vertical scanning interferometry (VSI) and scanning electron microscopy (SEM). The points were placed in a 3x3 grid with 22.5 mm between each point. The dissolution was evaluated by exposing the coated samples to cell media for 7 days. The obtained extracts were diluted (neat extracts (1:1), and 3 two-fold dilutions (1:2, 1:4 and 1:8)) and used to measure ion levels with inductively coupled plasma (ICP-OES) and to assess indirect biocompatibility in vitro using the tetrazolium dye MTT and L929 fibroblast cells.

Results and Discussion
The XPS results revealed compositional gradients with Si ranging between 27.4-32.8 at.%, Cr 4.1-10.9 at.%, Nb 3.5-8.4 at.%, N 41.8-46.8 at.% and O 10.9-14.6 at.%. SEM revealed coating thicknesses between 320-590 nm, and interlayers approx. 50 nm thick. Images displayed an overall smooth surface with an average roughness, Ra, of 5.6 to 9.3 nm, similar for all points. Grooves from polishing and occasional features at the microscale were observed, likely formed during deposition. The ICP results showed a reduction in Co ions from the substrate in the coated samples compared to uncoated. The cell viability results suggest that fibroblasts tolerated the neat extracts and its dilutions (1:1, 1:2, 1:4) obtained from the coated samples in a dose dependent manner.

Conclusion
The findings from this study suggest that the differences in composition did not affect the surface properties. The material characteristics indicate that silicon nitride has a promising potential to be used as a coating in metallic implants to improve corrosion resistance and reduce ion release, warranting further biological evaluation.

Acknowledgement
Page 803 of 2028
This project has received funding from the European Union’s Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 812765 and from the European Union’s Seventh Framework Programme (FP7/2007-2013), grant agreement GA-310477(LifeLongJoints).

References
Biomaterials-on-chip: an alternative method to screen the biological properties of biomaterials

Sarah-Sophia D. Carter¹, Abdul-Raouf Atif¹, Michael Pujari-Palmer², Laurent Barbe¹, Maria Tenje¹, Gemma Mestres¹

¹ Uppsala University, Materials Science and Engineering, Science for Life Laboratory, Uppsala, SE; ² Swedish University of Agricultural Sciences, Clinical Sciences, Uppsala, SE

Introduction
In order to reach the clinic, biomaterials need to be thoroughly evaluated, which requires reliable in vitro methods. However, current in vitro methods, which have provided significant insights into the biological response to biomaterials, have shown to be inadequate for accurately predicting in vivo outcomes [1]. For this reason, the development of alternative in vitro approaches with increased physiological relevance is rapidly advancing. One such approach is the use of microfluidic technology, which allows cells to be cultured while confined in micrometric channels and, simultaneously, be perfused with cell culture medium, thereby enabling an environment closer to the in vivo bone niche.

In this work, two microfluidic systems were developed and used to evaluate the biological properties of different biomaterials for bone repair. A medical grade titanium alloy (Ti₆Al₄V) and calcium-deficient hydroxyapatite (CDHA) were integrated on the respective systems and characterized for a period of 10 days using, among others, imaging and biochemical assays. In subsequent sections, these microfluidic systems are referred to as titanium-on-chip and CDHA-on-chip.

Experimental Methods
The titanium-on-chip consisted of a double-sided tape channel, sandwiched between a conventional microscopic glass slide and cover glass, the latter comprising a laser cut hole to which the titanium disc was docked (Figure 1A). For the CDHA-on-chip, a calcium phosphate cement (CPC) was prepared by mixing α-tricalcium phosphate with 2.5% w/v Na₂HPO₄(aq) at a liquid-to-powder ratio of 0.65 ml/g. The CPC was poured into a polydimethylsiloxane (PDMS) channel and subsequently set for 10 days in a 0.9% w/v NaCl(aq) solution, thus transforming into CDHA. Afterwards, the PDMS was bonded to glass, leaving a 0.5 mm channel gap through which the cell culture medium could flow (Figure 1B).

On the titanium-on-chip, MC3T3-E1 pre-osteoblast cells were seeded at 45,000 cells/cm² and incubated under static conditions for 4 hours, prior to starting flow (2 µl/min). As a control, MC3T3-E1 cells were seeded on titanium discs kept in a well plate (titanium off-chip). A similar approach was used for the CDHA-on-chip, however, in this case an initial cell seeding density of 50,000 cells/cm² was used and the static incubation was set to 2 hours. The CDHA-on-chip was initially used to characterize the ionic exchange on-chip, using flow rates between 2-14 µl/min. Based on the results from this preliminary study, cell studies on the CDHA-on-chip were performed using a flow rate of 8 µl/min.

For both systems, cell viability, proliferation and differentiation were monitored over a period of 10 days using calcein/propidium iodide, lactate dehydrogenase assay and alkaline phosphatase (ALP) assay, respectively. For cell proliferation and cell differentiation studies, the analysis was performed using cell lysates.

Results and Discussion
MC3T3-E1 cells were successfully maintained (viable) on both titanium-on-chip and CDHA-on-chip, as was shown by a vast majority of living cells after 10 days of culture (Figure 1C, D). Interestingly, regardless of the biomaterial or microfluidic system, the general trend observed was that cell proliferation dominated on-chip experiments, to the detriment of cell differentiation when compared to the static condition (data shown for titanium-on-chip, Figure 2) [2].
A possible explanation for this finding is a depletion in cell-secreted factors when maintaining the cells under continuous dynamic culture conditions. However, in order to make conclusive statements, further work is needed.

**Conclusion**

Overall, this work demonstrates the importance of optimizing *in vitro* cell culture conditions, particularly for novel miniaturized and microfluidic applications, and raises awareness of how these conditions may affect biomaterial testing outcomes, thus illustrating the potential of alternative methods that can more closely approach physiological conditions.

**Acknowledgement**

This work was supported by the Research Council for Sustainable Development FORMAS [#2016-00781, 2016]; the Swedish Research Council (Vetenskapsrådet) [#2017-05051, 2017]; and the Göran Gustafsson’s Foundation [ID #1841, 2018]

**Figure 1**

(A) Schematic of the different layers of the titanium-on-chip and (B) photograph of the assembled CDHA-on-chip. Hoechst/calcin/propidium iodide stained MC3T3-E1 cells after 10 days of culture on (C) the titanium-on-chip and (D) CDHA-on-chip. Scale bars correspond to 200 µm.

**Figure 2**

Cell proliferation and differentiation of MC3T3-E1 cells after 1, 5 and 10 days of culture on the titanium-on-chip and titanium discs off-chip.

**References**


3:45 p.m. – 5:15 p.m.

Track 05

ESB-SFB S | ESB-Society For Biomaterials (SFB) Joint Symposium: Immunomodulatory Biomaterials

Chairs
Andrés Garcia
Georgia Institute of Technology, US
Abhay Pandit
National University of Ireland, Galway, Galway, IE
Rocio Corrales-Orovio (YSF)
Pontificia Universidad Católica de Chile, Santiago, CL
Biosynthetic Hydrogels for Islet Engraftment and Immune Acceptance

Andrés J. García

Parker H. Petit Institute for Bioengineering and Bioscience, George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, US

Hydrogels, highly hydrated cross-linked polymer networks, have emerged as powerful synthetic analogs of extracellular matrices for basic cell studies as well as promising biomaterials for regenerative medicine applications. A critical advantage of these synthetic matrices over natural networks is that the biophysical and biochemical properties of the material can be tuned with high control and precision. For example, bioactive functionalities, such as cell adhesive sequences and growth factors, can be incorporated in precise densities. We have engineered poly(ethylene glycol) [PEG]-maleimide hydrogels that support improved pancreatic islet engraftment, vascularization and function in diabetic models. Two biomaterial strategies will be discussed. We have developed proteolytically degradable synthetic hydrogels, functionalized with vasculogenic factors, engineered to deliver islet grafts to extrahepatic transplant sites via in situ gelation. These hydrogels induce differences in vascularization and innate immune responses among subcutaneous, small bowel mesentery, and epididymal fat pad transplant sites with improved vascularization and reduced inflammation at the epididymal fat pad site. This biomaterial-based strategy improves the survival, engraftment, and function of a single pancreatic donor islet mass graft compared to the current clinical intraportal delivery technique. In a second application, we have developed a localized immunomodulation strategy using hydrogels presenting an apoptotic form of Fas ligand (SA-FasL) that results in prolonged survival of allogeneic islet grafts in diabetic mice. A short course of rapamycin treatment boosts the immunomodulatory efficacy of SA-FasL-hydrogels, resulting in acceptance and function of allografts over 200 days. Survivors generate normal systemic responses to donor antigens, implying immune privilege of the graft, and have increased T-regulatory cells in the graft. Current studies focus on evaluating this immunomodulatory strategy in a large animal model of type 1 diabetes. This localized immunomodulatory biomaterial-enabled approach may provide an alternative to chronic immunosuppression for clinical islet transplantation.
ESB-SFB S-KL02

Taking control of innate immunity with nanobiologic therapeutics

**Willem J.M. Mulder**¹,²

¹ Radboud University Nijmegen Medical Center, Department of Internal Medicine, Radboud Institute of Molecular Life Sciences (RIMLS) and Radboud Center for Infectious Diseases (RCI), Nijmegen, NL; ² Eindhoven University of Technology, Laboratory of Chemical Biology, Department of Biomedical Engineering and Institute for Complex Molecular Systems, Eindhoven, NL

Immunotherapy is revolutionizing the treatment of diseases. Most immunotherapy strategies engage the adaptive immune system. In recent years, emerging evidence has shown that the innate immune reprogramming induces long-term changes through metabolic and epigenetic programming of myeloid cells, including monocytes, neutrophils and macrophages. This de facto innate immune memory is also referred to as trained immunity. Therefore, targeting myeloid cells and their progenitors is a powerful ‘trained immunity-regulating framework’ to govern the delicate balance of immune homeostasis, priming/training and tolerance. This Presentation will showcase how nanobiologic-based immunotherapies can be applied to achieve long-term therapeutic benefits in detrimental immune-driven diseases, including cancer, cardiovascular and infectious diseases, as well as to prevent organ rejection after transplantation.
Immunomodulatory IL-23R antagonist peptide coatings to prevent infection of percutaneous devices

John A. Pizarek¹², Nicholas G. Fischer¹, Conrado Aparicio¹

¹ University of Minnesota, Minnesota Dental Research Center for Biomaterials and Biomechanics, Minneapolis, US; ² Naval Medical Leader and Professional Development Command, Lieutenant Commander, United States Navy Dental Corps, Bethesda, US

Introduction
Percutaneous devices are some of the most common medical devices in use, yet device infection remains ubiquitous. Left untreated, infection may lead to device failure, costly additional surgical interventions, and possible mortality. The concomitant exploding use of antibiotics has led to rising antimicrobial resistance and formation of dysbiotic biofilms. Thus, percutaneous device infection remains a foreboding healthcare burden in critical need of alternative solutions [1].

Bacterial invasion of peri-implant surface leads to a keratinocyte innate inflammatory response. This results in production of pro-inflammatory cytokines, secretory immune molecules, that modulate an immune response by recruitment, activation and proliferation of specialized immune cells, such as macrophages. Specifically, the IL-17/IL-23 cytokine inflammatory axis is highly active in infected percutaneous dental implants compared to healthy implant sites [2]. Peptide nanocoatings offer highly tailorable presentation of immunomodulatory motifs to regulate such inflammatory axes [3]. We evaluated the keratinocyte response to an IL-23 receptor antagonist (IL-23Ra) peptide [4] coating for proliferation, production of IL-17/23, and cytokine secretome regulation. Conditioned media was collected to analyze macrophage polarization to M1 vs. M2 macrophages. Regulating the IL-17/23 pathway may reduce the induction and recruitment of aforementioned inflammatory cells and promote integrity of the soft tissue interface of percutaneous devices at sites of bacterial infection [5].

Experimental Methods
Model percutaneous device titanium surfaces were covalently functionalized with IL-23Ra. Randomized sequence IL-23Ra peptides (Random) and non-coated nanorough titanium disks (etched titanium; eTi) served as controls. Coatings were physicochemically analyzed by X-ray photoelectron spectroscopy (XPS), water contact angle, and peptide fluorescent labeling and visualization. Peptide surface density was quantified using a NanoOrange peptide quantification kit.

Keratinocyte proliferation was measured colorimetrically and with phallolidin/DAPI staining. Keratinocyte-conditioned media and cell lysates were collected and analyzed using a 36-target cytokine array for functional protein association network analysis (STRING). ELISAs were used to analyze levels of IL-23 and IL-17 in the presence and absence of pro-inflammatory Porphyromonas gingivalis lipopolysaccharide (LPS).

Human monocytes were isolated and cultured with keratinocyte-conditioned media at different ratios in macrophage differentiation media. Control monocytes were differentiated to M1 and M2 macrophages as controls. Macrophages were analyzed by immunofluorescence for CD68 and iNOS (M1 markers) and CD163 and CD206 (M2 markers). ANOVA and Tukey post-hoc tests assessed statistical significance (p<.05).

Results and Discussion
Physicochemical analysis demonstrated the successful immobilization of the peptide nanocoatings on titanium (XPS results in Figure 1a).
The IL-23Ra-coated titanium significantly increased proliferation of the keratinocytes (phalloidin/DAPI immunofluorescence at 1 day in Figure 1b) and significantly decreased IL-17 and IL-23 levels, both without (IL-17A results in Figure 1c) and with LPS stimulation (IL-17A results in Figure 1d), compared to controls. STRING analysis revealed a significant shift toward pro-reparative cytokine production on IL-23Ra peptide coated disks compared to controls.

Keratinocyte-conditioned media collected from IL-23Ra polarized macrophages toward M2 more than controls.

**Conclusion**

Our results supported the use of IL-23Ra-coatings to reduce the pro-inflammatory IL17/23 pathway, along with a reduction of keratinocyte pro-inflammatory cytokines production and polarization of macrophages toward M2. Immunomodulatory peptide nanocoatings may promote soft tissue healing around percutaneous devices and thereby reduce the burden of care of percutaneous devices.

**Acknowledgement**

This work was supported/funded by NIH/NIDCR R01DE026117 (CA), F30DE029105 (NGF), and a 3M Science and Technology Fellowship (NGF). JAP is a military Service member [or employee of the U.S. Government]. This work was prepared as part of JAP official duties. Title 17, U.S.C., §105 provides that copyright protection under this title is not available for any work of the U.S. Government. Title 17, U.S.C., §101 defines a U.S. Government work as a work prepared by a military Service member or employee of the U.S. Government as part of that person's official duties. The views expressed in this review are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government.

**Figure 1:** Successful synthesis and biological activity of IL-23Ra peptide nanocoatings.

- a) XPS full-spectra for control etched titanium (eTi), randomized peptide (Random), and IL-23Ra (Antagonist).
- b) Immunofluorescent microscopy of keratinocytes cultured on IL-23Ra at 1 day (phalloidin is blue, DAPI is grey, and the scale bar is 100 μm).
- c) IL-17A production by keratinocytes cultured on eTi, Randomized, and IL-23Ra.
- d) IL-23 production by keratinocytes cultured on eTi, Randomized, and IL-23Ra under LPS stimulation.

**References**


Page 811 of 2028

Human Cell-Assembled extracellular Matrix (CAM) does not trigger the innate immune system and shows long-term in vivo stability

Laure Magnan1, Fabien Kawecki1, Gaëlle Labrunie1, Julien Izotte2, Sébastien Marais3, Marie-Pierre Foulc4, Michaël Lafourcade4, Nicolas L’Heureux1

1 University of Bordeaux / Inserm, BioTis - Laboratory for the Bioengineering of Tissues, Bordeaux, FR; 2 University of Bordeaux, Animal facility A2, Bordeaux, FR; 3 University of Bordeaux / CNRS / Inserm, Bordeaux Imaging Center (UMS 3420 / US4), Bordeaux, FR; 4 Société de Recherche Rescoll, Bordeaux, FR

Introduction

Cell-assembled extracellular matrix (CAM) has been used to produce vascular grafts without exogenous materials.1-2 These grafts were produced by rolling sheets of CAM that were fused in a bioreactor over many months of culture. We have now developed a textile-inspired approach that uses yarn of CAM that are assembled in vascular grafts by weaving.3 This approach avoids the need for fusion/bioreactors, offers directional and local control over mechanical properties, is automatable, and allows the production of graft 3X faster. While CAM-based grafts performed well in clinical trials, the in vivo remodeling and inflammatory response of this truly “bio” material has not yet been investigated. In this study, human CAM yarns were implanted subcutaneously in nude rats to investigate the innate immune response to this extracellular matrix processed into yarn.

Experimental Methods

Normal human skin fibroblasts were isolated from surgical waste (abdominoplasty). The epithelium was removed (500 mg/ml thermolysin) and the dermis was digested with collagenase (0.40 U/ml). After centrifugation, fibroblasts were plated into tissue culture flasks (10^4 cells/cm^2) in DMEM:HamF12 (1:1) with 20% fetal bovine serum (FBS – FetalClone III, Hyclone). Cells were passaged 3 times and tested for mycoplasma infection by PCR before storage in liquid nitrogen. To produce sheets (10x18 cm), cells were cultured in media containing 500 µM sodium ascorbate for 8 weeks in a T-225. MATURE sheets were rinsed in distilled water and frozen (-80°C). To produce yarn, sheets were thawed and cut into ribbons (typically 5-mm-wide) using a multi-blade system. In To produce twisted yarn (threads), one extremity of the rehydrated ribbon was attached to the rotating rod of a custom twisting device, while a manipulator pinched the other extremity. The calibrated motor was turned on for a calculated duration to twist the ribbons at the desired number of revolutions per cm of length (here 7.5). Yarn was then air-dried under the hood, spooled, and stored at -80°C. Upon thawing, this material is termed “devitalized”.4 Decellularized materials was obtained by incubating thawed sheets in decellularized (8 mM CHAPS, 1 M NaCl, 25 mM EDTA, 0.12 M NaOH) for 6 hours followed by extensive rinsing. Gamma-sterilization was performed on spooled dry yarn (25 kGy, Steris (Marcoule, France)). Tensile testing (Criterion® 43; MTS; Berlin, Germany) was performed with a 250 N force sensor, pre-stretched at 0.1 N and pulled until rupture at 20 mm/min.

Results and Discussion

The impact of processing steps relevant to yarn manufacturing (devitalization, decellularization, gamma sterilization, and twisting) was evaluated in vitro and in vivo. Both histological and mechanical effects were observed. Histology revealed that decellularization was successful and that gamma sterilization affected the matrix. Mechanically, both gamma sterilization and twisting reduced the force at failure of the yarn by 25% to 30% (p < 0.001 and 0.0001). The ultimate tensile stress (UTS) was significantly increased by decellularization and even more by twisting (p < 0.0001). After subcutaneous implantation in nude rats, all yarns were still present after six months, and were integrated into a non-inflamed loose connective tissue with a CAM tissue repopulated by fibroblastic cells and blood vessels. Two weeks after implantation, yarns caused minor peripheral inflammation except for gamma sterilized yarn that triggered
a more intense host response dominated by M1 macrophages. However, strips of a membrane of processed bovine collagen clinically available (BioMend®; 0107; Zimmer Biomet Dental, Rungis, France) was aggressively degraded showing that the nude rats had a functional innate immune system that recognized and attacked “damaged” ECM. Yarn mechanical strength was decreased two weeks after implantation except for the more compact “twisted” yarn. While the strength of other yarns were stable after initial remodeling, the gamma-sterilized yarn continued to lose mechanical strength over time and was significantly weaker than devitalized (control) yarns at six months.

**Conclusion**

This is the first study to formally demonstrate that devitalized human CAM is very long-lived in vivo and does not trigger a sustained degradative response but, rather, is very slowly remodeled. This data supports a strategy to produce human textiles from CAM yarn for regenerative medicine applications where a scaffold with low inflammation and long-term mechanical properties are critical.

**Acknowledgement**

This work was supported by the French “Ministère de la Recherche et de l’Enseignement Supérieur”; the chair senior of the “Initiative d’Excellence de l’Université de Bordeaux (IdEx Bordeaux)”; the French’s “Agence Nationale de la Recherche (ANR)” (grant number: ANR-16-CE18-0024-01); and the European Research Council (ERC Advanced grant 785908). We thank Sylvie Rey, Robin Siadous, Niki Sarika, Yoann Torres, Agathe Grémare, Lionel Couraud, and the “CIC-IT Biomatériaux et dispositifs médicaux implantables” for their technical supports.

---

**References**


Chitosan/poly(γ-glutamic acid) nanoparticles synergize with IFN-γ to fight breast cancer

Flávia Castro¹², Marta L. Pinto³, Catarina L. Pereira¹², Karine Serre⁴, Mário A. Barbosa¹²⁵, Karim Vermaelen⁶⁷, Fátima Gartner¹⁸, Raquel M. Gonçalves¹²⁵, Olivier de Wever⁷⁹, Maria J. Oliveira¹²

¹ i3S - Institute for Research and Innovation in Health, University of Porto, Porto, PT; ² INEB - Institute of Biomedical Engineering, Porto, PT; ³ CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, PT; ⁴ IMM - Institute of Molecular Medicine João Lobo Antunes, University of Lisbon, Lisbon, PT; ⁵ ICBAS - Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, PT; ⁶ Tumor Immunology Laboratory, Department of Pulmonary Medicine and Immuno-Oncology Network Ghent, Ghent University Hospital, Ghent, BE; ⁷ CRIG - Cancer Research Institute Ghent, Ghent University, Ghent, BE; ⁸ iPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, Porto, PT; ⁹ LECR - Laboratory Experimental Cancer Research, Department of Human Structure and Repair, Ghent University, Ghent, BE

Introduction

Interferon-γ (IFN-γ) has been identified as a key factor for antitumor immunity and response to immunotherapy [1]. However, most of the clinical trials reported a limited potential of IFN-γ as single therapy, highlighting the importance of evaluating its potential when combined with immunotherapies.

We described chitosan (Ch)/poly(γ-glutamic acid) (γ-PGA) nanoparticles (NPs) able to modulate immature and immunosuppressive antigen-presenting cells (APCs) to an immunostimulatory profile, leading to T cell activation and impairing APCs-mediated cancer cell invasion [2]. Further, we recently showed that Ch/γ-PGA NPs synergize with conventional radiotherapy, decreasing breast tumor growth and lung metastasis in an orthotopic breast tumor mice model, by decreasing local and systemic immunosuppression [3]. These immunomodulatory abilities of Ch/γ-PGA NPs point them as appealing adjuvants to IFN-γ-based therapies. We have previously shown that Ch/γ-PGA NPs incorporating IFN-γ were able to induce an immunostimulatory profile on APCs, hampering cancer cell invasion in vitro. This effect was mainly mediated by Ch/γ-PGA NPs, but the incorporation of IFN-γ potentiated the effect observed [4]. Therefore, the current study addressed the potential synergistic effects of Ch/γ-PGA NPs combined with IFN-γ using an immunocompetent 4T1 orthotopic breast tumor mouse model.

Experimental Methods

NPs were prepared by co-acervation method and characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM). 1x10⁶ 4T1-luciferase cells, a mouse breast tumor cell line, were injected orthotopically in the mammary fat pad of BALB/cByJ. Animals were divided in four groups: non-treated (control), treated with Ch/γ-PGA NPs, with IFN-γ or with the combination of both treatments (NPs+IFN-γ). After 7 days, when the tumors reached 94.1±32.1 mm³, animals were subcutaneously injected, adjacent to the tumor, 6 times for 2 weeks with Ch/γ-PGA NPs, IFN-γ or with both (NPs + IFN-γ). Tumor volume was measured using a caliper and calculated as (length × width × width)/2 (mm³) and tumor progression was followed by bioluminescence imaging every week. Animals were sacrificed at day 28. Tumor burden, lung metastasis formation and immune cell profile were explored. Tumor size was normalized, considering the initial size, prior any treatment.

Results and Discussion

The DLS and TEM results indicated that Ch/γ-PGA NPs presented a constant size (206.8 ± 3.2 nm) with a reduced polydispersion index (0.18 ± 0.09), a positive zeta potential (18.4 ± 0.5 mV) and a spherical shape. Regarding in vivo studies, treatments had no significant impact on mice weight nor liver or kidney structure, confirming their safety. While non-treated animals had progressive tumor growth (514.7 ± 61.2 %) and developed lung metastasis, NPs- and IFN-γ-treated animals significantly decreased primary tumor burden (286.5 ± 41.6 % and 262.0 ± 43.4 %,
respectively). Remarkably, when both treatments were combined, breast tumor growth was blocked (107.8 ± 25.5 %). This impairment was associated with a reduction in splenomegaly, a decrease in the percentage of splenic myeloid-derived suppressor cells and an increase in antitumoral CD4+IFN-γ+ population. Notably, animals from the combinatorial treatment (NPs+IFN-γ) presented lower bioluminescence in the lungs than other groups, which indicate lower metastatic burden. Additionally, NPs+IFN-γ-treated animals had lower levels of the systemic pro-tumoral cytokines (IL-3, IL-4, IL-10) in comparison to non-treated animals.

**Conclusion**

Overall, these results suggest that Ch/γ-PGA NPs potentiate and synergize with IFN-γ to reduce tumor progression, opening new perspectives to be used in anticancer strategies.

**Acknowledgement**

This work was financially supported by FEDER – Fundo Europeu de Desenvolvimento Regional funds through the COMPETE 2020 – Operacional Programme for Competitiveness and Internationalisation (POCI), Portugal 2020, by Portuguese funds through FCT – Fundação para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e do Ensino Superior in the framework of the project “MAGICIAM-a MAcrophaGe Immunomodulatory-delivery system to prevent Cancer Invasion And Metastasis” (PTDC/BTM-SAL/31859/2017) and by Fund for Scientific Research Flanders (FWO-Vlaanderen).

**References**


3:45 p.m. – 5:15 p.m.

Track06

**N13 | Advances in Additive Manufacturing and Biofabrication**

**Chairs**

*Cecilia Persson*
Uppsala University, Uppsala, SE

*Marcy Zenobi-Wong*
ETH Zürich, CH

*Manuel Delgado Caceres (YSF)*
University Regensburg Medical Centre, Experimental Trauma Surgery, Department of Trauma Surgery, Regensburg, DE
Application of Image Analytics to Assess the Degradation Rate of 3D Bioprinted Scaffolds

Ghayadah Alkharusi\textsuperscript{1,2}, Nicholas Dunne\textsuperscript{1,2}, Suzanne Little\textsuperscript{3}, Tanya Levingstone\textsuperscript{1,2}

\textsuperscript{1} Dublin City University, School of Mechanical & Manufacturing Engineering, Whitehall, IE; \textsuperscript{2} Dublin City University, Centre for Medical Engineering Research, Whitehall, IE; \textsuperscript{3} Dublin City University, School of Computing, Whitehall, IE; \textsuperscript{4} Dublin City University, School of Mechanical & Manufacturing Engineering, Whitehall, IE

Introduction

The new revolution of 3D printing technologies has led to the development of bioprinting processes capable of fabricating scaffolds for regenerative medicine applications. This technology integrates living cells and biomaterials within a precisely controlled system to create complex structures that cannot be achieved using existing scaffold fabrication processes. The ultimate goal of bioprinting is to be able to produce tissues and organs in the laboratory for use in surgical therapies and transplants [1]. The application of Artificial Intelligence (AI) methodologies for the optimisation of the bioprinting process has the potential to bring about rapid advancements towards the achievement of this goal. Within this study, a computer vision method will be developed to explore scaffold degradation rate. This approach will be compared to standard scaffold degradation quantification methods i.e. calculation of changes in scaffold weight and dimensions. These methods will be applied to assess the influence of tannic acid (TA) concentration and crosslinking time on the degradation rate of bioprinted collagen scaffolds.

Experimental Methods

Collagen bioinks were prepared by combining Porcine tendon type I collagen (SYMATESE, France) with 0.5 M acetic acid (Merck, Ireland) at concentrations of 4\% and 6\% (w/v). The collagen solution was homogenised for 30 min at 4\(^\circ\)C. Collagen scaffolds were then printed using the Inkredible\textsuperscript{+} bioprinter (Cellink, Sweden) with a 22G cannulated needle. The scaffolds were crosslinked with tannic acid (TA, Merck, Ireland) at concentrations of 4\% and 6\% (w/v) for 15 or 37.5 min at 37\(^\circ\)C. Scaffolds (dimensions of 10\(\times\)10\(\times\)3 mm, infill density of 15\%) were printed using the following parameters: printhead and printing stage temperature -20\(^\circ\)C, air pressure -235\(\pm\)10 kPa and printhead speed - 10mm/s.

Degradation rate of the scaffolds was studied by immersing them into 3 mL PBS solution (pH 7 at 37\(^\circ\)C) for up to 14 days. The degradation rate was determined by quantifying changing in scaffold weight (D_Weights) and dimensions (D_Dime) daily. In addition, images of the scaffolds were taken daily using a microscope camera and scaffold degradation was assessed by segmenting the object and calculating the change in area (D_Comp). Fig.1 illustrates the methodology used to capture the images and analyse the scaffold degradation rate. The code used image segmentation to determine the scaffold area in the image (pixels) and convert it to SI units (m\(^2\)). The area degradation rate was computed by calculating a daily percentage change in scaffold area. The relative error between the D_Weights, D_Dime and D_Comp methods was then determined.

Results and Discussion

Fig.2(A-D) demonstrates the average degradation rate of the scaffolds (n=4) measured using the three different methods for the different bioink formulations. The results show that degradation can be observed as a uniform change in the scaffold dimensions. In terms of average rate of change in degradation, similar levels were recorded for the experimental and computational methods (D_Weights=6.6\%, D_Dime=7.3\% and D_Comp=6.8\%). This shows that there is a similarity between these methods in terms of measuring the degradation rate. Calculation of the relative error for each group showed a small difference between D_Dime and D_Comp results (7.4\% for Group 1). However, there was a greater difference in relative error between D_Weights and D_Comp.
results (22.7% for Group 1). This is due to the lack of information recorded for changes in scaffold internal architecture using the D_Dime method. This indicates there is higher precision when using the D_Comp method to measure scaffold degradation.

These results show that the use of computer vision provides a fast and efficient method for quantifying the degradation rate of bioprinted scaffolds, while removing the need to handle the scaffolds required for other methods for assessing degradation. Furthermore, the results show that increasing the crosslinking time from 15 to 37.5 min results in a reduction in the degradation rate of collagen scaffolds.

**Conclusion**

Image segmentation has been successfully applied to measure the degradation rate of 3D printed scaffolds and demonstrates that it is a potentially reliable method to use in determining the degradation rate of bioprinted scaffold. However, it is clear from the results, that internal feature changes of a scaffold, such as pore size, were difficult to obtain due to limitations with the 2D imaging technique. The study shows that increasing the crosslinking time reduces the degradation rate of collagen scaffolds. Further work will explore the ability of a more complex computer vision model to relate external changes in the shape to internal degradation. Specifically, the next stage is to develop the study to use machine learning methods to derive computer vision models that can convert the observed values into predicted weights.

**Acknowledgement**

This work was conducted with the financial support of Science Foundation Ireland (SFI) Centre for Research Training in Artificial Intelligence Grant No. 18/CRT/6223, EU funded H2020 project GIOTTO Grant No. 814410 and Insight SFI Centre for Data Analytics Grant No. SFI/12/RC/2289_P2, co-funded by the European Regional Development Fund.

**References**

Biocompatible DLP resins based on poly(ester/carbonate) urethane acrylates

Rong Wang, Tobias Kuhnt, Ramiro Marroquin-Garcia, Agustina Aldana, Lorenzo Moroni, Matthew Baker

Maastricht University, MERLN Institute; Complex Tissue Regeneration Department, Maastricht, NL

Introduction
Among the additive manufacturing techniques, light-based fabrication like Digital Light Processing (DLP) results in high-resolution and fast printing times. DLP has been recently explored in regenerative medicine owing to high accuracy to print complex scaffolds like vascular branches [1, 2]. However, the development of customizable biodegradable and biocompatible resins remains a bottleneck. Scalable resins with proven biocompatibility and biodegradability are necessary in order to further develop custom tissue engineered implants. Of particular concern, there exist few resin platforms with tailorable cell adhesion, tailorable degradation rates, or with intermediate and elastomeric mechanical properties. We recently reported a series of customizable resins based on poly(caprolactone-co-trimethylene carbonate) (PCT) and poly(caprolactone-co-lactide) (PEU), end-capped with lysine isocyanate and acrylate to address these aims [3,4].

Experimental Methods
PCT and PEU copolymers were synthesized and end-capped using acrylated l-lysine isocyanate (LDI-HEA) as previously reported [3,4]. In the PCT series, the copolymers compositions were varied from 80:20, 75:25 and 70:30 caprolactone (CL) to trimethylene carbonate (TMC) at a constant molecular weight (4k g/mol); in the PEU series, the copolymer molecular weights were varied (1k–10k g/mol) at a constant composition 75/25. All copolymers were characterized by nuclear magnetic resonance (1H-NMR and 13C-NMR), gel permeation chromatography (GPC) and differential scanning calorimetry (DSC). For DLP printing, resins were prepared by adding 2-(2-ethoxyethoxy)ethyl acrylate (EOEOEA) (30 wt%) and TPO (1 wt%). Mechanical properties were determined via a combination of rheology and tensile testing. Cell viability and morphology was determined using human dermal fibroblast (HDF) adhesion assays and the PCT copolymers were tested in vivo in a pig model for subcutaneous inflammation.

Results and Discussion
The copolymer composition, degree of polymerization and molecular weight were determined by 1H-NMR and GPC. All co-polymers showed good dispersities (D) in the range of 1.1 to 1.6, and reflected both the targeted composition and molecular weight. The polymers were able to be formulated into resins with their performance as a resin dictated by the melting temperature of the polymer. In addition, we were able to utilizing a heating bath to overcome limitations of printing at room temperature. The scaffolds all showed mechanical properties in the intermediate regime (MPa). The 3D printed scaffolds showed variation in HDF adhesion as a function of the molecular weight and polymer composition. The PCT scaffolds showed a lack of significant inflammatory response in a short-time-frame subcutaneous implantation in a pig model, and the polymers showed controllable degradation based on polymer composition.

Conclusion
The synthesis of the PCT and PEU copolymers has allowed us to explore these DLP polymerizable resins with controllable properties and cell adhesion. Currently, we are exploring adding in stimuli responsive elements and controllable bioactivity into the scaffolds. This platform provides a tailorable starting point for future studies in the translation of biodegradable and tailor-made scaffolds for tissue regeneration. The cytocompatibility and
biodegradability, as it was reported, together the current results lend these polymers well to further in vitro and in vivo testing of DLP printed scaffold for soft and medium-soft tissue regeneration.

Acknowledgement

Portions of this research have been made possible with via support of NWO (Innovation Fund Chemistry, project “DynAM” under project agreement 731.016.202), and the Dutch Ministry of Economic Affairs, the Province of Limburg, and the Brightlands Materials Center.
N13-04

An Ideal Photoresin for Fast Volumetric Bioprinting Based on Thiol-Norbornene Photoclick Crosslinking

Riccardo Rizzo, Dominic Ruetsche, Hao Liu, Marcy Zenobi-Wong

ETH, Department of Health Sciences and Technology, Zurich, CH

Introduction

In last two decades, light-mediated biofabrication techniques have increasingly gained interest thanks to the development of novel photochemical strategies and hardware solutions. Recently, volumetric printing (VP) (also known as volumetric additive manufacturing or computed axial lithography) has emerged as a powerful tool to overcome major limitations of printing methods based on layer-by-layer processes (i.e. digital light processing (DLP), stereolithography (SL), extrusion). This light-based technique enables the generation of low-defects centimeter-sized complex 3D objects within seconds, thus significantly reducing the printing time and light exposure. To date, common (meth)acrylate photoresins (i.e. Gel-MA) represent the standard materials for VP. In this work we introduce an optimized photoresin based on gelatin-norbornene (Gel-NB) for VP and volumetric bioprinting (VBP) as well as an efficient and scalable method to produce such material which overcome the current limitations resulting from the use of chain-growth based photoresins.

Experimental Methods

Gel-NB was synthesized in a pH-controlled alkaline buffer with the sequential addition of carbic anhydride (CA). Various Gel:CA w/w were used in order to target different degree of substitution (DS), which were estimated by $^1$H-NMR in the presence of an internal standard (DSS). Gel-NB synthesis scalability was assessed for batches of 2, 10 and 50 g. Gel-NB based photoresins were prepared by mixing with thiolated crosslinkers and 0.05% LAP photoinitiator. Oscillatory photorheological analysis was performed to assess the influence on rheological properties of DS, polymer content, thiol:ene ratio and type of thiolated crosslinker. Printing was performed with a commercially available volumetric printer (Readily3D). Muscle cells (C2C12) and fibroblast (NHDF) were used for VBP. Cell viability was assessed with Live/Dead staining, while immunofluorescence of sarcomere myosin heavy chain was performed to study tissue maturation of bioprinted skeletal muscle constructs.

Results and Discussion

The use of highly reactive thiol-ene step-growth chemistry poses several benefits over the chain-growth polymerization mechanism, starting from the more rapid crosslinking kinetics which resulted in the fastest VP reported to date (~10 s). On the other hand, highly efficient thiol-norbornene crosslinking enables the use of lower polymer concentration, which is crucial to generate a more cell-permissive environment and of lower polymer degree of substitution (DS), which is beneficial for the retention of native polymer bioactive and biophysical properties. The use of Gel-NB in combination with 4-arm-PEG-thiol (PEG4SH) and other thiolated linkers have also shown remarkable tunability over rheological properties, with photocrosslinked hydrogel stiffness varying from ~40 Pa to 15 kPa. In addition, we developed a simple, fast and accessible to non-expert synthesis method to produce Gel-NB with notable scalability (2-50 g) and broad, tailorable degree of substitution (3-50%). We demonstrated the high versatility of Gel-NB/PEG4SH photoresin formulation to print various complex 3D objects with high throughput by using Gel-NB concentration down to 2.5% (Figure 1a-d). Finally, we demonstrated the potential of Gel-NB based resins for VBP (Figure 1e-g). Thanks to the soft matrix obtained with low polymer concentration and the fact that the extremely efficient thiol-norbornene crosslinking drastically reduces light-exposure and radical formation compared to chain-growth systems, we obtained excellent cell viability (~100%) upon printing. In addition, we showed VBP of cell-laden perfusable models as tissue-on-a-chip proof of concept. Free-form complex skeletal muscle models were also...
bioprinted showing cell proliferation, spreading and differentiation towards contractile myotubes on the soft gelatin-based matrix.

**Conclusion**

In this work, we presented a simple, inexpensive, and scalable method to produce Gel-NB which can contribute to a widespread use of such efficient material for light-based bioprinting techniques. In particular, here we demonstrated that Gel-NB based photoresins exhibit ideal biophysical and photochemical properties for VBP. With potential application ranging from tissue engineering and regenerative medicine to soft robotics, the use thiol-norbornene photo-click crosslinking hold great promise for VBP future advances.

**Acknowledgement**

Dominic Ruetsche and Hao Liu contributed equally to this work. MZW acknowledges ETH Grant application ETH-3819-1 for their support. The authors further acknowledge the assistance from ETH (ScopeM) imaging facility.

---

**References**


N13-05

Micro-extrusion induced orientation of collagen fibrils embedded in hyaluronan and bioinks chondrogenic property

Matteo D'Este¹, Andrea Schwab¹, David O. Eglin¹², Flurina Staubli¹

¹ AO Research Institute Davos, Regenerative Orthopedics, Davos Platz, CH; ² Ecoles des Mines Saint Etienne, IMT, Campus Santé Innovations, Saint-Priest en Jarez, FR

Introduction

3D Bioprinting is providing the possibility to produce engineered tissues based on 3D computer models with desired shapes and with gradients in composition and cell type. This is mostly achieved at macroscopic level and up to the ~10⁻¹ mm scale. However, methods to control microscopic architecture with extrusion-based printing are seldomly reported. Still, tissues physical and biological properties critically depend to this architecture. In this study, we introduce a technique to control distribution and orientation of type 1 collagen (col) in fibrillar form embedded within a hyaluronan (HA) bioink matrix via extrusion-based 3D printing. Cell-free and cell laden constructs were prepared and characterized, analyzing the influence of this controlled microscopic anisotropy on cell behavior and chondrogenic differentiation.

Experimental Methods

Tyramine modified HA (THA) was combined with acidic col at two polymer ratios (THA:col at 12.5:2.5 mg/ml and 16.7:1.7 mg/ml). THA was enzymatically crosslinked with horseradish peroxidase and 18 to 24 ppm hydrogen peroxide [1] and col fibrillogenesis was induced via pH shift to neutrality at 37°C. shear thinning and viscoelastic properties of THA, col and composites were characterized via rheometry. THA-col was extruded with a RegenHU 3D Discovery™ controlling col fibrils alignment. Col orientation was visualized via Second Harmonic Generation (SHG) and immunostaining/confocal microscopy. Images were processed with image J (NIH) to quantify fibrils orientation. Human bone marrow derived mesenchymal stromal cell (hMSC) aggregates were embedded within the bioink to evaluate cell migration and orientation, assessed by F-actin and DAPI staining (Fig 1 C). In vitro chondrogenic behavior of hMSC embedded in casted THA-col (chondrogenic media containing 10 ng/ml Transforming Growth Factor β1) was evaluated via histology, gene expression (COL1A1, COL2A1, COL10A1, RunX2, SOX9, Aggrecan ACAN) and proteoglycan quantification. Chondrogenic differentiation was compared to hMSC pellet as a gold standard.

Results and Discussion

A workflow starting from liquid precursors with simultaneous col fibrillation and HA crosslinking was developed to achieve uniform distribution of col fibrils within the HA-based viscoelastic matrix [2]. Shear-induced fibrils alignment along the printing direction was shown by immunohistochemistry, immunofluorescence and SHG (Figure 1 A, B). As expected, alignment increased for reducing nozzle diameter (Fig 1 C, D). The shear thinning of THA was preserved in the THA-col composite at the investigated mixing ratios. THA-col showed 2-fold increase in storage modulus compared to THA. Cell migration was stimulated by the presence of col. Actin filament staining showed cytoskeleton alignment along the fibrils orientation, determined by the printing direction (Fig 1C). Production of proteoglycan rich extracellular matrix during hMSC chondrogenesis was observed after 21 days in vitro culture of hMSC embedded in THA-col (figure 2B). Chondrogenic associated genes COL2A1 (>100-fold), ACAN (>100-fold) and SOX9 were upregulated in all samples 21 days of culture (Fig 2). Compared to hMSC pellets, hypertrophic markers COL1A1, COL10A1 and RunX2 were less upregulated in the THA-col hydrogels with higher ratio of SOX9/RunX2 in both THA-col samples (~10-fold) compared to no upregulation in the pellet group (~1-fold).
The field of biofabrication has achieved significant advances in controlling constructs shape and composition. However, mechanical and biological properties of tissues depend also on the specific spatial arrangement of fibrillar components, structural molecules and biological factors. Introducing control over the microscopic architecture of these components is key to recapitulate complex tissue structure and morphology.

**Conclusion**

In this work, we have introduced a method to obtain THA-col composite with macroscopic homogeneity and microscopic heterogeneity mimicking the matrix architecture of animal tissues. Ongoing studies are assessing the effect of the fibrils orientation on chondrogenesis and new matrix deposition. The possibility of printing matrix components with control over microscopic alignment brings biofabrication one step closer to capturing the complexity in tissues.

**Acknowledgement**

This work is part of the osteochondral defect collaborative research program supported by the AO foundation. The Graubünden Innovationsstiftung is acknowledged for its financial support.
hMSC spheroids chondrogenesis in the casted bioink.
Gene expression of hMSC spheroids (5 Mio cells/ml) embedded in THA-Col at different polymer ratios. The results are presented on day 7 and 21 relative to day 1 of the same condition. N = 2 biological replicates, 2 technical replicates each donor. * = p <0.05; ** = p < 0.001; *** = p < 0.001 (relative to day 1 of same condition).

References
N13-06

Composite biomaterial-ink based on hyaluronan and nano hydroxyapatite for biofabrication of bone graft substitutes delivering chemically modified RNAs

Daphne van der Heide¹,², Elena Della Bella¹, Huipin Yuan³, Florence De Groot-Barrère³, Martin J. Stoddart¹, Matteo D’Este¹

¹ AO Research Institute Davos, Davos, CH; ² Institute for Biomechanics, Department of Health Sciences and Technology, ETH Zürich, Zürich, CH; ³ Kuros Biosciences Bv, Bilthoven, NL

Introduction

Normally, most bone injuries heal without complications, however, there are an increasing number of cases where bone lesions result in delayed healing or non-union. The gold standard treatment, autografting, has severe drawbacks, due to donor site morbidity and limited availability [1]. Furthermore, bone graft substitutes containing growth factors have been associated with dose-related safety concerns. Additionally, the current clinically available bone graft substitutes lack control of spatial architecture to anatomically match defect sites after complex fractures. Therefore, the aim in this study was to engineer a biomaterial-ink as a bone regenerative therapeutic approach combining osteoinductive calcium phosphate and matrix biopolymers for delivery of chemically modified RNAs (cmRNAs) inducing nerve, vessel and bone formation using 3D-printing (Figure 1). To this end, a biomaterial ink based on hyaluronan derivatized with tyramine (HA-Tyr), combined with a range of nano hydroxyapatite (nanoHA) particles, was developed.

Experimental Methods

NanoHA was produced by Kuros Biosciences Bv and characterized via SEM and TEM imaging [2]. The optimized biomaterial-ink consisted of 3.5% w/v HA-Tyr (degree of functionalization = 6%, prepared according to a previous described method [3]) with 0.1 U/mL horseradish peroxidase (HRP), 0.17 mM (5.78 ppm) H₂O₂, and 0.02% w/v Eosin Y. Rheological measurements were performed, starting from the biomaterial-ink pre-polymer solution in the absence of nanoHA to avoid measurement artefacts. All tests were carried out with a cone-plate geometry of 25 mm diameter, gap of 0.049 mm, at 20°C. The gelation time (n = 4) was measured triggering the enzymatic crosslinking with H₂O₂, and continuously monitoring the storage (G’) and loss modulus (G’’). After 30 minutes, the same samples were illuminated for additional 30 minutes from the bottom by means of a light source with a wavelength of 505 nm. The oscillatory strain sweep (n = 3) was performed between 0.001% and 100% strain, with frequency 1 Hz. The biomaterial ink was then combined with nanoHA. A parametric study was carried out to characterize the biomaterial ink’s printability and cohesion for increasing nanoHA content. Biomaterial-inks with nanoHA content between 0 and 30% w/v were tested for swelling (n = 4). Each sample was casted in cylindrical shape with a ø 8 mm and a thickness of 3 mm, incubated in PBS at 37°C to allow swelling and then weighed after 0, 4, 6, 24, 48, 72 and 168 hours. The swelling ratio was calculated using the following formula: swelling ratio = ((weight after swelling – weight at 0 hours) / weight at 0 hours) x 100%. The range of compositions identified will be further assessed for cytotoxicity and in vitro osteogenesis (gene expression, protein production and mineralization) using human Mesenchymal Stem Cells (hMSCs).

Results and Discussion

HA-Tyr biomaterial-ink has two independent crosslinking mechanisms. The enzymatic crosslinking was used for obtaining a soft extrudable gel with a 2.5-fold increase in G’ compared to the prepolymer (Figure 2A). The photocrosslinking achieved a 11.4-fold increase in G’ and was used for final shape fixation. The damping factor (tan δ) was 0.58 at 1% strain, indicating good printability (Figure 2B) [4]. SEM and TEM showed that nanoHA particles have a...
needle-shaped morphology and a size of 10-50 x 50-400 nm. Extrusion of HA-Tyr with a range of nanoHA particles, resulted in the formation of a continuous strut, with good shape retention and without waviness up to 30% w/v nanoHA. Swelling was dependent on nanoHA concentration, with highest swelling ratio of the formulation containing 10% w/v nanoHA (Figure 2C). The extruded filaments stayed intact and with no nanoHA leaching over a time period of 3 weeks, indicating that visible light crosslinking occurs also in presence of non-transparent nanoHA (Figure 2D).

**Conclusion**

With this study we have identified a 3D-printable composite HA-Tyr/nanoHA biomaterial-ink suitable for combination with cmRNAs that has significant potential for biofabrication of bone graft substitutes.

**Acknowledgement**

This work was created in the frame of the project cmRNABone that has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 874790.

### References


Coaxial bioprinting as a strategy to apply differentiation factors in a spatially defined manner inside osteochondral tissue substitutes

David Kilian¹, Silvia Cometta¹, Anne Bernhardt¹, Rania Taymour¹, Julia Emmermacher¹², Tilman Ahlfeld¹, Michael Gelinsky¹, Anja Lode¹

¹ TU Dresden, Centre for Translational Bone, Joint and Soft Tissue Research, Faculty of Medicine, Dresden, DE; ² TU Dresden, Institute of Natural Materials Technology, Faculty of Mechanical Engineering, Dresden, DE

Introduction
A challenge for biofabrication of osteochondral tissue substitutes is to spatially define the differentiation pattern of encapsulated stem cells or pre-differentiated cell types towards osteogenic/chondrogenic lineage in one construct. We developed a system based on coaxial (core-shell) bioprinting to combine a bioink carrying human chondrocytes (hCC) or pre-osteoblasts (hOB) with a biomaterial ink and specific factors in defined compartments of one strand. This allowed independent and spatial adjustment of ink compositions for cell support (shell) and release kinetics of bioactive molecules.

Material selection was based on the potential of an alginate-methylcellulose blend for in vitro chondrogenesis in mineralized tissue substitutes [1] and a human plasma-supported bioink for in vitro osteogenesis [2]. The release kinetics of factors were controlled via Laponite, disc-shaped nanoclay particles with a high binding affinity for various molecules applied in skeletal bioprinting applications before [3].

Experimental Methods
3D plotting was performed using the multichannel-3D bioprinter BioScaffolder 3.1 (GeSiM mbH, Germany) equipped with a module for coaxial printing (Fig.1A). Inks applied for bioprinting were based on 3 % alginate with 9 % methylcellulose (3-9-0) [4], and 3 % alginate with 3 % methylcellulose (3-3-X) with varying Laponite content (X=0-12%).

In preliminary experiments a model cell line of human mesenchymal stem cells (hTERT-MSC) was applied. Cell viability in non-coaxial, monophasic scaffolds of varying Laponite content and in core-shell scaffolds with Laponite-supported inks in a cell-free core, and cells embedded in a 3-9-0-shell was compared after Calcein staining of DiD-prelabelled cells via confocal fluorescence microscopy. Indirect cytotoxicity of monophasic Laponite scaffolds was studied with hTERT-MSC cultivated in 2D. Rheological data (shear stress, mass flow etc.) were used to analytically determine a model for flow parameters inside the printing nozzle for the applied bioinks [5].

For differentiation and co-cultivation experiments in core-shell scaffolds, primary hCC and hOB were extracted from human femoral head after informed consent of patients undergoing hip replacement surgery. Cells were applied in the shell material (3-9-0 for hCC, 3-9-0+plasma for hOB) while the respective factors (TGF-β3, BMP-2) were delivered via the core of coaxial strands (Fig.1B). Specific medium compositions for hOB and hCC differentiation in monoculture, as well as for bi-zonal hCC-hOB co-culture (Fig.1C) were defined. Viability of the cells was studied over 3 weeks of differentiation in mono- and co-cultures. In monocultures, chondrogenesis and osteogenesis were examined on mRNA expression levels of respective markers and on biochemical level investigating the secretion of collagen type II and sulphated glycosaminoglycans, and ALP respectively.

Results and Discussion
The developed inks allowed the control over release kinetics in a Laponite-dependent pattern, demonstrated in cell-free, monophasic conditions for albumin, lysozyme, dexamethasone and BMP-2. However, a lower cell viability of encapsulated hTERT-MSC in monophasic scaffolds with increasing Laponite concentration was found compared to Laponite-free bioinks while this drop was prevented by applying the cells in a 3-9-0 shell compartment surrounding...
the Laponite pastes in the core. No intrinsic cytotoxicity of scaffolds with Laponite concentrations of 0-9 % was identified. Modeling and evaluating flow parameters during monophasic extrusion through a 410 µm-needle with higher Laponite content revealed that the shear stress was not increased in these conditions. Since high viscosity led to a lower mass flow in concentrations above 3% (3-3-6; 3-3-9), the analytically determined maximum flow and shear stress were even lower in these inks compared to 3-3-0 and 3-3-3.

During differentiation of hCC in core-shell scaffolds, the majority of the cells survived the fabrication process and treatment, which led to increased mRNA levels of collagen type II in response to TGF-β3 supplied via core ink 3-3-3, while for 3-3-3 only and for 3-9-0 with TGF-β3, no effect was observed. Investigating the osteogenesis of hOB in the shell of monoculture scaffolds showed that the cells reacted to BMP-2 in the 3-3-3 core reservoir by ALP production. In co-culture conditions, both cell types survived with a viability rate that was not affected compared to the monoculture conditions (mean viability at day 7, hOB: 70 %, hCC: 60 %, n>3) while by day 21, the hOB had formed a cellular network in the respective zone and at the zonal interface (Fig.1D).

**Conclusion**

Coaxially separating the compartments for cells and factors allowed the free adjustment and balancing of respective ink requirements – a concept that can be easily transferred to other co-cultivation systems. This study provides a basis for fabricating and understanding 3D bioprinted, multi-cellular tissue substitutes and for co-cultivation of hCC and hOB.

**Acknowledgement**

The authors would like to thank the European Social Fund ESF and the Free State of Saxony for the financial support in the course of the young researchers group *IndivImp*.

**Figure 1: Coaxial bioprinting of bi-zonal osteochondral co-culture scaffolds**

- a) Coaxial printing module allowing the extrusion of a bioink in the shell (left) and a factor-laden core (right, stained in blue for demonstration purposes) by GeSiM BioScaffolder 3.1.
- b) Concept for coaxial Factor supply in vertically stacked zones in a scaffold laden with hCC and hOB.
- c) Horizontally combined bi-zonal, 3D plotted core/shell scaffold.
- d) Viability of DII-labelled hOB and DiD-labelled hCC at the interface of a biphasic core-shell scaffold after 21 days of co-culture, total z-stack thickness 60 µm, scale bar = 500 µm

**References**

[5] Emmermacher et al., Biofabrication 2020
Influence of Hot Isostatic Pressing on the corrosion resistance of Mg-4wt%Y-3wt%Nd processed by Laser - Powder Bed Fusion

Hanna Nilsson-Åhman¹,², Pelle Mellin¹, Cecilia Persson²

¹ Swerim AB, Kista, SE; ² Uppsala University, Department of Material Science and Engineering, Uppsala, SE

Introduction
Magnesium (Mg) alloys have recently gained increased attention as a biodegradable metal implant. They can be biocompatible and have superior mechanical properties compared to the biodegradable polymeric implants used today. However, poor formability and excessive corrosion rates have limited the clinical implementation of Mg alloys to an extruded Mg-Y-Nd alloy[1]. Additive manufacturing through Laser - Powder Bed Fusion (L-PBF) allows for the production of patient specific implant designs. Hot Isostatic Pressing (HIP) is a common method applied after printing to obtain fully dense materials for improved mechanical and corrosion properties. However, the amount of work done on L-PBF of Mg alloys remains limited. Esmaily et al [2] found an improved corrosion resistance after HIP of a Mg-Y-Nd alloy processed by L-PBF, as evidenced by surface activity and H₂ evolution over 24h. However, the influence of HIP on the long term corrosion properties and the part morphology after corrosion have not been evaluated. Herein we show that HIP can be highly detrimental to the long-term corrosion properties of a Mg alloy processed by L-PBF.

Experimental Methods
Spherical powder of alloy Mg-4wt%Y-3wt%Nd, particle size 24µm - 67µm (NMD GMbH) was processed by L-PBF (EOS M290). Samples measuring 10*10*30mm were produced with laser power of 200W, scanning speed of 1111 mm/s, layer thickness of 30µm, and hatch distance of 0.1 mm. Some samples were subsequently HIPed (QIH9, Quintus), at T= 520 °C, P=105 MPa and t=3h. The porosity was evaluated before and after HIP, using Archimedes density testing, and image analysis (ImageJ). The microstructure was characterized by light optical microscopy (LOM, Leica DM IRM, Leica microsystems), scanning electron microscopy (SEM, Sigma 300VP, Zeiss) and electron backscatter diffraction (EBSD, Aztec crystal). Secondary phases were characterized using X-ray diffraction (XRD, D8 Discover, Bruker) and electron diffraction spectroscopy (EDS, Aztec 5.0).

The corrosion media used was Dulbecco’s Phosphate Buffered Solution (Sigma Aldrich). Samples with sizes of 10*10*3mm were prepared from the as-built (AB), HIPed and extruded material. The surfaces were subsequently grinded down to 2500 grit. The electrochemical activity of the samples was characterized by potentiodynamic testing (Versa studio). The corrosion rate in immersion for 28 days was measured by H₂ evolution and weight loss. The chemical composition of the corrosion products and morphology of the corroded surfaces was evaluated after 28 days using LOM, SEM, XRD and EDS.

Results and Discussion
The density of the AB samples was 99.5%, and a density of above 99.9% was achieved after HIP. Secondary phases in the form of Mg-RE (RE=Y and/or Nd) intermetallics were present in all materials, but in varying size and amount. Flakes of Y₂O₃, and Zr precipitates were present in the AB and HIPed material. The Mg-RE intermetallic particles grew during HIP, and the neighboring areas were depleted from alloying elements. In the extruded material, a higher amount of the alloying elements is dissolved in the matrix, and no Y₂O₃ or Zr particles are present. The average grain size of the AB material was 3.5µm, 12.4µm for the HIPed, and 10µm for the extruded material. The potentiodynamic testing showed a lower corrosion current for the HIPed material compared with the AB material, but higher than the extruded material. The AB samples also had the highest H₂ evolution, followed by the HIPed, and
lastly the extruded samples. However, the mass loss for the AB material was 5± 1%, for the HIPed material 15±4%. The extruded material showed no significant mass loss.

LOM images after 28 days of immersion (Fig. 1 a-c) exhibited a rough surface with a white layer of corrosion products for the HIPed samples, consisting mainly in hydroxides and phosphates, and having a thickness of several microns. The surfaces of the AB and extruded samples remained smoother, largely consisting in a cracked oxide/hydroxide layer of a couple of microns.

For the HIPed samples there was an accumulation of detached pieces and corrosion products at the bottom of the test tube, which explains the discrepancy between the weight loss and the H₂ evolution. The poor corrosion properties of the HIPed material can be related to the zones depleted in alloying elements, making them more susceptible for localized attack [3], and the growth in grain size [4]. The superior corrosion properties of the extruded material can be related to the absence Y₂O₃ and Zr, and a larger amount of alloying elements dissolved in the matrix [5].

Conclusion

HIP improved the electrochemical properties of the surface but was highly detrimental to the long term corrosion properties. The change in microstructure played a more important role than the increase in density. Further optimization of the L-PBF process or subsequent heat treatments is needed for making the implementation of Mg-4wt%Y-3wt%Nd as a biodegradable metal implant possible.

Acknowledgement

The authors are grateful for assistance by Lena Thorsson (Exmet AB), and the financial support from the Swedish Foundation for Strategic Research (SSF, project nr FID17-0028), and the Swedish Governmental Agency for Innovation Systems (VINNOVA, project nr 2019-05259 and 2019-00029).

References

5:45 p.m. – 6:30 p.m.

Track01

**Int AW | International Award 2021**

Chair
**Matteo Santin**
University of Brighton, Centre for Regenerative Medicine and Devices, Brighton, GB

**Int AW-01**

**Cells, Viscoelasticity and Medical Devices**

**David Mooney**

*Harvard University, School of Engineering and Applied Sciences, Cambridge, US*

The stiffness of biomaterials is increasingly appreciated as a key variable in the response of adherent cells and interacting tissues. However, most current work ignores that tissues are viscoelastic, with time-dependent mechanical behavior. We have demonstrated that the rate of biomaterial stress relaxation has dramatic effects on stem cell fate, and recently found it impacts multiple cell types in the tumor microenvironment, including cancer cells, monocytes, and T cells. Inspired by the importance of viscoelasticity to native extracellular matrix, new medical adhesives and medical devices are being synthesized from energy dissipative hydrogel networks.
6:30 p.m. – 7:00 p.m.

Track 01

Social Activity 6 | Music Evening

Chair
Meriem Lamghari
Raquel M. Gonçalves

i3S - Institute for Research and Innovation in Health, University of Porto, Porto, PT; INEB - Institute of Biomedical Engineering, Porto, PT; ICBAS - Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, PT

University of Porto “Tuna”

Performance by a University of Porto “Tuna” from Abel Salazar Institute of Biomedical Sciences, University of Porto, Porto, Portugal

Tuna Académica de Biomédicas was created in September 2003 by a group of students and former students of the Abel Salazar Institute of Biomedical Sciences, University of Porto, Portugal. The ambition was to unite a group of friends around a tradition as unique as the Portuguese concept of “Tuna Universitária” and create an ideal of fraternity that would last for generations, always prioritizing music in order to adequately represent the concept of tuna, ICBAS and the Porto city.

Thus, in these 16 years of existence, the group has been growing, in number and in quality, currently surpassing 100 members, all students or professionals already working in Medicine, Veterinary Medicine and Aquatic Sciences. Among numerous awards won in Tuna Festivals across the country and in Spain, we also organize our own festival - TABernal - which has 9 successful editions, in addition to having already held 6 European Tours, passing through countries like Spain, France, Switzerland, Germany, Luxembourg and Italy, and 2 National Tours. In 2005 we launched our 1st CD - Memórias de um Tuno - which has already been published in 4 editions and thousands of copies have been sold. In 2017 we released our 2nd CD - Um Porto para o Mundo.
<table>
<thead>
<tr>
<th>Time</th>
<th>Track01</th>
<th>Track02</th>
<th>Track03</th>
<th>Track04</th>
<th>Track05</th>
<th>Track06</th>
<th>Track07</th>
<th>Track08</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:30</td>
<td>PL3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Opening</td>
</tr>
<tr>
<td>10:00</td>
<td>ESB GA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Biomechanics 91</td>
</tr>
<tr>
<td>10:30</td>
<td>ESB General Assembly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Anatomo-functional bases of arthroses in sports</td>
</tr>
<tr>
<td>11:00</td>
<td>BREAK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:00</td>
<td>K09</td>
<td>X10</td>
<td>S14</td>
<td>S15</td>
<td>N14</td>
<td>N15</td>
<td>N16</td>
<td>Biomechanics 92</td>
</tr>
<tr>
<td></td>
<td>New Tools to Study Physiology and Disease</td>
<td>Supramolecular Peptide Assemblies as a Platform for Antifouling, Antimicrobial and Antiviral Materials</td>
<td>Innovative Approaches to Develop 3D Nanostructured Devices for Bone and Osteochondral Regeneration</td>
<td>Regeneration of the Intervertebral Disc: The Battles that Biomaterials Will Have to Win</td>
<td>Electropun and Electrowritten Biomaterials</td>
<td>Nanobiomaterials</td>
<td>Hydrogels and Smart Biomaterials 1</td>
<td>Swimming Biomechanics</td>
</tr>
<tr>
<td>12:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:00</td>
<td>CC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Biomechanics 93</td>
</tr>
<tr>
<td>13:30</td>
<td>Awards &amp; Closing Ceremony</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Oral Presentations C1</td>
</tr>
<tr>
<td>14:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Biomechanics 94</td>
</tr>
<tr>
<td>15:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Oral Presentations C2</td>
</tr>
<tr>
<td>16:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Biomechanics 95</td>
</tr>
<tr>
<td>17:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SBB General Assembly and Award Ceremony</td>
</tr>
<tr>
<td>18:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
9:30 a.m. – 10:15 a.m.

Track01

PL3 | Plenary Lecture 3

Chair
Cristina L. Martins
INEB - Instituto de Engenharia Biomedica, i3S- Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, PT

PL3-01

Molecular and colloidal building blocks to assemble structured biomaterial scaffolds in situ

Laura De Laporte\textsuperscript{1,2,3}

\textsuperscript{1} RWTH University Aachen, ITMC, Aachen, DE; \textsuperscript{2} DWI - Leibniz Institute for Interactive Materials e.V., aachen, DE; \textsuperscript{3} University Hospital RWTH Aachen, AME/AMB, aachen, DE

We apply polymeric molecular and nano- to micron-scale building blocks to assemble soft 3D biomaterials with anisotropic and dynamic properties. Microgels and fibers are produced by technologies based on fiber spinning, microfluidics, and in-mold polymerization. To arrange the building blocks in a spatially controlled manner, self-assembly mechanisms and alignment by external magnetic fields are employed. Reactive rod-shaped microgels interlink and form macroporous constructs supporting 3D cell growth. On the other hand, the Anisogel technology offers a solution to regenerate sensitive tissues with an oriented architecture, which requires a low invasive therapy. It can be injected as a liquid and structured \textit{in situ} in a controlled manner with defined biochemical, mechanical, and structural parameters. Magnetoceptive, anisometric microgels or short fibers are incorporated to create a unidirectional structure. Cells and nerves grow in a linear manner and the fibronectin produced by fibroblasts is aligned. Regenerated nerves are functional with spontaneous activity and electrical signals propagating along the anisotropy axis of the material. Another developed platform is a thermoresponsive hydrogel system, encapsulated with plasmonic gold-nanorods, which actuates by oscillating light. This system elucidates how rapid hydrogel beating leads to a reduction in cell migration, while enhancing focal adhesions, native production of extracellular matrix, and nuclear translocation of mechanosensitive proteins, depending on the amplitude and frequency of actuation.
Aligned nerve growth in Anisogel

Aligned growth of primary neurons from chick embryonic dorsal root ganglion inside a PEG-based Anisogel (purple). The Anisogel provides orientation cues by its magnetically aligned rod-shaped microgels in green. The microgels are fixed by a surrounding biofunctionalized PEG hydrogel.

References


10:15 a.m. – 11:15 a.m.

Track01

ESB GA | ESB General Assembly
11:45 a.m. – 1:15 p.m.

Track01

K09 | New Tools to Study Physiology and Disease

Chairs
Arti Ahluwalia
University of Pisa, Pisa, IT
Fernando Monteiro
University of Porto, Porto, PT
Sofia Guimarães (YSF)
INEB, Porto, PT
K09-KL01

Organs-On-a-Chip: A New Tool for the Study of Human Physiology

Ben M. Maoz¹²

¹ Tel Aviv University, Biomedical Engineering, Tel Aviv, IL; ² Tel Aviv University, Sagol School of Neuroscience, Tel Aviv, IL

Micro-engineered cell culture models, termed Organs-on-Chips, have emerged as a new tool to recapitulate human physiology and drug responses. Multiple studies and research programs have shown that Organs-on-Chips can capture the multicellular architectures, vascular-parenchymal tissue interfaces, chemical gradients, mechanical cues, and vascular perfusion of the body. Accordingly, these models can reproduce tissue and organ functionality and mimic human disease states to an extent thus far unattainable with conventional 2D or 3D culture systems. In this talk, we will present two approaches of using this technology. The first, will demonstrate how drug can be tested by linking of 8 human-Organ-on-a-Chip and showing results that are comparable to clinical data. Furthermore, we demonstrate how to exploit the micro-engineering technology in a novel system-level approach to decompose the integrated functions of the neurovascular unit into individual cellular compartments, while retaining their paracellular metabolic coupling. Using individual, fluidically-connected chip units, we have created a system that models influx and efflux functions of the brain vasculature and the metabolic interaction with the brain parenchyma. This model reveals a previously unknown role of the brain endothelium in neural cell metabolism: In addition to its well-established functions in metabolic transport, the brain endothelium secretes metabolites that are directly utilized by neurons. This discovery would have been impossible to achieve using conventional in vitro or in vivo measurements.
References


Biomimetic advanced *in vitro* models of glioblastoma multiforme

Daniele De Pasquale, Gianni Ciofani

*Italian Institute of Technology, Center for Materials and Interfaces, Pontedera, IT*

Introduction

Glioblastoma multiforme (GBM) is one of the most lethal cancers. Currently, GBM treatment consists of invasive techniques with strong side effects and poor outcome. Thus, there is an urgent need to find effective anticancer approaches and to improve *in vitro* research towards reliable tests for a better prediction of therapeutic efficacy and possible side effects *in vivo*.

Conventional 2D *in vitro* models poorly represent the tumor microenvironment and the complex interactions occurring when a drug has to be targeted. Thus, they fail at being a reliable model to describe anticancer treatments *in vivo*. Recently, *in vitro* research has focused on developing biomimetic models able to recapitulate some peculiar aspects of cancer microenvironment.

In this work, we present alternative approaches to study GBM, based on biomimetic models for advanced *in vitro* studies. In particular, we propose an innovative GBM spheroid model that can be controlled remotely with a simple magnet. Magnetically-driven cage-spheroids (MDCSs) offer the possibility to easily manipulate cancer cell spheroids, to control their size to standardize large-scale *in vitro* studies in order to better describe treatments efficacy, and to place GBM spheroids in complex *in vitro* constructs.

Experimental Methods

GBM primary cells derived from patients’ biopsies were cultured, amplified, and later seeded in MDCSs. MDCSs are hexagonal prisms of 120 µm in diameter realized by two-photon polymerization technique (Nanoscribe) with a photoresist (IP-L 780) doped with iron oxide nanoparticles to impart magnetic features. Viability tests (WST-1) were performed to compare MDCSs with conventional cultures derived from the same patient’s cells. Immunohistochemistry investigations for proliferative and stemness-related markers (Ki-67, SOX2, CD133) were carried out. The movement of MDCSs in desired positions driven by an external magnet was also characterized.

Results and Discussion

MDCSs present good magnetic responsiveness, as they faithfully follow the direction imposed by an external permanent magnet; moreover, cultures in MDCSs show the typical spheroids stratification, with an external proliferation layer and a semi-quiescent core. Cells in MDCSs also showed the expression of stemness-related markers. No differences were detected between conventional spheroids and MDCSs in terms of cell viability.

Conclusion

We can conclude that constructs obtained with MDCSs show identical biological features compared to conventional spheroids, yet allowing for a remote and easy control of their movement in complex systems, a feature not offered by conventional 3D cultures.

Acknowledgement

The research leading to these results has received funding from AIRC under IG 2020 - ID. 24454 project – P.I. Ciofani Gianni.
Figure 1
A) Scanning electron microscope images of cage-like scaffolds; bright field B) and scanning electron microscope C) image of MDCss supporting glioma primary cells.

References


Modular hydrogels reveal impact of type I innate lymphoid cells on intestinal organoids

Geraldine Jowett1,2, Michael Norman1, Tracy Yu1, Suzette Lust1, Christian Lorenz2, Ricardo da Silva1, Joana Neves2, Eileen Gentleman1

1 King’s College London, Centre for Craniofacial and Regenerative Biology, London, GB; 2 King’s College London, Centre for Host-Microbiome Interactions, London, GB; 3 King’s College London, Department of Physics, London, GB

Introduction
Pathological matrix remodelling plays a central role in many human diseases, but is challenging to study as in vitro models often cannot replicate the complex 3D cell-matrix interactions that drive pathologies. Here, we describe a 3D model of the gut that allowed us to uncover an unexpected role for a rare immune cell type called Type-1 innate lymphoid cells (ILC1) in driving epithelial proliferation and gut fibrosis in patients with inflammatory bowel diseases. ILC1 are enriched in patient mucosa with active inflammatory bowel disease (IBD), but the impact of this accumulation remains elusive, and α-IFNγ therapeutics against their signature cytokine lack clinical efficacy. We hypothesise that ILC1 accumulation in IBD is not simply a driver of inflammation, but rather that ILC1 play a role in driving disease pathology itself. Therefore, the aim of this work was to use reductionist models based on intestinal organoid-ILC1 co-cultures to uncover how ILC1 impact the intestinal epithelium and matrix.

We used molecular dynamics simulations to design PEG hydrogels that cross-link quickly, but can still mimic the stiffness of normal intestinal tissue. We then co-cultured encapsulated human intestinal organoids with ILC1, and using a combination of atomic force microscopy force spectroscopy and multiple particle tracking microrheology, found that ILC1 drive intestinal matrix remodelling through a balance of MMP9-mediated matrix degradation and TGFβ1-driven fibronectin deposition. Our findings demonstrate the potential of using hydrogels in disease modelling, and open the possibility of unravelling how pathological matrix remodelling contributes to disease.

Experimental Methods
We established co-cultures of murine small intestinal organoids (SIO) with ILC1, and human induced pluripotent stem cell (iPSC)-derived intestinal organoids (HIO) with patient ILC1. We also developed a functionalized, PEG-based synthetic hydrogel system designed to form efficient networks at low polymer concentrations (Fig. 1).

Results and Discussion
Pico-SMARTSeq2 transcriptomics on SIO co-cultures revealed that IFNγ sensitises epithelial cells to Fas-mediated apoptosis. However, ILC1 also drive expansion of the epithelial stem cell crypt through p38γ phosphorylation and aberrant Cd44v6 expression, which is unexpectedly regulated by ILC1-derived TGFβ1, not IFNγ. We next established that human ILC1 also secrete TGFβ1, and drive CD44v6 expression in both HIO epithelium and the surrounding mesenchyme, though notably this phenotype is only recapitulated by ILC1 from patient biopsies with active inflammation. As TGFβ1 is a master regulator of fibrosis, the leading indicator for surgery in IBD, we next characterised the ability of ILC1 to regulate matrix remodelling. We created modifiable PEG hydrogels that cross-link quickly but at low stiffnesses and harnessed this platform to perform microrheology and atomic force microscopy on encapsulated HIO. We show that ILC1 drive matrix stiffening and degradation, which we posit occurs through a balance of MMP9 degradation and TGFβ1 induced fibronectin deposition.

Conclusion
Our synthetic organoid co-culture system enabled us to tease apart an important role for intestinal ILC1 in epithelial and matrix remodelling, which may drive either wound healing or fibrotic pathologies in IBD (Fig. 2). Moreover, this...
controlled 3D microenvironment provides a broader platform for dissecting interactions between complex human iPSC-derived tissues and rare cell subtypes in development and disease.

**Figure 1: PEG hydrogel design**

PEG-based hydrogels are formed using two sequential and orthogonal click reactions. PEG-4NPC is first conjugated with degradable, adhesive, or non-adhesive/non-degradable peptides, and then reacted 1:1 with PEG-4VS. Stiffness is altered by changing polymer concentration. Ligand density/degradability are controlled by varying the percent of their respective conjugates.

**Figure 2: ILC1-human intestinal organoid co-cultures**

Schematic showing how patient-derived ILC1 co-cultured with human iPSC-derived intestinal organoids impact the epithelium by driving intestinal crypt expansion through CD44v6. ILC1 also drive both extracellular matrix secretion (matrix stiffening) and MMP-mediated degradation of the hydrogel (matrix softening).
K09-04

Designing a new targeted dendritic nanosystem for nucleic acid delivery to neuronal cells: the combination of fully biodegradable dendrimers and proteins

Ana P. Spencer¹,², Pedro Mota¹, Sara Silva-Reis¹, Ana Jesus¹, Victoria Leiro¹, Ana P. Pêgo¹,²,³

¹ i3S/INEB- Instituto de Investigação e Inovação em Saúde/Instituto de Engenharia Biomédica, Universidade do Porto, Porto, PT; ² Faculdade de Engenharia da Universidade do Porto (FEUP), Universidade do Porto, Porto, PT; ³ Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Porto, PT

Introduction

With 500,000 new cases worldwide per year, spinal cord injury (SCI) is one of the most serious medical conditions with substantial consequences to the patient, with the total loss of functionality in the most severe cases. Recently, gene therapy has been proposed as a powerful treatment of SCI. However, it requires the development of effective and clinically suitable delivery vectors with the ability to compact and protect nucleic acids. Cationic dendrimers are especially attractive nanobiomaterials due to their unique and tunable features: globular, nanosized, and very branched architecture, with the presence of several functional groups in the periphery, which allow their functionalization with different ligands simulating the multivalency of many biological systems [1]. Despite these remarkable properties, the non-biodegradability of the most commonly used dendrimers can lead to cytotoxicity due to the bioaccumulation of synthetic materials [1]. Thus, a new family of fully biodegradable (fb) PEG-dendrimers has been recently proposed and patented by us to act as nucleic acid delivery vectors [2-4]. Our PEG-dendrimers showed a great ability to complex and protect siRNA in compact nanoparticles (dendriplexes) that showed physicochemical properties very suitable for cellular uptake without causing any toxic effect in neuronal cells [4]. Here, we describe the preparation and characterization of a new targeted dendritic nanosystem, based on our amine-terminated fb PEG-dendrimers complexing siRNA, and functionalized with the non-toxic receptor-binding domain (HC) of tetanus neurotoxin as targeting molecule, which confers neurospecificity to the system [5]. This was designed to carry nucleic acids to neuronal cells, ultimately seeking their application in the development of new therapeutics to treat SCI patients.

Experimental Methods

The fb amine-terminated PEG-dendrimers were successfully synthesized and characterized by NMR and FTIR. Dendriplexes were prepared at different N/P ratios (N=number of primary amines in the dendritic structure; P=number of phosphate groups in the RNA backbone) ranging from 5 to 80 by adding siRNA to different volumes of dendrimer solution and characterized regarding siRNA retention capacity (SYBR®Gold accessibility assay and polyacrylamide gel electrophoresis), size and polydispersion index (PdI) by dynamic light scattering and surface charge by laser Doppler electrophoresis. Subsequently, dendriplexes were functionalized with the selected targeting moiety – HC protein. Briefly, the purified HC was functionalized with a heterofunctional PEG spacer (PEG:HC ratio of 1) and tethered to the reactive functional group at the PEG end of dendrimers/dendriplexes. The targeted dendriplexes were characterized as previously mentioned. The capacity of non-targeted dendriplexes to bind and be internalized by cells was assessed by flow cytometry and confocal fluorescence microscopy. Neuronal cells (ND7/23 and HT22 cells) and embryonic dorsal root ganglion (DRG) explants were incubated with dendriplexes carrying Cy5-tagged siRNA and then analyzed by the mentioned techniques.

Results and Discussion

A new fb PEG-dendrimer has been successfully synthesized until the 3rd generation [3]. The assembled siRNA dendriplexes were characterized in terms of their physicochemical properties, showing low nanosizes (<60 nm) and...
PDI (<0.3). These non-targeted dendriplexes demonstrated a great capacity to deliver siRNA in neuronal cell lines through clathrin-mediated endocytic mechanism beginning to appear inside cells after 6h of incubation. In addition, siRNA delivery capacity has been demonstrated in more complex and hard-to-transfect biological models, such as DRG (Figure 1). After incubation, the dendriplexes’ distribution is identified mainly within the cell body of neurons (center of DRG). Dendriplexes’ uptake by neurons was confirmed in the cross-section image showing the Cy5 fluorescence within βIII-tubulin (neuronal marker)-stained cells. Targeted dendriplexes were formed using different HC concentrations and showed good physicochemical properties (low nanosizes and PDI). Currently, the cell specificity of the HC functionalized nanosystems is under investigation.

Conclusion

Based on our PEG-dendrimers, a new targeted fb PEG-dendritic nanosystem was developed to deliver nucleic acids to neuronal cells. The encouraging physicochemical properties of dendriplexes make them a great candidate for neuronal cellular uptake. The new fully biodegradable dendritic nanosystem proposed shows great promise to contribute to a long-awaited therapeutic strategy for SCI patients.

Acknowledgement

SFRH/BD/137073/2018 (FCT); PTDC/NAN-MAT/30898/2017; PTDC/MED-NEU/28336/2017 (iDNArt); and NORTE-01-0247-FEDER-033399 (siRNAC).

References

Collagen/nano-apatite scaffolds as osteosarcoma 3D culture models to investigate the resistance mechanism to cold plasma therapy

Miguel Mateu-Sanz, Juan Tornin, Maria Pau Ginebra, Cristina Canal

Technical University of Catalonia (UPC), Biomaterials, Biomechanics and Tissue Engineering Group (BBT), Dpt. Materials Science and Engineering, Escola d'Enginyeria Barcelona Est (EEBE), Barcelona, ES

Introduction

Osteosarcoma (OS) is the main primary bone cancer, and most current therapies are not completely efficient [1]. Recently, Cold Atmospheric Plasmas, which consist in ionized gases composed by UV–visible radiation, electromagnetic fields and a wide variety of reactive species, have been validated as anti-cancer agents against OS [2]. This type of plasmas can be directly applied over OS cells or can be used to produce reactive species in aqueous solutions, which are believed to be the main cause of the anti-cancer effects [3]. Despite showing promising results, these studies have been obtained in 2D cultures that do not suitably represent tumor complexity. It is known that the bone environment plays a crucial role in the maintenance of drug-resistant Cancer Stem Cells (CSC) and in the progression of OS. CSC are subpopulations of tumor cells resistant to traditional therapies and they are responsible for tumor relapses. The aim of this work is to develop a suitable 3D culture system to produce distinct OS tissue-engineered models which allows to record the effects of cold plasma therapy on a relevant tumor microenvironment, considering their ability to eliminate CSC populations.

Experimental Methods

Composite scaffolds were prepared by freeze-drying of Collagen Type 1 (Col1) with hydroxyapatite nanoparticles (nHA) prepared by biomimetic routes. The Col1/nHA scaffolds were characterized in terms of porosity, nanoparticle distribution, mechanical properties, swelling and stability. Different human OS cell lines (MG-63 [4], G-292, SaOS-2 and U-2 OS) were seeded in the scaffold and their adhesion, proliferation and osteomimicry were characterized by DNA quantification (PicoGreen assay), fluorescence confocal microscopy and qPCR of osteogenic markers. Plasma-activated Ringer’s saline (PAR) was obtained by treating Ringer’s saline with a plasma jet (kINPen® IND) for times up to 240 seconds (s) and used to treat the tumors generated. The resulting cell viability was evaluated (DNA quantification and LIVE/DEAD assay) and compared to 2D cultures. Gene expression of OS progression and stemness-related markers were evaluated by qPCR in the 3D models exposed to PAR.

Results and Discussion

The macroporous scaffolds presented high porosity close to 86% (with most pores of 60 μm diameter) and high specific surface area of 22.38 m²/g. nHA were uniformly distributed in the scaffold (Figure 1A) which displayed high swelling ratio and good stability during two weeks with a weight loss of 10.82% ± 2.86% [4]. The Col1/nHA scaffolds allowed OS cells to proliferate, interact with the 3D matrix (Figure 1A) and increase osteomimicry. Following treatment with PAR, cell viability was reduced below 14.09 ± 2.61% with 120 s PAR of treatment in 2D, while in 3D models cell viability was above 44.73 ± 2.45% in 240 s PAR (Figure 1B). This was related to the reactive oxygen and nitrogen species generated in PAR in a time-dependent manner. Plasma treatment decreased the expression of osteogenic markers OPN and ALP under 0.5 ± 0.13 and 0.45 ± 0.1-fold change for the longest PAR treatment, while strongly enhancing (more than 5-fold change) the expression of stem-like related genes SOX-2, OCT3/4 or NANOG in a dose-dependent manner. Moreover, PAR treatment promoted in MG-63 an increase (2-fold change) of the sarcospheres (spherical, clonal expanding colony of CSC) formed from 3D-OS cultures [4]. Combination of PAR treatment with a specific inhibitor abrogated these negative effects in OS stemness, increasing the sensibility of OS
cells to plasma-derived reactive species, suppressing the remnant cell viability and giving hints on the possible mechanism implicated in the resistance to PAR described above.

**Conclusion**

The Col1/nHA scaffold developed provides a suitable substrate to generate a wide range of 3D OS tumor models *in vitro*, allowing to investigate the effects of reactive species generated by cold plasma therapies in a relevant context of the disease. The tumor microenvironment and presence of stem cell-like subpopulations allowed to propose an undescribed mechanism employed by OS cells to resist PAR induced-lethality in 3D cultures by the induction of stem properties. This study provides new insights to develop an effective treatment based in cold plasmas for OS therapy.

**Acknowledgement**

This project has been primarily funded from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation program (grant agreement No. 714793). The authors acknowledge MINECO for PID2019-103892RB-I00 project. The authors belong to SGR2017-1165. Support for the research of MPG and CC was received through the ICRA Academia Award for Excellence in Research, funded by the Generalitat de Catalunya.

---

**References**


An endochondral ossification strategy using dynamically cultured liquefied and multilayered microcapsules

Inês J. Fernandes, Sara Nadine, Sónia G. Patrício, Clara R. Correia, João F. Mano

University of Aveiro, Chemistry, Aveiro, PT

Introduction
Bone tissue presents an incredible intrinsic self-repair ability. However, large bone defects may result in cessation of the regenerative process. Therefore, bone tissue engineering (TE) strategies have been explored to obtain the successful repair of large bone defects by recapitulating two distinct mechanisms of bone formation/healing, namely endochondral (ECO) or intramembranous ossification (IMO). Although IMO has been the standard bone TE strategy, the ECO process is being increasingly explored because of the well vascularized bone tissue that is obtained. ECO begins with the chondrogenic differentiation of recruited mesenchymal stem/stromal cells (MSCs) forming an initial cartilaginous template, followed by hypertrophic differentiation that will result in the release of osteogenic and angiogenic factors. Such biomolecules give rise to newly deposited bone tissue but also helps tissue vascularization.

Inspired by this mechanism, we propose a co-culture system composed by cartilaginous 3D microtemplates (MT), umbilical cord MSCs (UCMSCs), and human umbilical vein endothelial cells (HUVECs), bioencapsulated in liquefied and multilayered microcapsules (MC). These MC are composed by (i) a multilayered membrane obtained through the layer-by-layer assembly of poly(L-lysine), alginate, and chitosan, (ii) a liquefied alginate core, and (iii) cells.

The cartilaginous 3D MT are previously cultured in vitro for 21 days, as well as the non-primed 3D MT, used as control. Then, such MT are co-cultured with UCMSCs and HUVECs within the MC in dynamic conditions for another 21 days. MC with cartilaginous 3D MT are cultured with (ECO) and without (ECO control) osteogenic differentiation factors. Also, MC with non-primed 3D MT are cultured with (IMO) and without (negative control) osteogenic differentiation factors. Our hypothesis is that the engineered and privileged microenvironment of such ECO MC would mimic the native bone creation and healing processes, leading to the in vitro production of close-to-native vascularized bone-like microtissues (Fig. 1VI).

Experimental Methods
The MSCs-derived cartilaginous 3D MT are produced at high-rates and cultured in vitro for 21 days in chondrogenic differentiation medium (Fig 1I). Non-primed 3D MT, cultured in basal medium, are used as control. Then, the MT are co-cultured with MSCs and HUVECs in an alginate solution (6x10^5 templates/mL and 5x10^6 cells/mL). Alginate microgels are generated by electrohydrodynamic atomization technique. For that, under influence of electrical forces (10kV), the alginate solution breaks up into droplets. After crosslinking in calcium chloride, microgels encapsulating cells are obtained (Fig. 1II). Then, layer-by-layer is performed using poly(L-lysine), alginate, and chitosan as polyelectrolytes to produce a 10-multilayered membrane surrounding the obtained microgels (Fig. 1III). Ultimately, the core is liquefied by chelation with EDTA for 5 min (Fig. 1IV). Afterwards, liquefied MC with non-primed and cartilaginous MT are cultured up to 21 days in basal or osteogenic differentiation media. The proposed MC are tested under dynamic culture conditions, using a rotary cell culture system (Fig. 1V).

Results and Discussion
Cell viability was maintained during all the experiments. The cartilaginous nature of the 3D MT cultured in chondrogenic medium was confirmed by collagen II immunofluorescence assay and glycosaminoglycans quantification. After 21 days of encapsulation, the ECO MC presented osteopontin (OPN) and hydroxyapatite (HA) staining, indicating the production of bone extracellular matrix (Fig. 2A and 2B). Furthermore, both ECO (Fig. 2A) and ECO control conditions showed an increased endothelial cell recruitment compared to IMO. The elemental...
analysis by scanning electron microscopy and energy-dispersive X-ray spectroscopy (SEM-EDS) showed a higher matrix mineralization in the ECO MC, being the only condition to present a calcium/phosphorous (Ca/P) ratio (1.71) close to the native HA ratio (1.67) (Fig. 2D-F). HA was widely dispersed and formed large nodule-like structures in the surface of the ECO microtissues (Fig. 2C). Although IMO MC presented OPN and HA staining, the nanocrystals of HA presented a lower Ca/P ratio (1.11).

**Conclusion**

In conclusion, ECO MC proved to be an effective strategy for the *in vitro* osteogenic differentiation. Furthermore, results show the relevance of using the cartilaginous 3D MT in bone repair, highlighting the advantage of ECO over IMO strategy. In the future, we intend to use the proposed system as an injectable and non-invasive TE strategy for bone regeneration applications.

**Acknowledgement**

The authors acknowledge the financial support given by the Portuguese Foundation for Science and Technology with the project “CIRCUS” (PTDC/BTM-MAT/31064/2017), and the European Research Council for the project “ATLAS” (ERC-2014-AdG-669858). This work was developed within the scope of the project CICECO-Aveiro Institute of Materials (UIDB/50011/2020 & UIDP/50011/2020).

---

**Figure 1**

Schematic representation of the creation of the ECO *in vitro* model. I. Production of the UCMSCs-derived cartilaginous 3D MT, using Aggrewell anti-adherence plates. II. Encapsulation of 3D MT with UCMSCs and HUVECs in alginate microbeads using the electrohydrodynamic atomization technique. III. Layer-by-layer deposition using poly(L-lysine) (PLL), alginate (ALG), and chitosan (CHT) as polyelectrolytes. IV. Core liquefaction in ethylenediaminetetraacetic acid (EDTA). V. *In vitro* culture of the produced microcapsules in four different conditions. VI. Hypothesis representation.
Figure 2
Bone ECM analysis of the IMO and ECO condition MC, at day 21 post-encapsulation; (A) OPN and CD31 immunofluorescence assay. Cells nuclei were counterstained with DAPI; (B) HA immunofluorescence assay; (C) SEM images of the microtissues formed within the MC; (D) SEM-EDS images identifying phosphorous and calcium deposition; (E) Comparation of the elemental analysis of the microtissues ECM by EDS mapping of P and Ca deposition; (F) Ca/P ratio analysis of the microtissues within MC in the four conditions (dotted horizontal line represents the Ca/P ratio of native HA).

References
Uptake and transport of milk-derived extracellular vesicles across the Caco-2 intestinal barrier model

**Josepha Roerig**¹, Laura Schiller², Cica Vissiennon², Michael C. Hacker¹,³, Christian Wölk¹, Michaela Schulz-Siegmund¹

¹ Leipzig University, Pharmaceutical Technology, Institute of Pharmacy, Medical Faculty, Leipzig, DE; ² Leipzig University, Institute of Medical Physics and Biophysics, Medical Faculty, Leipzig, DE; ³ Heinrich Heine University Duesseldorf, Institute of Pharmaceutics and Biopharmaceutics, Duesseldorf, DE

**Introduction**

Extracellular Vesicles (EVs) are membrane-enclosed nanostructures which gain increasing interest for their role in intercellular communication as natural nucleic acid carriers (e.g. miRNA). We have chosen bovine raw milk as a source for EVs as these milk EVs withstand the harsh conditions of the gastrointestinal system making them interesting for oral drug delivery purposes¹. Moreover, bovine milk is cost-efficient and largely available to shed light in EV characteristics and their uptake mechanisms. This work aims to critically study uptake and transport properties of milk EVs in vitro. Therefore, uptake is compared to liposomes (DPPC/Chol) in intestinal cells. For transport experiments a standardized protocol considering the distinct EV requirements was established and apparent permeability coefficients (P_{app}) reflecting the transport rate were calculated.

**Experimental Methods**

**EV Isolation:** A differential centrifugation protocol was applied to bovine raw milk. The supernatant was loaded onto a Sephacryl S-500 (GE Healthcare, USA) column as described by Blans et al.² The presence of EVs was evaluated by dot blot analysis with antibodies against the EV-marker CD63, CD9, Tsg101, and the milk whey contaminant casein. EV-containing fractions were pooled and investigated for morphology and absence of aggregates using transmission electron microscopy (TEM).

**Total Protein and Lipid Concentration:** The total protein content was determined using a Bradford assay with absorbance measured at 610 nm. The lipid content was estimated using a sulfo-phospho-vanillin (SPV) assay as described by Visnovitz et al.³. Absorbance was measured at 540 nm.

**Nanoparticle Tracking Analysis:** To determine the EV size distribution and particle concentration, nanoparticle tracking analysis (NTA) with a Nanosight LM10 (Malvern Instruments) was used. Three videos of 60 seconds were recorded for analysis.

**EV Fluorescence Labelling:** EVs were labelled with 5 µM Vybrant DiO (membranes), 20 µM CellTrace CFSE (intravesicular proteins), or 10 µM SYTO® (RNA). Excess dye was removed with ultrafiltration columns. Dye controls were prepared with buffer instead.

**V and Liposome Uptake into Caco-2 Cells:** Enterocyte-like Caco-2 cells differentiated over 21 days on PET inserts (0.4 µm). Cells were incubated with stained EVs for 15 min to 6 h. For comparison with liposome uptake, cells were incubated with the same particle number of DPPC-Chol (70/30 mol/mol) liposomes. Cell uptake was evaluated with a confocal laser scanning microscope (Leica, Germany). To account for different labelling efficiencies of EVs and liposomes, a fluorescence correction factor was calculated.

**EV Transport across the Caco-2 Monolayer:** Caco-2 cells grown on 3 µm PET inserts were used for transport studies. Stained EVs were added to the donor compartment and fluorescence measured in the acceptor compartment to calculate the P_{app} transport rate.

**Results and Discussion**
Bovine milk was subjected to a SEC resulting in reproducible elution profiles (Figure 1A). EVs eluted in the first peak well separated from the main whey contaminant casein as confirmed by dot blot. TEM confirmed the presence of vesicular structures with a characteristic cup-shaped appearance after uranylacetate treatment (Figure 1B). The mean size was measured as 190.8 (±3.3) nm by NTA.

To characterize the EV isolation yield, protein content, lipid content and particle concentration were measured (Table 1) as recommended by guidelines from the International Society for EVs.

<table>
<thead>
<tr>
<th>unit</th>
<th>method</th>
<th>result (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>proteins</td>
<td>Bradford</td>
<td>12.73 (± 0.95) µg/mL</td>
</tr>
<tr>
<td>lipids</td>
<td>SPV</td>
<td>7.00 (± 0.01) µg/mL</td>
</tr>
<tr>
<td>concentration</td>
<td>NTA</td>
<td>6.72 ∙ 10^{11} (± 1.1 ∙ 10^{11}) mL^{-1}</td>
</tr>
</tbody>
</table>

Table 1. EV yield after SEC isolation.

Three different labelling approaches showed comparable results by microscopic evaluation after 15 min to 6 hours indicating a fast EV internalization into Caco-2 cells. Dye controls exhibited no signal, so excess dye removal seemed to be successful. A novel fluorescence correction factor was implemented to compensate for differences in membrane labelling of EVs and liposomes. EVs outperformed liposomes regarding uptake efficiencies. Z-stacks revealed internalization of EVs whereas liposomes (DPPC/Chol) rather remained attached to the cell surface (Figure 2).

The transport rate was quantified for the first time as $P_{app} = 2.4 – 6.5 \cdot 10^{-6}$ cm/s.

**Conclusion**

Size-exclusion chromatography offers a reproducible method to isolate bovine milk-derived EVs of a good purity. The fast uptake of EVs in intestinal Caco-2 cells makes them promising for oral drug delivery purposes. Additionally, the variety of analytical methods applied creates a basis to further investigate the beneficial properties associated with EVs compared to liposomes to set a basis for rationally designed EV therapeutics. $P_{app}$ values reflecting the transport rate obtained with this model will add to the comparability of EV bioavailability testings.

**Acknowledgement**

We are grateful to Prof. A. Aigner and A. Ewe (Rudolf-Boehm-Institute, Leipzig) for NTA access, to H. Kalwa (Clinical Pharmacology, Leipzig) for access to CLSM imaging, and to Gerd Hause (Biozentrum, MLU-Halle/Wittenberg) for the TEM images.
Figure 2.
Z-stacks of Caco-2 cells incubated with DiO (green) labelled EVs (left) and liposomes (right) are shown. White arrows point towards internalized fluorescence signal, blue arrows towards extracellular fluorescence signal attached to the cell surface.

References
11:45 a.m. – 1:15 p.m.

Track02

K10 | Supramolecular Peptide Assemblies as a Platform for Antifouling, Antimicrobial and Antiviral Materials

Chairs
Geoff Richards
AO Foundation, AO Research Institute Davos, Davos, CH
Daniel Rodriguez Rius
Technical University of Catalonia, Dept. Materials Science and Engineering, Sant Adrià del Besòs, ES
George M. Vlasceanu (YSF)
Faculty of Medical Engineering, University Politehnica of Bucharest, Bucuresti, RO
K10-KL01

Supramolecular peptide assemblies as a platform for antifouling, antimicrobial and antiviral materials

Meital Reches\textsuperscript{1,2}

\textsuperscript{1} The Hebrew University, Chemistry, Jerusalem, IL; \textsuperscript{2} The Hebrew University | Center for Nanoscience and Nanotechnology, Jerusalem, IL

The lecture will present bio-inspired functional coatings that are spontaneously formed by short peptides. These peptide-based coatings self-assemble on metals, oxides and polymers under mild conditions without any need for a curing step. The coating can serve in many applications. One application is preventing biofouling - the undesirable adhesion of biomolecules and organisms to surfaces. This process leads to numerous adverse phenomena including hospital-acquired infection, blockage of water desalination facilities and food contamination. We showed that this coating prevents the first step of biofouling, which involves the adsorption of bioorganic molecules to the substrate. Moreover, the coating significantly reduces the attachment of various organisms such as bacteria and fungi to surfaces. Another function that these peptide-based coatings can mediate is the adhesion of mammalian cells to implants. This function is important for the integrating of implants into the human body. Finally, we showed that these peptides self-assemble in solution into particles that adsorb and release active compounds that synergistically reduce the number of bacteria and viruses on the surface.

Acknowledgement

M.R acknowledges the support of the Israel Ministry of science and Israel innovation authority.
K10-02

Silicon Nitride, A Bioceramic for Bone Tissue Engineering: A Reinforced Cryogel System with Antibacterial and Osteogenic Effects

Seunghun S. Lee¹, Leanid Laganenka², Xiaoyu Du¹, Wolf-Dietrich Hardt², Stephen J. Ferguson¹

¹ ETH Zurich, Institute for Biomechanics, Department of Health Sciences and Technology, Zurich, CH; ² ETH Zurich, Institute of Microbiology, Department of Biology, Zurich, CH

Introduction
Silicon nitride (SiN, [Si₃N₄]) is a promising bioceramic for use in a wide variety of orthopedic applications. Over the past decades, it has been mainly used in industrial applications such as the space shuttle engines, but not in the medical field due to scarce data on the biological effect of SiN. More recently it has been increasingly identified as an emerging material for dental and orthopaedic implant applications. Although a few reports about antibacterial property and osteoconductivity of SiN have been published, to date, there have been limited studies of SiN-based scaffolds for bone tissue engineering. Here, we developed a silicon nitride reinforced gelatin/chitosan cryogel system (SiN-GC) by loading silicon nitride microparticles into a gelatin/chitosan cryogel (GC), with the aim to produce a biomimetic scaffold with antibacterial and osteogenic properties. In this scaffold system, the GC component provides a hydrophilic and macroporous environment for cells while the SiN component not only provides antibacterial properties and osteoconductivity, but also increases the mechanical strength of the scaffold, to provide enhanced mechanical support for the defect area and a better osteogenic environment.

Experimental Methods
First, we analyzed characteristics of SiN-GC groups with different SiN concentrations such as interconnected porosity, mechanical properties and swelling ratio, then we checked apatite forming capacity in simulated body fluid (SBF) and protein adsorption capacity. Next, we started in vitro experiments and investigated an antibacterial effect of SiN-GC against Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) by checking bacterial proliferation rate and attachment to the scaffold. Then, we seeded MC3T3-E1 pre-osteoblast cells to study the cellular activity by examining viability, cell proliferation and morphology. Also, we seeded cells on SiN-GC to investigate mineralization and osteogenic gene expression (ALP, COL1, RUNX2 and OCN) by Alizarin Red S (ARS) staining and RT-qPCR. Finally, we developed a bioreactor to culture cell-laden scaffolds under cyclic loading (1 hour/day of cyclic compression with 1 Hz of frequency and 10% of strain) to mimic physiological conditions and analyze osteogenic effect of SiN-GC with ARS staining and RT-qPCR.

Results and Discussion
Characterization of SiN-GC demonstrated that incorporation of SiN in the scaffold led to higher elastic modulus, apatite formation in SBF and protein adsorption capacity while keeping the similar level of highly interconnected porosity as GC has which allows easier cell migration and nutrition flow. For antibacterial experiment, we confirmed that all SiN-GC groups showed significantly reduced bacterial proliferation and attachment compared to GC and increase of SiN concentration in SiN-GC groups led to improved anti-bacterial effect. In addition, for in vitro experiment with MC3T3-E1, the groups with higher SiN concentrations exhibited improved cell proliferation and enhanced morphology. Finally, results from ARS staining and RT-qPCR showed the improved mineralization and upregulated osteogenic gene expressions from SiN-GC compared to GC under both static and cyclic loading condition in bioreactor. Furthermore, we confirmed that scaffolds with higher SiN concentrations resulted higher mineralization and enhanced osteogenesis.

Conclusion

Page 858 of 2028
Overall, we confirmed the antibacterial and osteogenic effect of a silicon nitride reinforced cryogel system and the results indicate that silicon nitride has a promising potential to be developed further for bone tissue engineering applications.

**Acknowledgement**

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 812765 and the grant LA 4572/1-1 from Deutsche Forschungsgemeinschaft.

**Graphical Abstract**

Schematic illustration of silicon nitride (SiN) reinforced gelatin/chitosan cryogel (GC) system (SiN-GC) study. After (SiN-GC) fabrication, characteristics and bioactivity of SiN-GC with different concentration were analyzed. Then, in vitro experiments were carried out to investigate anti-bacterial properties, cellular activity and osteogenic effect under static and cyclic loading condition by bioreactor.
Lure and kill lethal ovitraps for tiger mosquito control based on biomimetic biomaterial based hydrogel and growth of entomopathogenic fungus *Beauveria bassiana* as biopesticide.

**Marco Friuli**¹, Marco Pombi², Riccardo P. Lia³, Claudia Cafarchia³, Christian Demitri¹

¹ University of Salento, Department of Engineering for Innovation, Lecce, IT; ² Sapienza University of Rome, Department of public health and infectious diseases, Rome, IT; ³ University of Bari, Bari, Italy, Department of Veterinary Medicine, Bari, IT

**Introduction**

Tiger mosquito (*Aedes albopictus*) is a vector of extremely serious pathologies for humans. Actually, traditional control methods (e.g. sprinkling of insecticides) presents more drawbacks (risk for human health and for environment) than efficacy, thus more targeted and specific are needed. Among them there are the precision pest management methods, relaying on “lure and kill” approaches based on semiochemical attractant or feeding stimuli (baits). However, *Aedes albopictus*, are semiochemicals and baits non-responsive, then the approach fails. A possible alternative for tiger mosquito are ovitraps and natural bioactive substances, including entomopathogenic fungi. Nevertheless, both methods have limits of applicability and effectiveness that, in relation to the higher costs, do not make them competitive with respect to insecticide spraying (low cost/effectiveness). Cost/effectiveness can be improved either by lowering costs and/or by increasing traps effectiveness (increasing lure). On the other hand, natural bioactive substances have a poor persistence at field, strongly influenced by the surface and the method of application. The work explores a connection between materials engineering and pest management to propose a new approach, called biomimetic, aiming to lure insects without semiochemical but rather imitating an insect-specific microenvironment. At the same time, biomaterials biocompatibility can be exploited to host inside the microenvironment a natural living bioinsecticide such as fungi, bacteria etc. Consequently, is possible to obtain the lure and kill effect bypassing both pesticide and semiochemical use.

The aim of the work was to create and optimize a lure and kill substrate acting as biomimetic oviposition substrate for tiger mosquito and as matrix for the growth and deliver *Beauveria bassiana* (an entomopathogenic fungus lethal for *Aedes albopictus*), with the aim to employ it inside a lethal ovitrap, suitable for use in large-scale control campaigns, industrially scalable and low-cost.

**Experimental Methods**

For the purpose, a macromolecular hydrogels was engineered and first tested for tiger mosquito in oviposition assay and then was evaluated as matrix for the survival, growth and delivery of *Beauveria bassiana*. The work focused first on identifying the main drivers of tiger mosquito’s oviposition and to reproduce them on the substrate. Furthermore, the effects of the material properties on the deposition and trap applicability were evaluated in order to optimize the substrate. Finally, oviposition in field condition was assessed by using a low-cost trap setup employing the materials that gave the best lab results. Successively, the compositions optimized for the lure were tested as a biocompatible matrix for the growth of *Beauveria bassiana* in order to assess the existence of a possible overlapping between oviposition and growth parameters. Furthermore, their effectiveness on *Aedes albopictus* eggs was tested through hatching tests using a 24 days old bioinsecticide, growth inside the matrix under controlled conditions. Finally a relation between fungal growth and material properties was explored.

**Results and Discussion**

Trials proved that a cellulose based physical hydrogel shows a superior efficacy in terms of reached eggs and lasting (up to 30 days vs few days) compared to common ovitraps and standard substrates Masonite and absorbing paper.
Furthermore, it has been verified that it can be effectively applied inside a low cost cardboard made trap. From oviposition assays pH, Salinity, turbidity and water evaporation resulted to influence the attractiveness of the substrate. Consequently, influencing parameters can be managed both with the composition of the hydrogel (e.g. preparation media, polymers choice or with the addition of humectants) but also by varying some properties of the gel such as viscosity or degree of crosslinking. This allows to regulate a parameter capable of amplifying (or suppress) lure directly through the engineering of the substrate. The tests verified the biocompatibility between hydrogel and *Beauveria bassiana*. A particular affinity was found between *Beauveria* and some compositions that showed to stimulate fungal proliferation. Furthermore, the lethality of the matrix (due to fungal and material action on the eggs and larvae) was verified. The highest lethal effect for *Bb/Gel* system was obtained using cellulose based compositions in particular preparations presenting high viscosity values. This was probably due to the presence of the fungus that seems to lead to a reduction in viscosity involving a lower trapping effect but also to a higher proliferation of infecting fungal structure in more viscous gels.

**Conclusion**
Trials proved that is possible to produce a cellulose based physical hydrogel acting both as oviposition substrate (based on biomimetic lure) and as biocompatible matrix *Beauveria bassiana*.

**Acknowledgement**
The work was supported in conceptualization and research by Gea srl-Settimo Milanese and the contribution of Unisalento Phd program.

**References**


K10-04

Pexiganan-A grafted chitosan microparticles & *Helicobacter pylori*: a bitter love story

Diana R. Fonseca1,2,3, Ana Moura1,2, Catarina L. Seabra1,2, Sandra Gomes1,2, Joaquim M. Oliveira4,5,6, Rui L. Reis4,5,6, Victória Leiro1,2, Berta Estevinho7, Cátia Teixeira8, Paula Gomes8, Paula Parreira1,2, M. Cristina L. Martins1,2,9

1 Instituto de Engenharia Biomédica, Universidade do Porto, Porto, PT; 2 i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, PT; 3 Faculdade de Engenharia, Departamento de Engenharia Metalúrgica e de Materiais, Porto, PT; 4 3B's Research Group, I3Bs - Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, PT; 5 ICVS/3B’s - PT Government Associate Laboratory, Braga/Guimarães, PT; 6 The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, PT; 7 LEPABE, Departamento de Engenharia Química, Faculdade de Engenharia da Universidade Do Porto, Porto, PT; 8 LAQV-REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Porto, PT; 9 ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, PT

Introduction

*Helicobacter pylori* (*H. pylori*) is a gastric pathogen that infects the stomach of 50% of the world’s population (≈ 4 billion people). *H. pylori* infection is the etiological agent of several gastric disorders and accounts for 90% of all diagnosed gastric cancers (5th most common and 4th deadliest cancer worldwide)1. *H. pylori* is one of the sixteen bacteria that ranks higher in what concerns antibiotic resistance and as a threat to human health1. Antimicrobial peptides (AMPs) are an interesting alternative to antibiotics due to their broad-spectrum of activity and low propensity to induce bacterial resistance. AMPs immobilization onto biomaterials is an advocated strategy to overcome some of the AMPs drawbacks in vivo, namely proteolytic degradation and aggregation with proteins2. MSI-78A, an analog of Pexiganan, is one of the few reported bactericidal AMPs against this gastric pathogen3. Within this work, MSI-78A grafted chitosan microspheres (AMP-ChMic) were developed to eradicate *H. pylori* infection in situ (surface of gastric epithelium cells). A pH-responsive system was designed to prolong the gradual gastric release of AMP-ChMic.

Experimental Methods

Chitosan, a natural cationic biomaterial, was chosen due to its mucoadhesive properties, which also favor the electrostatic interactions with the negatively charged *H. pylori* membrane4. Chitosan (acetylation degree of 6%) was purified and, to prevent its dissolution in gastric acidic conditions, crosslinked with genipin (2.5 mM). Chitosan microspheres (ChMic), ranging from 2 to 7 μm in diameter, were produced by spray drying technique. Afterwards, a heterobifunctional spacer (NHS-PEG-MAL) was grafted onto the ChMic (PEG-ChMic) for MSI-78A immobilization with controlled orientation (MSI-78A-SH; modified on C-terminal with a terminal cysteine). Low acyl gellan gum (LAGG) hydrogels (1.5% w/v) were tested as possible ChMic carriers. LAGG hydrogels were prepared according to Pereira et al.5 and ChMic (1 mg/mL) were added. After the gelation process, small discs were cut with a puncher (diameter of 9 mm). To evaluate mass and diameter changes, as well as the ChMic release, stability tests were done over one week (37°C, 150 rpm) and in different pHs (from pH 2.6 to pH 7.0).

Results and Discussion

AMP-ChMic were successfully engineered, as shown by Fourier Transform Infrared spectroscopy analysis. The yield of the immobilization reaction was set at 91% (indirect determination by UV-Vis spectrophotometry). The developed AMP-ChMic were stable in acidic and neutral pH, supporting this approach for gastric settings. AMP-ChMic were bactericidal against *H. pylori* J99 (highly pathogenic human strain) at lower concentrations than the free peptide in...
solution (=277 μg vs 512 μg, respectively), even in simulated gastric conditions. AMP-ChMic killed *H. pylori* through membrane destabilization and cytoplasm release. Also, bacteria attraction to chitosan promoted this interaction and enabled that ≈10 bacteria were killed by 1 single microsphere.

Concerning the pH-responsive system, the mass loss was higher in pH 2.6 than in neutral conditions (pH 7.0), which indicates a different behavior according to the pH of the medium, as expected. The AMP-ChMic delivery occurred in all conditions tested, demonstrating the potential of this strategy for ChMic delivery in situ.

**Conclusion**

This microsphere-based innovative strategy boosted the activity of MSI-78A and AMP-ChMic demonstrated high potential for *H. pylori* infection management. Also, the pH-responsive delivery system engineered for microspheres release is expected to further boost efficiency *in situ*. Altogether, this is a promising approach within the scope of non-antibiotic therapeutic strategies against this gastric pathogen.

**Acknowledgement**

PTDC/CTM-BIO/4043/2014 & SFRH/BD/146890/2019

**References**


K10-05

Bacteria Material relationship – it’s complicated! Bacterial behaviour in Pluronic based bioinks for Living Therapeutic Devices

Shardul Bhusari¹,², Shrikrishnan Sankaran¹, Aránzazu del Campo¹,²

¹ INM - Leibniz Institute for New Materials, Dynamic Biomaterials, Saarbruecken, DE; ² Saarland University, Chemistry Department, Saarbruecken, DE

Introduction

Engineered living materials have driven a major shift in the paradigm of materials research in the recent years. The incorporation of living cells within a non-living matrix is emerging as a new approach for drug delivery. Such living therapeutic materials include genetically programmed living organisms for performing enhanced functions like productive, adaptable, on-demand drug delivery with replenishable repositories.¹,² However, much is yet to be understood in the effects of encapsulation on bacterial performance. To address this issue, we used a bacterially-orthogonal hydrogel system based on Pluronic F127, in which network stabilization could be established with both non-covalent and covalent interactions that could be proportionally tuned. The interplay between the dynamic viscoelastic properties of the pluronic and the functional performance of the encapsulated bacteria was studied. The effect of the dynamic mechanical properties of the surrounding biocompatible and scale-up friendly hydrogel matrix on the growth behaviour, microcolony growth kinetics, viability and functionality of the encapsulated microbial cells was studied providing essential cues for living materials applications. We demonstrate the development of this tunable hydrogel system to encapsulate genetically engineered E. coli bacterial strain, in core-shell 3D bioprinted scaffolds, leading to a light-regulated, localized, tunable and prolonged drug/protein release.

Experimental Methods

Pluronic F127 polymer was chosen as a bioink. Pluronic hydrogel forms physically assembled gel networks at room temperature and its diacrylated derivative, pluronic diacrylate, can be chemically crosslinked upon UV light exposure using a free-radical photoinitiator. Hydrogel matrices with a range of viscoelastic properties were obtained by mixing pluronic and pluronic diacrylate in varying proportions. The dynamic mechanical properties were studied using rheology. A light-regulated dVio drug-producing E. coli strain was engineered by incorporating the genes related to the metabolic synthesis of the drug into a light responsive optogenetic plasmid.² Bacteria was encapsulated in 3D-bioprinted core-shell constructs and the effect of change in the crosslinking density and the resulting mechanical properties on the bacterial growth rate, drug/protein production and viability was studied using epifluorescence and confocal laser scanning microscopy.

Results and Discussion

Bacteria in physically crosslinked Pluronic hydrogels grew unidimensionally, as a chain with cells predominantly arranged along their longitudinal axis (Fig 1a). In contrast, in covalently crosslinked Pluronic Diacrylate hydrogel, the growing chain buckled already in the 2nd or 3rd division cycle, and the dividing bacterial population formed rounded colonies (Fig 1b). These results evidence that the nature of the crosslinks of the hydrogel network influences the growth of the encapsulated microorganisms. The compressive forces imposed by the network as the bacterial colony grows act as physical modulators of bacteria proliferation in the confined state. 3D printed core-shell fibres with violet colored dVio drug producing bacteria were fabricated (Fig 2). The bacteria were viable in the 3D scaffolds stored at various temperature conditions for over 14 months.

Conclusion

We studied the effect of the dynamic mechanical properties of the surrounding biocompatible and scale-up friendly hydrogel matrix on the growth behavior, microcolony growth kinetics, viability and functionality of the encapsulated
microbial cells. This would provide fundamental insights into the mechanisms by which these parameters can be controlled for living materials applications. Also, genetically engineered bacteria were successfully encapsulated inside 3D bioprinted core-shell hydrogel system. We demonstrate the possibility of controlling the bacterial growth by tuning the mechanical properties of the hydrogel and drug release in situ using an optogenetic strategy.

Bacterial behaviour in viscoelastic confinement

Figure 1: Relaxation modulus of the Pluronic and Pluronic Diacrylate hydrogels mixed in varying proportions. The inset shows the bacterial Live Dead assay (green = live, red = dead) highlighting the growth morphology and viability of the bacteria in a) Pluronic (Plu) and b) Pluronic diacrylate (DA 100) hydrogel constructs.

3D bioprinted scaffolds for living therapeutic devices

Figure 2: Spiral 3D printed core-shell fiber containing light responsive violet colored dVio. a) Optical image and b) epifluorescence image highlighting the drug production. (Scale = 2 mm)

References


Development of antimicrobial polycaprolactone wound dressings loaded with niclosamide by melt electrowriting

Clara M. Guarch Perez1, David Martinez Perez2, Augusto Vazquez Rodriguez3, Frits Van Charante4, Livia Ferrari3, Martijn Riool1, Tom Coenye4, Antonio Felici3, Wojciech Święszkowski2, Sebastian A.J. Zaat1

1 Amsterdam UMC, University of Amsterdam, Department of Medical Microbiology and Infection Prevention, Amsterdam institute for Infection and Immunity, msterdam, NL; 2 Warsaw University of Technology, Faculty of Material Science and Engineering, Warsaw, PL; 3 Aptuit S.r.l., an Evotec Company, Discovery Microbiology, Verona, IT; 4 Ghent University, Laboratory of Pharmaceutical Microbiology, Ghent, BE

Introduction
Globally, the annual costs for wound care are projected to rise up to 3.5 billion euro in 2021 [1]. Wounds provide an ideal environment for bacterial colonization which often leads to chronic infections. The majority of wound infections are caused by Gram-positive bacteria such as Staphylococcus spp. Notably, the presence of bacterial biofilms has been linked to the induction and persistence of an inflammatory state of wounds and delayed wound healing, responsible for high rates of morbidity and mortality in patients. Moreover, the increase of antimicrobial resistance compromises the antibiotics available to treat the infections in the clinics, and alternatives for antibiotics are required. Wound dressings loaded with antimicrobial agents are emerging as a novel solution to prevent and treat wound infections while improving the healing process [2]. Under this rationale, we developed polycaprolactone wound dressings loaded with niclosamide, a repurposed molecule, originally used as an oral antihelminthic drug for treatment of parasitic infections [3], using the melt electro-writing (MEW) technology. MEW allows to create any type of shape and is solvent-free, making it easier to develop products towards clinical application. Poly-caprolactone (PCL) was selected for its elasticity, hydrophobicity and good mechanical properties and niclosamide was chosen as the antimicrobial agent for its potent antimicrobial activity against multidrug resistant Staphylococcus spp and its thermostability allowing its use in MEW.

Experimental Methods
The ink was prepared by the solvent casting method. PC-12 (medical grade PCL) and niclosamide were dissolved in chloroform and poured in a crystallizer glass. After overnight evaporation, the films were transferred at a low-pressure chamber (50 mbar) for three days. Films were cut in small pieces and loaded into the melt electrowriting syringe. The syringe was pre-heated at 70-80°C for 2 hours. Samples were printed at an angle ranging from 0 to 90 degrees and 0.5 mm distance between fibers. In vitro release studies of niclosamide from PCL were performed using 2 different systems (submersion and transwell model) to better predict the in vivo release kinetics. For both systems, PCL-niclosamide dressings were incubated in 1 mL of phosphate buffer solution with 2 % Tween 80 at 37°C and 120 rpm for 1, 3, 5, 7, 24 and 48 hours. The in vitro antimicrobial activity of the dressings was characterized against S. aureus strain ATCC 25923 and the multidrug resistant S. aureus strain LUH 14614, using a modified Kirby-Bauer method and a 4 and 24 hours bacterial adhesion assay. Moreover, biofilm formation on dressing fibers was visualized at both time points by scanning electron microscopy (SEM). The dressings were also evaluated in an in vitro wound model to evaluate effectiveness for the intended application. The wound model consists of an artificial dermis made primarily from collagen and hyaluronic acid which is partially submerged in a medium supplemented with plasma and blood to simulate the air liquid interface. The artificial dermis was infected with S. aureus strain LUH 14614, the dressing placed on top, and incubated for 4 and 24 hours. CFU counts were obtained from both the dermis and the dressing.

Page 867 of 2028
Results and Discussion
The submersion release profiles showed a burst release of 95% after 1 h followed by low release in the next 3 and 5 hours above the minimum inhibitory concentration (MIC) of niclosamide. Interestingly, the transwell release system showed a sustained release profile over time all above the MIC of niclosamide (Figure 1A). The niclosamide loaded PCL dressings significantly reduced the bacterial colonization of the dressings at 4 and 24 hours for both *S. aureus* strains (Figure 1B). These results were visually confirmed by SEM (Figure 1C). In the wound model, the niclosamide dressings showed significantly lower colonization with bacteria compared to unloaded dressings with 2 log less CFU recovered for the 4 hours and 3.5 log less for the 24 hours experiment. The number of bacteria in the dermis itself was also significantly reduced with a 1.5 log reduction of CFU for the 4 hours and a 2.5 log reduction for the 24 hours experiment (Figure 1D).

Conclusion
In conclusion, the niclosamide loaded PCL dressings produced by MEW technology are a promising combination for the prevention of *S. aureus* infection *in vitro* and with the dermis model. As a next step, we will assess the antimicrobial efficacy of the dressings in a murine skin wound infection model.

Acknowledgement
This research was funded by the research project PRINTAID, the EU Framework Programme for Research and Innovation within Horizon 2020 - Marie Sklodowska-Curie Innovative Training Networks under grant agreement No. 722467.

References
Advantages and limits of surface viscoelasticity as a drug-free strategy for the design of new antimicrobial biomaterials

Annabelle Vigué¹, Dominique Vautier¹, Bernard Senger¹, Youri Arntz¹, Vincent Ball¹, Amine Ben Mlouka², Julie Hardouin², Thierry Jouenne², Lydie Ploux¹,³

¹ INSERM/University of Strasbourg, U1121 Biomaterials Bioengineering, Strasbourg, FR; ² CNRS/Université Rouen Normandie/INSA Rouen Normandie, UMR6270 Laboratoire Polymères, Rouen, FR; ³ CNRS, Strasbourg, FR

Introduction
Fighting microbial biofilms on biomaterials is usually addressed by incorporating antimicrobial agents. Nevertheless, as usual in the natural life, intrinsic properties of the material surface can also be a complementary approach. They may drastically reduce the quantity of adhered microorganisms, and these remaining microorganisms can be treated with classical antimicrobial agents. Mechanical properties of material surfaces recently emerged as a possible way to impact biofilm formation [1-5]. However, many questions have not been elucidated so far, and the biological or physico-chemical nature of the underlying mechanisms is still being discussed. We have here especially studied the effects of surface elasticity on microbial behavior, metabolisms and biofilm formation, and attempted to provide significant insight regarding the biological or non-biological origin of the observed phenomena.

Experimental Methods
Materials based on silicone and hyaluronic acid have been considered as typical non-hydrogel and hydrogel biomaterials without microbicide activity. Their viscoelasticity has been varied from a few (for hydrogels and the softest non-hydrogel) to hundreds of kPa (for the stiffest non-hydrogel). Surface properties, including mechanical and hydration, have been thoroughly characterized regarding both the bulk and the surface of the material. The study has been conducted with yeast (Candida albicans) and bacteria species (Escherichia coli -E. coli-). Microbial mobility, adhered or retained quantity, production of pili-like structures as well as the proteome of the adhered bacteria have been specifically investigated by using confocal fluorescence microscopy, modeling and proteomic analyses.

Results and Discussion
A general impact of the viscoelastic properties on adhesion, retention and mobility of cells has been confirmed for both material types, which was usually maintained but reduced for longer-term culture (up to one week). However, the microbial behavior significantly differed on hydrogels and non-hydrogels with even an opposite relationship to elasticity for the adhered quantity at short- and long-term culture. Mobility results highlighted different subpopulations of E. coli cells with different displacement speeds, which varied both with viscoelastic properties and material, and were attributed to different strength of interaction between cells and surface. Cell mobility was drastically modified when inoculated cells were first killed, suggesting the predominant role of biological rather than physico-chemical mechanisms. In that regard, several proteins associated to adhesion, mobility or/and biofilm formation were differently produced according to the material nature, but also to their viscoelastic properties, some of them being known for their involvement in the capacity of E. coli to sense their mechanical environment.

Conclusion
Finally, the overall results offer new insights regarding microbial behaviors and biological mechanisms in response to variations in viscoelasticity of materials. Even though they confirm that viscoelasticity is an additional surface property that can be optimized to tune biofilm formation on materials, they also temper the impact of viscoelasticity in contrast to other properties by comparing microbial behaviors on materials of similar elasticity.

References
11:45 a.m. – 1:15 p.m.

Track03

S14 | Innovative Approaches to Develop 3D Nanostructured Devices for Bone and Osteochondral Regeneration

Chairs
Simone Sprio
National Research Council of Italy, Institute of Science and Technology for Ceramics, Faenza, IT
Anna Tampieri
National Research Council of Italy, Institute of Science and Technology for Ceramics, Faenza, IT
Clarissa Tomasina (YSF)
Maastricht University, Maastricht, NL

To raise the discussion on new bio-inspired concepts in materials science, chemical approaches and innovative fabrication technologies capable to overcome the current limitations and yield significant advances in the field of development of 3-D scaffolds associating bio-relevant, often contrasting, properties, such as bioactive chemistry, porosity, nanostructure and damage-tolerant mechanical performance.
3D printing synthetic bone grafts: challenges and opportunities

Maria-Pau Ginebra¹, Yago Raymond¹, Joanna Konka¹, Cristina Canal¹, Raul Benitez², Antonio Riveiro³, Juan Pou⁴, Jordi Franch⁵, Maria-Cristina Manzanares⁶, Montserrat Espanol¹

¹ Universitat Politècnica de Catalunya, Dept. Materials Science and Engineering, Group of Biomaterials, Biomechanics and Tissue Engineering, Barcelona, ES; ² Universitat Politècnica de Catalunya, Biomedical Engineering Research Center, Barcelona, ES; ³ Universidad de Vigo, Department of Materials Engineering, Applied Mechanics and Construction, Vigo, ES; ⁴ Universidad de Vigo, Department of Applied Physics, Vigo, ES; ⁵ Universitat Autònoma de Barcelona, Veterinary School, Barcelona, ES; ⁶ Universitat de Barcelona, Department of Pathology and Experimental Therapeutics, Barcelona, ES

The development of additive manufacturing technologies has enabled the implementation of personalized medicine strategies in the field of bone regeneration. Calcium phosphates (CaP) are among the best performing materials for this application, due to their similarity to the mineral phase of bone. Different additive manufacturing techniques can be used to print CaP 3D bone scaffolds. Among them, microextrusion-based direct ink writing (DIW) consists of extruding a pseudoplastic ink through a nozzle, in order to generate, layer by layer, the desired 3D structure. Different ink formulations have been proposed, most of them requiring a post-printing sintering step. However, self-setting ceramic inks have also been developed, in which the consolidation of the ceramic scaffold is not based on a high-temperature sintering process, but on a cement-like reaction that leads to the hardening of the structure at body temperature. They offer several advantages. In contrast to high-temperature sintering, this mild consolidation setting results in a very small shrinkage, and moreover they can be combined with biological molecules or even cells. Porosity is known to be a decisive factor in the performance of scaffolds for bone regeneration. Not only total pore volume, pore size and pore interconnectivity are relevant parameters. Also pore morphology plays an important role. Numerous studies have demonstrated that the concavity of a surface is a determining factor in its ability to trigger bone formation. We recently demonstrated that calcium deficient hydroxyapatite (CDHA) foams, with an open network of concave pores induced a significantly higher amount of bone in a canine model, both ectopically and orthotopically, than the same CDHA obtained by DIW, which resulted in arrays of cylindrical rods with convex surfaces [1,2]. Thus, although robocasting allows controlling the macroporosity and pore shape of printed scaffolds, the fact that microextrusion results in convex surfaces may be a limitation as far as bone regeneration and osteoinduction are concerned.

In this talk I will describe two strategies that provide greater versatility in the development of calcium phosphate bone grafts by DIW. They consist of either tailoring the internal porosity of the individual strands or modifying the external shape of the extruded filaments. The final goal is to create concavities in the otherwise convex surfaces of the 3D-printed scaffolds fabricated by DIW.

The first strategy is based on the incorporation of microspheres of a sacrificial material in the calcium phosphate ink. This allows creating an additional level of porosity, with concave morphology. Gelatin is a good candidate material when using self-setting inks, as it can be dissolved in the aqueous medium while the hardening reaction takes place. The formation of the characteristic network of needle-like hydroxyapatite crystals is not hindered by the addition of gelatin. The partial dissolution of the microspheres results in spherical pores throughout the filaments and exposed on their surface, increasing filament porosity up to 70%. Moreover, the presence of retained gelatin also has implications for the mechanical properties of the scaffold, reducing the strength but simultaneously conferring an elastic behavior, despite the high content of ceramic as a continuous phase.

3D printing with non-circular nozzles is the second strategy, which adds new degrees of freedom to DIW. Using nozzles with complex morphologies, e.g. star-shaped, not only allows to increase the specific surface area of the
structure, but also to have concave surfaces on the filaments. On the other hand, it requires that the inks exhibit fast elastic recovery, to ensure good shape fidelity of the printed filaments, and some restrictions arise in terms of the printing process. As for the impact of these morphological changes on the \textit{in vivo} behavior of the scaffolds, the results obtained in a study comparing the performance of scaffolds with star-shaped strands to conventional scaffolds with cylindrical strands in a rabbit model revealed that strands with concave surfaces enhance bone regeneration and have better osteoconductive properties, guiding the newly formed bone faster towards the core of the scaffold. Taken together, these results provide relevant inputs for the design of DIW patient-specific scaffolds with superior clinical performance.

\textbf{Acknowledgement}

The authors acknowledge the Spanish Government for financial support through the PID2019-103892RB-I00/AEI/10.13039/501100011033 project. They also thank the Generalitat the Catalunya for funding through projects 2017SGR-1165 and BASE3D 001-P-001646 co-funded by European Regional Development Funds. YR acknowledges the Spanish Government for the Ph.D. grant DI-15-08184. MPG and CC acknowledge the Generalitat de Catalunya for the ICREA Academia Award.

\textbf{References}


S14-02

Effect of cold atmospheric plasma-assisted surface modification of β-TCP with collagen and bone morphogenetic protein-2 mimetic peptide on osteogenic stem cell differentiation

Günnur Onak Pulat, Utku K. Ercan, Ozan Karaman

İzmir Katip Çelebi University, Biomedical Engineering, İzmir, TR

Introduction

Bone defects caused by trauma, disease, or surgical formations are challenging health issues worldwide. Therefore, there is a high demand for bone tissue engineering solutions such as synthetic grafts to treat bone defects. β-tricalcium phosphate (β-TCP), a synthetic bioerodible ceramic, has a degradable structure and high biocompatibility. Nevertheless, the absence of biological cues on synthetic β-TCP arises the need for further surface modification. β-TCP, which is produced at high temperatures, must be modified with bioactive molecules as a post-manufacturing process commonly by using highly concentrated solutions with physical adsorption methods. Absorbed molecules on β-TCP are not guaranteed to sustain cellular response during bone regeneration while covalently bound bioactive molecules remain on the surface until cell interactions and have a longer retention time after implantation [1]. On the other hand, using peptides as bioactive molecules rather than proteins have some advantages such as easier manipulation during grafting, high resistance to pH or temperature changes, low risk of immunological response, and chemical synthesis that provides precise control over the chemical composition of the peptide. Both bone morphogenetic protein-2 derived peptide (BMP; NSVNSKIPKACCVPTELSA) and collagen mimetic peptides (CMP; GTPGPQGIAGQRGVV) are a promising group with great therapeutic potential among bioactive peptides and known to increase cell attachment and the ability of cells to differentiate into osteoblastic cells. Herein we investigate a new strategy to modify surfaces of β-TCP with cold atmospheric plasma (CAP) to enhance bioactive peptide conjugation. Within the proposed novel approach, we assessed the surface modification of β-TCP ceramics with CAP to induce hydroxyl (-OH) groups and BMP-2 derived peptide (BMP) and collagen mimetic peptides (CMP) were immobilized to β-TCP graft. The proliferation and differentiation of human mesenchymal stem cells (hMSCs) on β-TCP, BMP/β-TCP, and CMP/β-TCP scaffolds were compared.

Experimental Methods

BMP sequence containing NSVNSKIPKACCVPTELSAI and CMP sequence containing GTPGPQGIAGQRGVV were synthesized with 4-Methylbenzhydrylamine (MBHA) resin. CAP was applied on β-TCP discs until maximum hydroxyl (-OH) groups are exposed by determining parameters roughly by FTIR. Peptide sequences were conjugated on β-TCP by silanization with APTES (1x10⁻² M) in anhydrous hexane followed by succinimidyl 3-maleimidopropionate (SMP, 2x10⁻³ M) in DMF and 1 mM peptide in DMF [2]. BMSCs were seeded on β-TCP, BMP/β-TCP, and CMP/β-TCP scaffolds and incubated in an osteogenic medium. Cell proliferation, ALP and DNA quantification assay, q-PCR analysis for collagen type I (COL-I), osteopontin (OPN), and osteocalcin (OCN) genes were evaluated on β-TCP, BMP/β-TCP, and CMP/β-TCP groups by 7d, 14d, 21d, and 28d to compare the effect on osteogenic differentiation of hMSCs. Immunofluorescent staining will be performed to stain COL-I, OPN, and OCN proteins.

Results and Discussion

FTIR results showed that CAP plasma was generated -OH groups with oxidative effect due to the presence of reactive oxygen species and the broad bands appeared at around 3600 and 3000 cm⁻¹ due to the presence of hydroxyl and alkyl groups. Furthermore, a change in the absorption bands at 1460–1200 cm⁻¹ was observed by the effect of nitrogen introduction caused by peptide conjugation. The cell number (Figure 1 A) and DNA content (Figure 1 B) of
all groups increased slightly with incubation time. The cell number and DNA amounts of CMP/β-TCP groups at each time point were higher than other groups. However, the ALP activity of all other groups incubated in osteogenic medium peaked at day 14 and returned to baseline level at day 28 (Figure 1 C). The ALP activity was maximum on CMP/β-TCP groups compared to other groups. The mRNA expression of osteogenic markers such as COL-I, OPN, and OCN were higher in CMP/β-TCP groups compared to BMP/β-TCP and β-TCP according to real-time-PCR amplification. Cell proliferation, DNA, ALP assay, and q-PCR analysis results indicated that CMP sequence is more effective than BMP sequences. The expression pattern of osteogenic markers such as COL-I, OPN, and OCN proteins will be also investigated by immunofluorescent staining to confirm the result.

**Conclusion**

The outcomes of this study would help to enhance biomaterial’s function with the most effective sequence on human MSCs on osteogenic differentiation for further modifications of the synthetic scaffolds for bone tissue engineering. Furthermore, a new strategy to modify surfaces of β-TCP with cold atmospheric plasma (CAP) could be used to enhance bioactive peptide conjugation.

**Acknowledgement**

The authors would like to thank TÜBİTAK (The Scientific and Technological Research Council of Turkey) through the Research Project 117S429 for providing financial support to this project.

---

**Figure 1**

(A) Cell number (B) DNA Content (C) ALP Activity of hMSCs seeded β-TCP, BMP-β-TCP, and CMP-β-TCP and incubated in osteogenic medium for up to 28 days. Error bars represent mean ± SE (n = 5) [significant differences were determined by one-way ANOVA [Newman-Keuls multiple comparison test, (*p<0.05, **p<0.01, ***p<0.001)].

**References**


Incorporation of nanoparticles into PEOT/PBT electrospun scaffolds for the treatment of bone fractures

Clarissa Tomasina¹, Giorgia Montalbano², Priscila Melo², Sonia Fiorilli², Paulo Quadros³, António Azevedo³, Chiara Vitale-Brovarone², Sandra Camarero-Espinosa¹, Lorenzo Moroni¹

¹ MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, NL; ² Department of Applied Science and Technology, Politecnico di Torino, Torino, IT; ³ FLUIDINOVA S.A., Rua Eng. Frederico Ulrich 2650, Maia, PT

Introduction
Osteoporosis is a bone disease that leads to the loss of bone mass and mineral density making the tissue brittle and increasing the risk of fractures. In particular, osteoporotic pelvic fractures require the patient to undergo extensive surgeries and long bed recovery.

To regenerate the fracture site, we employed solution electrospinning, a technique able to generate nanofibers that resemble the bone extracellular matrix, and that allows also the creation of composite mats by embedding particles during the process. Mesoporous bioactive glasses (MBGs) and hydroxyapatite (HA) particles have both proved to promote bone regeneration, offering a suitable platform for the delivery of ions for osteoporosis treatment such as strontium.

Hence, we fabricated a composite scaffold made of PEOT/PBT with MBGs and HA doped with strontium to boost bone regeneration in pelvic osteoporotic fractures.

Experimental Methods
Two types of MBGs (MBG_SD and MBG_SG doped with 10% strontium) and HA (nanoHA non doped and doped with 50% strontium) were sonicated using a horn sonicator and the morphology, hydrodynamic radius and ion release were characterized by transmission electron microscopy (TEM), dynamic light scattering (DLS) and inductively coupled plasma-mass-spectrometry (ICP-MS), respectively.

For scaffold fabrication, 300PEOT55PBT45 was dissolved at a concentration of 28 wt% in a mixture of chloroform: hexafluoro-2-propanol (75:25) for 24 hours and particles were consequently added to the polymer in concentrations ranging from 5 w/v% to 15 w/v%, and left stirring for 1 hour. Initial electrospinning tests were conducted at a distance of 10-15 cm using a plate collector with voltage and flow rate ranging from 18,5 to 20 kV and 150 to 50 ul/h, respectively. For investigation of the morphology, composition and particle distribution, electrospun scaffolds were imaged using a Jeol JSM-IT200 InTouchScope scanning electron microscope (SEM) at V=10kV equipped with an energy dispersive X-ray analyzer (EDX). Furthermore, Fourier-transform infrared spectroscopy (FTIR) was also performed to remark the presence of particles at different concentrations in the mats.

Mechanical tests were performed on fibrous mats by uniaxial tensile strength using a TA ElectroForce equipped with a 45 N load cell.

Finally, scaffolds were evaluated for their biocompatibility through in-vitro tests and seeded with hMSC at passage 5. hMSCs were cultured for 35 days in maintenance and osteogenic media. At day 14 and 21, ALP assay was performed as an early indicator of osteogenic activity. At day 35, we performed Alizarin red for calcium deposits, immunofluorescence for collagen I, osteocalcin and osteopontin, SEM and qPCR.

Results and Discussion
Particles were sonicated from 5 to 10 min using the horn sonicator, resulted well dispersed after 6 min, and were stable in the electrospinning solvent. MBGs and HA were then embedded in the scaffolds after sonication up to 12,5 w/v% and 15w/v% respectively. In both cases, at the maximum concentration, the particles showed small aggregates.
up to 10 um. The scaffold fibers resulted to have a diameter of $620.07 \pm 111.7$ nm and $356.3 \pm 70.14$ nm for MBGs and HAs, respectively, which was expected considering the size of the two particles. Their incorporation in the scaffolds was further confirmed by EDX and FTIR. Mechanical tests revealed a young modulus below 10 MPa for the scaffolds.

hMSC were cultured on the scaffolds during 35 days, showing at day 14 and 21 higher levels of ALP expression in the composite scaffolds compared to the ones without particles. At day 35, a dense extracellular matrix coverage in composite samples through SEM in osteogenic media as well as an increase in calcium deposits through alizarin red. These results, together with qPCR analysis, prove the biocompatibility and bioactivity of the fibrous mats and their ability to support cell adhesion.

**Conclusion**

PEOT/PBT was successfully spun with two different sizes of MBG and HA particles which were embedded in the mats until 12.5 w/v% and 15 w/v%. The composite scaffolds were morphologically, chemically and mechanically characterized as well as investigated in-vitro for their bioactivity. This characterization highlights the potential for these scaffolds to promote bone regeneration in osteoporotic fractures.

**Acknowledgement**

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 814410.
Engineered 3D-printed vascularized hydrogels for efficient presentation of growth factors

Oana Dobre¹, Ruoxiao Xie², Liliang Ouyang³, Molly M. Stevens², Manuel Salmeron-Sanchez¹

¹ University of Glasgow, Centre for the Cellular Microenvironment, Glasgow, GB; ² Imperial College London, Department of Materials, Department of Bioengineering, Institute of Biomedical Engineering, London, GB; ³ Tsinghua University, Department of Mechanical Engineering, Beijing, CN; ⁴ University of Glasgow, Centre for the Cellular Microenvironment, Glasgow, GB

Introduction

Bone is the second most transplanted tissue after blood and can regenerate by itself to a certain degree, but in cases of critical size defect, an intrinsic capacity of self-regeneration and consequently bone repair is delayed and impaired. This type of lesion is termed non-union bone fracture and requires additional treatment with bone grafts materials in order to restore pre-existing function¹. There is therefore an urgent unmet clinical need for personalized scaffolds that promote the regeneration of large bone defects. Different material-based strategies are being explored to improve the efficiency of growth factor (GF) delivery by recapitulating the characteristics of extracellular matrices for applications in regenerative therapies, including large bone defect repair and regeneration caused by trauma, disease or tumor resection². Vascularization is important for bone grafts to properly integrate at the injury site however it is being underestimated in current management strategies¹.

The aim of this study is to develop a bioink system by incorporating PEGylated full-length laminin (LM) into gelatin methacryloyl (GelMA). The incorporation of LM allows increased availability of cell and GF binding domains. LM is an extracellular protein composed of three chains α, β and γ, that plays a role in multiple cellular process. LM has 16 isoforms with different affinities for various GFs. The abundance of every LM isoform is tissue-dependent³, suggesting that LM has tissue-specific roles. To demonstrate the application of this bioink system in biofabrication and regeneration, 3D vascularized bone tissue will be fabricated by printing LM/GelMA hydrogel with mesenchymal stem cells (MSCs) and human umbilical vein endothelial cells (HUVECs) via extrusion-based bioprinting.

Experimental Methods

3D LM-based bioinks containing different concentrations of PEGylated human LM, GelMA and a photoinitiator (see Figure 1A, B and C) were printed using a multi-nozzle 3D Bioprinter⁴. Then the printed structures were crosslinked via photopolymerization as shown in Figure 1D. Mechanical properties of the bioinks with and without LM with different concentrations of GelMA were characterized by shear bulk rheology. hMSCs and HUVECs with the addition of specific growth factors such as vascular endothelial growth factor (VEGF-A165) and bone morphogenetic protein-2 (BMP-2) were incorporated into the LM bioinks to evaluate cell cytotoxicity, study their release kinetics and potential to drive vasculogenesis and osteogenesis.

Results and Discussion

We investigated the possibility of 3D LM/GelMA to promote bone regeneration by delivering growth factors in a controlled manner. To do this, 3D LM/GelMA with tuneable stiffness and different concentrations of LM 332 were successfully printed to mimic the native bone microenvironment. Other properties such as GF release kinetics and biocompatibility of the bioinks were evaluated to determine if our system could be used as an alternative biomaterial for bone regeneration. Protein (immunofluorescence) and gene expression (qRT-PCR) results of co-culturing hMSCs and HUVECs encapsulated in 3D LM/GelMA bioinks loaded with BMP-2 and
VEGFA-165 showed upregulated expression of osteopontin (OPN) and endothelial cell adhesion marker CD31 after 14 days.

**Conclusion**

These novel bioinks combine LMs with GFs, which are powerful signalling molecules that direct cell response, promoting the regeneration of target tissue that can be used to engineer in vitro tissue models. We report on the development and characterization of 3D LM/GelMA hydrogels with tuneable mechanical and highly efficient growth factor presentation for potential applications such as regeneration of bone defects with an adequate vascularisation.

**Acknowledgement**

This work was completed with support from the Engineering and Physical Sciences Research Council (EPSRC) Grant EP/P001114/1.

---

**Figure 1.**

LM bioinks chemistry. A. LM structure with hypothesized GF binding sites; B) Gelatin methacryloyl structure; C) Photoinitiator (irgacure) structure; D) LM/GelMA gel formulation schematic drawing.

**References**


S14-05
Spheroid laser printing as a tool for high throughput creation of pre-micro vascularization patterns

Charles Handschin\textsuperscript{1,2}, Clotilde Jugie\textsuperscript{1,2}, Leo Comperat\textsuperscript{1,2}, Nathalie Dusserre\textsuperscript{1,2}, Marie-laure Stachowicz\textsuperscript{1,2}, Hugo De Oliveira\textsuperscript{1,2}, Jean-Christophe Fricain\textsuperscript{1,3}

\textsuperscript{1} Inserm, U1026, BioTis, Accélérateur de recherche technologique BioPrint, Bordeaux, FR; \textsuperscript{2} Université de Bordeaux, U1026, BioTis, Bordeaux, FR; \textsuperscript{3} CHU Bordeaux, Services d’Odontologie et de Santé Buccale, Bordeaux, FR

Introduction
It is well established that the survival and integration of large biofabricated tissues is directly associated with the capacity to achieve rapid oxygenation and nutriment supply. However, the effective fabrication of hierarchical vascularized networks remains a main challenge in the fields of tissue engineering and biofabrication. Many strategies have been developed to overcome this barrier. In particular, numerous approaches have been deployed to design and biofabricate vascularization networks at the macro or mesoscopic scale (Datta et al., 2017). However, the complex architecture of vascularized networks at the microscopical scale has been less studied. This is partly due to the lack of direct applications, but is mostly a result of the technical complexity required to achieve its fabrication and control its evolution from the original design to the resulting function in 3D (Richards et al., 2017). With the advent of new microscopic 3D cell models and advanced micro tissues, the ability to control biofabricated microvascularized networks appears not only relevant but increasingly required.

In this context, we propose a new method to bioprint 3D pre-microvascularisation models, based on the replication of simple endothelial cell (EC) geometries in a microenvironment containing fibroblasts. Bioprinting ECs in coculture with fibroblasts has been largely used in the past. One of the main limitations of previously proposed models lies in a loss of the geometry resolution, at the microscopic scale, during maturation (Kolesky et al., 2014). Here, we used a laser-assisted bioprinting technology to deposit small EC spheroids (tens of micrometers in diameter), in a controlled fashion, within a biocompatible hydrogel (Hakobyan et al., 2020). By controlling the positioning of the spheroids in different 3D conditions, we evaluated the pertinence of this method in terms of cell maturation and geometry resolution.

Experimental Methods
A laser-assisted bioprinter (Novalase) was used in this study (Guillemot et al., 2010). The instrument integrated a nanosecond laser (1064 nm, 30 ns, 7 W, 1-100 kHz), an optical scanner (SCANgine 14, ScanLab), a system of sample positioning, and a graphical user interface that was used to simultaneously control all these elements and to generate various patterns. Human umbilical endothelial cells (HUVEC), expressing a Red Fluorescent Protein, were cultured and amplified in standard conditions, then trypsinized and concentrated at 70.10^6/mL to create the bioink. 33 µL from this bioink were spread on a 30 mm diameter glass plate, previously coated with a thin gold layer and playing the role of a donor. As a receiver, 150 µL of 5% (w/v) methacrylated gelatin (GelMa) were spread on a thin glass slide before bioprinting. The consistency of the GelMa was critical to enable the 3D microinjection of the spheroids inside the hydrogel, since more rigid hydrogels only lead to surface printing. A special compromise was found between the laser pulse energy and the printing distance (donor-receiver) as to control the depth of the spheroid deposition inside the GelMa. Typical parameters were a pulse energy of 60 µJ and a printing distance of 700 µm. To study HUVEC maturation after printing, human skin fibroblasts (HSP) were cultured, amplified and loaded at various concentrations within the GelMa (i.e. 5, 10, 15, 20 and 25 millions/mL) prior to printing. The observation of
the resulting maturation was conducted through confocal microscopy, 15 days post printing. Stacks of images were analyzed using Imaris to reconstruct the pre-vascularization in 3D.

Results and Discussion
We first assessed the spheroid volumes as a function of laser energy. We showed that we were able to control the size of the bioprinted spheroids. We thus adjusted the parameters to get a spheroid diameter about a few tens of micrometers. In order to facilitate the analysis, 10 to 15 lines of spheroids, spaced by 2 mm, were printed for each sample. This allowed us to easily quantify and compare the number and the directionality of the pre-vascularization connections generated in different conditions. The highest concentration of HSFs tested induced a decrease in the number of connections, resulting in better connection interactions between individual spheroids within the printed lines, and along the line direction. Keeping these optimized conditions, we finally exploited the bioprinting high throughput capability and versatility to create more complex geometries.

Conclusion
We have established a new approach to biofabricate microvascularized networks with a printing resolution close to 50 µm. These networks can be replicated on demand, with any desired geometry. This innovative laser-assisted bioprinting technique enabled us to increase by a factor of 10 the post-printing resolution compared to 2D laser printing, while allowing the deposition of very high localized cell concentration in 3D, a key parameter to support cell maturation in 3D.

Figure 1
3D Imaris reconstruction of HUVEC bioprinted spheroid.

References
3D bioprinting of vascularized bone with inherent spatial controlled release of osteogenic and angiogenic factors

Meric Goker, Pinar Y. Huri
Ankara University, Biomedical Engineering, Ankara, TR

Introduction
Bone tissue is capable of self-repair after a fracture or a damage. Yet, critical-size defects may remain unrepaired and filler materials is used in order to support the defect site structurally and mechanically [1]. In this study, 3D printed vascularized grafts were produced with and inherent spatiotemporal growth factor delivery system, where ASCs were utilized as the only cell source to produce the complex structure. The ASC osteogenic differentiation, and vascular network formation by the endothelial cells within the ASC population was provided by controlled spatial release of BMP-2 and VEGF, respectively. Growth factor-laden microparticles were placed in predetermined positions during 3D printing for the controlled formation of mineralized tissue and vessels (Figure 1).

Experimental Methods
The 3D model was uploaded to the 3D Bioplotter (Envisiontec) which has two simultaneous print heads. One of the heads was used to 3D print poly(ε-caprolactone) (PCL) to provide the mechanical stiffness. The other print head was used to print alginate which carries microparticles that are loaded with BMP-2 and VEGF. Release kinetics from the hybrid grafts was studied by using a model protein. 3D printed samples were seeded with ASCs, cultured in vitro and then fixed on days 7, 14 and 21 for immunofluorescence staining for osteopontin and CD31.

Results and Discussion
Print parameters of the PCL/alginate hybrid scaffolds were optimized including linear speed of the head, pressure, temperature, and concentration of the polymer. Highest resolution for the 3D print was achieved with 2mm/s linear speed and 0.5 bar pressure for alginate. Different concentrations varying from 1% to 4% w/v were tested for determining the printability of capsule-laden alginate solution. It was observed that 2% w/v is preferable as alginate concentration in terms of printability and embeddability of capsules.

Results of immunofluorescence staining from the same sample reflected that vascularization in the canal section and osteogenesis in the rest of the scaffold was successfully induced due to spatiotemporal delivery of growth factor cues (Figure 2).

Conclusion
With this work, it was aimed to fabricate an off-the-shelf construct produced from simpler components (PCL as the only load bearing component, alginate as the bioink material and ASCs as the single source of cells) to produce a vascularized bone graft. A novel approach was proposed, and 3D printed to mimic the complex architecture of the tissue. The spatial delivery of BMP-2 and VEGF was applied by positioning capsules release them within the specific regions of the 3D printed construct. In conclusion, this work offers a novel yet simple vascularized bone graft production procedure that can possibly be adapted to the clinic in the future.

Acknowledgement
This study was funded by The Scientific and Technological Research Council of Turkey (Project No: 119S131). We would like to thank METU BIOMATEN for CLSM use.
Figure 1
Design of the scaffold architecture; red areas represent osteogenic tissue formation within alginate fibers from BMP-2 release where white canals portray alginate fibers that generate angiogenesis by the aid of localized delivery of VEGF.

Figure 2
CLSM images of dual growth factor delivered taken from the same scaffold on day 14 under (a) and (c) 5x magnification (Scale bar = 200µm), (b) and (d) under 100x magnification (Scale bar = 50µm). Channels; red: Osteopontin, green: CD-31, blue: DAPI.

References
Laser Directed Energy Deposition: new perspectives in biomaterials fabrication for the dental prosthetics field

**Oscar Barro**¹², Felipe Arias-González³, Fernando Lusquiños⁴, Rafael Comesaña¹⁵, Jesús del Val¹, Antonio Riveiro¹⁵, Félix Gómez-Baño², Juan Pou⁴

¹ University of Vigo, LaserOn Research Group, Vigo, ES; ² Corus-Fegoba, A Coruña, ES; ³ Universitat Internacional de Catalunya, School of Dentistry, Barcelona, ES; ⁴ SERGAS-UVIGO, Galicia Sur Health Research Institute, Vigo, ES; ⁵ University of Vigo, Materials Engineering, Applied Mechanics and Construction Department, Vigo, ES

**Introduction**

Dental restorations have experienced a great development throughout the last decades. Historically, the most employed materials have been gold and its alloys. However, due to the high cost of these materials, non-precious metallic alloys like Co-Cr alloys appeared. Nowadays, Co-Cr alloys are the predominant preference for dental fixed restorations in the dental industry. There are several techniques currently employed for manufacturing these restorations, being the milling technique the one that gives better mechanical properties. However, in the recent years, some additive manufacturing technologies have emerged in this industry due to the benefits of the free form generation. Co-Cr alloys are expensive materials, therefore, additive manufacturing technologies allowing reduction of material waste, are very interesting alternatives in this field. The Laser Directed Energy Deposition (LDED) is a technique which can be implemented in the dental industry, merging the advantages of Additive Manufacturing technologies and the high overall performance of the materials generated by this technique.

The main objective of this study is to evaluate the viability of the Laser Directed Energy Deposition (LDED) technique as a manufacturing alternative for the metallic structure of the dental prostheses. To do so, LDED has been compared with the manufacturing techniques currently employed in the dental industry: lost wax casting, milling from commercial disks and Selective Laser Melting (SLM).

**Experimental Methods**

Four manufacturing techniques were employed to manufacture the samples: lost wax casting, LDED, milling from commercial disk, and SLM.

Specimens obtained by casting (CAST) were obtained using a centrifugal casting machine by melting ingots of Heraenium Pw alloy with a casting torch.

Specimens obtained by Laser Directed Energy Deposition (LDED) were generated using a proprietary LDED manufacturing device with a high power near-infrared continuous wave laser used as energy source. Co-Cr Starbond Easy Powder 30+ powder was used as precursor material. The near-net-shape specimens were machined until final dimensions in a commercial CAD/CAM milling system. The angle between tensile tests directions and building direction was 90°.

Milled specimens were obtained from Co-Cr Kera® dental discs using a CAD/CAM milling machine. The angle between tensile tests directions and axial disc direction was 90°.

Selective Laser Melting (SLM) specimens were generated using an EOS M 270 machine, with a high power near-infrared continuous wave laser used as energy source. The material employed was Co-Cr EOS SP2 powder. The angle between tensile tests directions and building direction was also 90°, as in the LDED alloy.

**Results and Discussion**

Microstructural analyses (Fig.1) showed that samples obtained from different techniques possess different microstructures with different segregation behaviors. CAST specimens showed a typical dendritic matrix with high
segregation size. LDED samples showed columnar grains with reduced intergranular segregation. Mill samples are composed by equiaxial grains with segregations aligned with the axis of the disc. SLM samples showed small cellular grains with a homogeneous segregation evenly distributed along the whole material. Mechanical results (Fig.2) demonstrate that the materials obtained by LDED possess a similar behavior of the milled disks material. Both materials performed better than the ones obtained by casting and SLM, absorbing nearly twice the energy before rupture, as well as a significant elongation after fracture in comparison with materials obtained by casting or SLM. It should be noted that all materials comply with the type 4 of ISO 22674 ISO standard for dental materials, which is recommended for dental fixed restorations.

Conclusion

LDED is an additive manufacturing technique that can be implemented in the restorative dental industry with high overall performance. This manufacturing process can compete directly with the best quality techniques, and taking the best part of each of them, mechanical properties from milling disk, and freeform generation from SLM processes.

Acknowledgement

This work was partially supported by the EU research project Bluehuman (EAPA_151/2016 Interreg Atlantic Area), Government of Spain [CLADDENT project _CDTI, RTI2018-095490-J-I00 (MCIU/AEI/FEDER, UE)], and by Xunta de Galicia (ED431C 2019/23, ED481D 2017/010, ED481B 2016/047-0).

The technical staff from CACTI (University of Vigo), SAI (University of A Coruña) and AGACYP (University of Vigo) is gratefully acknowledged.

Figure 1

SEM micrographs showing a longitudinal section of each fabrication technique: CAST (a), LDED (b), MILL (c) and SLM (d). Images at x100 (1) and x500 (2) of magnification.

Figure 2

Mechanical properties of the different techniques: yield strength (a), ultimate tensile strength (b), Young modulus (c), modulus of toughness (d), elongation after fracture (e), and microhardness (f). Error bars indicate the standard error of the mean.
11:45 a.m. – 1:15 p.m.

Track04

S15 | Regeneration of the Intervertebral Disc: The Battles that Biomaterials Will Have to Win

Chairs
Mauro Alini
AO Research Institute, Davos Platz, CH
Raquel M. Goncalves
University of Porto, Porto, PT
Cristina Lopez Serrano (YSF)
Université de Bordeaux, CBMN UMR5248, Pessac, FR

Learning Objectives:
- Biomaterials that mimic the IVD extracellular matrix
- Biomaterials that induce rejuvenation of the microenvironment
- Biomaterials that control inflammation in IVD
- Exploring the pluripotency of resident cells
Understanding the Regenerative Response Induced by Biomaterials Systems: Insight into the Role of Glycosylation

Abhay Pandit

National University of Ireland, Galway, CÚRAM-SFI Research Centre for Medical Devices, Galway, IE

Biomaterials are no longer considered innate structures and using functionalisation and biofabrication strategies to modulate the desired response whether it is a host or implant is currently an important focus in current research paradigms. Fundamentally, a thorough understanding of the host response will enable us to design appropriate strategies. The input from the host response needs to be weighed in depending on the host disease condition. Our current inputs have been through a deliberate understanding of glyco-proteomics based tools that we are developing in our laboratory.

In addition, biomaterials themselves provide immense therapeutic benefits which need to be accounted for in the design paradigm. Using functionalisation strategies such as enzymatic and hyperbranched linking systems, we have been able to link biomolecules to different structural moieties. The programmed assembly of biomolecules into higher-order self-organized systems is central to innumerable biological processes and the development of the next generation of scaffolds. Recent design efforts have utilized a glycobiology and developmental biology approach toward both understanding and engineering supramolecular protein and sugar assemblies.

Acknowledgement

Financial support of Science Foundation Ireland (SFI), funded under the European Regional Development Fund through Grant number 13/RC/2073_P2, EU Horizon 2020 Programs (Grant No:825925).

References


The iPSpine project: smart biomaterials escorting iPS cells to rejuvenate the degenerate disc

Marianna A. Tryfonidou

Utrecht University, Faculty of Veterinary Medicine, dept Clinical Sciences, Utrecht, NL

Over 40% of the cases of low back pain is due to degeneration of the intervertebral disc. In the Horizon 2020 iPSpine project, 21 partners joined forces to develop an advanced therapy medical product (ATMP) for the treatment of chronic low back pain caused by disc degeneration. For this strategy, induced pluripotent stem (iPS) cells are differentiated into notochordal-like cells (iPS-NCs). Notochordal cells (NCs) reside only in human juvenile discs. They have been shown to have high regeneration capacity. Biomaterials have in this strategy an essential role: they are designed to carry and also to provide iPS-NCs with the necessary cues and physically support them in the hostile degenerate disc environment. For this purpose, we follow a strategy inspired by nature. New (semi)synthetic biomaterials are developed based on components of naturally derived NC-derived biomaterials that have already been shown to have regenerative properties. iPS-NCs escorted by these biomaterials should thrive within the degenerate disc. Hereby iPSpine hopes to achieve biologic disc repair and ultimately offer improved quality of life for millions of patients, through long-lasting reduction of low back pain.

These technological advances are addressed within this multidisciplinary consortium. The project brings essential elements together in a chain of translation. This includes biomaterials and biomedical scientists working closely together with regulatory experts, biologists, and clinicians. Cell-biomaterial interactions are evaluated in a set of in vitro models with increasing complexity. Ultimately, bioreactor technology mimicking loading of the human intervertebral disc during daily life and proof-of-concept studies in large animal models of disc degeneration will be employed to test the potency of the iPS-NCs escorted by the biomaterials inspired by nature. During the keynote, I will elaborate on cornerstones of this iPSpine concept, the results of the consortium thus far, with particular focus on how the iPSpine consortium selects the best performing biomaterials in a collaborative effort.

Acknowledgement

This project received funding from the European Union's Horizon 2020 research and innovation program iPSpine under the grant agreement #825925 (www.ipspine.eu)
Younger decellularized matrices induce adult nucleus pulposus extracellular matrix *de novo* synthesis

Morena F. Fiordalisi\(^1,2,3\), Joana R. Ferreira\(^1,2,3\), Marta L. Pinto\(^4\), Cláudia Ribeiro-Machado\(^1,2\), Mário A. Barbosa\(^1,2,3\), Raquel M. Gonçalves\(^1,2,3\), Joana Caldeira\(^1,2\)

\(^1\) i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, PT; \(^2\) INEB – Instituto de Engenharia Biomédica, Porto, PT; \(^3\) ICBAS- Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, PT; \(^4\) CNC-Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, PT

**Introduction**

Intervertebral disc (IVD) degeneration and the consequent low back pain affect over 80% of the world's population\(^1\), constituting a tremendous socio-economic burden and largely impairing patient's life quality. Current therapies include conservative approaches (which only relieve pain and control inflammation) and surgical procedures (which have limited success and several associated risks). With the lack of effective and long-term treatments, tissue engineering strategies, particularly the use of decellularized extracellular matrix (ECM)-based biomaterials, has emerged with undeniable promise for IVD regeneration\(^2\). We have shown that fetal nucleus pulposus (NP) ECM is enriched in pro-regenerative proteins (Collagen type XII and type XIV), when compared to adult and aged tissues\(^3\). Thus, we evaluated the potential of fetal decellularized NP (dNP) – based scaffolds to promote ECM synthesis *ex vivo*.

**Experimental Methods**

IVDs collected from fetal (~8 months of gestation), young (~1 year old) and old (~12 years old) bovine tails, obtained from local abattoirs, were dissected and NP punches decellularized using an optimized SDS 0.1% - based protocol. After decellularization, NPs were characterized for: presence of cells and DNA content (DAPI staining and DNA quantification, respectively); biochemical composition (glycosaminoglycan (sGAG) quantification and Alcian Blue/Picro-Sirius Red staining, western blotting for the fetal proteins Collagen type XII and type XIV); structural organization (cryo-scanning electron microscopy) and biomechanical properties (rheology). Afterwards, NP cells were isolated from adult bovine IVDs, as previously described\(^4\), and used for the recellularization of dNPs from different ages. NP cells were seeded on top of the matrices and cultured *ex vivo* for 7 days, under hypoxia (6% O\(_2\) and 8.5% CO\(_2\)). After 24 hours of seeding, repopulation efficiency was assessed by DNA quantification. Metabolic activity was also monitored during culture using the resazurin assay. At day 7, the response of NP cells was evaluated in terms of cell survival (Live/Dead), and ECM production (collagen type II and aggrecan) at both the molecular and protein level. Finally, collagen changes were analyzed by Picro-Sirius Red staining, under polarize light and quantification of birefringent fibers.

**Results and Discussion**

An optimal decellularization method for bovine NPs from different ages was developed. This was efficient in removing cells and DNA while preserving native ECM architecture and composition, namely collagen (in particular, Collagen type XII and XIV) and to some extent sGAG. Fetal native NPs were shown to be softer than young and old ones and this trend was preserved after decellularization. Acellular matrices from different ages were able to support adult NP cell culture after recellularization. Nevertheless, fetal and young ECMs were characterized by higher DNA content after 24 hours of cell seeding and increased cell viability at day 7 of *ex vivo* culture, as compared to old ones. Bovine NP cells cultured on the fetal scaffolds showed the greatest collagen type II and aggrecan gene expression levels. At the protein level, collagen type II decreased, while aggrecan increased in both fetal and young scaffolds, when compared to old ones. This may indicate increased matrix remodeling. Finally, after repopulation, fetal ECMs showed...
an increased ratio of thicker (mature)/thinner (immature) collagen fibers than young and old ones. The biomechanical properties of repopulated matrices are currently being investigated.

**Conclusion**

Decellularized matrices from different ages were able to support adult NP cell culture. However, softer fetal NP – based scaffolds were shown to have an increased capacity to promote ECM de novo synthesis. This is the first report demonstrating a higher pro-regenerative potential of fetal NP matrices, thus opening new avenues for the development of minimally invasive IVD therapeutic strategies.

**Acknowledgement**

Supported by FCT: funds FCT/DL 57/2016/CP1360/CT0005-JC, PhD grant (PD/BD/135544/2018-MF) and project: (UIDB/04293/2020). This work was also financed by QREN and by a grant from the ON Foundation.

**References**

S15-04

Characterising Behaviour of Biomaterials Using Viscoelastic Models

Samantha Hayward, Anthony Miles, Patrick Keogh, Sabina Gheduzzi

University of Bath, Department of Mechanical Engineering, Bath, GB

Introduction

Biological materials typically exhibit non-linear viscoelastic behaviour, and this includes isolated intervertebral disc specimens (ISDs) [1, 2]. Loaded cyclically, ISDs produce non-linear, S-shaped hysteresis loops characterised by stiffening effects [1]. Fully defining the viscoelastic characteristics of spinal specimens under load is critical to understanding their biomechanical behaviour.

A method to tune non-linear viscoelastic models to the load-displacement behaviour of spinal specimens is proposed which improves on that introduced by Panjabi (1991) [2]. Viscoelastic models have been used to describe creep response of many materials under quasi-static or impact loading [3,4], but their use for cyclic loading is less documented.

The model comprising a non-linear Kelvin element is implemented as proof of concept (Figure 1), with the implication that this method could be applied to a variety of viscoelastic biomaterials to achieve their full characterisation.

Experimental Methods

Three two-vertebrae thoracic segments were obtained from porcine spines. The segments were dissected with musculature and all posterior elements removed. During testing, the specimens were wrapped in moist tissue followed by plastic wrap to prevent disc dehydration. A preload of 500N was applied with a 30-minute equilibration period prior to 6 axis stiffness testing at a constant rate of 0.1 Hz with displacement (u/θ) amplitudes (Figure 2) appropriate for the spinal level of specimens.

The proposed model was implemented in MATLAB R2020b using the SimScape library (Figure 1). The coefficients required to tune the model to the test data were determined using an optimization cost function defined by sum of squared errors (SSE) with a non-linear least squares optimization method and a Trust-Region-Reflective algorithm [5].

Results and Discussion

Figure 2 shows an example of the resulting fit obtained by the model for a single loading cycle in axial rotation. Fits of similar quality were observed for tension-compression, anterior-posterior shear, and lateral bending, though decreased fit quality was noted for flexion-extension and medio-lateral shear due to variability in experimental data.

The mean % difference between area enclosed by the model and experimental curves is below 4%, indicating the model's ability to represent dissipated energy.

Lower values of the optimization cost function and absolute maximum residuals at solution indicate a better model fit. These values can be used to compare the fit obtained for different specimens and by various model configurations.

Conclusion

The similarity between the model and experimental curves demonstrates potential for using MATLAB’s SimScape to characterize viscoelastic behaviour of biomaterials. Further work should include additional model configurations and a larger sample size of data.

Acknowledgement

The authors wish to express their gratitude to Enid Linder Foundation for their continued support of this PhD project.
Figure 1
Proposed viscoelastic model as implemented in SimScape

Figure 2
Example of fit obtained by the model in axial rotation

References
Disclosing new therapeutic targets in disc degeneration: insights from a pro-inflammatory intervertebral disc organotypic culture

Joana R. Ferreira¹,²,³, Catarina Meireles¹, Jaime Freitas¹,², Mário A. Barbosa¹,²,³, Meriem Lamghari¹,²,³, Graça Almeida-Porada⁴, Raquel M. Gonçalves¹,²,³

¹ i3S, Porto, PT; ² INEB, Porto, PT; ³ ICBAS, Porto, PT; ⁴ WFIRM, Winston-Salem, US

Introduction
Cell therapies are emerging as regenerative therapies to treat intervertebral disc (IVD) degeneration, particularly those using Mesenchymal Stem/Stromal Cells (MSC). Yet, the effects of MSC on target IVD cells is still unknown, precluding their fully clinical translation. Due to their close similarity to human, bovine organotypic cultures can be used to disclose MSC mechanisms of action in degenerated IVD. As such, using a standardized bovine IVD organ culture under pro-inflammatory conditions [1] we have demonstrated that MSC exert an immunomodulatory effect through paracrine intercellular communication [2]. A similar effect was observed with the MSCs secretome, that further potentiated aggrecan deposition [3]. However, IVD cellular therapeutic targets are still poorly understood. In this study we propose to investigate IVD's cell target profile of an MSC-based immunomodulatory therapy for IVD.

Experimental Methods
Bovine nucleus pulposus (bNP) were isolated from 1-yr old animals immediately after sacrifice, needle-punctured and cultured in the presence of IL-1b, as described [1]. After 48h, the NP cell phenotype was evaluated by flow cytometry, as described [4]. bNP gene expression was evaluated in IVD organ control cultures vs IL-1b-stimulated conditions, up to 14 days. The distribution and co-localization of bNP cell surface markers in response to IL-1b, were evaluated by immunofluorescence staining. IL-1b-stimulated bNP cell surface marker distribution and gene expression were evaluated upon treatment with MSC secretome.

Results and Discussion
IL-1b-stimulation of bNP organ cultures significantly increased CD44 surface expression from 40 to 80%. This was confirmed at the transcriptomic level, with an upregulation of CD44 gene expression, accompanied by CD14, IL-6 and IL-8. All the CD44+ bNP cells co-express CD14 and CD45, while only 60% of the CD44- bNP cells are CD14+CD45+. Fresh bNP cells were sorted based on CD44 expression, revealing that CD44+ bNP cells express more IL-8 and TIMP1, while CD44- cells express higher levels of MMP2, MMP3, ADAMTS5, TIMP2, AGG and COL2. When treated with MSC secretome, bNP inflammatory markers (IL-6, IL-8, PGE2) decreased, and was accompanied by reduced CD14 gene expression, but not CD44. These results are currently being investigated at the protein level.

Conclusion
The results obtained suggest that CD44+ bNP cells are associated to the inflammatory process, while CD44- cells reflect extracellular matrix's dynamics. This study discloses CD44 as an important therapeutic target on the degenerative IVD, revealing a sub-population of bNP cells with a macrophage-like signature, possible to be targeted by MSC secretome therapy for IVD.

Acknowledgement
This work was financed by EUROSPINE TRF (2017_05) through the project “Disc degeneration-, immune- and neuro-modulation" and by FCT–Fundação para a Ciência e a Tecnologia, in the framework of JRF PhD grant (PD/BI/128357/2017).

References

[3] Ferreira JR et al. (2021) "IL-1b-pre-conditioned Mesenchymal Stem/stromal Cells’ secretome modulates the inflammatory response and aggrecan deposition in intervertebral disc". eCM (Accepted for publication)

N14 | Electrospun and Electrowritten Biomaterials

Chairs
- Riccardo Levato
  Utrecht University, Utrecht, NL
- Paula A. Marques
  University of Aveiro, PT
- Gregor Miklosic (YSF)
  AO Research Institute Davos, Regenerative Orthopaedics, Davos, CH
Controlling angiogenesis via functionalized electrospun scaffolds: H$_2$S donors and VEGF peptide mimetics

Tianyu Yao$^1$, Rebeca Rivero$^1$, Ryan Carrazone$^2$, Lorenzo Moroni$^1$, John Matson$^2$, Matthew Baker$^1$

$^1$ Maastricht University, Maastricht, NL; $^2$ Virginia Tech, Chemistry, Blacksburg, US

Introduction
Control over angiogenesis of tissue engineering implants remains a major challenge. In order to drive and control angiogenesis, one must mimic both the physical and chemical cues found in the native extracellular matrix (ECM). While most successful angiogenesis approaches use powerful natural materials (fibrin, Matrigel) and growth factors (VEGF), a simple and fully synthetic approach is lacking. Electrospinning allows a simple and scalable approach to creating nanofibers like found in the native ECM; however, electrospun scaffolds need closely engineered chemical functionality to control angiogenesis. By engineering in small functional handles into electrospun scaffolds, we envisioned that we could incorporate either small peptide mimetics of VEGF, or novel H$_2$S releasing agents in order to stimulate angiogenesis. These simple synthetic systems can provide a route toward controlled angiogenesis.

Experimental Methods
Small (2k) bifunctional polymers of PCL were synthesized with either azide or acrylate end groups for further functionalization. By mixing in small amounts of these functional polymers into higher molecular weight PCL (80k), controlled compositions of functionalized electrospun fibers could be created. Fluorescence dyes were used to test the functionalization of the fibers post electrospinning, and then series of functional scaffolds were made with either a QK mimetic peptide or NTA, a novel H$_2$S releasing agent. HUVECs were cultured on the functional scaffolds and proliferation, metabolic activity and endothelial markers were measured. Promising scaffolds were then tested in the chick chorioallantoic membrane assay (CAM).

Results and Discussion
Mixing in the functionalized polymers was able to create a series of electrospun fibers with different density of functional groups, but similar fiber diameters and morphologies. In the scaffolds with the acrylate functionality, light-activated thiol-ene chemistry was able to controllably functionalize and pattern the scaffolds. In the scaffolds with the azide functionality, azide-alkyne cycloaddition was successful in controlled functionalization of the scaffolds. Both the QK peptide (thiol-ene) and the NTA (azide-alkyne, H$_2$S donor) functionalized scaffolds showed a dose-dependant response in vitro for the proliferation and growth of HUVECs. Also, both the QK peptide and the NTA scaffolds showed an increase in vessel growth in the CAM assay, with the NTA scaffolds showing slightly better early results.

Conclusion
Both the scaffold with the peptide mimetic QK and the newer gasotransmitter H$_2$S releasing agent showed promise to control angiogenesis in vitro and in ovo. Though a direct comparison is difficult, early results showed that the H$_2$S approach is surprisingly powerful compared alongside the QK mimetic. This study shows the potential power of the poorly studied gasotransmitters in control of angiogenesis in tissue engineering scaffolds.

Acknowledgement
Province of Limburg, China Scholarship Council
Functionalization of electropun scaffolds with H2S donors stimulate angiogenesis
Hydrogel-based bioinks for cell electrowriting of well-organized living structures with micrometer-scale resolution

Miguel Castilho\(^1\), Riccardo Levato\(^1\), Paulina Nunez Bernal\(^1\), Mylène de Ruijter\(^1\), Christina Sheng\(^1\), Joost van Duijn\(^1\), Susanna Piluso\(^1\), Keita Ito\(^2\), Jos Malda\(^1\)

\(^1\) University Medical Center Utrecht, Orthopeadics, Utrecht, NL; \(^2\) Eindhoven university of technology, Biomedical, Eindhoven, NL

Introduction
Since its first appearance in the early 21st century, bioprinting technologies have come a long way to create biological structures for regenerative medicine and in vitro drug testing. Such applications require the creation of functional biological structures. To do so, it is desirable to provide a microenvironment suitable for the growth of desired cell types. While conventional 3D bioprinting technologies excels at creating larger, anatomical like structures (from 100 of microns to the cm scale), it lacks the ability to recreate the architecture of the niche surrounding living cells at a microscale (submicron up to tens of micron). Attempts have been made to create such cellular microenvironments by using non-conventional biofabrication processes, such as electrospinning and electrohydrodynamic jetting processes. However, due to the lack of biologically relevant material platforms, neither of these techniques could simultaneously meet the requirements to reproduce the intrinsic morphologies and local composition of cellular microenvironments, i.e. the three-dimensional (3D) patterning, the deposition of fibers with micron/ sub-micron size diameters and the maintenance of high cell viability. Here, we describe the precise patterning of cell-laden, microscale biomaterial fibers via a new hydrogel-based bioink platform for Cell ElectroWriting (CEW).

Experimental Methods
Two photocrosslinkable bioinks based on proteinaceous polymers, i.e., gelatin and silk fibroin, were developed using visible light-mediated thiol-ene click reactions and di-tyrosine oxidation photo-chemistries. Both bioinks were optimized for gelation kinetics, viscosity and electrical conductivity in order to obtain stable fibers with reproducible fiber diameters and ordered 3D structures. Their processing compatibility was systematically investigated according to key electrowriting process parameters (e.g. voltage, collection speed, dispensing pressure) using an in-house built electrohydrodynamic set-up. Subsequently, swelling and mechanical characteristics of CEW scaffolds were investigated and benchmarked against scaffolds of the same composition prepared by conventional extrusion-based printing. Finally, the ability to pattern gelatin and silk fibroin hydrogels into 3D organized microfiber networks with encapsulated cells was assessed. For this, bone marrow-derived mesenchymal stromal cells (MSCs) were used, and cell viability, distribution and morphology was studied.

Results and Discussion
This process offers opportunities to mimic the hierarchical structure and composition of native tissues, which cannot be realized with the existent conventional bioinks and biofabrication technologies. We engineered two novel photo-responsive hydrogel bioinks based on protein-based polymers with different gelation chemistry, i.e., gelatin and silk, that are compatible with electrodeposition principles and simultaneously support and maintain living cells. The rapid photo-mediated crosslinking mechanisms, electrical conductivity and viscosity of these two engineered bioinks allow the fabrication of 3D ordered fiber constructs with small pores (down to 100 µm) with different geometries (e.g. squares, hexagons and curved patterns) of relevant thicknesses (up to 200 µm). Importantly, the biocompatibility of the gelatin- and silk fibroin-based bioinks enable the fabrication of cell-laden constructs with reduced fiber sizes (5 to 40 µm), while maintaining high cell viability post-printing. Another important implication of our results is the
potential development of multiple tissue type microenvironments as the investigated stem cells possess the ability to differentiate towards different lineages (e.g. myoblasts, tendon and neural-like cells) which we are currently exploring.

**Conclusion**

In summary, the unique biocompatibility of both the gelatin and silk materials, the promising mechanical performance when compared with conventional extrusion bioprinting, the reduced cell-laden fiber sizes and the unprecedented resolution and patterning precision, open promising avenues for the high-resolution printing of labile biological moieties and living cells.

**Acknowledgement**

This research was supported by the Gravitation Program “Materials Driven Regeneration”, funded by the Netherlands Organization for Scientific Research (024.003.013) and also the strategic alliance University Medical Center Utrecht–Technical University Eindhoven.
Development of a new driven system for melt electrowriting technologies

Amit Chandrakar, Lorenzo Moroni, Paul Wieringa

Maastricht university, MERLN, Maastricht, NL

Introduction
Here we introduce a new auger-driven polymer melt extrusion system for Melt electrowriting (MEW), improving the consistency, reproducibility, and versatility of this exciting new technology. MEW is a manufacturing technique of producing ultra-fine fibers using electrical instabilities from polymer melts contrary to electrospinning (ESP) process that uses polymer solution. In MEW, the polymer melt delivery to the nozzle outlet is typically provided by a pressure-driven system; this gives the system good control over the flow rate of the polymer melt through a nozzle and improved startup time, compared to a piston-driven piston. However, with trying to process high molecular weight polymers, the resulting increase in polymer melts viscosity requires unachievable extrusion pressure on the order of MPa. It is also challenging to extrude polymer melts through a small nozzle and maintain a consistent small flow rate. To ensure a consistent polymer flow, the raw material is typically preheated overnight to ensure polymer melt homogeneity and to remove any pockets of air, thus ensuring an uninterrupted supply of polymer can be maintained. However, for a thermally sensitive polymer, preheating for a prolonged time can degrade the polymer, drastically change its viscoelastic properties and the mechanical and molecular properties of the resulting polymer scaffold. Due to the above drawbacks of the current pressure-driven system, we designed a new auger-driven polymer melt extrusion system for MEW.

Experimental Methods
The auger was designed to achieve a minimum and consistent flow rate. Auger modelling and simulation is performed with the help of COMSOL. Here, the manufacturing limitation of fabricating an auger screw is also taken into account. After simulation and optimizing different auger parameters, the auger dimensions were finalized, as shown in figure 1. The commercially available K8200 3d printer was modified, and an auger-driven melt head is integrated into the printer, as shown in Figure 2A. The polymer flow rate of polycaprolactone (45kDa) exiting the nozzle at different speeds and temperatures was recorded and a good correlation between simulation and experimental results were observed. The auger-driven MEW system was then characterized and compared with pressure-driven MEW system at different flow rates while keeping all other MEW parameters constant. We saw no significant variation between pressure and auger-driven systems on the measured fiber diameters, ranging from 5µm to 30µm, and our new auger system displayed excellent manufacturing reproducibility.

Results and Discussion
In MEW, the diameter of the fabricated scaffold depends both on flow rate and initial jet thickness. With a lower flow rate and small jet thickness, one can expect a lower filament diameter. With this new auger-driven system, we could extrude and control the molten polymer via a small nozzle, resulting in nanofiber production and making a hybrid scaffold consisting of nano and micro scaffold using a single run and single melt head for the first time, as shown in the figure 2B. We also observed that the new system was also effective at removing air and vapor pockets within the molten polymer via compression and upward pressure due to centrifugal force during extrusion and spinning. This was noticeable for the PLLATMC polymer, which has a hydroscopic nature that leads to vapor bubbles within the polymer melt. This polymer also cannot be maintained at a high temperature without significant polymer degradation, precluding an overnight preheating step. When this polymer is extruded via the pressure-driven system, we observed a regularly interrupted jet spinning and with noticeable air pockets within fibers. In contrast, our auger-driven system...
generated smooth uniform fiber. This confirmed our ability to supply the MEW process with an uninterrupted polymer supply and use raw materials without preheating.

Our computational modelling determined that our system was theoretically capable of generating consistent polymer melt flow rates that were invariant to melt viscosity. We were therefore inspired to extrude highly viscous material of viscosity greater than 25,000 Pa.s via an auger driven system, something which is currently not possible with any other drive system used for MEW. Successful deposition was achieved, resulting in fibers diameters on the order of 50 to 60 µm; while larger than typical MEW fibres, this was attributed to the polymers rapid solidification rate.

**Conclusion**

New designed auger driven MEW system provides several advantages compared to the pressure-driven system while maintaining the same fiber consistency as the pressure-driven system. Auger driven system can significantly increase the complexity of the scaffold fabricated by MEW. Future work will focus on tuning the auger rotation motion to the fabricated scaffold by varying fibre diameter in a single run.

**Acknowledgement**

We acknowledge the funding and support of the INTERREG Northwestern Europe BONE Project (NWE497). https://www.nweurope.eu/projects/project-search/bone-bio-fabrication-of-orthopaedics-in-a-new-era/

---

**Fig. 1. Screw geometry outline**

A) Conceptual design of an auger with different pitch, width and height of the screw channel is considered for simulation. B) Due to the non-symmetric profile of an auger screw, a complete 3d model is simulated in COMSOL. C) Optimized auger design corresponding to screw profile resulted in smallest flow rate.

---

**Fig. 2. Custom-made MEW printer**

A) Modification of K8200 printer by introducing collector plate and shielding all the electric hardware against the high voltage by acrylic plastic with the integration of auger driven melt head into modified K8200 printer. B) Hybrid manufacturing of scaffold containing Nano and Micro filaments, made in a single run with a single melt head.
N14-04

Melt electrowritten fiber scaffolds as in vitro culture systems for nervous tissue engineering

**Ezgi Bakirci**1, Dieter Janzen2, Annalena Wieland3, Andrei Hrynevich1,5, Biranche Tandon1,4, Natasha Schaefer2, Pamela L. Strissel3, Reiner Strick3, Carmen Villmann2, Paul D. Dalton1,4

1 University of Würzburg, FMZ, Würzburg, DE; 2 University Hospital Würzburg, Institute of Clinical Neurobiology, Würzburg, DE; 3 University Hospital Erlangen, OB/GYN, Laboratory for Molecular Medicine, Erlangen, DE; 4 University of Oregon, Eugene, US; 5 University Medical Center Utrecht, Utrecht, NL

**Introduction**

Nervous tissue has a wide and complex environment that provides many biochemical and topological stimuli, which define the advanced functions of the tissue. The biochemical and mechanical properties of the nervous tissue microenvironment play an essential role in both neuron regeneration and cancer progression. A new manufacturing concept to help replicate this environment *in vitro* is additive manufacturing (AM). One such AM technology, melt electrowriting (MEW), provides control over microscale structures. The modification of fibers with different biochemical cues makes MEW scaffolds very suitable for 3D culture systems.

**Experimental Methods**

Square and octagonal-shaped MEW scaffolds were modified with biochemical cues, including RGD and IKVAV peptides, while a custom-built MEW printer was used to produce *in vitro* culture systems. Medical grade polycaprolactone (PCL), (Purasorb PC 12, Purac Biomaterials, Netherlands) was melted to 77 to 85°C depending on the scaffold's design. The printing parameters were 25G nozzle, 2 to 3 bar applied air pressure, 6 to 7 kV applied voltage, and 4.5 mm collector distance. The fibers were coated with peptides using a methodology previously reported. Peptide content was measured with Pierce Peptide assay and specific peptide bonding examined with FTIC-conjugated peptides using a confocal microscope. Glioblastoma cells U-87 MG (ATCC® HTB-14™) and primary mouse astrocytes were used in the study. The cell morphology was analyzed by ImageJ and MIPAR software.

**Results and Discussion**

The scaffold coating stability was confirmed with FTIR, a peptide assay, and confocal imaging after 4 days. The fluorophore/peptide assays showed intensity significantly higher with FTIC- and peptide-conjugated scaffolds than NaOH treated and unmodified PCL controls, while U87 cells and astrocytes showed different cell morphology on the peptide-coated scaffolds. We examined the impact of the design and biochemical cues on *in vitro* culture systems in terms of the area and circularity of the cells. The glioblastoma cells showed more neurosphere-like colonies on non-coated PCL scaffolds. The cells on octagonal-shaped scaffolds have larger cell areas compared to square pores, either with or without peptides. The circularity of the cells is more visible on non-coated square and octagonal shaped scaffolds 0.70 and 0.76, respectively. The length of the cell processes increases when peptide-coated rather than on non-coated scaffolds. Primary astrocytes showed a stellate cell morphology on octagonal-shaped scaffolds. The in vitro systems fabricated by MEW showed promising accuracy and flexibility to alter the 3D microenvironment of cells.

**Conclusion**

The influence of peptide-coating suggested that MEW scaffolds can be used as in vitro systems to understand the complex in vivo behavior of U87 cells and astrocytes due to the adjustable fiber diameter and the capacity of modification of the fiber with different biochemical cues.

**Acknowledgement**
This work was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)—Project number 326998133—TRR 225 (sub-project B01).


References
Adaptive toolpath g-coding for well-defined fibrous architectures produced by melt electrowriting for size-relevant and mechanically robust bioengineered myocardial constructs

Andrei Hrynevich1,2,3, Gerardo Cedillo-Servin1,2, Madison J. Ainsworth1,2, Joost van Duijn1,2,3, Jos Malda1,2,3, Miguel Castilho1,2,4

1 University Medical Center Utrecht, Regenerative Medicine Centre Utrecht, Utrecht, NL; 2 University Medical Center Utrecht, Department of Orthopaedics, Utrecht, NL; 3 Utrecht University, Faculty of Veterinary Medicine, Department of Equine Sciences, Utrecht, NL; 4 Technical University of Eindhoven, Department of Biomedical Engineering, Eindhoven, NL

Introduction
Melt electrowriting (MEW) is an additive manufacturing technology capable of producing high-resolution fibre scaffolds. Recently, MEW scaffolds have been described as promising delivery substrates for cardiomyocytes in bioengineered myocardial constructs (BMC). Scaffold microarchitecture and mechanical properties have to be precisely tailored to recapitulate myocardial organization and allow for scaffold deformation under myocardium contraction. However, MEW fibre scaffolds typically show printing defects that increase with each added layer, such as wall slanting and printing path distortion, which alter scaffold architecture and mechanical performance and ultimately reduce control over the induced cell behaviour, especially in thick scaffolds. To address this challenge on printing accuracy, this study aimed to develop an adaptive toolpath g-code for fabricating melt-electrowritten fibrous scaffolds with well-defined and stretchable geometries with clinically relevant thicknesses.

Experimental Methods
MEW poly(ε-caprolactone) scaffolds (0.25, 0.5, and 1 mm thick) with hexagonal and auxetic shaped pores were designed in MATLAB using two different repetitive units, namely, basic and diagonal. Next, an adaptive toolpath g-code based on in-line adjustment of printing parameters (voltage, collector distance and speed) and printing path micro-shifting was developed to minimise fibre wall slanting. The micro-shifting approach compensates the natural smoothening of printed fibre path corners by gradually displacing corner pinnacles. The obtained scaffolds were analysed by scanning electron microscopy, and their mechanical performance was evaluated under uniaxial tensile testing. Finally, in order to study the effect of pore and repetitive unit geometry on in vitro cell alignment and construct mechanics, hexagonal and auxetic scaffolds with basic and diagonal repetitive units were combined with 5% GelMA hydrogels loaded with fibroblast/cardiomyocyte mixtures and cultured in vitro for 21 days. During culture, contraction rate and cell alignment were monitored by brightfield microscopy, and mechanical properties of the seeded scaffold after 21 days were evaluated under uniaxial tension at 25% strain, in order to approximate physiological strain levels.

Results and Discussion
MEW hexagonal and auxetic architectures (Figure 1) yielded a wide range of anisotropic mechanical responses, including an increase in stiffness of up to 100% from the orthogonal to the parallel tensile orientation. Scaffold cross-sectional area was used as an indicator of wall slanting, and it was reduced by up to 25% with path micro-shifting and by up to 32% with collector speed adjustments. The effect of printing path modification on cell alignment and contraction was demonstrated, since cell-seeded fibre/hydrogel scaffolds with basic repetitive units showed an increase in tensile stress by about 35% with respect to diagonal scaffolds. Considering that pore shape is crucial for cardiomyocyte alignment, the scaffold’s mechanical properties were shown to be adjustable by adjusting the printing path and the repetitive unit geometry, while keeping pore shape and size constant. This will allow for tailored manufacturing of scaffolds that comply with different infarction locations and myocardium layers.
Conclusion

Altogether, the findings of this study push the current thickness and resolution limitations in MEW of complex geometries and provide strategies for adjusting the mechanical properties of MEW scaffolds. This approach allows for complex optimisation of anisotropic BMC mechanics and microarchitecture for enhanced function and delivery of induced pluripotent stem cell-derived cardiomyocytes to the infarcted heart.

Acknowledgement

This work was supported by the European Union H2020 program under grant agreement 874827 (BRAV3). The authors acknowledge Tomasz Jüngst for support with SEM imaging and Inge Dokter for support with cell culture.

Figure 1. Melt-electrowritten fibrous scaffolds with hexagonal and auxetic geometries
(A-B) Schematic representations of the basic (A) and diagonal (B) repetitive units for melt electrowritten (MEW) scaffolds with hexagonal pores. (C-F) Scanning electron micrographs of hexagonal basic (C,E) and diagonal (D,F) MEW scaffolds. Scale bars: (C,D) 200 μm and (E,F) 50 μm. (G) Schematic representation and (H) optical micrograph of a MEW scaffold with auxetic pores. Scale bar: (H) 2 mm.
Electrospun, Biomimetic Tympanic Membrane Implants with Tunable Mechanical and Oscillatory Properties for Myringoplasty

Lukas Benecke1, Zhaoyu Chen2, Ines Zeidler-Rentzsch2, Matthias Bornitz2, Chokri Cherif1, Marcus Neudert2, Dilbar Aibibu1

1 Technische Universität Dresden, Faculty of Mechanical Engineering, Institute of Textile Machinery and High Performance Material Technology (ITM), Dresden, DE; 2 Technische Universität Dresden, Faculty of Medicine Carl-Gustav Carus, Clinic of Otorhinolaryngology Head and Neck Surgery, Ear Research Center Dresden (ERCD), Dresden, DE

Introduction

Tympanic membrane (TM) perforations can be caused by multiple reasons like chronic middle ear inflammations (COM), explosions, or traumata. Worldwide 32 million people suffer from COM alone, half of which have severe consequential damage to their hearing. TM defects are commonly repaired using autologous materials like perichondrium, fascia, or cartilage. In addition to damaging the donor site, these materials lack the mandatory mechanical and oscillatory properties needed for a complete restoration of the patients' hearing ability. Therefore, new artificial materials that meet the biomechanical and biological requirements for replacing the human TM are necessary to greatly improve the quality of life of millions of affected people worldwide.

The human TM is a funnel-shaped membrane mainly consisting of collagen and elastin fibers. Radial, circumferential, and random fiber alignment can be found in three different layers with a total thickness between 30 – 150 µm. Its fiber morphology and structure have a major impact on its oscillation properties. Therefore, the aim of the presented study was to develop biomimetic membranes that match the acousto-mechanical properties of human TM, and suitable technologies to realize those. Electrospinning was performed to generate randomly oriented nano- to microsized fibers. By manipulating the electrical field through the integration of additional electrodes, isolators, and/or relative movement between electrode and collector radial and/or circumferential fiber orientation was achieved. Additional precrystallized microfilaments were incorporated into the fiber mats to enhance mechanical properties. A model was developed using the finite element method (FEM) and validated to predict influences of electrospinning parameters and structural design on the acousto-mechanical properties. At first, the in medical applications like sutures or drug-release systems well established synthetic polymer polycaprolactone (PCL) was used to generate the TM implants due to its suitable mechanical and biological properties. Additionally, silk fibroin (SF) of the silkworm bombyx mori was blended into the material to further enhance mechanical and cell adhesion properties.

Experimental Methods

The generated membranes were characterized by tensile tests, acoustic vibration tests, enzyme catalyzed degradation studies, rheometry, cell culture, as well as light and scanning electron microscopy. Silk fibroin spinning solutions were additionally characterized by FTIR and gel electrophoresis to evaluate influences of the degumming process and electrospinning solvents on protein degradation and β-structure content (crystallinity).

Results and Discussion

The studies showed that by manipulating the electrical field biomimetic fiber structures can be achieved via electrospinning. Radial and circumferential fiber orientations were generated. By tuning spinning solution concentration, SF-content and spinning parameters the fiber diameter could be adjusted between 30 nm and 2,5 µm, therefore being able to mimic the natural collagen fiber dimensions. Tensile tests showed that by altering these parameters a broad range of E-moduli can be set from 10 MPa – 100 MPa, which exceeds the range of the E-modulus of human TM that lies between 20 MPa – 60 MPa. Various fiber arrangements and the incorporation of
precrystallized fibers in defined patterns to further enhance mechanical properties were investigated by the developed FEM-model. Especially the oscillation properties were simulated to match those of human TM. Therefore, the position of the first resonance frequency is a good indicator of accordance. We were able to develop membranes with an exceptional good match compared to natural TM specimen after reconstruction (Fig. 1). The amplitude of oscillation was found to be higher by approximately a power of ten for the plain ungraded 2D membranes, which would result in rise in hearing volume of ~10 dB. By replicating the typical 3D-funnel shape of the TM and incorporating precrystallized filaments, this value could be lowered. Finally, we demonstrated the biocompatibility and degradability of the developed SF-PCL-implants by culturing primary keratinocytes and bronchoalveolar epithelial cells on the membranes as well as by enzymatically catalyzed degradation studies over two weeks, using protease XIV and lipase.

**Conclusion**

In conclusion, by modifying the electrospinning technology to mimic the fiber morphology of the human TM and its 3D-funnel shape (Fig. 2), we were able to produce drum implants that can be tuned to match the mechanical and especially acoustic properties of the native tissue, using SF-PCL-blends. Furthermore, the material displays excellent cell adhesion and proliferation properties. No cytotoxic effects were observed during the cell culture studies. Surgeons specifically praised the handling of the new TM-implants due the similarity between the wetting behavior of silk and the native TM.

**Acknowledgement**

The authors would like to express appreciation for the funding of the research projects 20533BR (MyringoSeal) of the DECHEMA through the AiF within the program for supporting the „Industrielle Gemeinschaftsforschung (IGF)” from funds of the Federal Ministry for Economic Affairs and Energy (BMWi) by a resolution of the German Bundestag.

![Figure 1: Oscillation properties of electrospun PCL-/SF-PCL-membranes compared to human TM specimen](image)

The oscillation properties of the generated electrospun membranes match those of the native human TM. Furthermore, by adjusting electrospinning parameters like voltage, solution concentration, or spinning distance the first resonance frequency and amplitude can be specifically modified.
Figure 2: Electrospun 3D-PCL-membrane with graded fiber structures in an ossicular chain model

Through modifications of the electrospinning setup, the production of 3D membranes with integrated areas of radially and/or circumferentially oriented fibers, as well as the incorporation of precrystallized filaments was enabled. Therefore, TM-implants with bimimetic structural design and adjustable acousto-mechanical properties can be generated.
N14-07

Delivery of a microRNA ‘cocktail’ by electrospun PVA/alginate/ciprofloxacin nanofibres: a genetic nanomedicine for impaired wound healing

Adrian D. Juncos Bombin¹, Helen McCarthy¹, Nicholas Dunne²,¹

¹ Queen’s University Belfast, School of Pharmacy, Belfast, GB; ² Dublin City University, Dublin, IE

Introduction

Delays in wound healing are a significant financial burden to the NHS with annual costs estimated to be £3.2 billion (1). Wound healing is regulated by microRNAs (miRs), which impact multiple aberrant genetic pathways (2). miR-21 and miR-132 can promote re-epithelialisation and anti-inflammatory responses by targeting different molecular pathways (3,4) and miR-31 promotes angiogenesis and re-epithelialisation (2). In this study, single-stranded RNA (ssRNA) encoding miR-21, miR-132 and miR31 were condensed into nanoparticles (NP) using the RALA peptide via electrostatic interactions (5). These NPs were then incorporated into a nanofibre wound healing patch which has been electrospun. The nanofibre blend consisted of 89% w/w Polyvinyl alcohol (PVA) as the main electrospun polymer, 10% w/w sodium alginate (ALG) to promote wound healing and 1% w/w ciprofloxacin (CIP) as a wide-spectrum antibiotic.

Experimental Methods

RALA was complexed with miRs at a range of different N:P ratios. RALA/miR NP cocktails were fluorescently labelled with Cy3 and Cy5 and used to transfect NCTC-929 fibroblasts (ATCC, USA) and HaCaT keratinocytes (CLS, Germany). Cells were imaged with a Nikon 6D Widefield microscope (Japan). Viability of individual and cocktail NP formulations was assessed in vitro via MTS assay (ThermoFisher, USA). PVA and ALG were dissolved in water at 9% and 1% w/v and mixed at a 1:1 v/v ratio. CIP was added at concentrations of 1 wt.% of total polymer weight. The blend solution was electrospun using a 20 kV electrospinning kit (Spraybase, Ireland) and crosslinked in a 1% v/v glutaraldehyde and 0.1% v/v HCl solution in methanol for 24 h. The antimicrobial activity of the fibres was tested via disk diffusion against Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 29213 methicillin-sensitive (MSSA) and S. aureus ATCC 43300 methicillin-resistant (MRSA). As-spun and crosslinked nanofibres were incubated in opti-MEM media (ThermoFisher, USA), which was subsequently used to incubate NCTC-929 and HaCaT cells for cytotoxicity measurements. RALA/miR NP were incubated with crosslinked nanofibres and NP release was studied in UltraPure water via the Quant-iT™ microRNA Assay Kit (ThermoFisher, USA). Cells were incubated with NP released from nanofibre patches, and functionality of internalised miR-31, miR-21, and miR-132 was studied by (1) qRT-PCR with miRNeasy Mini Kit (Qiagen, Germany) and TaqMan™ MicroRNA Reverse Transcription Kit (ThermoFisher, US); (2) VEGF-A Human ELISA Kit (ThermoFisher, US), and (3) wound scratch assay with wound scratch culture inserts (Ibidi, UK).

Results and Discussion

RALA condensed miR into NP ≤200 nm with a zeta-potential of ≥10 mV at N:P ratio ≥ 4 (Figure 1a), ideal for cellular uptake (5). A significant increase in cell metabolic activity was detected in NCTC and HaCaT for both individual miR NPs and cocktail NP formulations (Figure 1b). Cocktails 1-3 were composed of two miRs, whereas cocktail 4 included the three miRs. NCTC cells were fluorescent under the optical microscope post-incubation with a miR cocktail containing labelled miR (Figure 1c), indicating efficient internalisation of NP which was confirmed by FACS analysis. As-spun ALG/PVA and ALG/PVA/CIP nanofibres were crosslinked (CL) with glutaraldehyde to confer stability in aqueous media, showing optimal morphology (Figure 2a). Average diameter was found to be 518.83 ± 101.23 and
552.37 ± 89.69 nm for CL ALG/PVA and ALG/PVA/CIP nanofibres. As-spun nanofibres were found to inhibit growth of *P. aeruginosa* and *S. aureus* (Figure 2b). Crosslinking impaired CIP release from the nanofibrous patch, thus creating an area of inhibition identical to the nanofibre patch. CL ALG/PVA nanofibres also inhibited microbial growth due to glutaraldehyde. Metabolic activity of NCTC and HaCaT was >80% when incubated with extraction media from CL nanofibres, indicating biocompatibility of the material (Figure 2c). RALA/miR NP containing 10 µg of total miR were incubated with CL ALG/PVA and ALG/PVA/CIP nanofibres. A burst release of >80% was obtained within the first 8 h with a 100% release achieved by 2 days (Figure 2d).

**Conclusion**

RALA/miR NP were successfully condensed and demonstrated excellent physiochemical characteristics for cellular uptake in fibroblast and keratinocyte. A range of *in vitro* studies have determined the optimal combination of RALA/miR NP to take forward *in vivo*.

An ALG/PVA/CIP nanofibrous wound patch has been prepared using electrospinning. This patch has shown antimicrobial properties, with crosslinking promoting a controlled CIP release from the patch. Moreover, it has been shown to be biocompatible with fibroblast and keratinocyte cell lines.

Taken together, this work proposes a holistic approach towards chronic wound healing based on the synergistic effects of a miR ‘cocktail’ genetic therapy delivered from a multifunctional, nanofibrous wound dressing that mimics the extracellular environment and protects against microbial infection.
Fabrication and characterisation of electrospun nanofibres
(a) ALG/PVA and ALG/PVA/CIP nanofibres were electrospun and crosslinked (CL) with glutaraldehyde, obtaining smooth and beadless nanofibres; (b) CL nanofibres inhibited microbial growth owing to CIP and glutaraldehyde; (c) CL nanofibres were shown to be biocompatible with fibroblast and keratinocyte cell lines; (d) RALA/miR NP were loaded into CL nanofibres and the release profile was studied, showing a burst release within the first 8 hours.

References
Surface potential a key parameter for electrospun polymer fibers scaffolds in tissue engineering

Urszula Stachewicz

AGH University of Science and Technology, Faculty of Metals Engineering and Industrial Computer Science, Krakow, PL

Introduction
Surface charge is one of the most important biomaterials property driving cell adhesion and tissue formation [1] and in electrospun scaffolds can be tuned by applying positive or negative voltage polarity during their production [2,3]. We study commonly applied in bone tissue engineering polycaprolactone (PCL) and piezoelectric polyvinylidene fluoride (PVDF) fibers with controlled surface potential verified in ambient and physiological conditions to investigate cell adhesion and proliferation.

Experimental Methods
PCL and PVDF fibers were electrospun applying 14-15 kV to the stainless needle keeping the constant distance of 20 cm to the collector and the solution flow rate at 0.5-1.5 ml·h⁻¹. Surface potential was examined using Kelvin probe force microscopy (KPFM) and via zeta potential measurement in SBF in pH 7.4. Cell proliferation and integration with fibers were verified with the scanning electron microscope (SEM) and 3D tomography based on SEM with focused ion beam (FIB-SEM).

Results and Discussion
Zeta potential measurements were found in correlation with the KPFM results showing higher surface potential for fibers produced with negative voltage polarity [4]. Higher surface potential increased the initial cell adhesion and enhanced their proliferation on PCL and PVDF scaffolds. Additionally, the 3D tomography has shown extended cell filopodia overlapping the electrospun PCL and PVDF fibers, whereas on porous PCL scaffolds cells internalized into the individual pores on fibers surfaces.

Conclusion
Electrospinning with positive and negative voltage polarities allows controlling surface potential and produce scaffolds without any additional surface modifications. The scaffolds made of PVDF and PCL fibers with the controlled surface potential promoting bone growth for next-generation tissue scaffolds tuning the nucleation process of collagen mineralization in osteoblasts culture for bone tissue regeneration.

Acknowledgement
This study was supported by funding from the National Science Centre in Poland, OPUS 17, No. 2019/33/B/ST5/01311.

References
Identifying key parameters for the transmission of visible light through electrospun nanofibrous PCL scaffolds for ophthalmic applications

Marcus Himmler\textsuperscript{1,2}, Dirk W. Schubert\textsuperscript{2}, Thomas A. Fuchsluger\textsuperscript{1}

\textsuperscript{1} University Medical Center Rostock, Ophthalmology, Rostock, DE; \textsuperscript{2} Friedrich-Alexander University Erlangen-Nuremberg, Polymer Physics and Processing, Erlangen, DE

Introduction
The cornea is the window of the eye and its transparency is essential for it. Recently, electrospun scaffolds have been discussed for the use in ophthalmic applications as wound dressings after corneal surgery or artificial DMEK grafts for treating patients with corneal endothelial cell pathologies. Concerning wound dressings, transparency is of crucial importance for the immediate improvement of the patients’ vision after surgery. In comparison to homogenous films, nanofibrous scaffolds not only reflect and absorb electromagnetic waves but scatter them. The scattering depends to a higher extend on the ratio of the wavelength to the dimension of the scatterer. Therefore, in this study light transmission through nanofibrous scaffolds is evaluated for varying fiber diameter in the nanometer range. Furthermore, the influence of refractive indices of the surrounding medium and scaffold thickness is examined. Eventually, based on theoretical considerations a formulation is provided for a better understanding of the underlying mechanism of light transmission through nanofibrous scaffolds.

Experimental Methods
PCL scaffolds were produced via electrospinning from the solvent system formic acid/acetic acid in the ration 7:3. Different PCL concentrations were used with 5 \text{ w/v}, 8 \text{ w/v}, 10 \text{ w/v}, 12 \text{ w/v}, 14 \text{ w/v} and 16 \text{ w/v}. For each solution, the working window depending on applied voltage, collector-to-needle distance and flow rate was defined to obtain a bead-free and homogenous fiber morphology. Based on these preliminary spinning experiments, scaffolds with varying thickness between 2 \text{ µm} to 50 \text{ µm} were fabricated for each concentration. Fiber diameter and scaffold thickness were measured using SEM images and a contact sensor, respectively. Transmission measurements were conducted using UV/Vis spectroscopy in the range of visible light from 380 nm to 780 nm. Therefore, the scaffolds were fixed in tissue carrier rings and then placed in an adjusted cuvette filled with ethanol or phosphate buffered saline (PBS). With the scaffold fully covered in medium, differences in the refractive indices of the surrounding medium could be evaluated. By plotting the light transmission of a sample type as function of the scaffold thickness for a fixed wavelength, an exponential decrease in light transmission could be identified following a Lambert-Beer law and a specific extinction coefficient could be calculated. The extinction coefficient was then further analyzed regarding the contributions of reflectance, scattering and absorption depending on the scaffold characteristics and ambient medium.

Results and Discussion
Transmission measurements from UV/Vis spectroscopy provide transmission versus wavelength curves for almost continuous wavelength values, covering the range of visible light from 380 nm to 780 nm. From the single measurements it can be seen, that the transmission is highly wavelength dependent for a given scaffold thickness. With increasing wavelength, the transmission of the electromagnetic wave increases. Merging the transmission measurements from one sample type confirms the exponential decrease in light transmission with increasing scaffold thickness. With a light transmission close to 100 \%, the transmission decreases to almost 0 \% for all scaffolds at a certain scaffold thickness. For comparison of different fiber diameter and surrounding medium, transmission values for interpolated 10 \text{ µm} scaffolds were taken from the charts. It is shown, that with increasing fiber diameter the transmission decreases. For the scaffolds from the lowest spinning concentration, fibers with a diameter of 35 \pm 13 \text{ nm}
nm were fabricated and resulted in a light transmission for a interpolated 10 µm membrane from 47.6 % at 380 nm to 72.0 % at 780 nm. In comparison, scaffolds fabricated from the highest spinning concentration provided significantly thicker fibers with diameter of 167 ± 35 nm and lower light transmission from 18.6 % at 308 nm to 54.8 % at 780 nm. Based on theoretical considerations, the ratio of reflectance, scattering and absorption shifts from high reflectance and low scattering for thin fibers towards low reflectance and high scattering ratios for thicker membranes. Absorption seems to be only a minor contribution to the extinction coefficient. Besides, it could be shown, that light transmission depends to a high extend on the difference in refractive indices, with increasing transmission for matching refractive indices.

**Conclusion**

Since for ophthalmic applications transparent grafts are sought, scaffolds should be designed as thin as possible and fabricated from polymers with refractive indices close to the refractive index of the human cornea. Concerning fiber diameter, smaller fiber diameters should be favored for maximizing graft transparency and thus improving the patients’ vision after surgery. From analyzing more than 200000 different experimental transmission data points it was possible to give a detailed physical model.
N15 | Nanobiomaterials

Chairs
Clara Mattu
Politecnico di Torino, DIMEAS, Torino, IT
João Rodrigues
University of Madeira, CQM - Centro de Química da Madeira, Funchal, PT
Daphne van der Heide (YSF)
AO Research Institute Davos, Regenerative Orthopaedics program, Davos, CH
N15-01

Directing stem cell phenotype commitment by sequestering transforming growth factor-β3 with epitope imprinted nanoparticles

Simao P.B. Teixeira¹,², Rui M.A. Domingues¹,², Pedro S. Babo¹,², Margarida S. Miranda¹,², Manuela E. Gomes¹,², Nicholas A. Peppas³,⁴, Rui L. Reis¹,²

¹ University of Minho, 3B's Research Group, I3Bs - Research Institute on Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, PT; ² ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, PT; ³ The University of Texas at Austin, Institute for Biomaterials, Drug Delivery, and Regenerative Medicine, Austin, US; ⁴ The University of Texas at Austin, Department of Biomedical Engineering, Austin, US; ⁵ The University of Texas at Austin, Department of Surgery and Perioperative Care, Dell Medical School, Austin, US

Introduction

Although growth factors (GF) have a very promising therapeutic potential, their exogenous administration has proven costly and ineffective. Recent extracellular matrix (ECM)-inspired biomaterial approaches have sought to sequester labile therapeutic molecules, thus regulating their activity and presentation via heparin, aptamers or other actives. However, additional limitations call for the development of cost-effective, scalable, and stable alternatives to the abovementioned systems with similar recognition specificity. Our hypothesis is that molecularly imprinted nanoparticles (MINPs) can fulfill these requirements.[1] As proof-of-concept, here we prepared and tested MINPs against transforming growth factor (TGF)-β3, a known regulator of stem cell chondro- and tenogenesis.

Experimental Methods

MINPs were produced by inverse microemulsion polymerization using different combinations of acrylic monomers and a TGF-β3 N-terminal epitope as template. MINP affinity and selectivity for TGF-β3 were evaluated in non-competitive (single GF) and competitive assays (platelet lysate), using dot blot and Western blot. Substrates functionalized with selected MINPs were used to culture adipose tissue-derived stem cells (ASCs) in basal conditions, and their biological performance was evaluated by gene expression and immunocytochemistry assays. Finally, MINPs were incorporated in ASC pellets and their influence studied by histological analysis.

Results and Discussion

Among the different formulations investigated, amine-containing MINP-2 showed higher affinity for rhTGF-β3 than their non-imprinted counterpart (NINP-2). Western blot demonstrated that MINP-2 selectively recognized and bound TGF-β3 in the hard protein corona over other GFs of platelet lysate, suggesting that the imprinting process significantly increases the capability to capture and retain the target GF. This process results in significantly higher expression of SOX9 by ASCs in MINP-2- than in NINP-2-functionalized substrates. SCX expression is also increased for MINP-2 at an earlier time point, but it significantly decreases over time. The trend observed in expression of these two transcription factors is consistent with the commitment of ASCs toward the chondrogenic lineage by activation of SMAD pathways through TGF-β3 signaling. Concomitantly, ASC pellets cultured with MINP-2 produced more abundant collagenous matrix than those with NINP-2, corroborating this chondrogenic behavior.

Conclusion

The results obtained in this study demonstrate that MINPs can modulate autocrine and paracrine stem cell signaling via GF sequestration, thereby directing their phenotypic commitment. The principles of this strategy may be applied for biomaterial functionalization and extended to other tissue engineering and regenerative medicine applications, thus benefitting from the advantages of MINPs in terms of stability and cost-effectiveness. Hence, in upcoming work we will explore this concept in biomaterial scaffolds with controlled topographical microenvironments, thereby
combining specific biophysical and biochemical cues to guide the tenogenesis of ASCs and tenogenic differentiation in bioengineered tendons.

Acknowledgement

The authors acknowledge ERDF for project NORTE-01-0145-FEDER-000021; EU’s HORIZON 2020 for ERC 772817, Twinning 810850-Achilles and Marie Skłodowska-Curie Actions 676338; FCT/MCTES for PhD scholarship PD/BD/143039/2018, project UTAP-ICDT/CTM-BIO/0023/2014 and PTDC/NAN-MAT/30595/2017. The authors declare no conflicts of interest.

MINPs significantly increase SOX-9 expression

Immunocytochemistry staining for transcription factor SOX-9 in human adipose-derived stem cells (ASCs) cultured on MINP-2 or NINP-2-coated coverslips (scale bars: 100 μm).

References

Antioxidant polydopamine nanoparticles in the treatment of autosomal recessive spastic ataxia of Charlevoix-Saguenay

Matteo Battaglini1, Alessio Carmignani1,2, Chiara Martinelli1, Stefano Doccini3, Fillipo M. Santorelli3, Gianni Ciofi1

1 Italian Institute of Technology, Smart Bio-Interfaces, Pontedera, IT; 2 Scuola Superiore Sant’Anna, The Biorobotics Institute, Pontedera, IT; 3 IRCCS Fondazione Stella Maris, Molecular Medicine for Neurodegenerative and Neuro-muscular Diseases Unit, Calambrone, IT

Introduction

Reactive oxygen species (ROS) are molecules that play a "double-edged sword" role in our metabolism: on one side they are pivotal components of several biological reactions, on the other, excessive production of ROS can lead to damages to important biological molecules with the consequent onset of pathological conditions. Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a neurodegenerative disease associated with mutations in the SACS gene.1,2 Some of the molecular hallmarks of the disease include mitochondrial impairments, reduced activity of the respiratory chain with a consequent overproduction, and over sensitivity to ROS.3,4 In this work, we evaluated the use of antioxidant polydopamine nanoparticles (PDNPs) as a countermeasure to the ROS-induced damage typical of ARSACS.5 In particular, we tested the effect of PDNPs comparing human dermal fibroblasts derived from healthy subjects and from ARSACS patients.

Experimental Methods

Polydopamine nanoparticles were prepared using a Stöber reaction5 and then characterized in terms of size (dynamic light scattering measurements), surface charge (Z-potential analysis), morphology (scanning electron microscopy - SEM-). The biocompatibility of PDNPs was evaluated on healthy fibroblasts combining Pico-Green analysis of DNA content and live/dead assay. The interaction of PDNPs with human fibroblasts, in particular their internalization and intracellular localization, was evaluated by combining confocal microscopy, SEM/focused ion beam (FIB) imaging, and flow cytometry. The protective effect of PDNPs upon both healthy and ARSACS patients derived fibroblasts against pro-oxidative insult caused by the treatment with TBH was measured in terms of ROS production and apoptosis/necrosis levels through flow cytometry, and in terms of mitochondrial functionality (in particular, mitochondrial morphology and membrane potential were analyzed in presence and absence of both PDNPs and TBH). Lastly, an in-depth proteomic analysis was carried out to assess the differential protein expression among all experimental conditions (namely healthy and ARSACS patient-derived fibroblasts with or without PDNPs and TBH treatment).

Results and Discussion

Characterization of the PDNPs dispersions demonstrates the presence of well-dispersed and homogeneous structures with an average size of 260 ± 35 nm (Figure 1). PDNPs showed good biocompatibility without any adverse cellular effect on human fibroblasts up to 250 µg/ml. Confocal analysis (figure 2), flow cytometry measurements, and SEM/FIB imaging confirmed the uptake of the particles by human fibroblasts. Flow cytometry analysis showed the ability of PDNPs to reduce the ROS production caused by an acute treatment (90 min) with 2.5 mM TBH, and to reduce the levels of both apoptotic and necrotic cells in presence of a chronic pro-oxidative stimulus (100 µM TBH for 24 h). PDNPs showed the ability to partially prevent the loss of mitochondrial morphology and membrane potential upon TBH treatment. Lastly, the proteomic analysis demonstrated how healthy and patient-derived cells have a different response to pro-oxidative stimuli, with the protein expression in patients’ cells being more affected by the
stimulus when compared to healthy fibroblasts. Finally, PDNPs showed the ability to partially prevent the effects caused by TBH treatment causing a partial recovery of the protein expression basal levels.

Conclusion
PDNPs showed good biocompatibility and high uptake levels by human dermal fibroblasts. Moreover, all our analyses indicate how PDNPs can partially prevent both the production and the molecular damage caused by ROS on healthy and ARSACS patient-derived cells. Future analysis involving a more complex in vitro cellular model of the disease and in vivo tests could potentially open the way for the exploitation of PDNPs as a countermeasure for the impairments caused by ARSACS disease.

Acknowledgement
This project has received funding from the Italian Ministry of Health, grant RF-2016-02361610. The authors gratefully thank Andrea Petretto and Martina Bartolucci for proteomic analysis, and Francesca Santoro and Valentina Mollo for SEM/FIB imaging.
Figure 2
Internalization of PDNPs by ARSACS patient-derived fibroblasts (in blue nuclei, in green particles, in red F-actin).

References


Interaction of antioxidant nanoparticles with myoblasts in simulated microgravity: possible strategies for muscle maintenance under mechanical unloading

Giada Graziana Genchi\textsuperscript{1}, Melike Belenli\textsuperscript{1,2}, Matteo Battaglini\textsuperscript{1}, Mirko Prato\textsuperscript{3}, Gianni Ciofani\textsuperscript{1}

\textsuperscript{1}Istituto Italiano di Tecnologia, Center for Materials Interfaces, Smart Bio-Interfaces, Pontedera, IT; \textsuperscript{2}Scuola Superiore Sant'Anna, Biorobotics Institute, Pontedera, IT; \textsuperscript{3}Istituto Italiano di Tecnologia, Materials Characterization, Genova, IT

Introduction
Muscle loss for disuse associated to mechanical unloading (like prolonged bed rest, and permanence in low Earth orbit) can be hindered through several measures ranging from physical exercise, to electrical stimulation and drug administration [1-3]. Physical constraints (like flight on space vessels) however may limit accessibility to devices or medications for muscle maintenance, and have motivated the exploration of novel approaches based on nanotechnology for effective and long-standing muscle loss prevention [4,5]. In this context, cerium oxide nanoparticles -also termed nanoceria-NC, characterized by self-renewing catalytic behavior that mimics activity of superoxide dismutase and catalase- can provide useful antioxidant support against oxidative stress-OS, recognized as a significant etiology player in muscle loss under mechanical unloading [6]. An investigation on OS induced in a muscle cell model by simulated microgravity-SM as a mechanical unloading condition and on NC interacting with muscle cells cultured under SM is therefore proposed, in view of a potential application of these nanomaterials to musculodegenerative conditions both on Earth and in space.

Experimental Methods
NC were synthetized by ethylene glycol-assisted direct precipitation. Briefly, Ce(NO$_3$)$_3$ $\times$ 6H$_2$O salt (5.16 gr) was dissolved in a 8% (v/v) ethylene glycol solution in water (100 ml). The solution was heated at 50°C, and then a 28%-30% NH$_3$OH solution in water was added dropwise under mild stirring until pH became 9.2. After 1 h of incubation, nanoparticles were collected by several cycles of centrifugation (at 8,000 g for 20 min) and resuspension in water. Nanoparticles were then desiccated and dispersed in water at a desired concentration by sonication at 8 W for 3 min. Transmission electron microscopy-TEM analysis was carried out to assess nanoparticle size, X-ray photoelectron spectroscopy were conducted to verify obtainment of CeO$_2$, and a colorimetric assay (Merck MAK187) was also done to quantify antioxidant property. Fetal-bovine serum coated nanoparticle dispersions were then obtained at a concentration of 100 µg/ml in a 10% FBS solution in water, and characterized in terms of colloidal stability by dynamic light scattering. C2C12 myoblasts (10,000 cells/cm$^2$) were seeded on Thermanox substrates positioned in ad hoc-prepared silicone multiwell plates (well size: 20 $\times$ 10 $\times$ 5 cm$^3$). Adhesion was allowed for 12 h and then cultures were administered a proliferation medium composed by high glucose DMEM, 10% FBS, 25 mM HEPES and antibiotics, either added or not with FBS-coated nanoparticles (0, 100 and 200 µg/ml NC). Cultures were sealed with adhesive foil, placed on the stage of a random positioning machine (Airbus) and rotated at 20 deg/s for 48 h. Control cultures with and without NC were also kept in static conditions. OS induced by 3D clinorotation was assessed on cultures stained with 2.5 µM CellROX Green reagent through flow cytometry. Nanoparticle internalization was investigated on fixed cells by confocal microscopy (after nuclei staining with 1 µM DAPI).

Results and Discussion
TEM analyses of uncoated nanoparticles demonstrated that the synthetic process yielded loosely aggregated, monodispersed nanoparticles (~5 nm size range) (Figure 1a). X-ray photoelectron spectroscopy-XPS revealed the presence of elements expected in the nanoparticle chemistry, with modest persistence of unreacted nitrate (Figure Page 921 of 2028
1b). Ce$^{3+}$ amounted to ~15%, whereas Ce$^{4+}$ to 85%. Raman spectroscopy confirmed the attainment of the desired CeO$_2$ chemistry. Dynamic light scattering analyses demonstrated a 1.4-fold increase of the nanoparticle hydrodynamic radius after coating, and a transition from a highly positive (+38 mV) to moderately negative zeta potential (-13 mV) after coating, still denoting good colloidal stability. Antioxidant property quantification demonstrated equivalence of 1 mg of NC to 17 nmol of Trolox. After 24 h of culture, C2C12 cultures exposed to SM underwent a mild OS (~3X) compared to cultures under static conditions, whereas no OS could be detected with the chosen assay after 72 h of culture, likely due to compensation mechanisms activated by cultures during clinorotation. Administration of NC at increasing concentrations apparently had no detrimental impact on cell viability within the observation period of 48 h, both in cultures exposed to microgravity and to normal gravity. Internalization occurred in both sample types, although at a lower extent in cells exposed to SM, denoting nanoparticle perinuclear accumulation (Figure 1c).

**Conclusion**
The chosen muscle model exhibited OS within a short time frame from exposure to SM for reasons yet to be ascertained. NC were internalized by C2C12 myoblasts under both normal gravity and SM. Further studies will aim at identifying nanoparticle internalization mechanisms and relevant time frames for NC to exert antioxidant activity against OS induced by mechanical unloading under SM.

**Acknowledgement**
The European Space Agency is gratefully acknowledged for supporting the present study by funding of the “InterGravity” project, contract 4000129652/20/NL/MH/ac. Dr. Rosaria Brescia (IIT Electron Microscopy Facility) and Ms. Sara Gorrieri (IIT Smart Bio-Interfaces) are also acknowledged for assistance in nanoparticle characterization.

![Figure 1](#)

**Bright-field TEM (BF-TEM) image of cerium oxide nanoparticles (a).** XPS wide scan of plain nanoparticles (b). Confocal microscopy scan on C2C12 myoblasts after incubation with nanoparticles (in red, visible due to light scattering) and nuclei staining with DAPI (in blue).

**References**
N15-04

PCL/PHB electrospun nanofibers decorated by Hybrid Melanin-TiO$_2$ nanoparticles: a bioinspired multifunctional wound dressing

Joshua Avossa$^{5,7}$, Giulio Pota$^1$, Giuseppe Vitiello$^{1,6}$, Antonella Macagnano$^7$, Anna Zanfardino$^6$, Michela Di Napoli$^4$, Alessandro Pezzella$^{3,8,9}$, Gerardino D’Errico$^{2,6}$, Mario Varcamonti$^4$, Giuseppina Luciani$^1$

$^1$ University of Naples Federico II, Department of Chemical, Materials and Production Engineering, Naples, IT; $^2$ University of Naples Federico II, Department of Chemical Sciences, Naples, IT; $^3$ University of Naples Federico II, Department of Physics “Ettore Pancini”, Naples, IT; $^4$ University of Naples Federico II, Department of Biology, Naples, IT; $^5$ Empa, Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Biomimetic Membranes and Textiles, St. Gallen, CH; $^6$ CSGI, Center for Colloid and Surface Science, Florence, IT; $^7$ National Research Council (CNR), Institute of Atmospheric Pollution Research (IAA), Rome, IT; $^8$ National Research Council (CNR), Institute for Polymers Composites and Biomaterials (IPCB), Naples, IT; $^9$ National Interuniversity Consortium of Materials Science and Technology (INSTM), Florence, IT

Introduction

Wound care has become a critical issue in modern society. Indeed, the extension of average life expectancy and consequent increase of aging-related diseases have raised the probability of a skin wound to develop chronic inflammation [1]. This has prompted the development of advanced wound dressing materials able to act as temporary skin substitutes and aid skin regeneration. An ideal construct for wound healing should adsorb wound exudate, promote cell proliferation and exhibit antimicrobial activity. Electrospinning has been emerging as a cutting-edge technique to produce non-woven high-specific surface gauzes able to mimic the extracellular matrix, due to the possibility to tune fibre’s morphological and structural properties [2]. In this context, Polycaprolactone (PCL) and Polyhydroxybutyrate (PHB) polymeric blends stand as a suitable choice to ensure a proficient balance among biodegradability, biocompatibility, easy processability, good stiffness and tensile strength as well as controlled hydrolytic degradation. However, PCL/PHB polymer blends are exposed to pathogen contamination and exhibit marked hydrophobicity, which limits their capacity to adsorb wound exudate. In the attempt to design new-generation biologically active moieties, valuable inspiration comes from nature. Notably, melanin, a class of negatively charged hydrophobic pigments, widespread in living organisms exhibit multifunctional properties and multiple biological roles, including photoprotection, anti-inflammatory, antioxidant action and potent antimicrobial [3]. These features can be markedly improved if melanin formation occurs in the presence of a ceramic nanostructured phase, acting as a catalyst and a structure-directing agent for the organization of the polymer. This approach was effectively exploited to design hybrid Melanin-TiO$_2$ nanostructures with significant antimicrobial activity even under environmental light [4]. In this work, Melanin-TiO$_2$ nanoparticles were electrospayed over PCL/PHB electrospun mats. The obtained dressings mats were investigated for morphology, hydrophilicity and in-vitro bioactivity. Finally, biological tests were also carried out to estimate antibacterial activity as well as cytotoxicity.

Experimental Methods

PCL/PHB mats were obtained by single-needle electrospinning procedure. Briefly, appropriate amounts of PCL and PHB were dissolved in a CHCl$_3$-Ethanol solution. The polymer solution was loaded into a glass syringe and connected to a syringe pump. After that, the fibrous layers were fabricated by applying 3 kV DC voltage between the syringe tip and the collector, at a pump feeding rate of 1400 μL h$^{-1}$. The fibres were deposited onto conductive filter paper, obtained by soaking into a PEDOT:PSS water solution. Melanin-TiO$_2$ nanoparticles were synthesized following a hydrothermal route previously described [4]. The electrospary of a Melanin-TiO$_2$ water-ethanol dispersion over one side of bare PCL/PHB mats resulted in Coated Melanin-TiO$_2$/PCL/PHB samples. Water-uptake behaviour and the bioactivity of the mats were tested in simulated body fluid (SBF) at 37°C to simulate physiological conditions. TiO$_2$-
Melanin NPs were deposited onto circular glass slides and put in contact with single colonies of Gram (+) and Gram (-) bacteria, to assess biocide activity. Cytotoxicity was evaluated towards human keratinocyte (HaCat) cells.

**Results and Discussion**

SEM images display the morphology of the electrospun fibres (Fig. 1). Bare PCL/PHB fibres are 1.9 μm in diameter and their surface appears smooth (Fig. 1a). On the contrary, Coated Melanin-TiO$_2$/PCL/PHB mats are 46 μm thick (Fig. 1c) and sprinkled with clusters of Melanin-TiO$_2$ nanoparticles (Fig. 1b), which produce a rough surface with marked hydrophilicity. Indeed, the water contact angle of bare PCL/PHB fibres is 118 ± 2°(Fig. 2a), confirming their hydrophobicity, while it could not be appreciated for the coated sample since the water drop spread on the surface as soon as it touched the fibres (Fig. 2b). The high surface area of the fibres is responsible for the huge weight gain (300 % wt) exhibited by both the samples, showing their ability to absorb wound exudates. FTIR spectroscopy assessed the bioactivity of Coated Melanin-TiO$_2$/PCL/PHB, suggesting the deposition of a hydroxycarbonatoapatite thin layer after soaking in SBF. Furthermore, Antimicrobial assays revealed biocide power of the coated mats against both Gram (+) and Gram (-) bacteria (Fig. 2c). Finally, the dressings exhibited no cytotoxicity towards HaCat eukaryotic cells, ensuring safety for future medical applications.

**Conclusion**

This study exalts electrospinning as a suitable strategy to produce high-absorbent gauzes from polymer blends. Moreover, coating the fibres with hybrid melanin-ceramic nanosystems lend the mats hydrophilicity, biocompatibility and marked wide-spectrum antimicrobial activity, paving the way to the design of smart multifunctional wound dressings.

**References**


N15-05

Hyaluronic acid based non-spherical nanoparticles by microfluidics for the active targeting of chemotherapeutics

Antonio Fabozzi¹, Francesca Della Sala², Assunta Borzacchiello², Luigi Ambrosio²

¹ Altergon Italia S.r.l., Morra De Sanctis (AV), IT; ² National Research Council, Institute for Polymers, Composites and Biomaterials, Naples, IT

Introduction

Hyaluronic acid (HA), a naturally-occurring anionic polysaccharide, is widely studied in active tumour targeting research for its ability to bind CD44 receptor, overexpressed in a varied set of cancer cells [1]. Shape of nanoparticles (NPs) is emerging as an important design parameter influencing considerably nano-biointeractions [2]. In literature, a manufacturing technique to generate non-spherical (NS) NPs, from the spherical (S) NPs, such as film-stretching method, has been described [3]. Unfortunately, the film-stretching method, based on the immobilizing spherical polymeric NPs in a thin plastic film, does not lead to gain reproducibility in physicochemical properties of NPs, as size distribution, polydispersity index (PDI) and consequently in vitro and in vivo drug delivery tests. Newly continuous-flow nanoprecipitation assisted by microfluidic systems, in contrast to conventional NPs synthetic methods, are widely investigated for their unique properties including precise control, rapid mass transfer, mixing efficacy, large reaction interfaces [4]. In the microfluidic system, the nucleation and growth steps for the NPs formation can be separated as a function of distance from the position where solution mixing occurs to obtain an absolute control of the particle size and morphology [5]. In this work, blank and irinotecan-loaded (IRI) NS and equivalent (E) S NPs composed of poly(lactic-co-glycolic acid) (PLGA) have been formulated and coated with HA by means of microfluidics for active tumor targeting. The stability of the formulated NS and ES blank and IRI-loaded NPs was evaluated by measuring their size and the in vitro IRI release. z-potential analysis and ELISA tests were further employed to investigate polymer assembly in NP formulations. Finally, in vitro cell uptake on L929 cell line was performed.

Experimental Methods

NS and ES blank and IRI-loaded NPs were prepared by a nanoprecipitation–aid assisted by microfluidic method. Briefly, for the NS and ES NPs organic phase (OP) PLGA (RG504H) 50:50) and poloxamers (PP) (PF68/PF127) (1:0.3:0.3) powder were solubilized at 3.0% (w/v). Afterwards, the dispersion was placed into a 15 mL tube connected to the micromixer chip having 12 mixing phases. In particular, PLGA/PP solution was introduced in the internal channel pumped at 250 μL min⁻¹. A solution of HA (803 kDa) at 0.08% (w/v), was used in the external channels with fluxes of 1250 μL min⁻¹ to set a flow ratio (i.e., internal flow/external flow) equal to 0.2. In the case of drug-loaded NPs, IRIN (1 mg) was solubilized in the OP. NPs sizes and z-potential were evaluated by DLS, and morphologies by TEM. Drug entrapment efficiency was evaluated by UV-vis and in vitro release kinetic of IRI by spectrophotometric assay. In vitro cell uptake of IRI-loaded NPs was performed on L929 cells.

Results and Discussion

TEM micrographs of NS and ES HA/PLGA/PP NPs fabricated by microfluidics are reported in fig 1. The NP HA/PLGA/PP show a regular and reproducible shape of an oblate ellipsoid (a=b>c) with dimensions 240.31x101.2 nm (fig. 1a), while their ES shown a diameter d=260 nm (fig. 1b). Single peaks and a PDI lower than 5 % have been found in all NPs size distributions, which was in agreement with TEM micrographs. In NS NPs HA addition resulted into a significant reduction of z potential from ∼−41.7 mV (ES HA/PLGA/PP) to ∼−51.7 mV. In the case of NS HA/PLGA/PP NPs, the drug entrapment efficiency (EE) was found to be 92.3%, while for their ES was 87.3% that is higher than EE obtained for the spherical HA/PLGA/PP synthesized by a traditional nanoprecipitation (62.7%).

Page 925 of 2028
Moreover, it was observed a decreasing for NP HA yield from 97.8%, for ES HA NPs, to 92.3%; in any cases the yield values of all NP formulation resulted higher than the traditional nanoprecipitation fabrication NPs method (48%). ELISA data revealed a loss of 6.4% w/w of HA in the supernatant. In vitro assay release profiles of IRI for ES and NS HA/PLGA/PP showed that IRI is completely released in less than two weeks, with >95% of the loaded drug eluted within 7 days. Cell uptake studies of ES and NS HA/PLGA/PP were performed on an L929 cell line up to 48h of incubation and reported in fig. 2. In both cases, internalization increases with exposure time, reaching a plateau after approximately 24 hours. Furthermore, from the analysis of biological assays it is clear that the NS HA/PLGA/PP NPs show a higher cellular uptake than their ES NPs, especially evident in the longer times of exposure. This trend of biological assay can be due to the greater surface area exposed by NS NPs.

Conclusion
We developed a novel non-spherical and equivalent spherical blank and IRI-loaded HA/PLGA/PP NPs by means of microfluidics. In vitro assays of HA/PLGA/PP NPs revealed that the non-spherical NPs allow better internalization than their ES NPs on the L929 cells. The obtained results highlight the potential application of novel non-spherical HA/PLGA/PP NPs for cancer therapy.

Acknowledgement
The authors acknowledge the research project "ADVISE DRUGS AND ANTI-TUMORAL VACCINES FROM THE SEA" - POR CAMPANIA FESR 2014-2020 AND WITH THE AXIS 1 OO.SS. 1.2.2 / 1.1 CUP B43D18000240007"

References
Effect of the zeta-potential of oxide particles in the thermal stability and hydrolytic degradation of polylactides

Iulia Caraseva, Jone Muñoz, Jose R. Sarasua, Ester Zuza

Basque Country University UPV/EHU, POLYMAT, Department of Mining and Metallurgic Engineering and Materials Science, Faculty of Engineering in Bilbao, Bilbao, ES

Introduction

Poly(L-lactide) (PLA) is one of the most studied and used biodegradable, bio-absorbable and thermoplastic aliphatic polyester derived from renewable products. These characteristics make PLA useful to be employed in the biomedical applications.

However, the processing of PLA presents still some challenges as it shows a poor thermal stability which must be improved. Many strategies such as copolymerization or blending have been proposed in literature [1-3]. In our work we are going to add inorganic particles to PLA, as previous reports have shown that the thermal degradation of PLA is affected by the presence of inorganic fillers, due to the chemical interaction between particle and matrix [4]. Moreover, we studied the effect of the inorganic particles on the hydrolytic degradation of PLA and PLA composites at simulated human body conditions. The understanding of the biodegradation process is vital for their proper use as biomedical devices.

The objective of the work is to foresee the thermal and hydrolytic behaviour of the composites based on previously measured zeta-potential of the particles. To reach this goal, we tested four different inorganic oxides; uncoated and coated with polydopamine (PDA) (if protection was necessary).

Experimental Methods

Composites of PLLA (Mw= 175,000 g/mol) with 1wt% of Bi$_2$O$_3$, Bioglass (BG), MgO, and 10 wt% of Fe$_2$O$_3$, Fe$_3$O$_4$ respectively were prepared by injection moulding. Additionally, Bi$_2$O$_3$, BG, MgO particles were coated with PDA; they are denoted as PDBG and PDBi$_2$O$_3$. For the in vitro hydrolytic degradation study films of PLA composites having 10 mm of diameter were totally immersed in Falcon tubes containing a phosphate buffered saline (PBS). Three samples for each composite were analysed. Each sample was placed in an oven at 37 °C during 6 months. The zeta-potential of the particles was measured with a Dynamic Light Scattering and the physical-chemical characterization was carried out by thermogravimetric analysis, differential scanning calorimetry, gel permeation chromatography and infrared spectroscopy.

Results and Discussion

The changes in molecular mass are compared for the different composites in order to assess the effect on the thermal degradation of the different fillers (see Table 1). Results reveal a higher decrease in molecular weight of composites with MgO, BG and Bi$_2$O$_3$ which are particles with a negative zeta-potential. However, when reversing the zeta-potential of those particles by coating them with PDA the thermal degradation is delayed. The same results are obtained with both iron oxides, which have a positive zeta-potential. Particles with negative Z-Potential could not be process above 1% of particle content due to high thermal degradation caused. Therefore, composites having particles with negative Z-potential are studied at 1wt% and those having positive Z-potential, including PDA-coated ones, at 10wt%.

Particles with negative Z-potential values degrade the PLA matrix whereas the positive one stabilized it. The degradation mechanism can be explained shortly attending the scheme shown in Figure 1. The ester group of PLA has a delocalized negative charge between its oxygens leading its carbon with a slight positive charge. This delocalized negative charge of the ester bond is stabilized when positive zeta-potential particles are coordinated with...
PLA, while the negative charge of the ester linkage is located separated between the two oxygens when the particles have negative zeta-potential, weakening the ester bond.

**Conclusion**

Negative Z-potential is found to be the responsible for thermal degradation. The polydopamine coating could be employed to reverse Z-potential sign and consequently get more thermally stable composites. These findings enable the control of the thermal degradation of the PLA matrix by knowing the zeta-potential of the particle. However, no relationship is observed between hydrolytic degradation and the zeta potential value, revealing that the chemical nature of inorganic particles has no influence in the hydrolytic behaviour.

**Acknowledgement**

Authors thank funding support from the Basque Government Department of Education, University and Research (consolidated research groups GIC IT-927-16) and Spanish Government MICINN (106236GBI00/AEI/10.13039/501100011033).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zeta-Potential</th>
<th>$M_{c, res}$ (g/mol)</th>
<th>$M_{w, res}$ (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>-</td>
<td>182225</td>
<td>91663</td>
</tr>
<tr>
<td>PLA/1wt% Bi</td>
<td>-14.1±0.2</td>
<td>109741</td>
<td>22550</td>
</tr>
<tr>
<td>PLA/1wt% Bi₂O₃</td>
<td>-7.9±0.3</td>
<td>143854</td>
<td>47142</td>
</tr>
<tr>
<td>PLA/1wt% MgO</td>
<td>-15.7±0.5</td>
<td>97269</td>
<td>19687</td>
</tr>
<tr>
<td>PLA/10wt% BGD</td>
<td>+6.3±0.5</td>
<td>115812</td>
<td>61503</td>
</tr>
<tr>
<td>PLA/10wt% Bi₂O₃</td>
<td>+27.5±1.2</td>
<td>129775</td>
<td>65294</td>
</tr>
<tr>
<td>PLA/10wt% MgO</td>
<td>+16.6±0.7</td>
<td>107269</td>
<td>21380</td>
</tr>
<tr>
<td>PLA/10wt% FeOₓ</td>
<td>+18.3±0.9</td>
<td>158021</td>
<td>42286</td>
</tr>
<tr>
<td>PLA/10wt% Fe₂O₃</td>
<td>+15.4±0.7</td>
<td>150026</td>
<td>40181</td>
</tr>
</tbody>
</table>

Table 1. Zeta-Potential, TGA and GPC results of PLLA with coated and uncoated particles.

**References**

Fine-tuning drug-free nanostructured lipid carriers (NLC) for *Helicobacter pylori* clearance

**Rute Chitas**\(^1,2,3\), Catarina L. Seabra\(^4\), Cláudia Nunes\(^4\), Paula Parreira\(^1,2\), Maria C. Martins\(^1,2,3\)

\(^1\) i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, PT; \(^2\) INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Porto, PT; \(^3\) ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, PT; \(^4\) LAQV-REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Porto, PT

**Introduction**

*Helicobacter pylori* (Hp) is a gastric pathogen that infects around half of the world’s population [1]. Hp is responsible for the development of several gastric disorders, being linked to 90% of gastric cancers [2]. The main treatments to counteract Hp infection consist in the administration of antibiotics and proton-pump inhibitors. However, these treatments are failing in up to 40% of the patients, mainly due to the increase of antibiotic resistance [2]. Drug-free nanostructured lipid carriers (NLC) have been studied for Hp eradication, showing bactericidal effect against Hp both in vitro/vivo [3,4,5]. The main goal of this work is to optimize NLC efficiency by fine-tuning its physicochemical characteristics, in order to achieve complete Hp eradication.

**Experimental Methods**

The previously developed NLC were composed by the lipids Precirol ATO5® & Miglyol-812® and Tween®60 as surfactant, having an average size of 200-300 nm. In this work the effect of size, surfactant and surface charge in NLC bactericidal activity was evaluated. In NLC preparation, the lipid content was maintained and different surfactants were tested: the non-ionic Tween®60 & Tween®80 (NLC60 and NLC80) to access the effect of a different surfactant; and the cationic Cetyltrimethylammonium bromide - CTAB (NLC CTAB) to access the effect of charge. NLC were produced by hot homogenization followed by ultrasonication. For size optimization, different sonication parameters were tested. NLC characterization was done by dynamic light scattering (DLS) and electrophoretic light scattering (ELS), to determine size and charge, respectively. Concentration was measured by nanoparticle tracking analysis (NTA). The effect of the different surfactants was assessed. Moreover, the effect of size was evaluated in NLC60 by testing three different sizes: a smaller size (<200 nm), the previously developed (≈260nm) and a bigger size (>400 nm). All NLC were tested in vitro against the human pathogenic Hp J99 strain. Additionally, NLC were tested against other gut bacteria, namely *Escherichia coli* ATCC®25922™ and *Lactobacillus acidophilus* -01 strains.

To access if a potential NLC protein corona would influence the nanoparticles bactericidal activity, NLC60 were incubated in different media: *phosphate buffered saline* (PBS), *Mueller Hinton Broth* (MHB) and MHB+10%(v/v) inactivated fetal bovine serum (FBS). Then, they were washed in PBS and tested against Hp. Also, in order to determine the adsorbed protein content, these NLC were analyzed by liquid chromatography–mass spectrometry.

**Results and Discussion**

The NLC60 and NLC80 had sizes between 140-486nm and 190-237nm, respectively. In terms of charge, both had negative surface charge between -25 to -30mV. NLC CTAB had sizes from 107-211nm and a positive surface charge around 25 to 76mV. All NLC stocks had a final concentration of 10\(^{13}\) particles/ml. NLC were tested against Hp J99 and, after 24h of incubation, all formulations achieved bactericidal effect. Regarding size optimization, NLC80 and NLC CTAB didn’t show a substantial alteration of size, having a variation of 47nm and 104nm, respectively. However, in NLC60 a significant variation of size was observed. Bigger NLC60 (>400 nm) showed a better bactericidal performance (when compared with the NLC60 (≈260nm)) and no bactericidal effect was seen for the other gut bacteria. NLC60 incubated in medium supplemented with 10% FBS showed less bactericidal activity than the same
NLC incubated in homologous medium without FBS. Mass spectrometry results showed that when NLC60 are incubated in medium with FBS, more proteins adsorb to the nanoparticles surface. Thus, the protein corona present in the nanoparticles leads to a decrease in NLC activity.

**Conclusion**

In conclusion, the NLC were successfully optimized in terms of size and charge. All formulations were effective against Hp. Preliminary results indicate that size and surface protein corona may influence the NLC bactericidal activity. Overall, these results further support the therapeutic potential of these nanoparticles for a non-antibiotic management of Hp gastric infection.

**Acknowledgement**

This work was financed by the project PyloriBinders-*Helicobacter pylori* specific biomaterials for antibiotic-free treatment/diagnostic of gastric infection (PTDC/CTM-BIO/4043/2014) and through the FCT PhD Programs and by Programa Operacional Capital Humano(POCH), specifically by the BiotechHealth Program (Doctoral Program in Molecular and Cellular Biotechnology Applied to Health Sciences).

**References**

11:45 a.m. – 1:15 p.m.

Track07

N16 | Hydrogels and Smart Biomaterials I

Chairs
Karine Anselme
Institute of Materials Science of Mulhouse, Mulhouse, FR
Helena S. Azevedo
Queen Mary - University of London, GB
Maria José R. Eischen-Loges (YSF)
Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine,
Maastricht, NL
Atorvastatin-loaded contact lenses: design, release profiles and cornea/sclera penetration

Ana Filipa Pereira-da-Mota, Maria Vivero-Lopez, Carmen Alvarez-Lorenzo, Angel Concheiro

University of Santiago de Compostela, Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, I+DFarma (GI-1645), Santiago de compostela, ES

Introduction

Statins, 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors, are a class of lipid-lowering drugs widely prescribed for regulation of cholesterol levels, and notably contributing to the prevention of cardiovascular diseases[1]. The oral administration of statins has been associated to beneficial effects on the eye conditions due to their pleiotropic effects, as anti-inflammatory, anti-proliferative and neuroprotective activities among others [2]. The present work relies on the hypothesis that hydrogels that can mimic the active site of HMG-CoA might exhibit enhanced affinity for statins. Thus, the aim of the work was to design for first time atorvastatin-loaded contact lenses using functional monomers that bear chemical groups that resemble those present in HMG-CoA and to optimize the loading and release of atorvastatin calcium while still preserving the properties required for contact lenses, mainly in terms of ocular tolerance, light transmission, and mechanical properties.

Experimental Methods

Non-imprinted and imprinted hydrogels were prepared from mixtures of 2-hydroxyethyl methacrylate (HEMA), ethylene glycol phenyl ether methacrylate (EGPEM), 2-aminoethyl methacrylate hydrochloride (AEMA) and methacrylamide hydrochloride (APMA) at various ratios. The hydrogels were characterized in terms of suitability as contact lenses (solvent uptake, light transmission, mechanical properties, and biocompatibility). Atorvastatin loading was carried out by soaking the dried discs in 10 mL of drug solution (0.04 mg/mL in ethanol:water 20:80 v/v) at room temperature under magnetic stirring. Atorvastatin release was monitored in 10 mL of simulated lacrimal fluid (SLF), at 37º C and under agitation. The most promising hydrogels were evaluated regarding cytocompatibility with Balb/3T3 fibroblasts, and ex-vivo accumulation/permeability of atorvastatin into/through porcine cornea and sclera tissues. The permeability experiments were evaluated following the BCOP test protocol in order to evaluate their capability of providing therapeutic amounts of atorvastatin to the eye structures. The amount of atorvastatin released from the hydrogels towards the donor chamber, and then diffused towards cornea and sclera tissues and the receptor medium were monitored by HPLC.

Results and Discussion

Dried discs rapidly sorbed SLF and the equilibrium was reached in one hour. The solvent uptake in atorvastatin solution was significantly higher (about 90%) than that recorded in SLF (about 50%), due to the addition of 20% of ethanol. All hydrated hydrogels were transparent in the visible range (400-700 nm), with transmittance values close to or above 90% and were considered non-irritant for the ocular surface. The elastic modulus obtained remained within the typical values for soft contact lenses. Addition of EGPEM did not further improve the loading. The uptake was remarkably higher for hydrogels prepared with AEMA and, particularly APMA (up to 11 mg/g loaded). Permeability studies through cornea and sclera revealed that the amount of atorvastatin in the receptor chamber was below the quantification limit. This means that atorvastatin mainly accumulated into the cornea and sclera tissues, but do not progress further.

Conclusion
We can conclude that the incorporation of APMA as functional monomer may endow contact lenses with sufficient affinity for the drug. Permeability studies through porcine cornea and sclera tissues revealed that the amount of atorvastatin accumulated could be effective to treat ocular surface diseases.

**Acknowledgement**

This research was funded by ORBITAL EU-funded Marie Sklodowska-Curie Innovation Training Network (MSCA-ITN), grant number – 813440.

**References**


Design of in situ crosslinkable protease-sensitive alginate 3D matrices via thiol–maleimide “click” chemistry

Mariana Magalhães¹,²,³, Nataliya Débera¹,², Rúben Pereira¹,²,³, Cristina C. Barrias¹,²,³, Silvia J. Bidarra¹,²
¹ INEB, Porto, PT; ² i3S, Porto, PT; ³ iCBAS, Porto, PT

Introduction
Alginate, a naturally occurring polysaccharide, has been widely used in the biomedical field, particularly to build three dimensional (3D) systems. Although alginate is a bio-inert biomaterial, it can be chemically modified to promote highly specific cell-ECM interactions [1]. We have previously showed that molecularly-designed alginate 3D matrices recap key features of native ECMs supporting tissue morphogenesis [2-4]. Here, we aim to develop a novel methodology to synthesize and characterize functional alginate hydrogels based on thiol–maleimide “click” chemistry. The potential of these peptides-conjugated hydrogels as ECM-like matrices for 3D cell culture was also evaluated.

Experimental Methods
Ultra-pure low viscosity alginate (ALG) with high guluronic content was modified with maleimide (MAL) by amidation of the carboxyl groups of alginate with the amine groups of 1-(2-Aminoethyl)maleimide. ALG was modified with variable amounts of MAL (ALG-MAL). Degree of substitution was assessed by ¹H-NMR. Thiol-flanked (bi-functional) protease-sensitive peptides (MMP-pep, CGPGIWGQC) and thiol-terminated cell-adhesion peptide (CGGGGRGDSP) were grafted to ALG-MAL via thiol-maleimide Michael addition click reaction. To confirm peptide double-end grafting and consequently crosslinking, dynamic viscosity of ALG solutions was analyzed by oscillation rheometry. Also, the extent of effective crosslinking was indirectly estimated by quantifying the amount of free thiols in dually crosslinked hydrogels by Ellman’s test. The amount of grafted RGD-pep was quantified using the BCA Protein Assay (Pierce). Gelation kinetics with different amounts of MMP-pep crosslinker was evaluated by single frequency oscillation assay. Primary human mammary fibroblast (hMF) were mixed with alginate and peptide solutions and hydrogels were casted as small discs. 3D cell response to different amounts of MMP-pep and RGD-pep were studied at different time points. Cell viability (live-dead assay) and morphology (F-actin staining) were assessed. Mechanical characterization of these cell-laden hydrogels was also performed.

Results and Discussion
Alginate was successfully functionalized with different amounts of maleimides (theoretical degree of substitution, tDS from 1 to 10%) that was qualitatively confirmed by ¹H-NMR. The presence of maleimide was identified by the appearance of a new peak ~6.9 ppm corresponding to the protons in the double bond of the maleimide group. The efficiency of the reaction was approximately 10%. We observed that high degrees of maleimides lead to poor solubility of alginate derivatives, so we only used derivatives with up to 3% of tDS. The addition of bi-functional MMP-pep should expectably increase the hydrogel viscosity due to the formation of a chemically crosslinked gel network. We observed an increase in viscosity and consequently gel formation between 120 and 480 µM of MMP-pep. Concentrations higher than 480 µM did not alter the viscosity of the solution, probably because at such high density, MMP-pep were preferentially bound only by one side, occupying the maleimides but without bridging two alginate chains. In fact, the presence of free thiols in these higher MMP-pep concentrations was confirmed by the Ellman’s test. hMF were successfully embedded within MMP-pep/RGD-modified alginate matrices, presenting an elongated morphology and forming extensive multicellular network, in contrast to control MMP-insensitive hydrogels, where cells remained essentially round.

Conclusion
A novel methodology for the synthesis of alginate polymer containing maleimide functional groups was established. The covalently grafted maleimides allow the biofunctionalization and in situ crosslinking of alginate by thiol-Michael addition reaction. The incorporation of proteases sensitive peptides significantly enhances 3D cell-cell interactions in alginate hydrogels, improving their performance as ECM-mimics.

Acknowledgement
Portuguese Foundation for Science for EndoSWITCH project (PTDC/BTM-ORG/5154/2020) and research contracts DL 57/2016/CP1360/CT0006 and IF/00296/2015.

References
Hybrid Multicomponent Laminaran/Platelet lysate-based hydrogels

Mehrzad Zargarzadeh, João F. Mano, Catarina Custódio

CICECO, University of Aveiro, Department of Chemistry, Aveiro, PT

Introduction
Hydrogels possess the capability of holding a large amount of water in a three-dimensional (3D) network and mimicking many features of the native extracellular matrix (ECM) which makes them attractive materials for biomedical applications[1]. Laminaran-based hydrogels are interesting due to their tuneable mechanical properties and high cytocompatibility[2]. Recently, photo-crosslinkable platelet lysates (PL)-based hydrogels have exhibited to support distinct human-derived cell cultures owing to their high content of bioactive molecules, such as cytokines and growth factors[3]. Aiming at taking advantage of all features of both PL and laminaran hydrogels, here we combine UV responsive laminaran-methacrylate and PL-methacrylate derivatives plus an adequate enzyme to fabricate a multicomponent hybrid hydrogel (GLMPL) (Fig1.a). This hydrogel is newly designed as a scaffold material for the sustained delivery of glucose produced via enzymatic degradation of laminaran and granting cell adhesin by presence of PL. Such innovation is expected to circumvent the limitations of the current hydrogels strategies that lack on nutrients diffusion and adhere motifs, boosting the application of hydrogels in diverse biotechnological contexts.

Experimental Methods
Methacrylated Laminaran (MeLam) and methacrylated PL (PLMA) were synthesized following the previous reports [2, 3]. The hydrogel precursor solution was prepared by dissolving different MeLam/PLMA mixtures (Fig1.b) in PBS at the final concentration of 10%. The resulting solution (40μL) was pipetted to cylindrical PDMS molds and afterwards irradiated by UV for 2 min. The fabricated hydrogels (LMPL) were investigated in terms of the cell’s adhesion and metabolic activity by encapsulating human adipose-derived stem cells (hASCs). In order to obtain sustained degradation and consequently, a gradual production of glucose over time in such hydrogels, glucoamylase (GA) enzyme was incorporated into the selected LMPL hydrogel solution before UV exposure. In vitro studies were performed on GLMPL hydrogels by culturing encapsulated hASCs in Dulbecco’s Modified Eagle Medium (DMEM) without glucose. As such, any difference in cells response could then be attribute directly to the presence of enzyme and consequently glucose accessibility for the encapsulated cells.

Results and Discussion
The metabolic activity of the hASCs in LMPL was determined by a CellTiter-Glo assay. ATP intracellular activity significantly increased in the function of increasing PLMA content (Fig1.c). As shown in Fig. 1.d, cells maintained their spherical morphology when encapsulated in 75% and 100% hydrogels after 21 days. In contrast, in the rest of the hydrogels, cells attached to the matrix and continued spreading over time. These effects were more pronounced for the 25% hydrogels in which cells form an interconnected network. Therefore, 25% formulation was chosen to fabricate multicomponent hybrid hydrogels. Live-Dead viability assay confirmed encapsulated hASCs, cultured in glucose free culture medium, retained their spherical shape, while in the presence of GA and consequently available glucose, cells stretched inside of the hydrogel (Fig1.e). DAPI/phalloidin staining have confirmed that cells readily elongated in GLMPL hydrogels and forming interconnected networks with neighbouring cells (Fig2.f).

Conclusion
In conclusion, these results, combined to the fact that most current bioscaffolds suffer from lack of nutrient diffusion and adhere motifs, clearly suggest the potential of this hydrogel in future developments of 3D structures in a wide range of biotechnological applications as an autonomous cell supporting system. This self-maintained and
biocompatible material could potentially be used as a closed culture system for living cells and any other microorganisms, as a novel platform for cell delivery, or to be used in bioreactors.

Acknowledgement
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020. Mehrzad Zargarzadeh acknowledges the doctoral grant SFRH/BD/143883/2019 and Catarina A. Custódio acknowledges the individual contract CEECIND/02713/2017.

References


Biomimetic hydrogels for bioprinting: combining dynamic and static networks

Ana A. Aldana¹, Francis L.C. Morgan¹, Sofie Houben², Marta Redondo¹, Louis Pitet², Lorenzo Moroni¹, Matthew B. Baker¹

¹ Maastricht University, Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, NL; ² Hasselt University, Institute for Materials Research, Hasselt, BE

Introduction
Hydrogels are promising candidates for the recapitulation of the native extracellular matrix. Yet recreating molecular and spatiotemporal complexity remains a challenge. Double network (DN) hydrogels have shown interesting properties to recapitulate the complexity of native tissues.[¹] Combining networks also show advantages in terms of remarkable mechanics, shear-thinning, and self-healing attributes. These properties combined with smart cross-linking strategies can make DN hydrogels great candidates for 3D bioprinting. 3D bioprinting allows the creation of a spatially defined object, but the field is currently dominated by static, unresponsive materials (synthetic and natural).[²] Here, we aim to create biomimetic DN hydrogels for bioprinting applications by combining dynamic and static bonding in the individual networks. Furthermore, these materials will allow us to explore the presentation of bioactive cues on either the static or dynamic network (within the same material) providing a handle to mimic the dynamic ECM complexity. We investigated the combination of oxidized alginate cross-linked by Schiff base reactions (OA, dynamic network) and polyethylene glycol diacrylate (PEGDA, static network) cross-linked by UV irradiation.

Experimental Methods
OA (10% theoretical degree of oxidation) [3] and PEGDA (20kDa) were synthesized and characterized by NMR and GPC. DN and single network (SN) formulations were prepared by adding varying proportions of each polymer: OA 0/2/2.5/3 wt% and PEGDA 0/10/15/20 wt%. One equivalent of hydrazide and Irgacure 2959 (1% w/v) were added in all formulations. Hydrogel (OA 2.5% - PEGDA 10%) formation was explored at various PBS (without Mg²⁺ and Ca²⁺) concentrations (0, 13.5, 30, 84 and 149 mM).

To investigate printability, we used the BioX bioprinter (CellInk, Sweden). Conical needles (ID 0.41 mm) were fixed to cartridges and printed via pressure. We then imaged each print (16 megapixel camera) and used ImageJ (NIH, USA) software to process them.

For cell culture experiments, 0.75 mM RGD (aminooxy-RGD or thiol-RGD for attaching to dynamic or static networks, respectively) was added to the hydrogel formulation to facilitate cellular adhesion. DN hydrogels were irradiated with UV light (365 nm, 30 s) to crosslink the static network. Human dermal fibroblasts (HDFs) were cultured under standard conditions on OA 2.5% - PEGDA 10% hydrogels.

Results and Discussion
Effect of PBS concentration on dynamic hydrogel formation. The pH and ionic strength were determined for each PBS solution. With an increase of PBS concentration, the hydrogel formulations (before photocrosslinking) change from soft and sticky to tough and brittle. Whereas OA single network formulations formed a gel after Schiff-base reaction (hydrazone bonds) only in presence of PBS. The results show that the ionic strength affects the dynamic crosslinking. For further characterization, hydrogels were prepared in 13.5 mM PBS solution.

Injectability and printability. The injectability of a small library of DN hydrogel formulations (OA 0/2/2.5/3 wt %; PEGDA 0/10/15/20 wt %) was tested. The overall polymer concentration affects the hydrogel properties. The higher the polymer concentration, the more pressure needed to extrude the gel through the nozzle and/or the higher nozzle inner diameter needed. OA 2.5 wt % and PEGDA 10 wt % showed the best balance between filament formation,
applied pressure and nozzle diameter, important for injection and bioprinting. Therefore, we selected a series of DN hydrogels based on the best formulation for further characterization (OA 2.5 wt % - PEGDA 10 wt %) The printability of DN formulations with varying amounts of OA(2/3 wt %) or PEGDA (0/5/10/15 wt %) were tested and the printing parameters were optimized.

**Self-healing.** We previously reported that the dynamic crosslinking of oxidized alginate with adipic acid dihydrazide gives self-healing properties to gels,[3] yet the effect of a second static network on this property is not known. Macroscopic self-healing of the hydrogels was tested (Figure 1.b). All DN hydrogel formulations were able to heal even at a high concentration of PEGDA.

**Cell response and structure relationships.** Human dermal fibroblasts (HDFs) were seeded on top of gels for 7 days to explore cell viability and morphology. HDFs were alive and migrated in the hydrogels (Figure 1.c), a crucial necessity for tissue maturation. By attaching RGD to dynamic or static networks, HDFs exhibited differences in morphology and spreading.

**Conclusion**

By combining dynamic and static networks, we developed hydrogels with better printability attributes than single networks and with tunable cell behavior by RGD conjugation. These complex dynamic hydrogels show potential for bioprinting applications and give unique opportunities to tune the artificial ECM environment. Currently, we are further characterizing the mechanical properties of the hydrogels, and developing these materials for applications in cell delivery and model tissue fabrication.

**Acknowledgement**

This research has been made possible via the support of NWO (Innovation Fund Chemistry, project “DynAM” under project agreement 731.016.202), and the Dutch Ministry of Economic Affairs.

---

**References**


Using SPAAC for the Synthesis of Versatile Covalent Hydrogels: Outperforming Them All?

Vianney Delplace1, Nathan Lagneau1, Pierre Tournier1, Boris Halgand2, François Loll1, Jérôme Guicheux1,2, Catherine Le Visage1,2

1 Inserm, UMR 1229, RMeS, Regenerative Medicine and Skeleton, Université de Nantes, ONIRIS, Nantes F-44042, FR; 2 CHU Nantes, PHU 4 OTONN, Nantes F-44042, FR

Introduction
Hydrogel design is a booming field of research. Hydrogels are the most suited scaffolds for cell encapsulation, owing to their properties that can best mimic the natural cell microenvironment. Yet, while many crosslinking strategies have been proposed to allow cell encapsulation, most of the existing approaches (i) require external stimuli or catalysts/activating agents, (ii) are not entirely bioorthogonal or compatible with physiological conditions, or (iii) have inherent limitations such as limited stability or slow gelation rate. Thus, a class of hydrogels that would be fully tunable, fast-gelling, biocompatible and, yet, easy to synthesize and use, remains to be designed. In this context, we hypothesized that using the strain-promoted azide-alkyne cycloaddition (SPAAC) «click» reaction as a bioorthogonal crosslinking mechanism would allow the synthesis of versatile polysaccharide-based hydrogels, addressing all design criteria and offering new opportunities.

Results and Discussion
We first investigated the development of SPAAC hyaluronic acid (HA)-based hydrogels, using azide and bicyclononyne (BCN) as complementary moieties. Each of the two components, i.e., HA-azide and HA-BCN, were easily synthesized in single-step reactions from commercially available compounds, with tunable degrees of substitution. Mixing the two HA components together, we successfully obtained hydrogels that form under physiological pH and temperature. Optimizing the HA molecular weight, component ratio, polymer content and substitutions, we designed tunable hydrogels that form within seconds to tens of minutes under physiological conditions, with a Young’s modulus tunable over orders of magnitude (0.5 to 40 kPa). Interestingly, we found that the crosslink density and polymer concentration of these gels can be finely tuned to obtain minimally-to-non-swelling materials with tunable stiffness, all stable over months. Finally, we demonstrated that all HA-based gels degraded enzymatically within hours to days. We further confirmed that SPAAC crosslinking can be easily applied to other polysaccharides, such as alginate or chondroitin sulfate, as a most versatile platform for hydrogel design.

Using a murine cell line (L929 fibroblast cells), we demonstrated excellent cytocompatibility of these gels (cell viability > 90%) via live/dead confocal imaging. We further investigated the effect of 3D encapsulation on the viability, metabolic activity and proliferation of human mesenchymal stem cells. These materials are now being investigated for a breadth of in vitro and in vivo applications, spanning from organoid culture to microgel design and bioprinting.

Acknowledgement
The authors thank the Fondation pour la Recherche Médicale (ARF201809007012; VD), the Nantes Excellence Trajectory program (NExT Junior Talent 2018; VD), and the Marie Skłodowska-Curie Actions program (MSCA-IF-RI 2019; VD) for their financial support.
N16-06

Novel thermosensitive hydrogel for the delivery of reactive species generated by cold atmospheric plasma

Xavi Solé-Martí, Cédric Labay, Francesco Tampieri, Maria-Pau Ginebra, Cristina Canal

Universitat Politècnica de Catalunya (UPC), Department of Materials Science and Engineering (CEM), Biomaterials, Biomechanics and Tissue Engineering Group (BBT), Escola d’Enginyeria de Barcelona Est (EEBE), Barcelona, ES

Introduction

In the last years, the use of cold atmospheric pressure plasma (CAP) to treat tumors has been widely investigated as a novel potential anti-cancer therapy [1]. CAP is an ionized gas that consists of a mixture of ions, UV photons, electromagnetic radiation, free electrons, radicals and excited molecules that, in presence of air and water, leads to an environment rich of reactive oxygen and nitrogen species (RONS). These CAP-generated RONS can be transferred to liquids by diffusion or generated inside them through secondary reactions, thereby generating a plasma-conditioned liquid (PCL). PCL represent a very interesting alternative to direct CAP treatment, because they may allow treatment of malignant tumors located in inner organs of the body by means of an injection. However, PCL injection in the tumor site may result in fast diffusion throughout the body due to extracellular fluids and blood flow. Therefore, the design of efficient vehicles that allow a controlled release of RONS to the diseased site is a fundamental requirement.

Injectable hydrogels that allow in situ gel formation have attracted much attention as drug delivery systems. Not only can they be conveniently injected with minimal invasive surgery, but also they are capable to fill irregular shaped defects, sometimes created after a tumor resection. Methylcellulose (MC) is a water-soluble cellulose derivative that can form a thermo-reversible hydrogel in water upon heating. At low temperature water molecules form a cage-like structure surrounding the methoxyl groups and thus solubilizing MC [2,3]. However, high temperatures cause the destruction of the cage structure of water and a three-dimensional hydrogel network is formed due to hydrophobic interactions of the methoxy groups of MC. Sol-gel transition of pure MC is ~60 °C, too high for in vivo purposes as an injectable hydrogel. However, the gel point can be altered by the addition of salts, based on the Hofmeister series [4].

The aim of this study is to develop a thermosensitive hydrogel with the ability to generate, store and release plasma generated RONS, and evaluate the effects of this release on cancer cells. To do so, we optimized the composition of the hydrogel to confer ability to form a gel at physiological temperatures while remaining in liquid phase at room temperature to allow the suitable formation of plasma-generated RONS. The physico-chemical properties of the hydrogel were characterized before and after plasma treatment and the in vitro effects of the RONS in the novel MC formulation were investigated against osteosarcoma.

Experimental Methods

MC solutions where prepared by dispersing MC powder in hot deionized water (approx. 80 °C) and then transferred into an ice bath under continuous stirring in order to allow the dissolution of the MC. The sol-gel temperature was tuned by incorporation of different concentrations of Na₂HPO₄ in a 1 wt % MC (1MC) solution and studied by rheology and microDSC analysis. Plasma treatment of MC was performed by means of a kINPen® plasma jet (1 L/min Ar gas flow, 10 mm gap). H₂O₂ and NO₂⁻ generated in the plasma-treated MC were determined by Amplex Red® and Griess methods, respectively. Also, the detection of OH radicals during plasma treatment was quantified with a coumarin probe. Chemical modifications of the MC backbone due to CAP treatment were investigated with nuclear magnetic
resonance (NMR) and size-exclusion chromatography. Cell viability was evaluated by metabolic activity quantification and Live Dead Assay was assessed by confocal microscopy.

Results and Discussion
With a gel point of ~ 36 °C, 3.5% Na₂HPO₄ in 1% MC (1MC3.5HP) was selected for plasma treatment. Plasma treatment times from 30 s up to 180 s were evaluated, leading to concentrations of ~170 μM and ~40 μM of H₂O₂ and NO₂⁻, respectively, for the longest plasma treatment (Fig 1). Hydroxylcoumarin production rate was 24 times lower in 1MC3.5HP compared to the 3.5% Na₂HPO₄ solution without MC. The gelling ability of the hydrogel was not affected by the CAP treatment. Cell viability at 24 h for the 1MC3.5HP samples decreased in a dose-response manner, down to 30% cell viability for longest treatment time (Fig 2. i). Confocal microscopy images also revealed compromised cell morphology after exposition to plasma-treated MC (Fig 2. ii).

Conclusion
MC hydrogels are able to generate, store and release plasma generated RONS. The anticancer effects of plasma-treated MC are time-dependent in MG63 osteosarcoma cells, showing a direct relation with the concentration of RONS at increasing plasma treatment time. It is the first time that a thermosensitive hydrogel is used as a delivery system of RONS generated by plasma, proving that plasma-treated hydrogels have a promising applicability in cancer therapy.

Acknowledgement
This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (Grant agreement Nº 714793). Authors belong to the SGR2017 1165.
Figure 2. Biological effects of plasma treated 1MC3.5HP hydrogel.

i) metabolic activity of MG63 osteosarcoma cell line. Cells in adherent culture were exposed to 1MC3.5HP treated by kINPen for 30, 60 and 180 seconds (P30, P60, and P180, respectively).

ii) Representative confocal images MG63 cells after 24 h exposition of plasma-treated 1MC3.5HP.

References
Thermoresponsive Single Wall Carbon Nanotubes reinforced Collagen/Chitosan Hydrogels Compatible for Bone Tissue Engineering

Kulwinder Kaur¹, Ciara Murphy¹²

¹ Royal College of Surgeons, Tissue Engineering Research Group, Department of Anatomy and Regenerative Medicine, Dublin, IE; ² Advanced Materials and BioEngineering Research (AMBER) Centre, ² Advanced Materials and BioEngineering Research (AMBER) Centre, Dublin, IE

Introduction
Mechanical strength is an important consideration in the design of biomaterial platforms for bone regeneration in load bearing defects. Hydrogels with in situ gelation properties are attractive biomaterials for minimally invasive tissue engineering applications. This is due to their ability to transition from liquid state to solid at physiologically temperatures, and suitability for administration via injection[1]. Whilst hydrogels provide an ideal platform for tissue regeneration in terms of biocompatibility, biodegradability and handling properties, their mechanical and structural properties often deem them unsuitable for bone[2]. The aim of this study was to formulate a chitosan-collagen (CS-COL) hydrogel, reinforced with carboxylated single wall carbon nano-tubes (SWCNTs) to develop mechanically robust hydrogels for load-bearing applications.

Experimental Methods
Hydrogel systems comprising of chitosan/collagen polymeric matrix reinforced with SWCNT were prepared by mixing acid solubilized chitosan with Type I collagen and different concentrations (0, 0.5, 1, 3 and 5 wt/v%) of SWNT in presence of β-glycerophosphate (β-GP), a gelling agent at pH~7.4. Thermoresponsive behaviour of the prepared hydrogels was confirmed by using rheology technique. Porous behaviour and change in structural properties with SWCNT was assessed by using different physiochemical techniques including X-ray Diffraction, Fourier Transform Infra-Red Spectroscopy, Scanning and Transmission Electron Microscopy. Quantitative analysis of mechanical strength was performed on Zwick Roell Materials Testing machine with 5N load. Effect of SWCNT on thermal behaviour of hydrogels was evaluated via Thermal Gravimetric Analysis (TGA), in vitro weight loss and mineralization was performed in phosphate buffer saline (PBS) and simulated body fluid (SBF) at physiological conditions respectively. To determine the compatibility of the prepared hydrogel systems for bone tissue engineering, osteoblast cell line MC3T3-E1 was used. In vitro cell cytotoxicity and proliferation was analyzed by using Lactate dehydrogenase activity (LDH) and Pico Green assay respectively.

Results and Discussion
All formulations were thermoresponsive and injectable in nature as confirmed by rheology analysis. It was speculated that addition of SWCNT led to porous structure (Figure 1), significantly increased mechanical strength from kPa to mPa (63% increase, Figure 2), high interconnectivity (85±0.5%) and crosslink density. The weight loss analysis indicated that the weight loss ratio can be varied by changing the concentrations of chitosan, collagen and SWCNTs. Physiochemical characterization results confirmed the presence of SWCNT at the expected diameter (0.85–1.30 nm) with strong molecular interactions between polymeric matrix (+ve zeta potential) and SWCNTs (-ve zeta potential) which led to the formation of more aligned and crystalline network with lower zeta potential values (Figure 1). Gelation mechanism of prepared hydrogels at physiological conditions was also elucidated. Results demonstrate that β-GP and temperature are the two most important driving factors for the transformation of hydrogels from solution to solid gel at physiological conditions. Hydrogels showed the presence of hydroxyapatite layer on the surface of the samples even after 1 day of incubation in SBF, which indicates the biocompatible nature of the prepared hydrogels. This was...
Further demonstrated by an absence of cytotoxicity and increased cell proliferation over 7 day culture period (Figure 2).

Conclusion
This study presents novel thermoresponsive, injectable and mechanically robust hydrogels formulations. For the first time, our results showed that β-GP and temperature are important for gelation at physiological conditions and the reinforcement of CS-COL hydrogels with SWCNT significantly change the structural morphology to more porous and aligned network which leads to high crystallinity and ultimate increased the mechanical strength from kPa to mPa which is closer to mechanical strength of the bone. So, these hydrogels are suitable for application as mechanically robust and injectable biomaterials in bone tissue engineering especially in load bearing area.

Acknowledgement
The author acknowledges Irish Research Council, Government of Ireland Post-Doctoral Fellowship (GOIPD/2019/793) for funding. Collagen materials were provided by Integra Life Sciences, Inc. through a Material Transfer Agreement.

References
Tuning dynamic hydrogels via molecular engineering: Equilibria constants in Schiff base hydrogels

Francis Morgan, Floor Ruiter, Lorenzo Moroni, Matthew Baker

Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, NL

Introduction
Robust control on the mechanical properties of hydrogels has become important for the development of new soft materials for tissue engineering and regenerative medicine. Dynamic cross-linking has emerged as a promising approach to tune hydrogel stiffness and impart both dynamic biomimetic properties and enhanced injectability and processability to the materials. (1) Recent studies have explored the fundamental relationship between molecular constants such as the equilibrium constant and material stiffness by varying the pH and simple changes to the molecular structure. (2) However, to successfully translate these findings more information is needed to fill out the toolbox available for material design under physiologic conditions. Here, we investigate the relationships between molecular equilibria and rate constants on the stiffness of alginate hydrogels formed through Schiff-base formation.

Experimental Methods
Alginate was modified via oxidation or covalent attachment of aldehydes to the backbone. Crosslinks with various modified amines were either purchased from Sigma-Aldrich, or synthesized via end modification of poly(ethylene glycols). Monomeric model amines were purchased or synthesized for determination of binding constants. Thermodynamic and kinetic measurements were performed via $^1$H NMR and/or UV-vis. Rheological properties were determined using a 20 mm cone-plate geometry on a DHR-2 rheometer (TA).

Results and Discussion
By molecularly engineering the interactions and the hydrogel architecture, we can varied physical constants like $K_{eq}$ and $k_1$ by several orders of magnitude by changing only the chemical environment of the amine and/or aldehyde participating in the reaction. The difference in stiffness corresponds well to the difference in measured equilibrium constants, emphasizing the importance of molecular parameters for dynamic systems. Furthermore, via using competitive cross-linkers we are able to uniquely tune the hydrogel. In some specific regimes, adding more cross-linker to the material can actually decrease the stiffness of the hydrogel, a unique property to dynamic hydrogels.

This work is a step towards the mainstream adoption of reversible Schiff-base formation as a stable platform for the design of novel biomaterial hydrogels leveraging molecular constants to achieve the desired mechanical properties.

Conclusion
The ability to control mechanical properties over several orders of magnitude without changing polymer content, molecular weight or temperature also has significant implications for numerous applications from tissue formation to biofabrication. Dynamically cross-linked hydrogels provide unique opportunities for tuning of materials properties, which are not available to covalently cross-linked materials. This work shows a path forward for rational design of dynamic hydrogel architectures, and uncovers unique opportunities.

Acknowledgement
The authors are gracious for funding from the research programme Innovation Fund Chemistry, which is partly financed by the Netherlands Organisation for Scientific Research (NWO) under TA grant agreement 731.016.202 (“DynAM”). The authors would also like to thank the Brightlands Materials Center and the Province of Limburg for their support of this work.
Designing dynamic covalent hydrogels based on reversible Schiff-base formation

A) Dynamic covalent chemistry leverages the relationship between molecular constants such as the equilibrium constant, and the resulting mechanical properties. B) General schematic for the formation of a secondary aldimine from an aldehyde and an amine-terminated functional group; variation in the R moiety allows us to tune the equilibria and rate constants for the reaction.

References
N16-09

Nanogels as smart drug delivery systems for the treatment of nonalcoholic fatty liver disease (NAFLD)

Emanuele Mauri¹, Manuele Gori¹, Sara M. Giannitelli¹, Pamela Mozetic², Nicolò Merendino³, Giuseppe Gigli²,⁴, Filippo Rossi⁵, Marcella Trombetta¹, Alberto Rainer¹,²

¹ Università Campus Bio-Medico di Roma, Department of Engineering, Rome, IT; ² National Research Council, Institute of Nanotechnology (NANOTEC), Lecce, IT; ³ Università degli Studi della Tuscia, Department of Ecology and Biology, Viterbo, IT; ⁴ Università del Salento, Department of Mathematics and Physics “Ennio De Giorgi”, Lecce, IT; ⁵ Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering “Giulio Natta”, Milano, IT

Introduction
Nonalcoholic fatty liver disease (NAFLD) represents a leading cause of chronic liver disease worldwide. Its multifactorial nature and its potential evolution from simple steatosis to the more severe steatohepatitis, eventually leading to cirrhosis and hepatocellular carcinoma[1,2], define a critical scenario, still demanding for effective therapeutic treatments. Hydroxytyrosol (HT) has been shown to contrast the development of hepatic steatosis through its lipid-lowering, antioxidant and anti-inflammatory activity. The efficient delivery of HT to hepatocytes remains a crucial aspect, which could be addressed by using smart nanocarriers.

Experimental Methods
We propose the design of traceable nanogels (NGs) as a promising tool to promote the intracellular HT release, avoiding metabolic alterations and the loss of the active molecule in the extracellular environment. NGs were synthetized from polyethylene glycol (PEG) and rhodamine-labeled polyethyleneimine (PEI), using a single o/w emulsion and solvent evaporation technique [3]. The nanosystems were characterized by a hydrodynamic diameter around 250 nm, and the HT loading was performed exploiting the NG swelling behavior from the dry state: in this way, we encapsulated HT within the NG meshes by steric hindrance, without any chemical modification of the drug that could affect its therapeutic potential. The performance of nano-encapsulated HT was evaluated in an in vitro model of hepatic steatosis (induced by an overload of free fatty acids, FFAs) and compared to that of pristine HT using high-content analysis tools [4].

Results and Discussion
NG specimens presented a sustained HT release over time and a significant decrease in the intracellular triglyceride accumulation was detected only when HT was delivered by the nanovectors, restoring cell viability and outperforming the efficacy of HT in its non-encapsulated form (Fig. 1).

Conclusion
The formulated nanogels represent a more promising strategy compared to the conventional drug administration routes for protecting liver cells from the development of NAFLD.
Fig. 1
(a) In vitro release profiles of HT delivered by NG at concentration of 0.1 mg/mL (white) and N at 0.5 mg/mL (black). (b) Representative 3D rendering of the confocal Z-stack micrographs of NG internalization (in red) in HepG2 cells. (c) Representative confocal micrographs of LipidTox HCS assay, showing intracellular triglyceride accumulation (in purple, false color). Scale bar = 50 μm. (d) Results of the HCS LipidTox assay. (e) MTT cell viability assay on HepG2 cells after 48h incubation with the different treatments with and without NGs.

References
1:15 p.m. – 2:00 p.m.

Track01

**CC | Awards & Closing Ceremony**

**Chairs**
Ana Paula Pêgo  
M. Cristina L. Martins  
Pedro L. Granja

Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Engenharia Biomédica (INEB), University of Porto, Porto, Portugal

**Program**

ESB2021 Awards for Best Oral and Best Poster Presentations given by Karine Anselme, Education Officer of the ESB Council

Photo Contest Award

Closing Address by ESB2021 Chairs

ESB2022 Presentation by Joëlle Amédée, Chair of ESB2022
Thursday, 9 September, 2021

09:45 a.m. – 9:00 pm.

Track08

Biomechanics by SIBB Symposium

Title: Sports biomechanics and biomedicine
09:45 a.m. – 10:00 a.m.

Track08

Biomechanics Opening | Welcome Ceremony
Biomechanics by SIBB Symposium: Sports biomechanics and biomedicine

Chairs
Enrique Navarro
Universidad Politécnica de Madrid, Health and Human Performance, Madrid, ES
Injuries have a high incidence in people who perform physical exercise in general and especially in both recreational and professional sports.

The aim of this table is to analyze and discuss risk factors and preventive and rehabilitation therapies that affect the joints of the human body and their relationship with sport in a different way.
Biomechanics 01-01
Enthesopathies in the shoulder
Karla B. Rodríguez

Unidad hombro y codo, Barcelona, ES

Biomechanics 01-02
Enthesopathies in the elbow
Joan Valenti

Clinica Molins de Rei, Barcelona, ES

Biomechanics 01-03
Enthesopathies in the wrist and hand
Jordi Font

Dirección médica. iMove Traumatologia, Barcelona, ES

Biomechanics 01-04
Enthesopathies in the hip
Marc Tey

Hospital Universitari del Mar, Barcelona, ES

Biomechanics 01-05
Enthesopathies in the knee
Paulo Amado

Unidade de Ortopedia e Traumatologia do Hospital Lusíadas Porto, Porto, PT
Biomechanics 01-06

Enthesopathies in the ankle and foot

Antonio Viladot Voegeli

Clinica Tres Torres, Barcelona, ES
Swimming performance depends on multiple factors that affect the different phases of competition. That is, the start, the swim, the turns and the arrival. The objective of this table is to present the state of the art on these factors on which the performance of a swimmer depends in a relevant way.
Biomechanics 02-01

Hydrodynamics

Daniel Marinho

University of Beira Interior/CIDESD, Covilhã, PT

Biomechanics 02-02

Swimming Biomechanics: combining statics and dynamics

Tomohiro Gonjo

Norwegian School of Sport Sciences, Oslo, NO

Biomechanics 02-03

Starting and Turning

Ricardo Fernandes

Spain

Biomechanics 02-04

Race analysis

Santiago Veiga

Polytechnic University of Madrid, Madrid, ES

Biomechanics 02-05

Questions and Discussion
3:00 p.m. – 5:00 p.m.

Track08

**Biomechanics 03 | Oral Presentations 01**

Chair

**Francisco J. Rojas**
University of Granada, Department of Physical Education and Sport, Granada, ES
Pedalling variability in cyclists of different competitive levels

Alba Herrero-Molleda¹, Pablo Floría², Juan García-López¹

¹ Universidad de León, Faculty of Physical Activity and Sport Sciences, León, ES; ² Universidad Pablo de Olavide, Physical Performance & Sports Research Center, Sevilla, ES

Introduction
Movement variability in sports has been extensively studied in the last few years. Previous cycling studies observed relationships between movement variability during pedalling and both competitive level and pedalling intensity. Expert cyclists showed a lower coordination variability than novice ones [4], and a decreasing in the muscle action variability as pedalling intensity increased [1]. Several approaches have been proposed in the literature to analyse movement variability highlighting the linear and non-linear measures [3]. However, it has been suggested that their behaviour could be different [5]. Therefore, the purposes of the present study were (1) to analyse the effect of the cyclists’ competitive level and pedalling intensity on the crank torque variability, and (2) to examine whether there would be a different interpretation when multiple-trial variability and complexity measurements are used to analyse movement variability.

Experimental Methods
Seventy-two cyclists participated in the present study (24.7 ± 5.4 yr, 69.0 ± 6.0 kg and 178.7 ± 5.0 cm). They were divided in three homogeneous groups (n= 24) of competitive levels (Level 1= club; Level 2= elite; Level 3= professionals), according to their cycling training volume per season (5000-15000, 15000-30000 and more than 30000 km, respectively) [2]. They performed three sets of 5-min submaximal pedalling (200, 250 and 300 W) at a constant cadence (90 rpm) with a 6-min rest in between. The tests were carried out on an electromagnetically braked cycle ergometer (Lode Excalibur Sport), using their own cycling shoes and bike geometries.

To assess the multiple-trial variability, an ensemble average curve from 20 complete cycles of crank torque series from the right leg (symmetry between both legs was assumed) were calculated for each cyclist and pedalling intensities, as well as mean and standard deviation of each data point on average curve. The average standard deviation across all points composing the average curve was calculated. Thus, the total variability of the continuous curve was represented as a single value. To examine the time-dependent structure of crank torque dataset, the sample entropy (SampEn) was calculated [3]. A two-way repeated measure of analysis of variance was performed on the SampEn and multi-trial variability values to test the effects of competitive level (between-participant factor) and pedalling intensity (within-participant factor) on movement variability.

Results and Discussion
The primary outcome was to demonstrate a clear effect of pedalling intensity on crank torque multiple-trial variability (i.e.; it increased) and complexity (i.e.; it decreased) (Figures 1 and 2). The decrease of SampEn as pedalling intensity increased (Figure 2) agree with previous studies that observed a low muscular activation variability when the pedalling power was increased [1]. Another important finding was that, as pedalling intensity increased, multiple-trial variability increased (Figure 1), while complexity showed an opposite trend (Figure 2). This is in accordance with previous studies that observed a different effect of exercise intensity on both variables [5], so these two parameters of movement variability must be interpreted in a different way. Non-linear analysis (SampEn) was more sensitive than linear one (multiple-trial variability) to detect the effect of pedalling intensity and performance level on crank torque variability. According to the results of SampEn (Figure 2), a small effect of competitive level on complexity was observed. It could be possible that high-level cyclists show an adaptation to their highest training volume, decreasing the SampEn. On the contrary, according to the results of multiple-trial variability (Figure 1), it could be...
possible that variability within the perceptual-motor system is not functional for cycling performance, being pedalling a task that does not need variability [4]. However, the fact that pedalling intensity was identical for all cyclists could support the hypothesis that the competitive level had an effect on SampEn, because Elite and Professional cyclists pedalled at a lower relative intensity than Club cyclists did (i.e.; their maximal aerobic power is presumably higher).

**Conclusion**

In conclusion, crank torque multiple-trial variability (standard deviation) and complexity (SampEn) are affected by pedalling intensity, which could be due to the changes in biomechanical constraints and to the minimal intervention principle. The SampEn analysis is more sensible than the multiple-trial variability analysis to detect the influence of pedalling intensity and cyclists’ competitive level. Taking into account the complexity results, it seems that the crank torque time series regularity increases (SampEn decreases) as competitive level increases, which could be due to an adaptation to the highest training volume. However, further studies should confirm this hypothesis using similar relative pedalling intensities.

**Acknowledgement**

The authors would like to thank the cyclists who participated in this study for their collaboration. Thanks also to the University of Leon for supporting a predoctoral grant (2021–25) and to the Spanish Council of Sports (CSD) for supporting the Spanish Cycling Research Network –REDICYM– (references 29/UPB/19 and 41/UPB/20).

![Figure 1:](image1.png)

**Figure 1:**

Multiple-trial variability values according to the cyclists’ competitive level (Club, Elite, Professional) and pedalling intensity (200, 250 and 300 W).

![Figure 2:](image2.png)

**Figure 2:**

Sample entropy values according to the cyclists’ competitive level (Club, Elite, Professional) and pedalling intensity (200, 250 and 300 W).

**References**


Inter-limb differences in bilateral CMJ peak force is not associated with inter-limb differences during unilateral CMJ height performance

Sergio Miras-Moreno¹, Alejandro Pérez-Castilla¹, Amador García-Ramos¹, Mar Cepero², Danica Janicijevic³, Juan Carlos De la Cruz¹, F.Javier Rojas¹

¹ University of Granada, Department of Physical Education and Sports, Granada, ES; ² University of Granada, Department of Teaching Body Language, Granada, ES; ³ University of Belgrade, Faculty of Sport and Physical Education, The Research Centre, Belgrade, RS

Introduction
The concept of inter-limb asymmetries has attracted considerable attention among researchers, physical therapists, and strength and conditioning professionals and it refers to the differences in mechanical performance of one limb with respect to the other [1]. This attention is warranted because inter-limb asymmetries may provide valuable information related to muscle function, injury risk, and decision regarding return to play. Numerous studies have compared the forces exerted by both limbs during jumping-based tasks [1,2,3]. Therefore, it is possible that the associations between the inter-limb differences in force production during the bilateral countermovement jump (CMJ) and the inter-limb differences in unilateral CMJ performance could be stronger in athletes who are frequently performing vertical jumps during their sport activity (e.g., basketball players) [2,4].

Previous studies have used the terms “limb preference” (i.e., the self-reported leg that subjects choose to complete a particular task) or “limb dominance” (i.e., the leg that provides a greater mechanical performance) as the criteria to analyze inter-limb differences following a jumping activity [5]. Therefore, the lack of agreement between the self-reported preferred leg and the dominant leg emphasizes the importance of considering this methodological factor when interpreting the jumping asymmetry profile of athletes [6].

Specifically, the main objective of the present study was to determine whether the inter-limb difference in unilateral CMJ height performance is significantly associated with the inter-limb difference in peak force production detected during the bilateral CMJ. A secondary aim was to elucidate whether the self-reported preferred leg is the extremity that contributes more to force production during bilateral and unilateral CMJs.

Experimental Methods
A repeated-measures design was used to explore the relationship between CMJ inter-limb asymmetries in force production and unilateral CMJ height performance in twenty-three amateur senior basketball players. In a single session, subjects performed eight unilateral CMJs (four trials with each leg) and four bilateral CMJs. Dual force platforms (Type 9260AA6; Kistler, Winterthur, Switzerland) were used for recording peak force values during bilateral CMJs and jump height during unilateral CMJs. The average value of the four trials of each CMJ type was considered for statistical analyses.

Results and Discussion
No significant differences between the legs were observed for the magnitude of any mechanical variable (p ≥ 0.215, ES ≤ 0.11), but they presented nearly perfect correlations (r ≥ 0.916; Figure 1). The inter-limb asymmetries reported during the bilateral CMJ peak force are not associated with the inter-limb differences in unilateral CMJ height performance (r = 0.149; Figure 2).

Many of the actions that are performed in basketball training and competitions (e.g., jumping, changing of direction, or pivoting) are generally performed unilaterally [4,5]. Therefore, it seems logical to expect inter-limb asymmetries in basketball players due to the specific demands of the sport [4]. It has been suggested that the evaluation of inter-limb asymmetries during different jumping tasks might provide useful information to reduce the risk of injuries and to
improve physical and sport-specific performance \cite{5}. However, an open research question is whether the inter-limb asymmetries should be preferably evaluated using bilateral or unilateral jumps. Therefore, considering the available scientific evidence, regardless of the studied population, clinicians and coaches should not expect a high agreement between the asymmetries detected in bilateral and unilateral CMJs. A possible explanation to this phenomenon appears to be related to neural factors instead of mechanical capabilities of the limbs. Therefore, the present study adds more evidence about the variable nature of inter-limb asymmetries, which do not only depend on the metrics and tasks, but also on the variant (unilateral or bilateral) of the task evaluated.

Another finding is that for most of the subjects the \textit{self-reported preferred leg} was generally weaker than the non-preferred leg during both bilateral and unilateral CMJs (self-reported preferred leg only revealed a higher performance in 7 out of 23 subjects for the unilateral CMJ height and in 6 out of 23 subjects for the bilateral CMJ peak force. Therefore, it seems that the subjective expression of the leg dominance cannot be used as a predictor of unilateral vertical jump performance because leg dominance may be in function of the type of activity \cite{5}.

\textbf{Conclusion}

These results encourage practitioners to evaluate asymmetries in tasks that resemble as close as possible the specific demands of their sport. The self-reported preferred leg should not be considered as the leg with higher vertical jump capacity. Therefore, it is necessary to objectively assess the leg that contributes more to vertical jump performance.

\textbf{Acknowledgement}

This study was funded by the Spanish Ministry of Science and Innovation (PID2019-110074GB-I00/SRA (State Research Agency)/10.13039/501100011033).
Relationship between unilateral CMJ height asymmetry and bilateral CMJ peak force asymmetry.

Figure 2. Relationship between the inter-limb differences in unilateral countermovement jump (CMJ) height and the inter-limb differences in bilateral CMJ peak force in men (empty circles) and women (full circles). $r$, Pearson’s product-moment correlation coefficient.

References
Deep Squat analysis: Functional Movement Screen criteria vs. biomechanical parameters related to global stability (preliminary results)

Pedro Aleixo¹, Sidnei Ramalho², João Abrantes³

¹ CIDEFES/CICANT/Universidade Lusófona de Humanidades e Tecnologias, Lisbon, PT; ² Faculdade Educação Física e Desporto/ULHT, Lisbon, PT; ³ CICANT/Universidade Lusófona de Humanidades e Tecnologias, Lisbon, PT

Introduction

The Functional Movement Screen is a screening system that identifies mobility and stability dysfunctions, as well as pain, during the performance of seven movement patterns exercises. This screening is fulfilled by visual observation and based on standardized criteria, scoring the exercises as: “0” if the subject presents pain during performance; “1” if the subject is unable to complete the exercise or is unable to assume the position to perform it; “2” if the subject is able to complete the exercise but must compensate in some way to perform it; “3” if the subject performs the movement correctly without any compensation [1]. However, according to Warren et al. [2], there is a research gap regarding the validation of the Functional Movement Screen’s criteria. In this way, a 3D motion analysis model may be very useful concerning the validation of criteria related to mobility and stability. The Deep Squat is one of the seven Functional Movement Screen’s exercises and assesses global stability as well as joint mobility of the lower limbs joints and shoulders [1].

Global stability indicates the ability to maintain adequate sustainability of the body along the movement [3]. According to a previous study [4], the relationship between cartesian coordinates of the center of mass projection (CoMp) and center of pressure (CoP) in the horizontal plan of reference may be an important way to analyze global stability. To the best of our knowledge, only one research [3] studied the relation between Deep Squat’s score and biomechanical parameters related to mobility during the Deep Squat’s performance (using a 3D motion analysis). Nonetheless, none research studied the relation between Deep Squat’s score and biomechanical parameters related to global stability. Thus, the aim of this study was to associate the Deep Squat’s scores (assessed according to the Functional Movement Screen’s criteria) with the values of the biomechanical parameters related to global stability.

Experimental Methods

For this observational study, the subjects were selected according to the following inclusion criteria: male; 18-40 years; undergo physical exercises at least three sessions per week for six months before assessment; absence of injuries at assessment time. The following exclusion criterion was also defined: Deep Squat’s exercises scored as “0”. The Deep Squat exercises were developed according to the Functional Movement Screen’s guidelines [1]. In order to achieve the Deep Squat’s scores, videos of three subjects’ performances in sagittal and frontal plans were collected. These videos were observed by two researchers with Functional Movement Screen’s certification and scored according to its criteria. A 3D motion analyses during the Deep Squat exercises were carried out using the Vicon® Motion Capture MX System – 9 MX cameras at 200Hz (Oxford metrics, UK; 7×1.3 MP; 2×2.0 MP). It was also used a force plate at 1000Hz (model BP400600, AMTI, Watertown, MA, USA), which was synchronized with the Vicon® System. Ten trials were collected from each subject. Biomechanical parameters related to global stability were assessed during the descendent displacement of the center of mass – determined from the Plugin Gait Full-Body model (from the instant that center of mass’s vertical values began to decrease to the instant that subject achieved the lowest vertical position of the center of mass): duration of the period (s); mean distance between CoMp and CoP during period (mm); mean medio-lateral distance between CoMp and CoP during period (mm); mean antero-
posterior distance between CoMp and CoP during period (mm). Kruskal-Wallis and Mann-Whitney tests were used to compare subjects with the same Deep Squat’s score and to compare different Deep Squat’s scores, respectively.

Results and Discussion
Six subjects were selected to this preliminary study: two were scored as “1”, two as “2”, and two as “3”. Figure 1 shows the CoMp and CoP displacements in the horizontal plan during the ten trials of a subject scored as “1”. The observed variability of the CoP displacements is also observed in the other five subjects. Table 1 presents data regarding the comparison between subjects with the same score. As can be seen, differences were observed regarding the biomechanical parameters related to global stability (p<0.05). Table 2 presents data regarding the comparison between different Deep Squat’s scores: no differences were observed in the biomechanical parameters concerning the comparisons between scores “1” and “2” and between scores “2” and “3” (p<0.05). To the best of our knowledge, this was the first study that studied the relation between the Deep Squat’s scores and the biomechanical parameters related to global stability. Data lead us to question the validity of the FMS to objectively measure global stability deficits.

Conclusion
Despite the small sample size, data point to FMS’s difficulties to objectively assess global stability in Deep Squat, however, more research is needed to answer this issue.
### Table 1 – Individual Deep Squat scores and biomechanical data – comparisons between subjects with the same score.

<table>
<thead>
<tr>
<th>SUBJECTS</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep Squat score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Biomechanical parameters</td>
<td>mean ± sd</td>
<td>mean ± sd</td>
<td>mean ± sd</td>
<td>mean ± sd</td>
<td>mean ± sd</td>
<td>mean ± sd</td>
</tr>
<tr>
<td>Duration (s)</td>
<td>8.0 ± 0.5</td>
<td>2.4 ± 0.4</td>
<td>4.1 ± 0.4</td>
<td>2.8 ± 0.5</td>
<td>3.4 ± 0.5</td>
<td>4.3 ± 0.5</td>
</tr>
<tr>
<td>Distance CoMp-CoP (mm)</td>
<td>93.7 ± 6.3</td>
<td>104.0 ± 8.6</td>
<td>93.8 ± 4.6</td>
<td>103.7 ± 7.6</td>
<td>93.4 ± 3.6</td>
<td>77.6 ± 3.4</td>
</tr>
<tr>
<td>Medio-lateral distance CoMp-CoP (mm)</td>
<td>9.5 ± 1.2</td>
<td>5.0 ± 1.3</td>
<td>5.0 ± 1.2</td>
<td>5.4 ± 0.7</td>
<td>4.0 ± 1.1</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>Antero-posterior distance CoMp-CoP (mm)</td>
<td>94.1 ± 4.0</td>
<td>104.3 ± 8.1</td>
<td>94.0 ± 4.0</td>
<td>104.0 ± 7.5</td>
<td>92.5 ± 1.6</td>
<td>79.0 ± 3.4</td>
</tr>
</tbody>
</table>

sd: standard deviation; †difference between subjects with the same score, p<0.05.

### Table 2 – Comparisons between different Deep Squat scores.

<table>
<thead>
<tr>
<th>Biomechanical parameters</th>
<th>SCORE 1</th>
<th>SCORE 2</th>
<th>SCORE 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± sd</td>
<td>mean ± sd</td>
<td>mean ± sd</td>
</tr>
<tr>
<td>Duration (s)</td>
<td>3.6 ± 0.5</td>
<td>3.2 ± 1.05</td>
<td>3.8 ± 0.63</td>
</tr>
<tr>
<td>Distance CoMp-CoP (mm)</td>
<td>99.1 ± 9.6</td>
<td>98.7 ± 7.8</td>
<td>85.2 ± 8.0</td>
</tr>
<tr>
<td>Medio-lateral distance CoMp-CoP (mm)</td>
<td>6.0 ± 2.7</td>
<td>5.2 ± 0.98</td>
<td>4.7 ± 1.1</td>
</tr>
<tr>
<td>Antero-posterior distance CoMp-CoP (mm)</td>
<td>100.1 ± 9.8</td>
<td>99.0 ± 7.8</td>
<td>89.5 ± 8.9</td>
</tr>
</tbody>
</table>

sd: standard deviation; †difference between score 1 and score 2, p<0.05; †difference between score 1 and score 1, p<0.05; †difference between score 2 and score 3, p<0.05.

### Table 1 and Table 2.

### References


Different muscle activation according to type of muscle contraction in male and female professional soccer players: preliminary results

Estrella Armada-Cortés, Gonzalo Torres, Javier Rueda, Alejandro San Juan Ferrer, Enrique Navarro

University Politécnica de Madrid, Health and Human Performance, Madrid, ES

Introduction

Injuries are one of the most prominent concerns in football. Male soccer players get 1.9 times more hamstring injuries than female soccer players, with a 12% absence rate in the season compared to a 6% for female counterparts [1]. Moreover, female soccer players have two times more quadriceps injuries than male soccer players, with an 8% absence rate compared to 3% for males [2]. There are basic exercises used to strengthen the hamstring and quadriceps muscles, and their study can provide information about the activation pattern of these muscle groups during the different execution phases of each exercise [3]. This study of the different exercises may be decisive for soccer players, considering the muscle group they are targeting, since recording the electrical activity of each of the muscular bellies would help reduce the time in the process of recovery from an injury and also help in its prevention [3].

Therefore, the main objective of this study was to determine if there are differences in the muscular activation according to the type of muscle contraction between professional female and male soccer players during the execution of a Bulgarian squat.

Experimental Methods

Muscle activation was recorded with surface electromyography (Trigno Wireless System, Delsys, Inc., Boston, USA) in 8 professional soccer players (age = 23.3 ± 3.5, height = 170.4 ± 3, weight = 63.9 ± 5.4), divided in two groups by sex (i.e., 4 males and 4 females). The players performed 5 repetitions of the Bulgarian squat with an external load of 30% of the body weight. Vastus medialis (VM), vastus lateralis (VL), rectus femoris (RF), semitendinosus (ST), and biceps femoris (BF) were analysed.

For the statistical analysis, SPSS 23.0 software (Armonk, NY: IBM Corp) was used, the dependent variable of the study was the electrical activity of each muscle belly (RMS) in relation with the total activation of hamstring muscle group [4]. A comparison of means was performed using the repeated measures ANOVA test, with the independent variables muscle with 5 levels (RF, VM, VL, BF, ST); phase with 3 levels (eccentric, isometric and concentric) and gender (male and female) as an inter-subject factor. The significance value was set at 0.05.

Results and Discussion

No significant differences were found between dominant and non-dominant leg (F1,19=0.10; p>0.05). Posteriori comparisons were made without taking into account the leg variable. Significant differences were found in muscle activation in each of the muscles, between phases and between the two sexes (F8,152=3.3; p=0.002; partial η2=0.14). Specifically, differences were found in the electrical activity (RMS) of the eccentric and isometric phases of the VM, being higher in boys than in girls (p=0.02 and p=0.005 respectively) (Figure 1).

Conclusion

In general, it seems there are not differences in the electrical activity (RMS) between professional female and male soccer players during the execution of a Bulgarian squat, except in the eccentric and isometric phases of the VM (i.e., Being higher in males than females). More research is needed with a larger population to clarify which are the differences of muscle activation between sex according to the type of muscle contraction in order to improve the prevention and recovery of musculoskeletal injuries.
Figure 1. Muscle activation according to the type of muscle contraction (A; eccentric phase, B; isometric phase, C; concentric phase) between sex during the execution of a Bulgarian squat. mV; milivoltios. *Significant difference

References
Biomechanics 03-06

Spatiotemporal analysis of the approach run phase in the 60 m indoor hurdles race

Pablo González-Frutos¹, Santiago Veiga², Javier Mallo³, Enrique Navarro²

¹ Universidad Francisco de Vitoria, Facultad de Ciencias de la Salud, Pozuelo de Alarcón, ES; ² Technical University of Madrid, Health and Human Performance Department, Madrid, ES; ³ Technical University of Madrid, Health and Human Performance Department, Madrid, ES; ⁴ Technical University of Madrid, Health and Human Performance Department, Madrid, ES

Introduction
The technical analysis of the hurdle’s races has been focused on two- and three-dimensional kinematic analyses of the hurdle unit phase [1,2]. In recent years, the approach run phase has been subjected to specific analysis [3], highlighting the large relationship of the time in this phase with the final time in both men (r = 0.91) and women (r = 0.92) [4]. In addition, elite-level athletes performed different step length, step time, support time and/or flight time during approach run phase (p < 0.05), either in men or women, compared to elite-level athletes [4]. The aim of this study is to analyze the relationships between spatiotemporal parameters with the approach run phase split time in eight steps hurdlers.

Experimental Methods
All the races were filmed during the 44th Spanish Indoor Championship and 12th IAAF World Indoor Championship (Valencia 2008) and analyzed with 2D-DLT [5]. The best result of the athletes who performed an approach run phase of eight steps (Males: n=55; Females: n=51) was included in the study. The variables calculated were approach run phase split time, step length, step width, step time, contact time and flight time.

Results and Discussion
The largest correlation with approach run phase split time was found in mean step time in both men (r = 0.89; p < 0.01) and women (0.94; p < 0.1). Moreover, approach run phase split time had small correlation (r = 0.28; p < 0.05) with mean contact time of men hurdlers, and moderate relationship (r = 0.48; p < 0.01) with mean flight time of women hurdlers. Although no other statistically significant correlations were obtained, the small relationships found in men for the average step length (r = 0.24; p = 0.07) and flight time (r = 0.25; p = 0.07) can be highlighted. Additionally, the mean step time showed a moderate relationship with contact time in men (r = 0.30; p < 0.05) and flight time in women (r = 0.46; p < 0.01).

Conclusion
Eight step hurdlers with a shorter time in the approach run phase obtained lower values in the passing time, both in men and women. This variable seems to be related to contact time in the case of men, and flight time in the case of women.

Acknowledgement
The authors would like to acknowledge José Campos as the coordinator of the biomechanical studies developed during the 44th Spanish Indoor Championship and 12th IAAF World Indoor Championship, as well as the Royal Spanish Athletics Federation (RFEA) and the International Association of Athletics Federations (IAAF) for promoting, facilitating access and location for data collection, and managing the stay during the championships.

References


5:15 p.m. – 7:00 p.m.

Track08

Biomechanics 04 | Oral Presentations 02

Chair

João Abrantes

Universidade Lusófona, Lisboa, PT
Dominant kicking limb kinematics between kicks in futsal. One subject case.

Gonzalo Garrido López, Alberto Galisteo Pedraz, Enrique Navarro Cabello

Universidad Politécnica de Madrid, INEF (Health and Human Performance Department), MADRID, ES

Introduction
Kicking is a determining action during a match. Defining kicking from a biomechanical way, it is a kinematic chain of the limb in which the player pretends to give the maximum linear velocity to the kicking surface (1). The maximum velocities of different joints or segments are obtained sequentially, proximal parts earlier than distal ones (1,2). There is not too much scientific information regarding futsal. Some studies consulted compare stationary ball kicks with rolling ball ones, kicks with different surfaces, or dominant or non-dominant limbs (3,4). However, we have not found studies about different ways of kicking in futsal. Although, there is more information about soccer kicking and other gestures. The main objective of this work was to compare the timing, linear velocities, and angular positions of the kicking leg joints during instep, side foot, and toe kick performed by a futsal player.

Experimental Methods
The subject was a futsal male player of 23-year-old, 1.81 meters tall, and a weight of 90 kilograms. His dominant leg was the right. At the time of the study, he had 7 years of experience playing futsal at the category of preferred of the Madrid Futsal Federation and was free of injuries. A motion capture system (VICON®) operating at 250 Hz was used. Ten shots were recorded (five with each leg) during three different ways of contact surfaces: instep, side foot, and toe kicks. The variables analyzed were the joints angles of the hip, the knee, and the ankle on different moments of the kick; and the maximum velocities of body segments obtained during the kick.

Results and Discussion
There have not been found differences between the normalized times of the kicks. The hip is the first joint to reach its maximum (33% ±3), followed by the knee (69%±1), ankle (90%±1), toe (89% ± 1), and ball (110% ± 2). Interestingly the times of joint ankle and the toe are not significantly different. Similarly, there were no differences in the joints velocities among kicks. The hip had 5.3 m/s ± 0.2, the knee had 10.1 m/s ± 012, the ankle had 16.8 m/s ± 0,1 and the toe reached 18.2 m/ ± 0.1. Also, the ball speed (26.4 ± 0.6) and the impact factor (1.5 ± 0,0) were not different between kicks.

However, joint angles variables were significantly different between kicks, showing a very high effect size in the sagittal, frontal, and transversal planes (all partial eta square above 0.3). At the sagittal plane (Figure 1), the knee did not present differences between kicks, while the hip during instep (p=0.000) and side foot (p=0.023) kicks showed 8.3° and 4.7° smaller angles of flexion than during toe kicks at the foot strike instant. Also, at the impact, the instep kicks had smaller flexion angles than toe kicks. Interestingly, the angles of hip flexion were similar during the instep and side-foot kicks. The way of kicking is affecting mainly the ankle flexion-extension, showing the more flexed position (p=0.000) during toe kicks. At the frontal and transversal plane (Figure 2), kicks present differences mainly around the anteroposterior axis of the hip joint, where toe kicks showed a lower angle of abduction (p=0.01) at toe-off, foot strike, and impact (Figure 2). About the longitudinal axis, the most remarkable difference is that the hip joint presented higher (p=0.001) internal rotation during the toe kicks than during instep (5.0°) or side foot (5.1°) kicks at the beginning of the kicking phase. The knee joint present also higher (p=0.031) internal rotation during the toe kicks at the beginning. The foot was more (p=0.001) supinated during instep kicks than inside foot kicks (13.5°) and toe kicks (18.4°), while side foot and toe kicks were not significantly different.

Conclusion
Page 974 of 2028
The player showed a similar pattern of movement during the kicks reaching joints and ball velocities with no significant differences. There has been found that the technique of the movement presented large differences between kicks. At the sagittal plane, the hip was less flexed, the ankle was more flexed during the toe kicks, while the knee was not affected. In the frontal plane, the hip is less abducted during the toe kicks clearly. Around the longitudinal axis, the hip showed bigger internal rotation at the beginning of the kicking phase. As expected, the foot behaved differently during kicks showing more supinated angles during instep and toe kicks than inside foot kicks.

Figure 1. Flexion (+) extension (-) angles of the hip, knee and ankle of the kicking leg.

Figure 2. Abduction (-)-adduction (+) of hip, internal(+)-external(-) rotation of hip and knee and supination(+)-pronation(-) of the foot.

References
Asymmetries in force production between unilateral and bilateral standing broad jumps in young senior amateur basketball players

Iago Rojas-Cepero¹, Alejandro Pérez-Castilla¹, Francisco Yuste-Hidalgo², Danica Janicijevic³, Sergio Miras-Moreno¹, Amador García-Ramos¹

¹ University of Granada, Department of Physical Education and Sport, Granada, ES; ² University of Granada, Department of Didactics of Musical, Plastic and Corporal Expression, Granada, ES; ³ University of Belgrade, Faculty of Sport and Physical Education, The Research Centre, Belgrade, RS

Introduction

The assessment of inter-limb asymmetries has been a frequent source of investigation in recent years in the fields of rehabilitation and strength and conditioning. Previous studies have suggested that lower inter-limb asymmetries may be associated with a lower injury incidence and a safer return to play. For that reason, it has been recommended that clinicians and coaches frequently evaluate inter-limb differences throughout a training cycle in order to ensure that their patients or athletes do not exceed an arbitrary “high risk threshold” (e.g.10-15%). Since multiple strength and jumping tests have been used to monitor the existence of inter-limb asymmetries, of special interest should be the identification of the most appropriate test and metric for an accurate diagnosis of inter-limb differences.

The standing broad jump (SBJ) likely is the test most frequently used for detecting inter-limb differences in the ability to apply force in a horizontal direction. The main goal of any variant of the SBJ is to jump as far as possible. The high applicability of the SBJ tests comes from the fact that a simple tape measure can be used to determine the main performance but to know in depth, the possible origin of asymmetries, analysis using force platforms is necessary. Due to the lack of similar studies, it seems reasonable to explore the consistency in the magnitude and direction of asymmetries between consecutive sessions during the unilateral and bilateral SBJs. To address the existing gaps in the literature, specifically, the aim of this study was to clarify whether single-leg performance and inter-limb asymmetries can be obtained with a higher between-session reliability during unilateral or bilateral SBJs.

Experimental Methods

Twenty-four amateur basketball players, 12 seniors male (age = 18.9 ± 1.8 years; mass = 80.2 ± 11.0 kg; height = 1.88 ± 0.08 m) and 12 female (age = 21.1 ± 4.2 years; mass = 70.6 ± 7.2 kg; height = 1.75 ± 0.06 m) team that played in a regional-level Spanish basketball club. The subjects completed two identical sessions which consisted of four unilateral SBJs (two with each leg) and two bilateral SBJs. All SBJ tests were performed on two parallel force platforms (Type 9260AA6; Kistler, Switzerland; 0.5 × 0.6 × 0.1 m) embedded in a wooden housing (1.1 ×1.0 × 0.1 m; see Figure 1). The horizontal Ground Reaction Force (GRF) data from each force platform were synchronously acquired with the BioWare® software (Kistler, Winterthur, Switzerland) at 1,000 Hz. Mean and peak values of force, velocity and power, and impulse were obtained separately for each leg using a dual force platform. Inter-limb asymmetries were computed using the standard percentage difference for the unilateral SBJ, and the bilateral asymmetry index-1 for the bilateral SBJ, using the equation proposed by Bishop et., (2018) ([dominant leg – nondominant leg]/[dominant leg + nondominant leg]*100).

Results and Discussion

All performance variables generally presented an acceptable absolute reliability for both SBJs (CV range = 3.65-9.81%) with some exceptions for mean force, mean power, and peak power obtained with both legs (CV range = 10.00-15.46%). Three out of 14 variables (mean force of the left leg and peak force of both legs) were obtained with higher reliability during the unilateral SBJ (CVratio ≥ 1.18), and 5 out of 14 (mean power and peak velocity of the right leg, as well as mean velocity, peak velocity, and impulse of the left leg) during the bilateral SBJ (CVratio ≥ 1.27).
Asymmetry variables always showed unacceptable reliability (ICC range = -0.40 to 0.58), and slight to fair levels of agreement in their direction (Kappa range = -0.12 to 0.40) except for unilateral SBJ peak velocity [Kappa = 0.52] and bilateral SBJ peak power (Kappa = 0.51), that showed moderate agreement for both SBJs. Increasing the sample size would likely result in a more concise results. As others authors aproached many measures of reliability are susceptible to the range of values in the sample, so increasing the sample size may also alter the reliability findings. Because of this lack of reliability, asymmetry measures should not be considered in decision-making performance or preparedness of basketball healthy young senior players.

**Conclusion**

These results highlight that single-leg performance variables can be generally obtained with acceptable reliability regardless of the SBJ variant, but the reliability of the inter-limb asymmetries in the conditions examined in the present study is unacceptable to track individual changes in performance due to the nature variability of asymmetry direction. Practitioners should use caution when considering the results (direction and magnitude) of asymmetry tests as they may not be as reliable. Further studies with a large number of basketball players of different ages and healthy or recovering from lower-limb injuries are needed to confirm asymmetries and to establish clinical guidelines for the assessment of bilateral strength asymmetry using SBJs.

**Acknowledgement**

This study was funded by the Spanish Ministry of Science and Innovation (PID2019-110074GB-I00/SRA (State Research Agency)/10.13039/501100011033).
Results

Figure 2. Individual comparisons between both testing sessions for the inter-limb asymmetry scores obtained for men (white circles) and women (black circles) during the bilateral standing broad jump variant.

References


Biomechanics 04-03

Analysis of reaction force and foot velocity in relation with the dominant leg in soccer kicking

Yecheng Zhang¹, Navandar Archit¹, Rueda Javier², Navarro Enrique¹

¹ Universidad Politécnica de Madrid, salud y rendimiento humano, madrid, ES; ² Universidad Europea de Madrid, Lecturer in Biomechanics, madrid, ES

Introduction
As the most important parameter of the supporting leg in kicking, the ground reaction force (GRF) of the supporting leg occurs in the leg cocking phase and continues until the kicking action ends[1]. After the supporting leg touches the ground, the swing leg transitions from the leg cocking phase to the leg acceleration, followed by ball impact and the follow through phase.[1]. At the end of the backswing phase, the length of the final step in the swing determines acceleration or deceleration of the foot and can significantly impact the ball and foot velocity[2]. This occurs when the hip flexion angular velocity rapidly increases and reaches a similar peak magnitude, after which, the angular velocity decreases until ball impact[3]. As described in previous research, the foot impact velocity is the most important factor affecting ball velocity[4]. There is a deceleration of the proximal segment and an acceleration of the more distal segment, as a result of which the linear speed of the knee was decreases to zero at impact (0.06 – 0.56 m/s) and the foot velocity increases to peak (11.76 – 20.3 m/s)[5].

According to previous studies, most studies on GRF have focused on analysing the differences in GRF between different genders, playing styles, and dominant non-dominant legs. However, the supporting leg and the kicking leg are related to each other, and elite athletes can achieve coordination of their legs. Therefore, the main purpose of this research is to Analysis of reaction force and foot velocity in relation with the dominant leg in soccer kicking.

Experimental Methods
Eighteen soccer players (age = 19.2 ± 0.5 years, height = 179.7 ± 5.3 cm, weight = 71.0 ± 5.9 kg) participated in the study, all of them men, belonging to the youth soccer team of a professional Spanish team. The players trained five days a week and played a match every week. The ground reaction force is collected by the Kistler and the Motivo capture os Vicon is used to collect players' kicking movements.

Results and Discussion
The reaction forces applied in the anteroposterior axis showed a consistent temporal pattern (Figure 1) that means that player is pushing forward with the foot applying maximum forces of 0.7 N/Bw between the 60% and the 70% of the kicking phase.

The lateral reaction forces showed that player applied forces from leg to right during a kick with the right leg due to the inclination of the body maximum forces reaching forces of 0.6 N/Bw between 20% and 30%.

The vertical forces showed that player push down the floor reaching forces around 2.5 N/Bw between 20% and 30% of the kicking phase.

There have not been found (Table 1) differences between dominant and no dominant legs at any of the ten instants analysed. However, the foot speed presented higher (p<0.05) velocities of at the dominant leg at the 50%, 60% And 70% of the kicking phase.

Conclusion
While there are differences on the velocity of the foot of the dominant and no dominant leg during kicking the reaction, forces are similar. It seems that reaction force don’t play a direct role related with the technique of kicking. However, the high forces applied are very huge reaching more than 3 times the body weight which it means the need of an
specific muscle strength training of the lower limbs. More research is needed for improving the knowledge about the behaviour of the support leg during kicking in soccer.

References


Biomechanics 04-04

Impulse, Power and Work predictors comparison on standard maximum vertical jump of elite and non-elite performers

Carlos B. Rodrigues1,2, Miguel V. Correia1,2, João Abrantes3, Marco B. Rodrigues4, Jurandir Nadal5

1 University of Porto, Doctoral Program in Biomedical Engineering, Porto, PT; 2 INESC TEC - Institute for Systems and Computer Engineering, Technology and Science, C-BER - Centre for Biomedical Engineering Research, Porto, PT; 3 Lusófona University - CICANT (Centre for Research in Applied Communication, Culture, and New Technologies), MovLab (Laboratory of Technologies for Interactions and Interfaces), Lisbon, PT; 4 Federal University of Pernambuco, Department of Electronic and Systems, Recife, BR; 5 Federal University of Rio de Janeiro, Biomedical Engineering Program, Rio de Janeiro, BR

Introduction

Stretch-shortening cycle (SSC) is a natural form of muscle action with concentric contraction immediately preceded by eccentric stretch to achieve more efficient submaximal actions and powerful maximal efforts [1]. Despite muscle SSC can be observed at lower limbs in gait and running, its higher expression and accessibility corresponds to standard maximum vertical jump (MVJ) with long countermovement (CM) and short CM at drop jump (DJ) for comparison without CM at squat jump (SJ) [2]. Standard MVJ have been increasingly used [3-5] with an open issue on the ability of non-elite performers to exploit long and short CM in relation to elite performers. Elite (E) and non-elite (NE) performers have been compared on standard MVJ based on kinematic and dynamic performance with an open question on impulse, power and work predictors comparison on E and NE.

Experimental Methods

Assessed subjects correspond to a group NE of six male degree sports students with (21.5±1.4) yrs, (76.7±9.3) kg mass and (1.79±0.06) m height without previous injuries, specific train or sport modality, and a group E of sixteen male players of the Portuguese national volleyball team ages (21.4±3.1) yrs, (85.2±5.8) kg mass and (1.93±0.04) m height. Each subject group performed a total of 3 SJ, CMJ and DJ from 40cm step repetitions with 2 to 3 minutes resting and instruction. During MVJ, ground reaction forces (GRF) were acquired with AMTI BP2416-4000CE force plate at 1000 Hz and best execution of each subject SJ, CMJ and DJ was selected according to maximum flight time. Vertical resultant force (RFz) was obtained from GRF and vertical impulse (Iz = \int RFz dt) from time integration of RFz during impulse phase. Vertical velocity (vz = Iz / m) of each subject center of mass was obtained as well as the net mechanical power (P = RFz \cdot vz) and mechanical work (W = \int P dt) developed during vertical impulse phase. Maximum vertical jump height (hi) was obtained from vertical impulse and (ht) from flight time. Selected variables from previous physical quantities were statistically compared at 5% significance with intra-subject paired T-test of mean differences from SJ, CMJ and DJ40 to 40cm step repetitions with 2 to 3 minutes resting and instruction. During MVJ, ground reaction forces (GRF) were acquired with AMTI BP2416-4000CE force plate at 1000 Hz and best execution of each subject SJ, CMJ and DJ was selected according to maximum flight time. Vertical resultant force (RFz) was obtained from GRF and vertical impulse (Iz = \int RFz dt) from time integration of RFz during impulse phase. Vertical velocity (vz = Iz / m) of each subject center of mass was obtained as well as the net mechanical power (P = RFz \cdot vz) and mechanical work (W = \int P dt) developed during vertical impulse phase. Maximum vertical jump height (hi) was obtained from vertical impulse and (ht) from flight time. Selected variables from previous physical quantities were statistically compared at 5% significance with intra-subject paired T-test of mean differences from SJ, CMJ and DJ pairs at each group NE and E, as well as inter-subject mean differences with independent samples T-test on SJ, CMJ and DJ between NE and E groups, Fig. 1-2.

Results and Discussion

NE and E presented higher hi on CMJ than SJ, both higher than DJ40 without statistical significative differences (ssd) between SJ and DJ40. As regards to ht, NE and E presented higher means at CMJ, with higher ht at SJ than DJ40 on NE and higher ht at DJ40 than SJ on E. NE and E presented higher ht than hi at SJ and CMJ, as well as as at DJ40 on E, but lower ht than hi at DJ40 on NE without ssd. On direct comparison between groups hi and ht presented on SJ higher means on NE than E without ssd, and lower means on NE than E at CMJ, with opposite results on DJ40 and higher NE than E on hi without ssd, and higher E than NE on ht.

CMJ presented at NE and E longer duration of the impulse phase (\Delta t imp) and downward subphase (\Delta t down), with longer \Delta t imp at SJ than DJ40 on NE and E, whereas NE presented longer \Delta t down at SJ than DJ40 without ssd and
E longer $\Delta t_{down}$ at DJ40 than SJ. NE and E presented longer upward subphase ($\Delta t_{up}$) than CMJ, both longer than DJ40. NE presented longer $\Delta t_{imp}$ and $\Delta t_{down}$ than E at SJ with shorter $\Delta t_{up}$ on NE than E at SJ and shorter $\Delta t_{imp}$, $\Delta t_{down}$ and $\Delta t_{up}$ at NE for CMJ and DJ40.

Vertical resultant force ($RF_z$) presented dominant higher values at DJ40 than CMJ, both higher than SJ, namely at the downward inversion $RF_z$ start up and the upward subphase $RF_z$ mean up, with higher values at NE than E on SJ and higher values at E than NE on CMJ and DJ40. Nevertheless, due to the balance between the force levels and the duration of the impulse subphases $I(RF_z)$ imp up presents higher values at CMJ on NE, as well as higher value on SJ than DJ40 and higher value at DJ40 than SJ on E, with NE presenting lower value than E on SJ, CMJ and DJ40.

As regards to developed mechanical power during impulse phase, despite $P_{mean imp}$ presents at NE and E higher value on SJ than CMJ and DJ, $P_{mean up}$ presents at NE and E higher value at DJ40 than CMJ, both higher than SJ. Also, NE presented lower $P_{mean imp}$ and $P_{mean up}$ than E.

In relation to developed mechanical work $W_{imp}$ during impulse and the ascending sub-phase $W_{imp}$ up, both NE and E presented lower values than SJ, DJ40, with lower $W_{imp}$ up at NE than E on SJ, CMJ and DJ. Nevertheless, $W_{imp}$ up presents at NE higher value than SJ at DJ40 and $W_{imp}$ up at E higher value on SJ than which can contribute for explaining differences on NE and E performances at SJ and DJ.

Conclusion
Despite vertical impulse has been applied as a dominant variable determining MVJ performance, complementarily developed power and mechanical work need to be considered as an energy transference metrics contributing for explaining differences on NE and E at SJ, CMJ and DJ.

![Fig. 1](image-url)

**Fig. 1**
SJ, CMJ and DJ40 selected variables from elite and non-elite performers inter-subject comparison
Fig. 2

SJ, CMJ and DJ40 selected variables from elite and non-elite performers intra-subject comparison

References
Scientific production in Sports Biomechanics. A vision from the doctoral theses completed in Spain

José Campos¹, Marcos Gutiérrez Dávila², José María Campos Coll³

¹ University of Valencia, Physical Education and Sport Sciences, VALENCIA, ES; ² University of Granada, Physical Education and Sport Sciences, Granada, ES; ³ I.E.E Districte Marítim, Physical Education, Valencia, ES

Introduction
In the literature there are no references of studies oriented in the evolution of doctoral theses in the specific field of Sports Biomechanics. The information available comes from global studies, which have been carried out in the field of Physical Activity and Sports Sciences, such as those carried out by Delgado and Medina (1997), Ponce de León, Gargallo and Loza (1998, and García García (2016). The purpose of this study is to carry out a review of the doctoral theses that have been read in Spain in the field of Sports Biomechanics during the period between the 1980-81 and 2018-19 academic course aimed at knowing the scientific production, the authorship and tutorial data, and the institutional references that give them academic coverage.

Experimental Methods
The sample has been composed of 233 theses, after a selection process with the use of inclusion / exclusion criteria and specific search terms. In order to analyse the data and its evolution over time, four academic periods were distinguished, considering normative issues and specific events that marked the evolution of the centers and doctoral programs in the field of Physical Activity and Sports Sciences in Spain. Descriptive statistics and contingency tables with Chi-Squared test and Haberman Standardized and adjusted residuals were performed.

Results and Discussion
The results have shown that the production of theses on this subject has been progressive and growing throughout the periods analysed, being the last period the one that more theses were read, both in public and private universities. In the second period, the percentage of theses read increased by 16% when compared to the first period. As for the third period, the increment was 10% when compared to the second period. Finally, a 11.5% increase was shown between the third and the fourth periods, which is the largest seen in the series (table 1).

The data collected shows that there is a total of 42 Universities in which Sports Biomechanics theses were read. Among them, 90% were public, whereas 10% were private.

In addition, and regarding the presence of University Departments in the above-mentioned field, it is worth highlighting the leading position of the Department of Physical Education and Sport. Both in the authorship, as in the direction of the theses, there is a majority presence of men. Despite the progressive and growing presence of women as authors of theses (15 theses in the second period, 17 theses in the third period and 39 theses in the fourth period), 69.5% of total are written by men (figure 1).

In the case of thesis tutorial, the presence of women is even more reduced, with a 81.5% of theses directed only by men, 5.2% directed by women only, and 11.6% directed by men and women working together. However, when analyzing its evolution throughout the four academic periods, it has been found that in the last period (2010/11 – 2018/19), 8 theses were directed by women only, and 19 theses were led between men and women. These, when added together, represent the 24.8% of the total theses read, which doubles those registered in the previous periods (11%-12%), resulting in a change of trend (X²: 9.506 y p: .05).

Another area of the study focuses on the evolution in the number of thesis directors. In this regard, the data shows that theses directed by one or two directors represent 89.9% of the total, while those led by three or more directors represent 9.9% of the total. That is, one of each ten theses read. When analysing its evolution in time, there is an increase in the tendency among the number of thesis directors (figure 2).
professors towards sharing the direction of their theses with other colleagues ($X^2: 45,250 \ y \ p: .000$). Specifically, in theses directed by 2, 3, or more directors, there is an increase, from 10 in the second period, to 81 in the fourth period.

**Conclusion**

The production of doctoral theses in Sports Biomechanics has contributed positively to the development and advancement of research in the field of Sports Sciences in Spain. However, its future will depend on the ability to overcome structural problems, such as the difficulty to access and lead projects in the National R+D+i Plans, its level of subsidiarity when compared to other areas of knowledge, and the orientation of research in both the applied field and high-performance sports services.

![Figure 1: Distribution of theses according to the sex of the authors by academic periods](image)

![Table 1: Evolution of theses in Sports Biomechanics](table)

References


Scope and contents of Research on Sports Biomechanics in Spain

José Campos Granell¹, Marcos Gutiérrez Dávila², José M. Campos Coll³

¹ University of Valencia, Physical Education and Sport, Valencia, ES; ² University of Granada, Physical Education and Sport, Granada, ES; ³ I.E.S. Districte Maritim, Physical Education, Valencia, ES

Introduction

One of the traditional aims of Sports Biomechanics has been analyzing sports technique as training support for athletes. This scientific discipline has, however, expanded its field of action over time, incorporating professionals from different fields to better deal of the systems that command the execution and control of human movement.

In the literature there are no references of studies oriented in the evolution of the contents of doctoral theses in the specific field of Sports Biomechanics. The information available from Spain comes from global studies (Delgado and Medina, 1997; Ponce de León, Gargallo and Loza, 1998; and García García, 2016).

The purpose of this study is to carry out a review of the doctoral theses that have been read in Spain in the field of Sports Biomechanics during the period between the 1980-81 and 2018-19 academic course aimed at knowing the evolution of their contents.

Experimental Methods

The sample has been composed of 233 theses, after a selection process with the use of inclusion / exclusion criteria and 32 specific search terms. In order to analyse the data and its evolution over time, four academic periods were distinguished, as well as four subject fields of study: Basic Biomechanics; Applied Biomechanics; Support Biomechanics; and Biomechanics and Technology. Descriptive statistics and contingency tables with Chi-Squared test and Haberman Standardized and adjusted residuals were performed.

Results and Discussion

The results show that the most common contents of the theses are those of Support Biomechanics and Applied Biomechanics, with a progressive growth that has escalated to a total amount of 107 and 98 theses respectively, followed by the field of Biomechanics and Technologies, with a total of 25 theses read. Finally, there is the field of Basic Biomechanics, which encompasses explanatory theories of human and sports movement and is composed of 3 theses (1.3% of the total). (figure 1).

Regarding the distribution of the established thematic subfields of the theses, in table 1 it can be seen that within the field of Support Biomechanics, the one with the highest representation is the Medical Area, which groups 73 theses (31.3% of the total). Next, the three subfields of Applied Biomechanics are located; (Analysis of sports technique (35 theses), Analysis of human movement (32 theses), and Various Analysis (31 theses), which represent 15%, 13.7% and 13.3% of the total theses respectively. In the last place, we can see the three subfields of Biomechanics-Technologies (Photogrammetry, with 7 theses; Dynamometry, with 8 theses and Other technologies, with 10 theses) that represent 3.1 %, 3.4% and 4.3% respectively of the total.

Nevertheless, it has not been possible to find that there are statistically significant associations between thematic fields and academic periods to justify the number of theses carried out over time.

In addition, the term that appears the most in the titles of the theses is “walking”. Specifically, it appears 61 times. Thus, this term is revealed as a cross-sectional element that is used in the Medical Area, as well as in Engineering, Physical Education and CAFD and also, linked to the three thematic fields considered.

Conclusion

The thematic fields in which theses have been carried out the most in the periods analyzed are Support Biomechanics and Applied Biomechanics. The former is related to studies whose independent variable and specific objective do
not respond to any biomechanical variable, such as specific training, a health program or a sociological, behavioral or psychological variable assessed by using specific techniques of Biomechanics. The latter is linked to studies whose independent variable is from the specific field of Biomechanics, such as the analysis of sports techniques, human movement, strength, or coordination.

This situation has continued to reinforce the applied nature of research over time, and also places Basic Biomechanics as a residual research field, given its orientation towards a more theoretical explanation of human and sports movement.

These trends also show that the field of Support Biomechanics has been growing progressively and expanding its field of action in several areas, such as Health, Occupational Biomechanics and Sports equipment and materials, thanks to a better access to more sophisticated and accurate measurement systems. Moreover, the evolution of the themes and contents of the theses in Sports Biomechanics help to better understand the effect that this scientific perspective may have had on the development of the sports sector in Spain. This evolution has also enhanced the formation of multidisciplinary research teams made up of professionals from various fields related to sports, health and engineering.

<table>
<thead>
<tr>
<th>SUB-FIELDS SUBJECTS</th>
<th>Normalization Period 80-81/89-90</th>
<th>Adaptation Period 90-91/89-00</th>
<th>Normalization Period 90-03/05-10</th>
<th>Internationalization Period 10-11/13-19</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>1-Bio-Basic</td>
<td>0</td>
<td>–</td>
<td>1</td>
<td>2,2</td>
<td>1</td>
</tr>
<tr>
<td>2.1-Bio-Applied_Analysis sport techniques</td>
<td>0</td>
<td>–</td>
<td>8</td>
<td>17,4</td>
<td>14</td>
</tr>
<tr>
<td>2.2-Bio-Applied_Movement analysis</td>
<td>2</td>
<td>20</td>
<td>10</td>
<td>21,7</td>
<td>7</td>
</tr>
<tr>
<td>2.3-Bio-Applied_Others</td>
<td>0</td>
<td>–</td>
<td>2</td>
<td>4,3</td>
<td>12</td>
</tr>
<tr>
<td>3.1-Bio-Support_Phyisiology</td>
<td>0</td>
<td>–</td>
<td>1</td>
<td>2,2</td>
<td>2</td>
</tr>
<tr>
<td>3.2-Bio-Support_Motor Control</td>
<td>0</td>
<td>–</td>
<td>1</td>
<td>2,2</td>
<td>4</td>
</tr>
<tr>
<td>3.3-Bio-Support_Medical area</td>
<td>6</td>
<td>60</td>
<td>14</td>
<td>30,4</td>
<td>22</td>
</tr>
<tr>
<td>3.4-Bio-Support_Others</td>
<td>0</td>
<td>–</td>
<td>4</td>
<td>8,7</td>
<td>3</td>
</tr>
<tr>
<td>4.1-Bio-Technol_Photogrammetry</td>
<td>1</td>
<td>10</td>
<td>3</td>
<td>6,6</td>
<td>7</td>
</tr>
<tr>
<td>4.2-Bio-Technol_Diamond/Aceleron</td>
<td>1</td>
<td>10</td>
<td>2</td>
<td>4,3</td>
<td>2</td>
</tr>
<tr>
<td>4.3-Bio-Technol_Others</td>
<td>0</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>100</td>
<td>46</td>
<td>100</td>
<td>68</td>
</tr>
</tbody>
</table>

Table 1: Evolution of doctoral theses read by subject fields and subfields
Figure 1: Evolution of doctoral theses production by subject fields

References
7:30 p.m. – 9:00 p.m.

Track08

Biomechanics 05 | SIBB General Assembly and Award Ceremony
2:30 p.m. – 4:00 p.m.

Poster floor

PS1 | Poster Sessions 1
PS1-01 | Biomaterials for Biofabrication
**Toward core-shell 3D printed photonic strain sensing medical device**

*David Eglin*¹², Christoph Sprecher², Christophe Marquette³, Edwin J. Courtial³

¹ Mines Saint Etienne, Biomaterials, Saint Etienne, FR; ² AO Research Institute Davos, Davos, CH; ³ University of Lyon, Platform 3D Fab, Villeurbanne, FR

**Introduction**

Medical devices are often required to function in contact with complex organ geometry under mechanical stress. Photonic strain sensing devices are being widely used for their diagnostic and therapeutic functions. In this study, we demonstrate the possibility to endow a 3D printed silicon structure with photonic strain sensing ability [1].

**Experimental Methods**

40, 60 and 63.5 w:w % hydroxylpropylcellulose (HPC, Mw circa 100 kDa): dH₂O solutions were prepared. The HPC solutions were characterized using a rheometer equipped with a camera (DHR, TA instrument). Coaxial 3D printing of a tubular structure was performed with the 63.5 w:w % HPC solution in the inner part and moisture curing silicon in the outer part using a customized Tobeca FDM printer with pressure driven coaxial extrusion head. The strain sensing ability of the printed structure was assessed under an optical microscope (Zeiss AxioVert 200M).

**Results and Discussion**

The viscoelastic properties and thixotropy of the HPC solutions are assessed (Fig 1). Changes of birefringence of HPC 63.5 w:w % under shear is imaged (Fig. 1). Three main regimes are observed in the flow sweep curve of 60 and 63.5 w:w % HPC solution indicatives of birefringent cholesteric liquid-crystalline solutions [2]. A first region, with an increase of viscosity and birefringence, related hypothetically to the setting-up of the formulation, a second region with a progressive extinction of the birefringence together with a decrease of viscosity and a final region with no changes in birefringence and logarithmic decrease of viscosity value. Upon application of high rotational shear, the HPC solutions viscosity drops, with 90% recovery observed after 11 ± 1, 181 ± 1 and 222 seconds for the 40, 60 and 63.5 w:w % HPC, respectively.

Coaxial micro-extrusion 3D printing of HPC (core) and silicon (shell) is performed, and after curing at room temperature in humid atmosphere for 2 days, stable rubbery 3D structure is obtained with conserved liquid crystal properties of the HPC (Fig. 2). Upon application of pressure on the silicon filaments, dark region under cross-polarized light suggests apparition of an isotropic HPC phase (Fig. 2).

**Conclusion**

Core-shell silicon/cholesteric HPC 3D printed structure could be produced with a facile coaxial micro-extrusion approach. The elastomeric structure can be deformed and extinction of the HPC birefringence observed. Further, characterization and fabrication optimization are required to validate potential applications as strain sensitive medical device.

**Acknowledgement**

DE and CS acknowledge the financial support of the AO Foundation.
Figure 1
Representative flow sweep curves (top right) and thixothropic curves (top left) of HPC solutions; 40 (orange), 60 (green) and 63.5 (blue) w:w %, at 25°C. Flow sweep and derivative curves of HPC solutions 63.5 w:w % at 25°C with images taken at specific time point (bottom).

Figure 2
Image of macroscopic HPC/silicon tubular structure fabricated with co-axial micro-extrusion (scale bar 1 cm) and cross-polarized images of coaxial HPC/silicon before and just after pressure application (scale bar 1 mm).

References
PS1-01-003

Stereolithography shows potential in additive manufacturing of synthetic trabecular bone structures

Ana Grzeszczak\(^1\), Susanne Lewin\(^1\), Olle Eriksson\(^2\), Johan Kreuger\(^2\), Cecilia Persson\(^1\)

\(^1\) Uppsala University, Department of Materials Science and Engineering, Uppsala, SE; \(^2\) Uppsala University, Department of Medical Biochemistry and Microbiology, Uppsala, SE

**Introduction**

Synthetic bone models are needed to train surgeons but also to test and design medical devices such as screws for fracture fixation. However, currently available models do not accurately mimic the trabecular bone and its complex structure [1]. This study aimed to investigate if stereolithography (SLA) additive manufacturing could produce synthetic trabecular bone models with high accuracy.

**Experimental Methods**

The synthetic bone models were printed by SLA (Formlabs Black resin, Form3 printer). The CAD-model had been generated from micro-computed tomography (micro-CT) synchrotron images of human trabecular bone [2]. The model was printed at the original scale (scale factor 1), and with upscaling factors up to 4.3. Structure replicability was assessed with micro-CT, and the mechanical properties were evaluated by compression and screw pullout tests. Dense cylinders of the printed material were also tested in compression for material characterization.

**Results and Discussion**

The elastic moduli obtained by compression of dense cylinders were approximately ten times lower than average values for human cortical bone. For the trabecular bone models with scaling factors below 1.8, micro-CT image analysis showed major artefacts due to printing and a low accuracy in trabecular thickness distribution (Fig. 1). Analysis of the total printed volume showed a difference to the original model higher than 50% for scale 1.5. However, this difference was less than 10% for scales 1.8 and above, although a refined overlap comparison with the original bone model showed that the scale 1.8 exhibited errors higher than 20%, implying printing inaccuracies of the smaller structures. The pullout strength of SLA-printed parts was higher than for existing synthetic models (Sawbones\(^\text{TM}\)) and cadaveric specimens, but within the same range as FDM-printed parts in poly(lactic acid) [2].

**Conclusion**

In conclusion, trabecular bone models with a scale factor of 1.8 or larger could be printed with acceptable accuracy, but models with smaller scale factors were not well represented. However, for the same 3D model, a higher resolution was achieved by SLA as compared to FDM [2].

**Acknowledgement**

The authors are grateful to Adam Engberg at U-PRINT: Uppsala University’s 3D-printing facility at the Disciplinary Domain of Medicine and Pharmacy for support and advice on the printers. This research was funded by Sweden’s Innovation Agency VINNOVA, grant number 2019-00029.
Fig. 1: Comparison of the CAD-model and the printed samples at different scales.
(a) Volumetric comparison of the µCT-image dataset of the original bone with the µCT-image dataset from the printed samples. The original bone is displayed in grey. The material that was printed but was not present in the original bone is displayed in green (extra material). (b) Cross-sections on the XY plane of the CAD-model and of the printed samples. (c) Cross-sections comparison on the XY plane. The CAD-model is displayed in white and the printed parts are displayed in green.

References
Effect of filament orientation and filler density on structural integrity and mechanical property of 3D printed PCL/Laponite construct for bone implants

Hongyi Chen¹, Jia Khong², Jie Huang¹

¹ University College London, Department of Mechanical Engineering, London, GB; ² University College London, Department of Medical Physics and Biomedical Engineering, London, GB

Introduction
3D printing has gained considerable attentions in tissue engineering due to its high efficiency, customizability for personalization of tissue engineered implants. Among the 3D printing techniques, direct ink writing (DIW) stands out as it operates under room temperature and allows the incorporation of biomolecules and cells in the ink. Mechanical property is a crucial aspect of a bone implant for load bearing applications, particularly high stiffness can lead to stress shielding and bone resorption. In this study, Laponite (LP) nanoclay was incorporated into polycaprolactone (PCL), a biodegradable polymer approved by FDA, to improve its bioactivity as well as to match the modulus of bone. Structural accuracy and integrity of the printed implant greatly influences its integration, mechanical properties and function. Micro-CT is an essential tool to analyse 3D structure and guide the manufacturing process of 3D printed products. Therefore, the effect of the 3D printing pattern and particle filling density, which are known to influence mechanical performance, was investigated, from the shape fidelity to modulus and strength of printed constructs.

Experimental Methods
50% m/v PCL was dissolved in DCM solvent with or without LP for making PCL based inks. Loading density of LP is up to 30 wt%. Flow sweep tests with shear rate ranging from 0.01 s⁻¹ to 100 s⁻¹ were conducted on the inks Discovery HR-2 and zero-shear viscosity was calculated. Dumbbell-shaped specimen (ASTM D638 Type IV scaled down 50%) for tensile testing was designed in two printing orientations (0° and 90°) as shown in Figure 1. The surface pattern and fracture morphology of the specimens were examined using SEM and the 3D structure of specimens were assessed using a micro-CT. Tensile tests were performed on the 3D printed specimens with ink and two orientations and their tensile properties were compared with independent t-test with significance level set at p < 0.05.

Results and Discussion
Shear response results show that the incorporation of Laponite has significantly enhanced the viscosity which provides resistance to post-printing deformation and shear-thinning property which facilitates extrusion (Figure 1A). For tensile properties shown in Figure 1BC, the addition of Laponite has improved Young’s modulus, but decreased ultimate tensile strength (UTS) of the structure due to higher porosity within the structure (Table 1) and agglomeration acting as stress concentration causing the formation of holes under tensile loading as shown in Figure 2B. Specimens printed with 90° orientation have higher tensile properties than 0° orientation due to the difference in filament bonding and porosity of the structure (Figure 1). For 0° orientation, nozzle travels longer between adjacent filaments which contributes to bigger exposure area and more solvent evaporation and solidification of filaments before contacting adjacent ones. This affects bonding between the cylindrical filaments. While in 90° orientation, filaments contact sooner leaving less exposure area and more time for post-printing deformation including the flattening and fusing of the deposited cylindrical filaments caused by gravity and capillary force. Although deformation is usually considered undesired for 3D printing, in this case it improves filament bonding. This can be observed in Figure 2(B): the ditches/interfaces between 5 filaments in 0° orientation are prominent while the filaments in 90° orientation are not visible and only shallow horizontal ditches due to uneven drying are observed. The space within Page 998 of 2028
the interfaces of cylindrical filaments and layers become air gaps/voids after deposition of the next layer. In Figure 2, voids in 0° orientation are higher in volume and distributed in parallel to the filaments printing direction in comparison to voids in 90° orientation which are located more to the sides and are likely results from air trapped at the turning points of the filaments. This results in stress concentrate at the interfaces and voids leading to the separation of filaments under tensile loading and lower mechanical properties for specimens printed in 0° orientation.

Table 1 Viscosity and porosity comparing the two inks with two orientations.

<table>
<thead>
<tr>
<th>Ink &amp; Orientation</th>
<th>Zero-shear Viscosity (Pa·s)</th>
<th>Porosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL 0°</td>
<td>370±90</td>
<td>3.2%</td>
</tr>
<tr>
<td>PCL 90°</td>
<td>0.1%</td>
<td></td>
</tr>
<tr>
<td>PCL/30LP 0°</td>
<td>9340±730</td>
<td>5.4%</td>
</tr>
<tr>
<td>PCL/30LP 90°</td>
<td>0.8%</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion

PCL/LP composites have been 3D printed using DIW. The addition of Laponite in PCL/30LP ink has enhanced viscosity, shear-thinning property and Young’s modulus but decreased UTS. Samples with 0° orientation have lower mechanical properties than 90° orientation due to lesser filament bonding and higher porosity caused by longer nozzle travelling time between adjacent filaments. This study has provided insight into the effects of the filament orientations and filler density on the mechanical properties of 3D printed solid structure for tissue engineering implants.

Figure 1

(A) Shear response comparing pure PCL and PCL/30LP inks. (B) Young’s Modulus and (C) ultimate tensile strength (UTS) of the samples comparing two inks and two orientations. PCL/30LP ink has higher viscosity, Young’s modulus but lower UTS. Specimens of 90° orientation have higher Young’s modulus and UTS with the same ink.
Figure 2
(A) Design of dumbbell shape with two orientations (0° and 90°). (B) SEM images (top view) on midsection of the dumbbell samples printed by PCL/30LP comparing the two orientations before and after tensile testing. (C) 3D visualization from micro-CT results of the surface and inner voids of the midsection comparing two orientations (scale bar is 1mm). Interface between filaments and layers are distinctive in 0° orientation. Voids in 0° are higher in volume and roughly distributed horizontally. Voids in 90° orientation are located more to the sides.

References
Bioprinting of a cell-laden hydrogel onto a titanium alloy Ti6-Al4-V ELI surface to mimic the bone tissue-implant interface

Marcin Kotlarz, Ana Ferreira-Duarte, Piergiorgio Gentile, Kenneth Dalgarno

Newcastle University, School of Engineering, Newcastle upon Tyne, GB

Introduction
After implantation of a metal implant, a cascade of cellular and extracellular biological events occurs at the bone-metal interface\(^1\). The surface of metallic implants serves as a powerful signaling cue for cells, facilitating early osseointegration by encouraging bone deposition on the surface and stabilising the interface. Here, we investigated the application of a bioprinting process called Reactive Jet Impingement (ReJI)\(^2\) to fabricate high cell density hydrogels onto metallic implants, with the ultimate aim of accelerating osseointegration by enhancing bioactivity at the bone-implant interface.

Experimental Methods
Additively manufactured titanium alloy Ti6Al4V ELI samples produced using the Electron Beam Melting process (Arcam Q10) were used as substrates for bioprinting. Three types of Ti6Al4V ELI samples that varied in surface roughness and surface morphology were investigated: as-fabricated (\(R_a = 14.5 \, \mu m\)), ground (\(R_a = 0.7 \, \mu m\)), and polished (\(R_a = 0.5 \, \mu m\)). Human TERT immortalised bone marrow stromal cells (at a nominal print cell density of 4 x 10\(^7\) cells/ml) were delivered to the metal surface in a collagen-alginate-fibrin hydrogel matrix. Cell-hydrogel-metal implant interactions were assessed using immunofluorescence and SEM. DNA content per construct was quantified with Picogreen assay. Cell mineralisation was demonstrated using Alizarin Red.

Results and Discussion
Cell viability was not affected by the bioprinting process. All three levels of surface finish supported the attachment of the cell-laden hydrogel. Topographical features of the Ti6Al4V ELI surface influenced cell organisation and cell-metal surface communication. Cellular migration from the hydrogel and interactions with the metal surface were visible from day 1. Cell proliferation rate remained stable over 21 days regardless of the Ti6Al4V ELI surface type. SEM observations and Alizarin Red revealed significant mineral deposition on the metal surface, confirming stem cell differentiation into osteoblasts and osteoblast mineralisation.

Conclusion
We showed the ReJI system application for developing cell-hydrogel-metal surface systems to mimic the bone-metal implant interface. The bioprinted cell-laden hydrogels supported the formation of a layer of functional bone-like tissue on the surface of the Ti6Al4V ELI implants.

Acknowledgement
The research was funded by the EPSRC Centre for Doctoral Training in Additive Manufacturing and 3D Printing (EP/L01534X/1) and DePuy Synthes.
Figure 1.
Reactive Jet Impingement set-up schematic. The ReJI system jets bio-ink materials using microvalves mounted in the custom-designed printhead. The printhead is integrated with a commercial JetLab 4 XL printer. The microvalves are connected to different bio-ink reservoirs and directed to jet bio-ink droplets at one another in mid-air where they react and form a gel that lands on a substrate of interest. Each microvalve is attached to a spike and hold drivers allowing for its opening and closing, and connected to the pneumatic system and the printer input.

Figure 2.
The types of surface finish of the Ti6Al4V ELI samples: As-fabricated, Ground, and Polished. (a) SEM microphotographs of the sample surface morphology. Scale bar: 100 µm. (b) SEM microphotographs of cell-laden constructs showing cell-hydrogel-metal surface interactions on day 1. The red arrows indicate cells, blue arrows the printed hydrogel matrix, green arrows the metal surface. Scale bar: 20 µm. (c) Immunofluorescence staining of hMSCs. Cell morphology at the interface on day 1. The blue staining indicates cell nuclei, red F-actin, and green vinculin. Scale bar: 50 µm.

References
PS1-01-009

3D printing of bio-synthetic based Nerve conduit: Fabrication and characterization

Thanusha Av, Maurice N Collins

UNIVERSITY OF LIMERICK, STOKES LAB, BERNAL INSTITUTE, LIMERICK, IE

Introduction
The utmost challenge in the field of neural tissue engineering is the development of peripheral nerve based conduits to treat the damaged nerves. Even though damage to peripheral nerves are not life threatening, it leads to loss of sensory and/or motor functions making daily activities uncomfortable and difficult. These afflicts > one million patients worldwide annually.

Experimental Methods
The overall purpose of this research work is to develop a three dimensional nerve conduit up to definite length utilising 3D printing technology. The nerve conduit has been printed using PCL and gelatine with bioactive components such as hyaluronic acid incorporated. Conducting polymer nanoparticles based on PEDOT and Polypyrrole have been applied via a layer-by-layer coating technique. The fabricated 3D nerve conduit has been physico-chemically characterized via swelling studies, degradation profiles, rheological measurements, mechanical testing and thermal analysis. In vitro characterization comprised of cell cytotoxicity and cell proliferation assays.

Results and Discussion
The fabricated nerve conduit was characterized and the outcome of the 3D nerve conduit plays an effective role in the treatment of nerve damage.

Conclusion
The acquired results demonstrate that the fabricated nerve conduit is biocompatible and a promising candidate for neural tissue engineering applications.

Acknowledgement
This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No. 801165, with co-funding through SSPC by its funding body, Science Foundation Ireland, grant no. 112/RC/2275_P2.

References
Tailorable composite bioinks containing zinc-substituted mesoporous bioactive glass

Vera Guduric\textsuperscript{1}, Niall Belton\textsuperscript{1}, Richard F. Richter\textsuperscript{1}, Anne Bernhardt\textsuperscript{1}, Janina Spangenberg\textsuperscript{1}, Chengtie Wu\textsuperscript{2}, Anja Lode\textsuperscript{1}, Michael Gelinsky\textsuperscript{1}

\textsuperscript{1} TU Dresden, Centre for Translational Bone, Joint and Soft Tissue Research, University Hospital Carl Gustav Carus and Faculty of Medicine, Dresden, DE; \textsuperscript{2} Shanghai Institute of Ceramics, Chinese Academy of Sciences, State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai, CN

Introduction

Bioactive glasses are used in dental and orthopedic surgery thanks to their osteoconductivity, osteostimulatory effect and high degradation rate. Besides that, mesoporous bioactive glasses (MBG) are specific for their highly ordered mesoporous SiO\textsubscript{2} channel structure and high specific surface area, making them suitable for drug, growth factor or bioactive ion delivery. MBG-scaffolds, containing 80, 5 and 15 mol% of silicon (Si), phosphorus (P) and calcium (Ca), respectively, showed good cytocompatibility after seeding of bone-derived cells. Therapeutic activity of MBG can be improved by substitution of Ca\textsuperscript{2+} in the glass network with bioactive metal ions inducing desired therapeutic effect. In order to make MBG particles usable for extrusion-based 3D printing of scaffolds, they could be combined with pasty biomaterial inks. In the present study we aimed to develop a bioink containing MBG, which is suitable for 3D (bio)printing and delivery of therapeutic metal ions for bone tissue engineering applications. Due to its antimicrobial effect and positive impact on osteoblastogenesis and activity of osteoblasts, Zn\textsuperscript{2+} was selected for substitution of Ca\textsuperscript{2+} in MBG. Already established bioink containing 3 wt% of alginate (alg) and 9 wt% of methylcellulose (MC) supporting mesenchymal stem cell viability was chosen for combination with MBG. We investigated various combinations of algMC ink with high content of MBG with different amounts of Zn\textsuperscript{2+} incorporated in the glass network.

Experimental Methods

Four different MBG were synthesized, containing 15 mol% of Ca (0ZnMBG), 5 mol% Zn and 10 mol% Ca (5ZnMBG), 10 mol% Zn and 5 mol% Ca (10ZnMBG) and 15 mol% Zn (15ZnMBG). Pore channel structure of all the MBG was evaluated by transmission electron microscopy and measuring their size with ImageJ. Composite inks were prepared by mixing MBG particles into algMC blends containing 3 wt% alg and 6 (3-6 ink) or 9 (3-9 ink) wt% of MC. Composites containing up to 10 wt% of the respective MBG in both blends were tested for rheological and printing properties and the amount of 7 wt% MBG was selected for mass flow rate measurement, bioprinting and ion release experiments. Vybrant DiD prelabelled immortalized human mesenchymal stem cells (MSC) were used for bioprinting of composite constructs, which underwent fluorescent microscopy observations and DNA quantification to determine cell number development over time. Bioprinted constructs were also used for the ion release examination using inductively coupled plasma-optical emission spectroscopy.

Results and Discussion

MBG containing 0, 5, 10 and 15 mol% of Zn (15, 10, 5 and 0 mol% Ca respectively) were successfully synthesized, keeping mesoporous structure of the glass and the same channel size. Since Ca\textsuperscript{2+} and Zn\textsuperscript{2+} are crosslinkers of alginate, adding of MBG into 3-6 and 3-9 algMC bioinks induced a pre-crosslinking effect, followed by an increased viscosity of resulting composites. Increasing amount of Zn in MBG (decreasing amount of Ca) resulted in lower viscosity of composites and significantly decreased shape fidelity of printed scaffolds, confirming that Zn\textsuperscript{2+} is weaker crosslinker of alg [3]. Addition of 7 wt% of MBG allowed the most appropriate mixing, rheological and printing properties of composites. When cell suspension was added, a drop in viscosity of inks negatively affected print fidelity of 3-6 bioink. Therefore, 3-9 composites were used for bioprinting of MSC. At the beginning of cultivation, Zn-modified
MBG induced significantly lower cell number than 0ZnMBG, but this effect was not observed from day 7 and as shown in Figure 1, 0ZnMBG and 15ZnMBG did not have a negative effect on cell number development. This is in contrary to observed cytotoxicity of Zn$^{2+}$ [5]. The reason for this is probably the presence of other bioactive ions (silicates, phosphates and Ca$^{2+}$) released from MBG-containing composites, having a beneficial effect on cells. After one day of culturing, Ca$^{2+}$ was released from both types of composites, but an uptake was observed from day 7 (Figure 2A). It is possible that the alginate was taking up Ca$^{2+}$ because of continuous crosslinking. Another reason might be the formation of hydroxyapatite layer, which would explain a constant uptake of P (phosphates) observed with both types of composites (Figure 2B). An uptake of phosphate could be also explained by precipitation of hopeite-like structure, highly depending on pH and Zn$^{2+}$ concentration. A release of Si from composites, corresponding to the degradation of MBG, was stable over time and slightly lower in the case of 3-9 composites. Release of Zn$^{2+}$ was constant over time as well, with higher released concentrations corresponding to its higher initial amount in the samples (Figure 2C).

**Conclusion**

This study shows a successful development of composite bio(ink) for bone tissue engineering with tailorabile release of bioactive ions.

**Acknowledgement**

The authors thank the German Research Foundation (DFG, grant GE1133/24-1) for financial support.
Ion release from composite scaffolds containing 7% of different MBG
Release of (A) Ca, (B) P (phosphates) and (C) Zn; Values are means ± SD, n = 3.

References
Tea Tree Oil loaded PHBV electrospun nanofibers for wound healing applications

Verônica Ribeiro dos Santos¹, Samara Domingues Vera¹, Gabrielle Lupeti de Cena², Ana Paula Bernardo da Silva³, Ana Paula Lemes³, Katia da Conceição², Alexandre Luiz Souto Borges⁴, Eliandra de Sousa Trichês¹

¹ Universidade Federal de São Paulo, Laboratório de Biocerâmicas - Instituto de Ciência e Tecnologia, São José dos Campos, BR; ² Universidade Federal de São Paulo, Laboratório de Bioquímica de Peptídeos - Instituto de Ciência e Tecnologia, São José dos Campos, BR; ³ Universidade Federal de São Paulo, Laboratório de Tecnologia em Polímeros e Biopolímeros - Instituto de Ciência e Tecnologia, São José dos Campos, BR; ⁴ Universidade Estadual Paulista, Laboratório de Bioengenharia - Instituto de Ciência e Tecnologia, São José dos Campos, BR

Introduction
Electrospun nanofibers have great potential in soft tissue engineering applications due to their great similarity with the extracellular matrix, promoting accelerated tissue healing and fewer infection risks. Recently, extracts and essential oils of plants have been associated with these nanofibers to promote specific properties. Tea Tree Oil (TTO, Melaleuca Oil), for example, is an antimicrobial and anti-inflammatory essential oil able to optimize wound healing [1]. PHBV (Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)) is an easily spinnable, biocompatible, and biodegradable natural biopolymer [2], being a great candidate for incorporate the TTO. The main objective of this work was to prepare different concentrations of TTO loaded PHBV electrospun nanofibers and to characterize their morphology, physical properties, and antibacterial activity.

Experimental Methods
PHBV pellets (Nature Plast, 2% HV) were dissolved in 1,1,1,3,3,3-Hexafluoro-2-propanol (HFP, Sigma-Aldrich) to obtain an 8 wt.% solution. After complete dissolution, TTO (PHYTOTERÁPICA) was added at 0, 5, 10 and 15% v/v, named of PHBV/0TTO, PHBV/5TTO, PHBV/10TTO and PHBV/15TTO, respectively. The solution was electrospinning at 14 kV with a 27G needle at 1.5 mL/h and 3.000 rpm.

Results and Discussion
SEM images (Figure 1) indicated smooth, uniform, and bead-free mats with an average diameter that decreased according to the increasing of TTO content, from 0,750 ± 0,030 nm (PHBV/0TTO) to 0,430 ± 0,027 nm (PHBV/15TTO). Also, PHBV nanofibers exhibited the plasticizing effect typically induced by essential oil [3]. XRD (Figure 2.a) patterns indicated typical peaks of PHBV, not showing any influence of TTO. DSC curves (Figure 2.b) indicated one peak and one shoulder commonly obtained for PHBV, indicating two crystal conformations (α orthorhombic cell and β planar zigzag)[4]. It was also observed the smoothness of the shoulder according to TTO increase, although the major peak remained between 173°C (PHBV/15TTO) and 175°C (PHBV/0TTO). Antimicrobial assays with E.coli, S. aureus, and C. albicans are in progress.

Conclusion
TTO loaded PHBV nanofibers were successfully prepared and presented great potential to be used in wound healing applications.

Acknowledgement
The authors would like to thank FAPESP (2019/10877-3) for the financial support of this work.
Figure 1
SEM images of TTO loaded PHBV electrospun nanofibers.

Figure 2
a) XRD patterns and b) DSC curves for TTO loaded PHBV electrospun nanofibers. Legend: *PHBV.

References
PS1-01-015

A Design of Experiment methodology to study the influence of production conditions in the behaviour of PCL/gelatin electrospun skin wound dressings

Sara Guerreiro, Joana Valente, Juliana Dias, Nuno Alves

Centre for Rapid and Sustainable Product Development, Polytechnic of Leiria, Leiria, PT

Introduction

Skin wounds associated with chronic diseases that compromise the healing process of tissues have been studied during the last years. Electrospun membranes can be used as wound dressing to enhance the regeneration of damaged tissues due to their intrinsic characteristics (e.g. porosity). Furthermore, they have been explored in drug-delivery systems given the ability of some materials for attaching therapeutic agents. Electrospinning uses electrical fields to stretch the drop of a solution that is continuously pumped to obtain fibers with diameter ranging from micrometres to few nanometres. This process can be used to obtain a physical barrier against infectious agents. The ability of this barrier in transmitting gas molecules depends on the structural arrangement of the nanofibres, and on appropriate hydrophobicity and degradation rate of the materials used. These are the requirements for the membrane to promote and enhance the healing process. At the same time, the versatility offered by electrospinning allows to set specific a drug-release profile for a given therapy. This can be accomplished by manipulating nature and concentration of the materials used. Gelatin and polycaprolactone (PCL) are examples of traditional polymers used to produce electrospun nanofibers given their attributes of biocompatibility and biodegradability, respectively.

Additionally, the high area-to-volume ratio and the low cost associated with the fabrication process of these membranes make electrospinning a cost effective and competitive technology. However, the high number of factors to consider during membrane production accounts for low reproducibility and high heterogeneity of the obtained electrospun membranes.

Experimental Methods

In this work, a statistical approach based on a Design of Experiments (DoE) methodology is proposed to optimize the production of electrospun membranes through a prediction model. The fiber diameter, the permeability of the membrane and its thickness were modelled using a Box-Behnken design where the applied voltage, the flow-rate, the needle’s working distance and the ratio of PCL/gelatin in the solution were defined as independent variables. In order to quantify their influence in the main membrane attributes, a time-domain analysis was performed. The diameter of the fibers was measured using ImageJ after scanning electron microscopy image acquisition. The permeability was calculated based on the mass of water lost in flasks sealed with the electrospun membranes and placed at 37°C for 24 hours. Finally, thickness of electrospun membranes was measured using a digital micrometre. In order to test the suitability of the electrospun membranes in working as controlled released systems, a release study using standard bovine serum albumin was implemented.

Results and Discussion

Statistical significance (p<0.05) was observed for the model accuracy in predicting the main electrospun membrane attributes (diameter, permeability and thickness) for more than 75% of the samples. Permeability showed to be the most reliable measure with R² of 83%. Additionally, the ratio of PCL/gelatin, the needle’s working distance and the flow rate showed to be the most relevant factors for explaining diameter, permeability and thickness, respectively. The developed models were used to guide the experimental methodology to obtain fibers with diameters up to 300 nm, subjected to the maximization of both, permeability and thickness. The membranes were produced under...
predicted conditions (50.10%; 0.82ml/h; 11.91cm and 14.11kV) and the release study was performed using this setup. The results suggest that the devised electrospun membranes successfully act as protein delivery systems, with a constant release rate of 1mg/h for the first 4 hours of contact.

**Conclusion**
Overall DoE showed to be a useful tool to optimize the electrospinning-based production process by allowing to reduce time and cost associated, with positive impact in research and industrial settings.

**Acknowledgement**
This work is supported by the Fundação para a Ciência e a Tecnologia (FCT) and Centro2020 through the following Projects: PAMI - ROTEIRO/0328/2013 ( Nº 022158); MATIS (CENTRO-01-0145-FEDER-000014); I-FILM (POCI-01-0247-FEDER-017921; Lisboa-01-0247-FEDER-017921); Bone2move (POCI-01-0145-FEDER-31146); and SpinningTNT (POCI-01-02B7-FEDER-069285).

**References**
Biomimetic and bioactive nanofibrous interfaces to modulate cell response

Gianluca Ciardelli$^{1,2}$, Michela Licciardello$^{1,2}$, Chiara Tonda-Turo$^{1,2}$

$^1$ Politecnico di Torino, Department of Mechanical and Aerospace Engineering, Turin, IT; $^2$ Politecnico di Torino, POLITO BIOMedLAB, Turin, IT

Introduction
Electrospun polymer mats have emerged for their great potential as biomimetic and bioactive interfaces thanks to their close structural resemblance to the extracellular matrix-ECM (which enhances the tissue growth), their high porosity that allows gas permeation, and the large surface to volume ratio which provides an open structure for cell adhesion. Nanofibrous mats are widely applied in tissue engineering application showing a high potential to facilitate the formation of artificial functional tissues. To date, biodegradable polymer nanofibres with different chemical and physical properties using both synthetic polymers and natural polymers have been applied in several tissue engineering approaches. In this work, the fabrication and characterization of natural-based nanofibers is discussed highlighting the role of biomimetic and bioactive cues in directing cell growth and fate.

Experimental Methods
Gelatin (GL, type A from porcine skin), chitosan (CS, molecular weight 100 – 200 kDa, deacetylation degree ≥ 95%) and collagen (CL, bovine type I) were used for nanofibers fabrication. Electrospinning protocols were optimized to achieve homogenous fibres with nanoscale size by using a “green electrospinning” method which avoids the use of environmental and cellular unfriendly solvents. Both randomly-oriented and aligned nanofibers were obtained mimicking the ECM architecture of different tissues. Developed nanofibrous matrices were loaded with active compounds such as antioxidant, piezoelectric, conductive and antibacterial agents to induce specific biological effects [1–5]. Complete morphological and physical-chemical characterisation was performed as well as biological in vitro tests.

Results and Discussion
The design of efficient scaffolds to sustain the cell outgrowth and its guidance towards appropriate targets is of paramount importance in reproducing the physiological environment to achieve tissue regeneration. Topographical stimuli can modulate cell organization as shown comparing cell morphology on random and aligned nanofibrous mats (fig. 1). The beneficial effect of morphological cues was combined with active agents such as antioxidant nanoparticles (nanoceria), piezoelectric particles (barium titanate), antibacterial agents (gentamicin sulphate, manuka honey, silver nanoparticles) and conductive polymers to evaluate the role of those cues in directing cell growth and fate [1–5]. Results confirmed the high versatility of the electrospinning technique to process different materials (fig. 2). Furthermore, a cell-specific response was observed when nanofibrous were doped with tissue-like cues [1–5]. The developed electrospun scaffolds allows to create a platform of interfaces able to modulate cell adhesion and growth in a physiological-like manner towards the creation of functional tissues.

Conclusion
Electrospun nanofibres are promising interfaces to enhance and modulate cell behaviour in the attempt to regenerate tissue in vivo as well as to reproduce organ in vitro to create in vitro organ models.

Acknowledgement
This work was financed by Compagnia SanPaolo, CRT Foundation and Piedmont Region (Italy).
Fig. 1. Representative scanning electron microscope (SEM) images of myoblasts cultured on a) randomly oriented chitosan nanofibres and b) aligned chitosan nanofibres (scale bar = 20 μm).

Fig. 1

Fig. 2. a) Gelatin doped with barium titanate particles (GL/BTNPs) electrospun membrane (scale bar = 10 μm); b) conductive electrospun membrane (scale bar = 10 μm); c) Alizarin red staining of MSCs after culture for 7 days on GL/BTNPs nanofibres (scale bar = 50 μm); d) Fluorescent microscopy images of fibroblasts (HFF-1) stained after 7 days of culture on conductive membranes (scale bar = 50 μm).

Fig. 2

References


PS1-01-021

Spatiotemporally functionalizable and mechanically tunable biomaterial for embedded bioprinting of tubular structures

Malin L. Becker, Maik R. Schot, Jeroen Leijten

University of Twente, Department of Developmental BioEngineering, TechMed Centre, Enschede, NL

Introduction

Embedded 3D bioprinting holds great potential as a versatile tool for the engineering of tissue replacements. Conventionally, the embedding bath has mostly acted as a removable support bath with its main function being to mechanically support the printed structure until solidification is achieved and the printed construct can be removed from the bath. Additionally, the use of sacrificial inks in combination with crosslinkable baths has been reported in order to create pre-vascularized perfusable tissue engineered structures. However, resulting tissue constructs featuring perfusable channel networks lack distinct mechanical and chemical properties and thus do not resemble their natural counterparts, which has limited their functional performance. Here, we tested our hypothesis that a dual crosslinking strategy could enable the embedded bioprinting of complex, integrated, and perfusable vascular networks that are mechanically and chemically distinct from the engineered tissues they are embedded within.

Experimental Methods

A dually crosslinkable polymer was created by functionalization of a dextran backbone with tyramine and biotin moieties. Physically crosslinked embedding baths were realized by exploiting the strong protein/ligand interaction between biotin and avidin. For the creation of tubular structures the bath was loaded with horseradish peroxidase (HRP) and a hydrogen peroxide (H₂O₂) containing gelatin based sacrificial ink was extruded into the bath. Local covalent crosslinking could be combined with bulk photocrosslinking to yield free standing or embedded tubular structures, respectively. Subsequently to crosslinking, the tissue’s biotin moieties can be utilized for on-demand biochemical functionalization, either of the bulk and/or the channel surfaces.

Results and Discussion

Rheological characterization of the physically crosslinked bath revealed shear-thinning and self-healing properties that were highly suitable for embedded bioprinting (Figure 1). Covalent crosslinking resulted in a three-fold increase of the storage modulus of the hydrogel constructs, which enabled the stabilization of printed channel networks. The wall thickness of the printed tubular structures could be adapted with varying H₂O₂ concentrations. The inside-out crosslinking resulted in a mechanical gradient with decreasing stiffness with increasing distance from the channel wall. The printing setup allowed for the creation of more complex vascular designs such as perfusable bifurcating tubular structures (Figure 1). Furthermore, the channel walls were demonstrated to be on-demand functionalizable with biotin-coupled moieties in a functional manner.

Conclusion

We report on a novel and dually crosslinkable hydrogel suitable for embedded bioprinting, in combination with an inside-out crosslinking approach resulting in perfusable structures that offer spatially distinct and modifiable mechanical properties, which resembles natural tissue designs. The resulting structures offer for on-demand functionalization with bioinstructive moieties to further guide tissue fate and function in a spatially controlled manner.

Acknowledgement

Financial support was received from the European Research Council (ERC, Starting Grant, #759425) and the Dutch Research Council (NWO, Vidi Grant, #17522).
Figure 1
Dynamic moduli of physically crosslinked gels in dependence on applied strain showing self-healing behavior (left) and printed bifurcating structure with fluorescent dye (pink) and crosslinked Dex-TAB (blue) (right).

References
Highly Oriented Electrospun Conductive Nanofibers of Biodegradable Polymers—Revealing the Electrical Percolation Thresholds

Muhammad A. Munawar¹,², Dirk W. Schubert¹,²

¹ Friedrich-Alexander-University Erlangen-Nuremberg, Department of Materials Science, Institute of Polymer Materials, Erlangen, DE; ² Bavarian Polymer Institute, Key Lab Advanced Fiber Technology, Fürth, DE

Introduction
The main goal of this study is to fabricate highly oriented electrospun conductive fiber composites (ECFCs) by using a special rotating collector electrode and allow an accurate determination of electrical conductivity. The determination of critical volume filler concentration/percolation threshold ($\varphi_c$) by using McLachlan and Kirkpatrick’s models for experimental conductivities of conductive fibers is another important objective. Since at percolation threshold, the conductivity of conductive fibers boost to several orders of magnitude, so it is very important to know for scientific community to have better understanding about conductivity behavior of conductive electrospun nanofibers for their right applications.

Experimental Methods
An electrospinning process with a special rotating collector electrode [1] is used to generate highly oriented electrospun conductive fiber composites (ECFCs) of biodegradable polymers and demonstrated in schematic Figure 1.. Polyethylene oxide (PEO), polycaprolactone (PCL) and polyactic acid (PLA) are blended with intrinsically conductive polymers (ICPs)—fillers of Poly 3,4 ethylenedioxythiophene (PEDOT) and polyaniline (PANI) [2]. The PEDOT doped with polystyrene sulfonate (PSS) anions and PANi doped with (+)-camphor-10-sulfonic acid (CSA) in their appropriate solvents, respectively. The processing parameters were varied from case to case and their ranges were; voltage (5-20 kV), flow-rate (0.1-2 mL/h), tip-collector distance (5-25 cm) and collector rotation (50-200 rpm). At the optimized processing parameters, the viscoelastic spinning solution ejected from spinneret and stretched in the form of a charged jet towards the grounded collector. The displacement of jets from spinneret to collector, the complete evaporation of solvent gave solid ECFCs on rotating wheel electrode. A detailed electrospinning theory can be found in the work from DW Schubert [2]. These highly oriented conductive fibers were collected on microscopic glass slide (GS) for the measurement of resistance $R$ using ohmmeter (Keithley 2400).

Results and Discussion
The straining of polymeric chains under mechanical stretching and thermal relaxing is seemed to be a significant factor for dynamic of percolation threshold in electrospun conductive nanofibers, as shown schematically in Figure 2. The ICPs-fillers (PEDOT and PANi) made agglomerations in their solvents (Figure 2a) and this was overcome by incorporating with their respective ionic surfactants (PSS & CSA) also called dopants or stabilizers, which can easily stabilize the ICPs particles against coagulation and precipitation (Figure 2b). The attraction between the positively charged ICPs and the negatively charged dopants or vice versa in the solvent systems led to coiled ICPs (Figure 2b). The ionic surfactants (dopants) which surrounded the coiled ICPs can create a barrier that block the charge transport within the ICPs. Therefore, blending with high molar mass polymer matrices (PEO, PCL and PLA) weakened the electrostatic interaction between ICPs and dopants, and hence helped in phase separation development (Figure 2c). The mechanical viscoelastic stretching of chains of ICPs, dopants and host polymers (matrices) disrupted the static charge interactions under high electrostatic forces during electrospinning process (Figure 2d & 2e). Therefore, the molecular chains started to reorganize themselves into more stretched structures, which essentially are responsible for high inter-chain conductivity in electrospun fibers (Figure 2f, 2g & 2h). During annealing process, the molecular chains of host polymers became more relaxed (expanded) and ICPs (filler particles)
acquired a certain degree of freedom to interconnect/reorganise themselves. This reorganising (intermingling) of ICPs created more paths and additional conductive channels, which essentially responsible for intra-chain conductivity in annealed ECFCs (Figure 2i, 2j, 2k, 2l & 2m) [3].

Conclusion
The existence of different interactions/compatibilities between polymer matrices and conductive fillers causes a dynamic $\phi_c$. The mechanical viscoelastic stretching under high electrostatic forces in electrospinning process introduces high inter-chain conductivity to electrospun fibers. The higher the inherent conductivity of intrinsically conductive polymers (ICPs) produce the higher conductive ECFCs and vice versa. The annealing process in electrospun fibers enhances the built up of additional conductive channels, which lead to superior electrical performance and lowering the percolation threshold and a hint is evident of a potential time-temperature superposition. Moreover, the conductivities of our fabricated ECFCs are suitable for a wide range of biomedical applications.

Acknowledgement
The authors thank the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)-Projectnummer 326998133-TRR 225 (subproject A07) for funding this work.

Figure 1. Schematic of electrospinning set-up which is used for producing highly oriented fibers.
Schematic of electrospinning set-up, used in this study and rotating wheel collector covered with electrospun fibers aligned to machine direction (MD) and SEM image showing high orientation of electrospun fibers.
Figure 2. Schematic for distribution of ICPs with host polymers within in electrospun fibers. Schematic for dispersion of ICPs doped with their respective dopants and blended with host polymers/matrices (a, b and c) and the mechanical viscoelastic stretching of molecular chains of ICPs, dopants and host polymers under high electrostatic forces during electrospinning process (d, e, f, g and h). The re-constructing of additional conductive channels and physical/chemical bonding upon annealing (i, j, k, l and m).

References
Anisotropic Conductive Melt Electrowritten Patches Combined with Electrical Stimulation for Cardiac Tissue Engineering

Yanping Zhang¹, Menglin Chen¹⁄²

¹ Aarhus university, Interdisciplinary Nanoscience Center (iNANO), Aarhus C, DK; ² Aarhus university, Department of Engineering, Aarhus C, DK

Introduction
Cardiac patches, three-dimensional (3D) tissue constructs of polymer scaffold embedded with cardiac muscle cells, represents a promising approach for the treatment of myocardial infarction (MI). However, recapitulation of the fibrillar structural organization, anisotropic mechanical behavior, and electrophysiology of native-like myocardial muscle remains a major challenge for engineering cardiac patches. Herein, we present an anisotropic conductive patch that conforms to the mechanical and bioelectrical properties of human myocardium.

Experimental Methods
Melt electrowritten (MEW) as an emerging technology combining electrospinning with 3D-printing, can realize tailored micro-architectures manufacturing. This unique capability of MEW was exploited to fabricate patches with controlled fibrous microstructures that can accommodate the strains and stresses exhibited by the human myocardium. Furthermore, a nanolayer of gold coating endowed the patch with electrical conductivity similar to that of human myocardium, and this patch served as an electroactive path to electrically stimulate cardiac muscle cells under exogenous electrical fields.

Results and Discussion
The results showed that the patches exhibit large biaxial deformations, and the structure of the patches can be fine-tuned to reflect anisotropic mechanical properties. Moreover, the patches guided HL-1 cardiac cells growth, proliferation, and alignment along the microfibers. Calcium imaging indicated that HL-1 cardiac cells appear synchronous contraction within 2 days after seeding on the patches. Coating gold on the patches led to the increased expression of cardiac-specific genes of HL-1 cells involved in muscle contraction and electrical coupling. In the following work, we will apply the electrical stimulation to promote the maturation of cardiac cells.

Conclusion
Overall, our anisotropic conductive patch holds great potential for cardiac tissue engineering.

Acknowledgement
We gratefully acknowledge Carlsberg Foundation (CF19-0300), Aarhus University Research foundation (AUFF-E-2015-FLS-7-27) and China Scholarship Council for support of the research.
Figure 1
A schematic outline of the preparation of 3D anisotropic conductive scaffolds and high-throughput stimulation device for cardiac tissue engineering

References
Design of hybrid peptide / polymer nanofibers for soft tissues regeneration

Coline Pinese, Karima Belabbes, Audrey Bethry, Gilles Suba, Xavier Garric

University of Montpellier, IBMM, Montpellier, FR

Introduction
Nanofibers are excellent supports for tissue regeneration thanks to their topography that reproduces the architecture of the extracellular matrix. Very commonly, nanofibers are made of PCL, PLA or PLGA and in these cases have a guiding role essentially.[1–3] Our objective is to create nanofibers which, in addition to this guiding role, could modulate the cellular behavior, in particular by introducing bioactive peptides in the intrinsic composition of the nanofibers biomaterial. We are considering sol-gel chemistry for this purpose, the goal being to assemble polymers and peptides like Legos® to create a bioactive tri-dimensional network constituting the nanofibers. For that purpose, we set up an original synthesis based on star-PLA bearing triethoxysilyl propyl groups (PLA-PTES) and bifunctional silylated peptides that react together via sol-gel process to create a bioactive network. [4,5]

Experimental Methods
We synthesized and functionalized the polymers and peptides according to protocols previously developed by our team. [4] Electrospinning was performed under fixed hydrolysis conditions by varying the molar mass of the polymers, the shape of the polymers and their ratio. The formation of the network was evaluated by FTIR and by quantifying the gel fraction of the network. The percentage of peptide incorporation (peptide-FITC) within the networks was performed by GPC double detection IR and fluorescence. The biological properties of the hybrid materials were then evaluated by deposition of HDFs on the nanofiber mats and by investigating their proliferation capacity according to the percentage of incorporated peptides.

Results and Discussion
First, we synthesized and characterized star-shaped PLAs functionalized with IPTES (StarPLA-PTES) and bifunctional silylated peptides that react together via a sol-gel process to create a bioactive network. Subsequently, we produced functional nanofibers by activating the sol-gel reaction during the electrospinning process. We studied the impact of different parameters on 1/ the formation of the three-dimensional polymer network composing the nanofibers and 2/ the rate of incorporation of the peptide into this network. During the fabrication of the nanofibers, the molecular weight of the polymer and the hydrolysis kinetics of the PTES functions had the most impact on the cross-linking between the polymers and on the incorporation rate of the peptide. The improvement of the peptide grafting rate to the polymeric network by using low molecular weight polymers is explained by the increase in the content of cross-linking groups (PTES). Finally, the potential of this new type of hybrid biomaterials created for soft tissue engineering was demonstrated by cells adhesion assays that showed a significant improvement of fibroblast adhesion on the peptide-containing nanofibers.

Conclusion
In conclusion, we demonstrated that poly(lactide), a degradable polymer wildly used for biomedical application could be crosslinked using sol-gel siloxane chemistry during electrospinning process, producing nanofibers composed by a bioactive three-dimensional network. This reaction takes place during the electrospinning process and offers the possibility to functionalize the nanofibers with peptides, bearing a siloxane function, with various properties and therefore adaptable to many applications.
Production of bioactive and degradable nanofibers by electrospinning of bifunctionalized star-shaped materials.

References


PS1-01-029

Development of chitosan / natural gums based bioinks for extrusion-based 3D bioprinting

Aurelia Poerio¹, Jean-Philippe Jehl¹, Sandrine Hoppe², Philippe Marchal², Joao Mano³, Franck Cleymand¹

¹ University of Lorraine, Institut Jean Lamour, Nancy, FR; ² Université de Lorraine, Laboratoire Réactions et Génie des Procédés, Nancy, FR; ³ University of Aveiro, Department of chemistry, CICECO, Aveiro, PT

Introduction
Due to its biocompatibility, nontoxicity and biodegradability, chitosan has become of great interest for medical and pharmaceutical applications in the form of films or hydrogels [1]. Nevertheless, its application as bioink for 3D bioprinting is limited due to the soft stiffness, which usually significantly reduces shape fidelity and resolution of the printed scaffolds. Different strategies can be adopted to improve the printability of bioinks through the increase of their viscosity such as the inclusion of additional molecules. Recently, natural gums have been extensively investigated as biomaterials for tissue engineering applications thanks to their similarity with the native extracellular matrix (ECM) and their capability of increasing solution viscosities even at small concentrations [2].

Experimental Methods
The aim of this work is the development and characterization of new bioinks based on chitosan and natural gums, such as guar gum (GG) and tamarind gum (TG) and the evaluation of their printability using an extrusion-based bioprinting process. The viscoelastic properties of the bioinks were evaluated and the bioprinted scaffolds were characterized by their morphology, swelling ratio, degradation in lysozyme and ability to release bovine serum albumin (BSA) as model protein.

Results and Discussion
The results show that the inclusion of GG and TG increases the viscosity of the bioink forming solutions improving their printability. The morphological characterization of the bioprinted scaffolds shows an highly porous internal structure that could provide a suitable and interconnected network essential for cell nutrition, proliferation, and migration. Furthermore, while constructs with no gums were completely degraded after 3 weeks, the inclusion of GG and TG slowed down the degradation process up to two months, in absence of crosslinkers. The construct containing TG also reduced the amount of BSA released suggesting their potential to be used for a more sustained release of the encapsulated factors. Furthermore, cellular studies to investigate the biocompatibility of the bioinks are in progress.

Conclusion
Taken together, these data show that these two natural gums have the potential to be successfully used for 3D bioprinting applications.

Acknowledgement
This study was supported by the French PIA project “Lorraine Université d’excellence”, reference ANR-15-IDEX-04-LUE

References
3D printed carboxymethyl cellulose scaffolds enriched with autologous growth factors for diabetic wound regeneration

Luis A. Diaz-Gomez¹, Iago Gonzalez Prada¹, Rosendo Millan², Angel Concheiro¹, Carmen Alvarez-Lorenzo¹

¹ Universidade de Santiago de Compostela, Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, I+D Farma (GI-1645), Facultad de Farmacia and Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, ES; ² Universidade de Santiago de Compostela, CEBEGA, Santiago de Compostela, ES; ³ Universidade de Santiago de Compostela, Pharmacology, Pharmacy and Pharmaceutical Technology, SANTIAGO DE COMPOSTELA, ES

Introduction
Wound regeneration in diabetic patients remains a clinical challenge. The delivery of autologous growth factors, such as platelet-rich plasma (PRP), is a promising strategy to enhance the regeneration ability in diabetic ulcers by increasing the deposition of collagen and promoting angiogenesis [1]. However, the direct administration of platelet concentrates in the wounded tissue might not ensure the adequate availability to local cell population [2]. Therefore, encapsulation strategies aiming to provide a controlled release of growth factors (GFs) are needed. In this work, we developed a strategy to prepare 3D printed personalized carboxymethyl cellulose (CMC) skin scaffolds loaded with autologous PRP to promote diabetic ulcer regeneration.

Experimental Methods
CMC (15 w/v%) hydrogels were autoclaved and then citric acid (15% w/w of CMC) was added to the dispersions. The obtained inks were printed using a commercial 3D printer to fabricate cylindric skin scaffolds (8 mm in diameter; 600 μm pore size). The scaffolds were freeze-dried for 24 h, crosslinked at 120 °C for 7 min, and then, immersed in a PRP solution previously activated by freeze-thaw cycles. The structure, mechanical properties, and GFs release of the obtained wound dressings were evaluated. In vitro cell proliferation and migration were also assessed. Finally, the ability of the dressings to promote wound regeneration was evaluated in a full-thickness cutaneous wound in a streptozotocin-induced diabetic rat model.

Results and Discussion
The rheological, morphological, and mechanical testing demonstrated that the developed CMC inks showed excellent printability and shape fidelity. Crosslinked dressings presented a remarkable swelling behavior able to promote the moist microenvironment necessary during the wound healing process, and GFs were released from the dressings over 7 days in a controlled manner. Migration assays confirmed that CMC and CMC-PRP dressings promoted mesenchymal stem cell (MSC) migration, while released PRP significantly enhanced cell proliferation. Compared with control treatment, CMC and CMC-PRP dressings resulted in a significantly accelerated wound closure rate in vivo, increased vascularization, and enhanced regeneration of granulation tissue.

Conclusion
CMC-PRP 3D scaffolds were successfully prepared using a straightforward procedure. The developed skin scaffolds may act as wound dressings to sustainably release autologous GFs and significantly enhance cell migration and proliferation. In vivo studies confirmed the ability of the CMC-PRP dressings to significantly enhance wound healing in a diabetic rat wound model. In summary, personalized CMC-PRP wound dressings may represent a promising therapeutic approach for the treatment of diabetic ulcers.

Acknowledgement
L.D.-G. acknowledges Consellería de Cultura, Educación e Ordenación Universitaria, for a Postdoctoral fellowship [ED481B2017/063]. This research was funded by MINECO [SAF2017-83118-R] and Xunta de Galicia [ED431C 2020/17].

References


Synthetic 3D fibrous scaffolds as animal free and low cost basis in 3D cellular applications

**Tobias Weigel**, Christoph Malkmus, Verena Schneider, Bastian Christ, Maximiliane Wußmann, Jörn Probst, Florian Gröber-Becker, Jan Hansmann

1 Fraunhofer Institute for Silicate Research (ISC), Translational Center for Regenerative Therapies (TLZ-RT), Würzburg, DE; 2 University Hospital of Würzburg, Dept. Tissue Engineering & Regenerative Medicine, Würzburg, DE; 3 University for Applied Sciences Würzburg/Schweinfurt, Department Electrical Engineering, Schweinfurt, DE

**Introduction**

Besides organoid/spheroid culture, 3D cellular constructs always require scaffolds as a fundamental building block. One example are hydrogel matrices, either isolated from organisms (animal or plants) or specifically designed as synthetic variants. Other possibilities are submicrometer sized fibrous tissues, mainly produced by decellularization of animal derived organs. Apart from ethical reasons, the mentioned scaffold possibilities have high batch-to-batch variations or are expensive to produce. Electrospun fibers, covering the diameter range between nano and micro, can be applied as a serious alternative achieving the mandatory three-dimensionality for cells. To gain the 3D structure of electrospun scaffolds with a sufficient cellular accessibility, we combine a high numbers of pores with large mesh openings. By incorporating high amounts of porogens in the scaffold during the spinning process, the required 3D properties can be generated with focus on stromal tissue applications.

**Experimental Methods**

Materials for the synthesis of electrospun 3D submicrometer fibrous scaffolds were polycaprolacton (PCL), polyamide 6 (PA6), polyacrylonitrile and silica. Applied water-soluble porogens were NaCl particles as well as microfibers of polyvinylalcohol or titanium-oxo-carboxo-complexes. Porogens were incorporated in the scaffolds by an alternating process. Porogen placement was performed by particle strewing or placing micro-fibrous mats during the paused spinning process or by unwinding microfibers during a continuous spinning process. The structural properties of the resulting 3D scaffolds were characterized by SEM, LM and CLSM. The suitability for 3D cell culture was tested with human primary cells (hdFs, hMSCs, heKs and different cellular organoids).

**Results and Discussion**

The removement of the porogens resulted in a highly porous scaffold with porosities higher 98.5 %. Depending on the porogen size and the collector speed, the pores demonstrated an elliptical morphology or were extended to form a kind of laminar scaffold composition. The fiber layers around the pores showed a loose network of single fibers with low numbers of contact points as well as mesh openings up to several 100 µm². Thereby, the pores separated the fiber layers and prevented the structural collapse resulting in spatial consolidation.

When seeding stromal cells on the scaffolds, the cell were able to migrate and populate the scaffolds with a population speed of around 100 µm per week. By suitable supplementation, the stromal cells were able to synthesize ECM and transform the synthetic structure to a biological tissue. Besides matrix expression, cells were also able to differentiate inside the fibrous scaffold.

By combination of several cell types in different scaffolds, more complex tissue models were generated. Starting with a synthetic scaffold based skin model with a dermis and epidermis layer, the model was extended with a subcutis layer.

Concerning further applications, additional cell types and co-cultures are tested on the extended electrospun matrices to classify the application range of potential tissue models.

**Conclusion**

Page 1025 of 2028
The incorporation of high amounts of porogens into electrospun scaffolds resulted in highly porous and for cells accessible scaffolds. First 3D cell experiments showed promising results towards the application of biological tissue generation.
Nano-engineered naturally-derived inks for bone tissue regeneration

Andreea I. Dinu¹, Alexandra I. Cernencu¹, Adriana Lungu¹, Horia Iovu¹, Sorina Dinescu²

¹ University Politehnica of Bucharest, Advanced Polymer Materials Group, Bucharest, RO; ² University of Bucharest, Department of Biochemistry and Molecular Biology, Bucharest, RO

Introduction
The popularity of additive manufacturing techniques in bone tissue engineering lies at an upward path, as more polymer formulations for extrusion based methodologies are developed to aid the effective regeneration of bone-tissue by printing a structure with the desirable shape, structural, chemical and biological characteristics. Taking advantage of both naturally derived polymers and nanostructured materials, we propose an innovative bio-inspired nano-engineered printing material that combines two types of nano-fillers, polyhedral oligomeric silsesquioxanes nanoparticles (POSS) and cellulose nanofibrils (CNF) with a proteinaceous-polysaccharidic matrix composed of methacrylated gelatin and amidated pectin by means of which we aim to fabricate a scaffold with applications in bone tissue engineering.

Experimental Methods
Ink formulations were synthesized and assessed herein by taking advantage of the fast-crosslinking potential of gelatin methacrylate and amidated pectin and the remarkable shear-thinning properties of oxidized nanocellulose. Prior to 3D printing, the polymeric formulations were studied closely to determine the suitable concentration for each component, as well as the best methods for blending the organic-inorganic POSS cages in the polymeric network. In this regard, physical mixing techniques were combined with dissolution in organic solvents while availing the surface chemistry of functionalized POSS compounds. The performance of the 3D printed structures after photo-cross-linking followed by enzymatic crosslinking was evaluated in terms of mechanical properties and swelling behavior. FTIR spectroscopy served as a tool for determining the compositional characteristics of the constructs before and after crosslinking. The morphology of the 3D-scaffolds was investigated focusing on the dispersion of POSS, the porosity and the geometrical features of constructs. Pre-osteoblasts were used to perform preliminary in vitro biological tests onto freeze-dried hydrogels.

Results and Discussion
The structural, chemical and biological properties were considered within a comprehensive study on the influence of polymer and nanoparticle concentration in relation with the chosen synthesis methods. By testing POSS with different functionalities we were able to determine the best approach and ensure that the designed formulation is highly homogeneous. Our results showed that the use of POSS did not hinder the swelling capacities of the freeze-dried scaffold and all samples maintained their hydrophilic character. The mechanical properties proved the great potential of the designed scaffold for bone tissue engineering and the preliminary in vitro biological tests performed using pre-osteoblasts showed an optimum biocompatibility. Accordingly, the resulted 3D scaffolds offer essential support for stem cell adhesion, differentiation and proliferation through the synergic roles of each wisely chosen component that ultimately emulate the natural extracellular matrix.

Conclusion
Novel bio-based ink formulations were designed as suitable for 3D printing inks by using POSS and CNFs as reinforcing agents in a hydrogel network. Therefore, by means of using different POSS compounds and a controlled polysaccharide:protein ratio, materials with enhanced biomechanical properties could be obtained. The composition of the bio-inspired formulations along with quick and efficient crosslinking properties imply that these inks are...
candidate biomaterials for extrusion based 3D printing. The 3D printable materials grant 3D networks with a high porosity that exhibits a good structural and functional resemblance with native (living) tissue.

Acknowledgement

The authors would like to thank for the financial support provided by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P1-1.1-TE-2019-0787, within PNCDI III.

References


Mechanically reinforced tri-layered composite scaffolds with a biomimetic composition for restoration of articular joint function following traumaticinjury

Mark Lemoine\textsuperscript{1,2,3}, John O’Byrne\textsuperscript{1,4}, Daniel Kelly\textsuperscript{2,3}, Fergal O’Brien\textsuperscript{1,2,3}

Introduction

Articular cartilage has limited self-healing capacity and damaged cartilage can lead to osteoarthritis, and to the need for joint replacement surgery. Regeneration of joint tissue presents a clinical challenge as the layered structure of the osteochondral tissue has depth varying composition, mechanical properties, and biomechanical functions. Our lab has developed multi-layered collagen based matrices that can regenerate both bone and cartilage \cite{1, 2} which have shown potential in a number of animal models and in human patients but their mechanical properties restrict their use to small joint defects. To overcome this limitation, this study focussed on developing a biomimetic multi-layered scaffold with both mechanical and biochemical properties tailored to the specific requirements of the complex native tissue. It achieved this by initially developing a process to produce a tri-layered 3D printed polycaprolactone (PCL) scaffold which was then combined with a layered collagen matrix containing ECM components known to direct bone and cartilage formation respectively. The aim of the study was thus to assess the mechanical properties of the scaffolds to ensure their efficacy as implants to restore function in large joint defects and the capability of the biochemical composition to direct bone and cartilage formation in the distinct layers.

Experimental Methods

By 3D printing PCL struts with varying alignment and size, innovative scaffolds that mimic the mechanical properties of the osteo and chondral layers of native tissue were developed and used to reinforce a biomimetic collagen matrix with a cartilage layer of collagen type I and II (0.25%w/v of both) and hyaluronic acid (0.05%w/v) (CI/II-HyA), an intermediate layer of collagen type I (0.5%w/v) and hyaluronic acid (0.05%w/v) (CI-HyA), and a bone layer of collagen type I (0.5%w/v) and nano-hydroxyapatite particles (0.5%w/v) (CI-nHA) (Fig. 1A). The mechanical abilities of the composite scaffolds to resist compression, shearing and delamination, and dislodgement from osteochondral tissue were tested to ensure implant safety in load bearing joints. The capacity of the tri-layered composite scaffolds to support layer-specific deposition by bone marrow stem cells of sulphated glycosaminoglycans (sGAG) for cartilage formation and calcium for bone formation, was assessed by histological staining and biochemical analysis.

Results and Discussion

Increased mechanical properties over culture time proved that functional restoration of osteochondral tissue is possible with this composite scaffold (Fig 1B). The high fixation strength (47.4kPa) and shear strength (0.93MPa) of the composite scaffold ensures that it can be securely anchored in a joint defect and withstand the shear forces in a moving joint. The tri-layered composite scaffolds supported layer-specific stem cell differentiation, with cell infiltration and cartilage matrix formation to a depth of 3mm, with mineralization in the bone layer (Fig. 1C). Quantitative analysis of deposited sGAG and calcium showed that differentiating cells in the bone and cartilage layers produced requisite matrix components specific for each layer (Fig. 1D).

Conclusion

This novel tri-layered composite scaffold was shown to support support layer-specific stem cell differentiation \textit{in vitro}, which is crucial for regeneration of osteochondral tissue. The mechanical properties of the composite scaffolds are
optimized to be suitable for load bearing joints to regenerate large defects to alleviate the need for joint replacement surgeries. In vivo assessment of the composite scaffold in an osteochondral defect model in goats is currently ongoing.

Acknowledgement
This work was funded by the European Research Council under ERC grant agreement no. 788753.

Figure 1
A) Scanning electron microscopy image of the composite scaffold with PCL struts highlighted in shades of blue. B) Increase in dynamic and aggregate moduli of the cartilage layer due to MSC deposition of ECM. C) Staining of sGAG (blue) and calcium (black) deposition after 42 days of culture, scale bar is 1mm. D) Increase in sGAG and calcium in cartilage and bone layers respectively with culture time. All data is presented as average ± standard deviation. ** = p< 0.01, *** = p< 0.001, **** = p<0.0001.

References

[2] Levingstone T., 2016, 'Cell-free multi-layered collagen-based scaffolds demonstrate layer specific regeneration of functional osteochondral tissue in caprine joints', Biomaterials, Volume 87, Pages 69-81,
Fabrication of hyaluronan-based matrices functionalized with laminin derived peptides, to sustain cellular adherence and axonal growth in bone regeneration applications

Quentin Muller¹, Romane Josselin², Daphne van der Heide³, Jean-François Le-Meins², Bertrand Garbay², Martin J. Stoddart³, Matteo D’Este³, Joëlle Amédée¹, Hugo Oliveira¹

¹ Univ. Bordeaux, Bioingénierie Tissulaire (BioTis), Inserm, U1026, Bordeaux, FR; ² Univ. Bordeaux, LCPO, CNRS, UMR 5629, Pessac, FR; ³ AO Foundation, AO Research Institute Davos (ARI), Davos, CH

Introduction
After blood, bone remains the most transplanted organ, used in cases of bone trauma and resection. Currently, surgeons are still faced with major hurdles: i) complexity, availability and morbidity associated with vascular fibula grafts, ii) insufficient vascularization/innervation and osteointegration for implanted biomaterials in the absence of autologous cells, iii) complexity/regulatory hurdles in the use of growth factors (i.e BMPs). Furthermore, tissue engineering cell-based approaches have also shown limitations: i) limited ability of autologous cells from non-healthy patients to proliferate and integrate with biomaterial systems, ii) tissue-engineered constructs are labor intensive to produce, low throughput and difficult to form into complex 3D geometries, iii) advanced cell therapies are associated with stringent regulatory hurdles. Also, for many years, bone tissue-engineering strategies were mainly focused on osteogenesis and shown limited clinical success. Only recently, focus has been drawn on the importance of neurovascular modulation to attain fully functional tissues [1]. Here, we explore the 3D fabrication of a cell-free scaffold, designed to support the intricate orchestration between vascularization, innervation and bone healing, in a regeneration scenario. We focused on a hyaluronan-based matrix functionalized with laminin derived peptides, characterized by rheology, able to be bioprinted [mstoddar2] and support endothelial and neuronal cell adherence and maturation. We present the main optimization steps for its development towards improved bone vascularization and innervation, and its impact on bone regeneration.

Experimental Methods
The tyramine-modified hyaluronan-based matrix (HA-Tyr) was serially crosslinked with a first step involving an enzymatic oxidation with H₂O₂ and horseradish peroxidase, and a second step consisting in visible light-mediated cross-linking (505 nm) with Eosin Y [2]. The first cross-linking was used to pre-jellify, and enable microextrusion bioprinting, and the second to set the bioprinted structure for final shape fixation. HA-Tyr was added to increasing peptide concentrations (i.e. 0, 0.5, 1 and 2 mg/mL), and the rheological properties evaluated before and after light cross-linking. The viscoelasticity properties and the tan δ value (G’/G”) were analyzed in the linear domain. The peptide is derived from laminin, an assembly of IKVAV-YIGSR with Tyr at each end, allowing covalent crosslinking with HA Tyr.

In vitro evaluation was performed on gels following both polymerization approaches by seeding Human Umbilical Vein Endothelial Cells (HUVECs), human bone Marrow Mesenchymal Stem cells (MSCs) or human Induced-Pluripotent Stem cell-derived sensory neurons (iPS-SN) on the top of the matrix. To evaluate the cellular metabolic activity and indirect cell viability, alamar Blue™ assay was performed, up to 7 days of culture. Phenotypical characterization was performed using cell-specific immunolabelling. Finally, the printability of the matrix (with different peptide concentrations) was evaluated for microextrusion on a 3D Discovery (RegenHU) bioprinting system.

Results and Discussion
The first crosslinking of HA-Tyr brought to the formation of a weak gel as expected, with an average G’=58 Pa and G”= 27 Pa. The addition of peptides showed to decrease the viscoelastic properties of the composite hydrogel, with...
decreased G’ and G” values. For the highest peptide formulation, 2 mg/mL, a loss of cohesion is observed. Nonetheless, all formulations with lower peptide concentrations, or without peptide, showed a tan δ (G”/G’) value between 0.4 and 0.6, in the optimal range for extrudability in terms of microextrusion bioprinting [3,4]. Following the second polymerization of HA-Tyr without peptide, we observed a higher crosslinking efficacy, with an average G’=576 Pa and G”=23 Pa. Again, we observed a lower elastic modulus when peptides were associated within the matrix. These results suggest that optimal peptide concentration will be established at concentrations lower than 1 mg/mL.

In terms of in vitro results, the addition of peptide enhances HUVEC and MSCs cell adhesion, metabolic activity and phenotype. Current studies are underway to establish the capacity of the developed matrices to support axonal growth of iPS-SN.

Conclusion
We showed the grafting of laminin-derived peptides supports cellular adhesion and phenotype. Further studies are ongoing and will improve the capacity of this biomaterial to sustain the interplay between angio/neurogenesis for bone regeneration.

Acknowledgement
This project is a part of the cmRNAbone project, who had received funding from the European Union’s Horizon 2020 research and innovation program under grant agreement No 874790.

References
Effect of calcium sulphate pre-crosslinking of alginate based bio-inks on humal limbal stromal fibroblast survival in 3D bio-printed constructs.

Anastassia Kostenko, Che Connon, Stephen Swioklo

Newcastle University, Newcastle Upon Tyne, GB

Introduction
Over the last decade, progress in three dimensional (3D) bioprinting has advanced considerably. The principle of 3D bio-printing involves integration of biomaterials, live cells and controlled motor systems for creating complex biomimetic constructs. Bio-ink is one of the most important components in the process of 3D bio-printing. Hydrogels are the bio-inks of choice for extrusion-based 3D bio-printing due to their ability to absorb large amounts of water, low cytotoxicity whilst creating structures similar to the native extracellular matrix. Bio-inks need to be sufficiently viscous to be dispensed as a free-standing filament and have appropriate strength and stiffness to maintain structural integrity after printing. Alginate has been used widely for 3D bio-printing due to its biocompatibility, tuneable properties, rapid gelation, low cost, and ability to be functionalized to direct cell behaviour. By tuning the physiochemical parameters of alginate-based bio-inks, improvements in print resolution, fidelity and growth characteristics of encapsulated cells can be achieved. Thus further optimizing the extrusion based bio-printing technique for therapeutic and drug discovery applications.

In this study, the viscosity of alginate-based bio-inks was increased compared to the control by pre-crosslinking with calcium sulphate (CaSO₄) to improve their suitability for the bioprinting process. Mechanical and structural properties of alginate-based bio-inks were characterized with the aim of mitigating the resolution problems associated with existing bio-inks used in extrusion based bioprinting. Subsequently, the survival of human limbal stromal fibroblasts (hLSFs) under standard cell culture conditions was compared between pre-crosslinked and control alginate-based bio-inks.

Experimental Methods
Medium viscosity, high β-D-mannuronic acid (MVM) alginate stock in ddH₂O was made up to 2.4%. Stock solution was then mixed in a 1:1 ratio with 0.01 M CaSO₄ in ddH₂O producing the final concentration of 1.2% pre-crosslinked alginate. The alginate CaSO₄ mixtures were mixed 50 times using two syringes and left to cure at room temperature for 3 hours prior to rheological analysis and later use as cell-laden bio-inks. Bio-inks were assessed using bio-printing and wet spinning. Wet spinning allows for standard quantification of the amount of cell-laden bio-ink extruded. Fibers can be produced with both the pre-crosslinked alginate and the liquid 1.2% control alginate. Wet spinning was performed at the rate of 1mL/minute dispensing 0.25mL of bio-ink into a 0.1M calcium chloride (CaCl₂) bath. Human limbal stromal fibroblasts (hLSFs) grown in standard serum containing medium were detached, mixed with either 1.2% alginate alone or 1.2% pre-crosslinked alginate (10% cell suspension, 90% bio-ink) and bio-printed in a lattice shape through a 30G polytetrafluoroethylene (PTFE) lined needle. CaCl₂ (0.1M) was added to the bio-printed constructs to post-crosslink the lattice and prevent the bio-ink from spreading. CellTiterGlo assay was used to assess hLSF viability over time.

Results and Discussion
CaSO₄ pre-crosslinking of alginate significantly increases the viscosity of the bio-ink, thus making it suitable for 3D bio-printing compared to the 1.2% MVM alginate control (p = < 0.0001; n = 3). Viability of encapsulated bio-printed
hLSF remained above 90% over 7 days in CaSO₄ pre-crosslinked alginate-based bio-inks in standard cell culture conditions. These results are consistent with hLSF viability assessed using the wet spinning model.

Conclusion
Pre-crosslinking of alginate-based bio-inks with CaSO₄ has a significant effect on the viscosity of the biomaterial influencing the final resolution of the printed structure. Encapsulation of hLSFs in pre-crosslinked alginate-based bio-inks does not have a detrimental effect (92% of encapsulated hLSFs are viable at day 7) on hLSF viability over 7 days in standard culture conditions.
Additive manufacturing of patient-specific prosthetic medical devices

Helda Pahlavani, Mohammad J. Mirzaali, Jie Zhou, Amir A. Zadpoor

Delft University of Technology (TU Delft), Department of Biomechanical Engineering, Faculty of Mechanical, Maritime, and Materials, Delft, NL

Introduction
Limb amputation has severe physical and psychological implications for the patients. Restoring normal function, comfort, and appearance are the main characteristics that should be taken into account when developing prosthetic devices. Patient-specific (PS) design offers many advantages with regard to satisfying the design requirements of such prosthetic medical devices (e.g., transtibial sockets).

Experimental Methods
These PS designs are based on the patient's anatomy obtained from various medical imaging modalities (e.g., computed tomography (CT) or optical scanning), thus, allowing for better fixation and improving the patient's comfort while enabling successful recovery. To aid in the PS design, computational models may be used to assess the functionality of these medical devices subjected to various physiological loading scenarios. These computational models can also provide further insight into the structural optimization and efficient material distribution of PS medical devices. Such an approach will lead to an optimized design with complex internal micro-architecture. The emergence of additive manufacturing (AM) techniques makes the high-quality manufacturing of these complex PS medical devices feasible. Due to the form-freedom offered by the AM technology and the availability of a wide variety of materials, it is now possible to design and fabricate various medical devices at a fast production speed and with minimized specialized manual labour.

Results and Discussion
Here, we developed a methodology connecting different steps (i.e., geometrical design, AM fabrication, and computational models) for the design of PS transtibial sockets. Towards this aim, the accurate geometries of the residual limb and internal bones were extracted from CT images. Next, the geometry of the socket was designed based on the geometry of the residual limb through computer-aided design (CAD) and all constructed 3D parts (i.e., bone, tissue, and designed socket) were imported into a finite element (FE) software for further analysis and structural optimization.

Conclusion
We showed that high-quality PS prosthetic devices are achievable through a combination of imaging, computer-aided design, computational modelling, and rapid prototyping using AM.

Acknowledgement
This work is part of the 3DMED project that has received funding from the Interreg 2 Seas program 2014–2020, co-funded by the European Regional Development Fund under subsidy contract No. 2S04-014.
PS1-01-045

Effect of hydrophobicity on the tumorigenesis of triple-negative breast cancer

David Angelats Lobo¹,²,³, Sònia Palomeras¹, Joaquim Ciurana², Paola Ginestra³, Elisabetta Ceretti³, Teresa Puig Miquel¹

¹ University of Girona, Department of Medical Sciences / New Therapeutic Targets Laboratory (TargetsLab) Oncology Unit, Girona, ES; ² University of Girona, Department of Mechanical Engineering and Industrial Construction / Product, Process and Production Engineering Research Group (GREP), Girona, ES; ³ University of Brescia, Department of Mechanical and Industrial Engineering, Brescia, IT

Introduction
Breast cancer is the second cause of death in both sexes, being triple-negative breast cancer the most aggressive, with a tendency to relapse between 2 to 3 years after the initial treatment. Cancer stem cells, a small subpopulation of cancer cells within the tumors, have been proposed as responsible for the growth, proliferation, and spread of the tumors within the body. Recently, the combination between engineering and biology allowed the fabrication of three-dimensional platforms, known as scaffolds, that helped to mimic the natural microenvironment of those cancer stem cells. Nowadays, the main research is focused on the study of those cancer stem cells, but there is still a lack of understanding of the tumorigenesis process in vivo.

Experimental Methods
In recent times, in 2013, a group of research produced a scaffold capable of mimicking the in vivo microenvironment where the tumors are found. Briefly, a polymeric solution containing methoxy-polyethylene glycol (mPEG), polylactic acid (PLA), and poly lactic-co-glycolic (PLGA) combined with the electrospinning technique, provided an interesting platform for the study of tumorigenesis.

Results and Discussion
This protocol has been reproduced in our group, showing a relationship between hydrophobicity and attachment to the tumoral samples.

Conclusion
This model may provide an interesting platform to study the aggressiveness of triple-negative breast cancer, and possible mechanisms to interrupt it.
Enzymatic Freeform 3D Biofabrication of Cell-laden Tubular structures

Marta A. Rocha, Maria V. Monteiro, Vítor M. Gaspar, João F. Mano

Aveiro University, Department of Chemistry, CICECO – Aveiro Institute of Materials, Aveiro, PT

Introduction
Reproducing human tissues composition, structure and architecture has gained a widespread interest in the tissue bioengineering and regenerative medicine field.[1] Due to monolayers incapacity to resemble human tissue features, three-dimensional (3D) human organ/tissue models have emerged as a promising alternative to better recapitulate the complexity of human tissues at cellular and organ level. To reproduce human tissue native components cells are commonly embedded in relevant biomaterials similar to that found in human microenvironment and assembled as bulk 3D in vitro models. Although such approach enables to reproduce important in vivo aspects, they cannot emulate complex tissues architecture. Recently, 3D bioprinting technique emerged as valuable tool to produce high-content 3D in vitro models assuming complex 3D structures. In this technique, cells are commonly embedded in relevant hydrogels to form a bioink and then printed according to the desired and pre-established arrangement following a computer assisted design software. Despite 3D bioprinting offers the ability to obtain complex 3D human-sized models, the establishment of printable bioinks that maintain the structure fidelity and resolution after the printing process remains a challenge. In order to improve the printability of hydrogel-based bioinks, researchers commonly modulate their rheological properties and crosslinking densities aiming to obtain more robust inks, which could affect cell viability, proliferation and differentiation. To face this issue, an emerging strategy is the use of supporting baths to provide a physical support during the 3D printing process, ensuring the shape fidelity maintenance of the printed structures with the desired spatial control. Such freeform bioprinting approach offers the possibility to print highly complex architectures such as hollow tubular constructs with high resolution without the demand of find or optimize fully printable hydrogels.[2] Commonly used supporting materials reside on microparticle systems, however such strategy is time-consuming, labor-intensive and influence cell maintenance and structure recovery. Alternatively polymeric-based baths are recently emerging as a more efficient approach to circumvent the drawbacks offered by traditional techniques ensuring the easily fabrication of high-resolution constructs.[2] Particularly, Xanthan gum-based baths already demonstrated be efficient in tubular constructs bioprinting with potential application for the assembly vascularized and/or ductal tissue/organ models.[2]

In this work, we exploit freeform bioprinting techniques to obtain highly complex and cell laden hollow tubular constructs crosslinked via enzymatic approaches. Complex freeform tubular models were obtained by direct 3D bioprinting of an ECM-mimetic ink comprising hydroxyphenyl propionic acid-modified gelatin (Gelatin-HPA) at different concentrations, in a xanthan gum supporting bath containing the crosslinking mushroom tyrosinase enzyme (MST).

Experimental Methods
Gelatin-HPA synthesis followed a carbodiimide/active ester-mediated coupling reaction. The chemical modification was performed overnight with HPA, DMF, NHS and EDC.HCl. Then, the obtained solution was dialyzed and freeze-dried. Gelatin-HPA conjugates crosslinking occurred by an enzymatic oxidative reaction mediated by MST, a naturally derived copper-containing enzyme [3,4]. The design of a representative 3D model of hollow-vessel like tubes was created in a CAD software. The 3D bioprinted constructs were manufactured by extrusion 3D bioprinting using a Cellink INKREDIBLE + Bioprinter (Cellink, Germany).

Results and Discussion
Prior to the 3D bioprinting process, the bioink concentration and 3D bioprinting conditions (i.e., temperature, pressure). In the case of gelatin-HPA bioink, which is a thermosensitive biomaterial, the highest resolution was obtained at $T = 30^\circ$C and a pressure between 30-40 kPa. A supporting bath comprising xanthan gum, cell culture medium and MST was used not only to achieve maximum printing fidelity, but also for gravitational support of the tubular structure during enzyme-mediated crosslinking (Figure 1, A). The structure was then easily retrieved and set to culture (Figure 1, B). Gelatin-HPA based-bioink has shown to be an appropriate formulation for freeform 3D bioprinting of hollow-vessel like tubes. Also, MST was revealed to be a successful crosslinking approach for obtaining 3D scaffolds with high resolution and structural stability.

**Conclusion**

Overall, the use of the support bath provided the ideal conditions for printing and crosslinking, while enabling constructs retrieval in a non-destructive manner. The bioactivity and processability of the enzymatic crosslinked bioink combined with the spatial manufacturing freedom and ease of scalability obtained from polymer supporting baths render this a promising approach for fabricating anatomic size living constructs for a myriad of biomedical applications.

**Acknowledgement**

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. This work was also supported by the Programa Operacional Competitividade e Internacionalização (POCI), in the component FEDER, and by national funds (OE) through FCT/MCTES, in the scope of project PANGEIA (PTDC/BTM-SAL/30503/2017). Vítor Gaspar acknowledges funding in the form of a Junior Researcher Contract under the scope of the project PANGEIA. Maria Monteiro acknowledges an individual PhD fellowship from the FCT (2020.07692.BD).

**References**

3D printed scaffold containing drug-loaded PLGA microspheres for bone tissue engineering
Habib Belaid¹², Catherine Teyssier², David Cornu¹, Vincent Cavailles², Mikhael Bechelany¹

Introduction
Breast cancer is the most common invasive cancer in women, and the second main cause of cancer death in women. Metastatic breast cancer, most frequently localized in bone, is causing considerable pain and high patient morbidity. The treatment of bone is challenging due to bone repair, and patients are often treated by implanting a passive artificial junction in addition to a systemic chemotherapy treatment.

3D printing is a powerful tool providing the ability to print bone substitute materials or “scaffolds” designed to mimic the extracellular matrix. The scaffolds need to be biocompatible and biodegradable with a highly porous and interconnected pore network. The mechanical properties have to match those of the tissues at the site of implantation. For patients with bone metastasis, the scaffold may also allow the controlled and local release of anticancer drugs.

One of the strategies to deliver these drugs by the scaffolds is to encapsulate them in microspheres such as Poly(lactic-co-glycolide) or PLGA.

The aim of this work is the development and characterization of novel biomimetic biodegradable 3D printing scaffolds allowing both bone regeneration and inhibition of breast cancer cell proliferation. To this aim, PLGA microspheres have been loaded with Raloxifene Hydrochloride (RH) and Alendronate (AL). These microspheres have been incorporated into a 3D scaffold fabricated using a Stereolithography 3D printer system with a Poly(propylene fumarate) (PPF) photopolymer.

Experimental Methods
To prepare drugs-loaded PLGA-microspheres of around 1 μm in diameter, a single or double-emulsion-solvent-evaporation method was used. PPF/DEF scaffold was fabricated using a Formlabs Form 2 printer equipped with a Class 1, 405 nm violet laser with a power of 250 mW and laser spot size of 140 μm. Axis resolution (i.e., layer thickness) used for printing was 50 μm.

Results and Discussion
The physicochemical properties of the nanocomposites scaffold have been fully characterized. Biological testing has been carried out in order to confirm the effects of encapsulated drugs on MCF7 cells proliferation. A cell viability test using MG63 cells has been conducted to determine the scaffold biocompatibility and its effects on cell proliferation. The successful cell adhesion was revealed by fluorescent microscopy, and MG63 bone differentiation was monitored by Alizarin red staining and semi-quantitative RT-PCR.

Conclusion
Altogether, our data showed that PLGA microspheres incorporated in PPF scaffolds could be suitable for the treatment of bone metastasis in breast cancer.
Graphical abstract. Fabrication of PPF/PLGA scaffold.
3D PRINTED HYDROXYAPATITE – PROPERTIES AND APPLICATIONS
AS SCAFFOLD MATERIAL FOR BONE REGENERATION

Skirmantas Norkus¹³, Osvaldas Grubys²³, Simona Raugalaite²³, Brigita Abakeviciene², Ramūnas Valiokas¹

¹ Center for Physical Sciences and Technology, Department of Nanoengineering, Vilnius, LT; ² Kaunas University of Technology, Department of Physics, Kaunas, LT; ³ Ortho Baltic, Kaunas, LT

Introduction
Recently bone grafts have been increasingly used around the world. Bone grafting frequency is indeed the second most frequent tissue transplantation worldwide, coming right after blood transfusion [1]. Over two million surgical surgeries are performed per year to treat bone tissue defects [1-2]. Bone tissue defects larger than 4-6 cm are critical and should be treated with bone grafts [2]. Critical-sized defects can be congenital or formed due to trauma, tumours, infections, and diseases such as osteoporosis, necrosis, or bone atrophy [1]. Four main types of bone grafting are used to treat bone tissue defects: autologous, allogeneic, xenogeneic, alloplastic. Autologous bone is currently considered the “gold” standard for treating bone tissue defects [1,3]. However, more and more attention is paid to alloplastic bone graft materials, as the use of these bone grafts could simplify operations and avoid the most common complications [1,3]. The major advantages of alloplastic materials include their high abundance relative to natural materials, no risk of disease transmission and the very low antigenicity. They can be made available in both resorbable and non-resorbable forms, and can be customized with varying levels of porosity and pore sizes [4].

Experimental Methods
The main objective of this study was to print hydroxyapatite (HA) scaffolds of complex geometry that could potentially be used to fill bone defects. One of the world’s latest 3D ceramic printing technologies LMC (Lithography-based ceramic manufacture) was used for printing hydroxyapatite scaffold. Using this technology, the production of printed hydroxyapatite scaffold essentially consists of two main parts: 3D printing and heat treatment. The geometry of the printed HA experimental specimens (see Fig. 1), scaffold porosity, scaffold pore size, and material density fluctuations were analysed using industrial computed tomography (µCT). In order to analyse the structural properties of HA samples, studies were performed using X-ray diffraction (XRD) analysis and Fourier transform infrared spectroscopy (FT-IR) analysis. The surface topography and roughness of HA samples were analysed using optical profilometry and scanning electron microscopy (SEM). The mechanical properties of HA scaffolds were evaluated by mechanical compression test.

Results and Discussion
The scaffold was designed using special software. The geometry of the scaffold used in the study was 10 x 10 x 10 mm with diamond-shaped lattice (see figure 1 a). The porosity of the designed scaffold was 89.05 ± 0.07%, the scaffolding thickness was 152.20 ± 0.40 µm, and the pore size – 784.80 ± 2.79 µm. Final part can be seen in figure 1 b. Investigations using industrial computed tomography (µCT) were performed to evaluate printing accuracy and other scaffold parameters. Comparing the 3D model with the manufactured scaffold, it was found that the deviations of the geometry of the manufactured scaffold from the 3D model range from -12 µm to 136 µm. These deviations can be explained by the fact that during the heat treatment, the material did not shrink as much as planned. Scaffold analysis showed that the porosity was 84.5 ± 0.42 %, the scaffolding thickness –175.45 ± 4.54 µm, and the pore size – 715.89 ± 7.56 µm. Volumetric analysis of the scaffold revealed that the scaffold did not exhibit any volume fluctuations.

In order to identify the crystalline phases of hydroxyapatite, XRD studies were performed on the printing material
(suspension), 3D printed object (green body) and after heat treatment (final product). XRD results of the performed studies are presented in Figure 2. According to JCPDS no. 00-009-0432 card, all peaks correspond to pure hydroxyapatite. The most intense reflection peaks of all the samples are at 31.8°, 34.1°, 32.2°, 25.9° and 49.5° 2θ angles. There are no significant differences between the suspension, printed and sintered samples. Calculated mean crystallite sizes of the suspension, printed, and sintered samples are 27.5 nm, 28.1 nm, and 30.6 nm respectively. In this work, the analyzes and results of FT-IR, SEM and optical profilometer will be further examined in more detail.

**Conclusion**

The study found that LCM ceramic printing technology is suitable for production of complex geometry scaffolds with high geometric accuracy and repeatability. In order to obtain even better results, more attention should be paid to the thermal treatment of hydroxyapatite. After XRD analysis, it was found that the hydroxyapatite scaffold formed by the LCM method has analogous crystalline phase properties as the hydroxyapatite objects produced by conventional methods. Using LMC technology, it is planned to produce more structures of different geometry, lattice and to study them in pre-clinical studies.

---

**Fig. 1. Scaffold**

Fig. 1. Scaffold: a) STL 3D model; b) Scaffolds after 3D printing and heat treatment; c) Microcomputed tomography (μCT) measurements. The color map represents deviation of scaffold after 3D printing and heat treatment in comparison with STL 3D model.
Fig. 2. XRD radiograms of hydroxyapatite suspension, green body and final product

References
Time-Temperature Superposition in Highly Oriented Electrospun Conductive Nanofibers of Biodegradable Polymers

Muhammad A. Munawar\textsuperscript{1,2}, Dirk W. Schubert\textsuperscript{1,2}

\textsuperscript{1} Friedrich-Alexander-University Erlangen-Nuremberg, Department of Materials Science, Institute of Polymer Materials, Erlangen, DE; \textsuperscript{2} Bavarian Polymer Institute, KeyLab Advanced Fiber Technology, Fürth, DE

Introduction
An electrospinning process with a special rotating collector electrode is used to generate highly oriented electrospun conductive fiber composites (ECFCs) of biodegradable polymers. Polylactic acid (PLA) are blended with intrinsically conductive polymers (ICPs)-filler of polyaniline (PANi). As the proportion of filler increases, the number of “conductive pathways” grows, and consequently the conductivity of the ECFCs also increases. A procedure is developed for calculating the volume fraction ($\phi$) of filler, density and conductivity of electrospun nanofibers. The conductivity as a function of filler fraction is shown and described by using a McLachlan equation for the first time to reveal the electrical percolation thresholds ($\phi_c$) of nanofibers. During the annealing process, a build up of additional conductive channels within ECFCs is responsible for increasing inter and intra-chain conductivities among fillers and matrices. Therefore, annealed ECFCs transport charge more efficiently due to an increase in continuous pathways, which lead to superior electrical conductivity and lowering the percolation threshold. Moreover, a time-temperature effect on percolation thresholds of ECFCs is evident.

Experimental Methods
The highly oriented electrospun conductive nanofibers of biodegradable polymers were fabricated successfully using a special collector electrode as depicted in Figure 1. The electrical conductivity $\sigma$ of ECFCs can be calculated by using the value of resistance $R$, which was measured using a two-point probe method.

Results and Discussion
We mainly studied the calculation of percolation threshold using the McLachlan equation on experimental conductivities of ECFCs with different annealing temperatures and annealing times. The percolation threshold decreased either with increasing annealing temperature or time. Finally, the contour plot (as shown in Figure 2) shows time-temperature effect on percolation threshold of electrospun nanofibers.

Conclusion
The annealing process in electrospun fibers enhances the buildup of additional conductive channels, which lead to superior electrical performance and lowering the percolation threshold $\phi_c$ and time-temperature superposition exists in electrospun nanofibers.

Acknowledgement
The authors thank the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)-Project number 326998133-TRR 225 (subproject A07) for funding this work.
Schematic of electrospinning set-up, used in this study and rotating wheel collector covered with electrospun fibers aligned to machine direction (MD) and SEM image showing high orientation of electrospun fibers.

Contour plot representation
The contour plot between time (t) in x-axis, temperature (T) in y-axis, and electrical percolation thresholds ($\phi_c$) in z-axis to show time-temperature superposition in electrospun nanofibers.
Bioprinting of microfluidic generated microgels based on a degradable and bioactivated POx/GelPA hybrid system

Ilona Paulus1, Benjamin Reineke2, Junwen Shan1, Stephan Hauschild2, Jörg Teßmar1, Stephan Förster2, Jürgen Groll1

1 University Würzburg, Department for Functional Materials in Medicine and Dentistry, Würzburg, DE; 2 Forschungszentrum Jülich, Jülich Centre for Neutron Science, Jülich, DE

Introduction
Biofabrication of living cells with a reproducible high cell survival and high special resolution is still a challenge in the biofabrication field. Substantial shear forces during common high-resolution printing processes can negatively impact cell viability, therefore the protection of printed cells against the shear forces during printing is necessary.[1] One conceivable way to enhance cell survival, cell viability and expand the biofabrication window is the encapsulation of living cells in microgels that act as a protective coating. Furthermore, with the application of these encapsulated cells as ink additive, it is possible to engineer multifunctional scaffolds with different cell types and uncouple cell micro- and macrome surrounded.[2] The fabrication of uniform and size controllable microgels can be achieved via microfluidics of in situ cross-linking polymers, which form mechanically stable hydrogels.[3] In this study, we prepared POx/GelPA-based microgels of controllable size using a custom-built microfluidic setup and crosslinked them via a visible light mediated thiol-ene reaction. The microgels were used and printed as additive in a GelPA/sPEG-based ink.

Experimental Methods
To synthesize the POx-based hydrogel precursor, 2-ethyl-2-oxazoline (EtOx) was randomly copolymerized with 2-(3-butenyl)oxazoline (ButEnOx) to yield polymers with a double bond to allow a subsequent functionalization. Afterwards the polymer was modified at the double bonds with free thiol groups for hydrogel formation according to an already published procedure.[4] For the bioactive component GelPA, the free amines of a gelatin with a low molecular weight were functionalized with free vinyl groups via NHS chemistry. The modified gelatin was further dialyzed against ultrapure water and freeze dried. The dissolved polymers were subsequently combined in a custom-designed microfluidic chip, which allowed the production of droplets of varying sizes with high uniformity. To crosslink the hydrogel precursor solution via thiol-ene reaction, a low concentration (0.5 wt%) of a UV/visible light sensitive photo initiator (LAP) was added. The droplets were stable crosslinked directly after their formation in the microfluidic chip. NIH/3T3 cells were used to demonstrate the successful encapsulation of cells in the aforementioned microgels.

Results and Discussion
For this application Poly(2-oxazoline)s are the ideal synthetic polymers, due to their excellent biocompatibility and synthetic availability. Moreover, the polymers provide an easy possibility for a side chain functionalization to control cross-linking degree of the obtained microgels.[4,5] Modified gelatin with its bioactivity and degradability can act here as cell-friendly component for a better viability of encapsulated cells. Both polymers were mixed and droplets were produced via microfluidics. It was possible to crosslink the hydrogel precursor solution droplets via visible light directly after the production to avoid coalescence of the droplets. The 3D-printing of the cell free microgels, after washing and purification, as additive in an ink could be shown in this study. Furthermore, the encapsulation of cells was already possible using this microfluidic approach. NIH/3T3 cells were mixed into the polymer precursor solution and cells were encapsulated during the microfluidic process.

Conclusion
We could successfully generate stable crosslinked POx/GelPA-based microgels via microfluidics and encapsulate living NIH/3T3 cells in these microgels. Additionally, it could be shown, that the printing of the cell free microgels as particulate additive in bioinks was still possible. In future studies the POx/GelPA-based microgels will be a used as protective coating of encapsulated cells during 3D printing process and subsequently provide a cell friendly microenvironment for the encapsulated cells. The ultimate applications for the bioactive microgel droplets are particulate bioinks for extrusion-based 3D-printing or as protective additive in conventional bioinks to ensure sufficient cellular protection and eventually the coextrusion of two or more different cell types.

**Acknowledgement**

This research has received funding by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Projectnumber 326998133 – TRR 225 (subprojects A06, A02)

![POx/GelPA-based microgels](image)

**Figure 1:** POx/GelPA-based microgels in A: Undecanol directly after production, B: in PBS-buffer after washing and C: used and printed as additive in a GelPA/sPEG-based ink

**References**

Development of a 3D in vitro hierarchized vascular model under active perfusion: a new model for the study of prostate cancer intravasation.

Lise Chagot, Chantal Medina, Leo Comperat, Nathalie Dusserre, Marie-Laure Stachowicz, Jean-Christophe Fricain, Hugo Oliveira

INSERM, U1026 ART BioPrint, Bordeaux Cedex, FR

Introduction
Prostate cancer is the third most frequent cancer diagnosed in men, with high incidence rates in Europe and North America [1]. Metastasis is the leading cause of prostate cancer-associated deaths and bone metastases can be found in most patients with castration-resistant prostate cancer [2]. The metastasis process of solid tumors starts with the migration of invasive cancer cells into the local microenvironment. Then, cancer cells intravasate into the vasculature or lymphatic system, disseminate through the circulatory system and extravasate into a secondary tissue and colonize it. The precise driving mechanisms responsible for each metastasis step remains relatively unclear [3]. Biofabrication, based on the precise deposition and assembly of materials and cells, can enable to mimic the structure and function of the desired tissue [4]. These complex 3D in vitro cancer models can provide new research tools to further decrypt complex mechanisms and open new therapeutic approaches. Here we have developed a new model to study prostate cancer intravasation, in flow conditions, through a hierarchized vascular model.

Experimental Methods

Cells models
Human umbilical vein endothelial cells (HUVECs) were isolated from fresh human umbilical cords and human skin fibroblasts (HSF) from human skin explants, using established protocols. Both were cultured under standard conditions, until passage 10.

PC3 cells, a human prostate cancer cell line, were obtained from ATCC and cultured under standard conditions, between passages 35 – 40. HUVECs and PC3 were transduced with lentiviral vectors coding for red and green fluorescent proteins, RFP or GFP, respectively. After printing, all cells were cultured in complete EGM2 MV medium.

Bio-inks

Pluronic was prepared from Pluronic F127 powder dissolved in milliQ water at a final concentration of 37%. Methacrylated gelatin (GelMA) was prepared from gelatin type A, obtained from porcine skin, and anhydride methacrylate at 140 μL/g gelatin was added for a methacrylation degree of 61.8 % (Average ±SD, n=9). GelMA was then used at a final concentration of 120 mg/mL in DMEM HG.

Methacrylated collagen (CollMA) was prepared from type 1 bovine collagen and anhydride methacrylate at 95 μL/g collagen. CollMA showed a methacrylation degree of 57.15 % (Average ±SD, n=3). The CollMA bio-ink was formulated at 4 mg/mL, by adding 10X DPBS, 1M NaOH, DMEM HG and LAP (0.1% w/v final) as a photoinitiator.

Bioprinting platforms

A multimodal commercial printer (Discovery 3D, RegenHU), equipped with two microvalve printerheads, two pneumatic microextrusion printheads, and a 365 nm UV curing source, was used.

Perfusion chambers

A personalized PDMS chamber was printed by microextrusion (Fig.1 A). This chamber was then sealed between two layers of Plexiglass and mechanically enclosed in a PETG 3D-printed system with screws. Culture medium active perfusion was ensured by a peristaltic pump (Ismatec) with a flow rate from 1 to 20 μL/min (Fig 1.C).

Bioprinting process

Page 1048 of 2028
The computer assisted design was created using the BioCAD software (RegenHU) (Fig1.B). Then, HUVEC and HSF (in coculture) and PC3 cells were bioprinted using two independent microvalve printheads. Following UV curing, Pluronic was deposited by microextrusion, as a negative mold for a perfusable tube. A second layer of GelMA was poured to cover all the printing and a final UV curing was performed to polymerize the whole structure. The sacrificial bioink, Pluronic, was removed by reducing the temperature and perfusing with cold DPBS. After poly-D-lysine coating, a suspension of HUVECs was added and let to adhere and to endothelialize the hollow tube. The system was then placed under active perfusion in standard culture conditions.

**Results and Discussion**

Gel rheology, bioprinting parameters and cell survival were optimized and evaluated. We could show that in optimal conditions a cell survival of 99.02% (Average ±SD, n=3) was obtained for HUVECs. HUVEC+ HSF bioprinting, tested at 10+10, 20+20, or 30+30 million/mL, showed optimal endothelial network maturation for 30+30 mio/mL, following 14 days of maturation.

Efficient macro to microvasculature connection was asserted by perfusing with Dextran-FITC 500kDa. PC3 cell association is ongoing, and the design of PC3 inkjet spots alongside HUVEC + HSF spots is being pursued. Maturation of the complex system is currently being evaluated using time lapse and confocal microscopy. Future studies will consider the validation of this biofabricated model in response to anticancer and anti-angiogenic drugs (e.g. Sunitinib treatment).

**Conclusion**

Here, we show the application of this modular hierarchized vascular system to model intravasation of prostate metastasis. Nonetheless, due to its inherent modularity it can easily be applied to other cancer models or to find use for the study of circulatory cancer cell extravasation. Also, active flow conditions allow to attain further physiological relevance, particularly important when modeling the metastasis mechanism.

---

**Fig. 1 : 3D in vitro perfusable model**

A. PDMS chamber. B. CAD printing design, Pluronic in blue, HUVEC in red, PC3 in green. C. Perfusion system with peristaltic pump, medium and PETG 3D-printed enclosing plates.

---

**References**


2:30 p.m. – 4:00 p.m.

Poster floor

PS1-02 | Biomaterials Characterization
Viscoelastic properties of organ-derived extracellular matrix hydrogels

Francisco D. Martinez Garcia¹,², Roderick H.J. de Hilster¹,³, Prashant K. Sharma⁴,², Machteld N. Hylkema¹,³, Janette K. Burgess¹,³, Martin C. Harmsen¹,²

¹ University of Groningen, University Medical Center Groningen, Department of Pathology and Medical Biology, Groningen, NL; ² University of Groningen, University Medical Center Groningen, W.J. Kolff Research Institute, Groningen, NL; ³ University of Groningen, University Medical Center Groningen, Groningen Research Institute for Asthma and COPD, Groningen, NL; ⁴ University of Groningen, University Medical Center Groningen, Department of Biomedical Engineering, Groningen, NL

Introduction
The extracellular matrix (ECM) is a non-cellular, heterogeneous mixture of macromolecules that provides cells with structural support. The ECM mechanics are commonly modelled using hydrophilic polymeric networks (i.e. hydrogels); with most studies focused solely on its elastic properties. Now it is recognised, that ECM mechanics are driven by a combination of elastic and viscous response, rendering the ECM viscoelastic. In this work, we compared the viscoelastic properties of organ-derived ECM hydrogels and mathematically modelled the data with a generalised Maxwell model of viscoelasticity

Experimental Methods
Adipose, cardiac left ventricle (LV) and lung tissue were decellularised and lyophilised. The resulting ECM was ground to a fine powder and 2% w/v hydrogels were produced by proteolysis with 10 % w/v porcine pepsin 0.01M HCL for 6 h (adipose), 8 h (LV) and 48 h (lung) [1 - 3]. The digested ECM was brought to 1x PBS, pH neutralized to 7.4 and gelated at 37°C. After 24 h of swelling at 37°C, ECM hydrogels underwent low-load compression testing (LLCT) at a 20%/s strain rate. The elastic modulus was determined during the first second of compression on the LLCT, while the viscoelastic properties were derived from the 100 s of stress relaxation at a constant strain of 20% [4].

Results and Discussion
The elastic modulus of Adipose-ECM (1.7 ± 0.6 kPa) differed from both Lung-ECM (4.98 ± 1.8 kPa) and LV-ECM (4.38 ± 1.73 kPa) (Fig. 1a). The time to reach 50% stress relaxation was fastest in Adipose-ECM (13.2 ± 15.68 s), followed by Lung-ECM (46.1 ± 10.37 s) and lastly LV-ECM (53.14 ± 8.23 s) (Fig 1b, 1c). Maxwell analysis showed that Adipose-ECM had two Maxwell elements, while Lung-ECM and LV-ECM had three, with differences in their relaxation time constants and their relative importance (Fig. 1d).

Conclusion
These data illustrate that irrespective of gel percentage, not only the stiffness of the ECM hydrogels was organ-dependent but also their viscoelasticity. Comparison of the molecular composition, such as polysaccharide and protein content, of the three ECM hydrogels may explain their respective physical properties.

Acknowledgement
The authors would like to thank Vasilena Getova, MD and Joris van Dongen, MD for their contributions in sample preparation and preliminary data acquisition.
Viscoelastic properties of adipose, cardiac left ventricle (LV) and lung ECM hydrogels LCT determined a) elastic modulus and b) time to reach 50% stress relaxation (SR). Fitting the data into a generalised Maxwell model showed the c) average model E(t)% in 50 s and d) the number of Maxwell elements and their relative importance. Data are presented as mean with standard deviation. P values are indicated *p <0.05 and ****p <0.0001 according to Brown-Forsythe and Welch’s ANOVA test followed by Dunnett’s T3.

References
Loco-regional skin toxicity of *in situ* forming depot administered subcutaneously at different flow rates in minipig

**Charlotte Peloso, Adolfo Lopez-Noriega**

*MedinCell, Research Applied to Development Unit, Jacou, FR*

**Introduction**

Despite the increasing interest of the scientific community for *in situ* forming depots (ISFD), little has been published regarding their loco-regional toxicity [1], which is one of the key parameters when developing an injectable drug product. MedinCell’s proprietary ISFD technology, trademarked as BEPO®, is based on bioresorbable PEG-PLA copolymers, mixed with an API into a biocompatible solvent [2]. Upon administration, the solvent will diffuse from the system and the copolymers will precipitate, forming a solid depot that will physically entrap the drug to be progressively delivered. To secure its proper administration and future compliance in clinic, local tolerability of BEPO® technology was investigated on top of the biocompatibility of the components themselves. In particular, injection flow rate was evaluated for both depot formation concerns and tissue tolerability. The aim of the present study was to assess the loco-regional skin tolerability of a model BEPO® vehicle (copolymers blend dissolved into solvent, without drug) when subcutaneously (SC) administered at different flow rates and volumes into Göttingen minipigs.

**Experimental Methods**

Two groups of four female Göttingen minipigs were injected either with 0.2 mL or 1 mL BEPO® vehicle. The tested vehicle had a high viscosity (> 600 mPa.s) and a particular polymeric composition with a slow degradation kinetics. Each animal received two manual injections of test item in SC in the *plica inguinalis*: one targeted at 1 mL/min and one targeted at 10 mL/min. Injection sites were systematically observed for macroscopic loco-regional skin reactions as well as ultrasound scanning, enabling longitudinal *in vivo* imaging of the depot. Observations were complemented by histopathological examinations (haematoxylin and eosin stain) at 72 h and 240 h post-injection.

**Results and Discussion**

On a macroscopic level, no skin local intolerance was observed at both injection flow rates and volumes. However, an induration was recorded over the 10 days. Because of the very thin skin and hypodermis present at the *plica inguinalis*, these inductions were visually evident, allowing their measurement with a caliper. At palpation, a firm mass was clearly identified and suggested as the implant itself. To confirm that this induration was mainly due to a mechanical swelling from the depot and not a loco-regional skin reaction, ultrasound imaging was performed. Implants were clearly detected and measured along the study. These measurements were comparable to macroscopic induration measurements, confirming it was mainly due to the mechanics of the implant and not a local reaction.

At the microscopic level, histopathological examinations were performed at both 72 h and 240 h post-injection. From 72 h, a pseudocyst with a well circumscribed empty space and thin fibrous capsule was highlighted. Over time, the pseudocyst developed into a well-defined encapsulated nodule, often multilocular. At 240 h, the observed nodule was composed of empty spaces limited by fibrous cords combined with mixed inflammatory cells, among which numerous multinucleated giant macrophages were noted. At both time points, the cavities most probably represent the location of the polymeric depots which dissolved during tissue processing, hence their empty appearance. Such reaction from pseudocysts to nodules represents a continuum characteristic of a Foreign Body Reaction (FBR). This innate biological response was expected, especially for this particular polymeric composition of the vehicle, as it was...
designed to degrade slowly (prolonged FBR). Again, the volume and/or rate of administration had no influence on the nature and severity of the induced local changes.

**Conclusion**

Local tolerability of the depot was highlighted by macroscopic observations and histopathological examinations of the injection sites. Ultrasound imaging helped to complete the observation, allowing to follow the morphological evolution of the depots during the course of the study. Taken together, these experiments showed an acceptable loco-regional response to the injected polymeric test item, designed to be an extreme case study (*i.e.* a vehicle of high viscosity and slow degradation kinetics), following low as well as high injection flow rates. The formation of a well-circumscribed subcutaneous nodule was observed along with a typical foreign body reaction (FBR) for all injections. The scoring of the injection site reactions was volume dependent, and no cases of treatment-emergent adverse events were reported, ruling out any matter of concern from a histopathological perspective. It can thus be considered that the ISFD PEG-PLA based-product is well tolerated locally, when injected subcutaneously at volumes ranging from 0.2 mL to 1 mL, and high injection flow rate.

Based on the loco-regional tolerance data noted in the Göttingen minipig, a human-relevant animal model for subcutaneous administration, we consider the ISFD BEPO® technology likely to be safe in human patients.

**Acknowledgement**

We thank Dr. Ulrik Westrup (Westrup Vet. Consulting, Lygby, Denmark) for ultrasound data acquisition and analysis as well as Dr. Loïc Longeart (Loïc Longeart Toxicologic Pathology, Vouvray, France), Pr. Marc Janier (Service of Nuclear Medicine of Hospices Civils de Lyon, Lyon, France) and Dr. Patrice Ravel (Cancer Research Institute of Montpellier, Montpellier, France) for their respective expertise in histopathology, imaging and statistics. The authors would also like to thank the Research Applied to Development Unit from MedinCell S.A., as well as Sophie Lelamer and Thibaut Deramoudt from the Preclinical Department.

References

PS1-02-067

2D/3D imaging of osteogenesis and macrophage polarization in magnesium-based alloys

Maryam Rahmati1, Sabine Stötzel2, Thaqif El Khassawna2,3, Kamila Iskhahova4, Florian Wieland4, Berit Z. Plumhoff4, Håvard J. Haugen1

1 University of Oslo, Clinical Dental Faculty, Biomaterials department, OSLO, NO; 2 Justus-Liebig University Giessen, Experimental Trauma Surgery, Giessen, DE; 3 University of Applied Sciences, Faculty of Health Sciences, Giessen, DE; 4 Helmholtz Zentrum Geesthacht, Institute for Materials Research, Division of Metallic Biomaterials, Geesthacht, DE

Introduction

Immune system responses are vital for tissue healing after injuries (1). The ability of a biomaterial to induce angiogenesis and macrophage polarization is a key element in defining its success (2). Magnesium (Mg)-based degradable alloys have attracted significant attention for orthopedic bone regeneration due to their biodegradability and potential for avoiding secondary removal surgeries (3). However, information regarding early inflammatory responses and osteogenesis to these alloys in vivo.

Experimental Methods

In this study, we investigated the early body responses to Mg 0.45 wt%Zn-0.45 wt%Ca pin-shaped alloy (known as ZX00 alloy) in juvenile rat femora 2, 5 and 10 days after implantation. We used 3D µCT and histological analyses to study new bone formation and early inflammatory responses at the implant-bone interface.

Results and Discussion

Our results suggested the potential of ZX00 alloy to stimulate the polarization of type 2 macrophages, 5 and 10 days after implantation, compared to the sham group. In addition, we observed an increased newly bone matrix formation at day 10, compared to day 5 and sham group in all time points, suggesting better bone formation in the ZX00 alloy group.

Conclusion

To sum up, our results supported the early osteoimmunomodulatory potential of the ZX00 alloy toward inducing the expression of anti-inflammatory type 2 macrophages and osteogenesis.

Acknowledgement

This work was supported by a project “Promoting patient safety by a novel combination of imaging technologies for biodegradable magnesium implants, MgSafe” funded by European Training Network within the framework of Horizon 2020 Marie Skłodowska-Curie Action (MSCA) grant number No 811226 (www.mgsafe.eu).

References


Comparative study between the effect of AgI and Ag_{2}O on in vitro bioactivity and antibacterial activity

Alexandra Feraru\textsuperscript{1,3}, Zsejke R. Tóth\textsuperscript{1,2}, Milica Todea\textsuperscript{1,4}, Dan C. Vodnar\textsuperscript{5}, Klára Magyari\textsuperscript{1}, Lucian Baia\textsuperscript{1,3}

\textsuperscript{1} Babeș-Bolyai University, Interdisciplinary Research Institute on Bio-Nano-Sciences, Cluj-Napoca, RO; \textsuperscript{2} University of Szeged, Department of Applied and Environmental Chemistry, Szeged, HU; \textsuperscript{3} Babeș-Bolyai University, Faculty of Physics, Cluj-Napoca, RO; \textsuperscript{4} Iuliu Hațeganu University of Medicine and Pharmacy, Faculty of Medicine, Cluj-Napoca, RO; \textsuperscript{5} University of Agricultural Science and Veterinary Medicine, Faculty of Food Science and Technology, Cluj-Napoca, RO

Introduction

From ancient times it is known that silver is effective against a wide range of microorganisms. Today, silver ions are used to prevent bacterial growth (sometimes controlled) in a variety of medical applications, including dental work, catheters, and wound healing. It is clear that we are exposed to a wide range of largely unknown uses of silver-containing products that are intended to function as antimicrobial biocides. Therefore, it is necessary to elucidate the antimicrobial activity of silver ions, which are widely used in these products [1]. Despite the prominent benefits of using silver in such applications, the possible effects on health as well as on the environment have raised concerns about their use and production, without a clear understanding of their behavior in biological systems. The release of silver into an environment, such as air or water, in experimental environments or in biological fluids, can produce transformations that alter its properties, influencing transport, bioactivity, and possible toxicity [2, 3]. A challenge in the field of bioactive glass with Ag content is the elimination of AgCl formation after immersion in simulated biological fluid (SBF). It is known that in aqueous media, the release of Ag\textsuperscript{+} ions takes place in the glass matrix, which interacts with chlorides, sulfides, or thiol groups and leads to the formation of AgCl. The literature does not specify a maximum permissible limit after which the composite is toxic, but certainly, large amounts of AgCl can have a negative influence on bioactivity, especially due to the fact that the release of this amount in the body cannot be controlled.

The purpose of this study is to identify the chemical transformations of silver, which was used to the obtaining of bioactive samples in two forms, as silver iodide and silver oxide, and their effect on bioactivity and antibacterial activity.

Experimental Methods

The bioactive glasses (60SiO\textsubscript{2}·32CaO·8P\textsubscript{2}O\textsubscript{5}) with AgI and Ag\textsubscript{2}O content were obtained by sol-gel process. AgI microcrystals were prepared by the addition of silver nitrate, sodium iodide, and PVP by the solvothermal method. Silver nitrate was used as silver oxide precursor.

Structural, morphological and optical investigations were performed using a Shimadzu XRD 6000 diffractometer, JASCO-V650 spectrophotometer with an integration sphere (ILV-724), Hitachi S-4700 Type II cold field emission scanning electron microscope, and X-ray photoelectron spectroscopy, SPEC PHOIBOS 150 MCD system. \textit{In vitro} bioactivity was evaluated seven days after the introduction of the samples into the SBF. The Gram-negative strain of \textit{Pseudomonas aeruginosa} was used to test the antibacterial activity of Ag-based composites.

Results and Discussion

After immersion in SBF the X-ray diffraction patterns indicated the existence of a self-assembled apatite layer in both systems, the difference being noticed by the presence of a signal associated with silver chloride for the Ag\textsubscript{2}O addition system. To provide information about these transformations, UV-Vis and XPS spectra were recorded. Both UV-Vis spectra show a maximum at about 370 and 420 nm, respectively, that can be attributed to the presence of silver nanoparticles with different size [4]. In the case of bioactive glasses with Ag\textsubscript{2}O appear a maximum at about 256 nm
that indicates the presence of AgCl. By adding the AgI microcrystals in the glass system can be eliminated the formation of AgCl, but the antibacterial effect remains moderate. In contrast, for the bioactive glasses with Ag₂O it was obtained good antibacterial activity against *Pseudomonas aeruginosa*, but in all samples the AgCl was present after immersion in SBF.

**Conclusion**

The sol-gel method has been successfully used to obtain bioactive glass composites with silver content using different silver sources. The result shows that the silver sources affect the samples’ in vitro bioactivity and bactericide effect.

**Acknowledgement**

This work was supported by a grant of the Ministry of Research and Innovation, CNCS - UEFISCDI, project number PN-III-P1-1.1-TE-2019-1138, within PNCDI III. A. Feraru acknowledges the funding provided by scholarship “Bursă Specială pentru Activitate Științifică” provided by the Babeș-Bolyai University.

![Comparison of BG-xAgI and BG-yAg2O composites after performing in vitro assays.](image)

**Fig. 1** Comparative evaluation of BG-xAgI and BG-yAg2O composites after performing in vitro assays.

**References**


Porcine Abattoir Blood as a Valid Model for In-vitro Hemocompatibility Investigations

Johanna C. Clauser¹, Judith Maas¹, Ilona Mager¹, Frank R. Halfwerk²,³, Jutta Arens¹,²

¹ RWTH Aachen University, Department of Cardiovascular Engineering, Institute of Applied Medical Engineering, Aachen, DE; ² University of Twente, Chair of Engineering Organ Support Technologies, Department of Biomechanical Engineering, Faculty of Engineering Technology, Enschede, NL; ³ Medisch Spectrum Twente, Thorax Centrum Twente, Enschede, NL

Introduction
The major challenge of cardiovascular and cardiopulmonary medical devices lies in their hemocompatibility, such as hemolysis, thrombogenicity and platelet activation. Consequently, extensive in-vitro and in-vivo blood testing is mandatory to ensure the application of safe devices. Device thrombogenicity can be evaluated by in-vitro human blood tests; however, they are often restricted by the availability and required blood volume. Instead, animal blood use is allowed by the current standards and thus common practice for in-vitro investigations.

In order to reduce the necessity of donation animals and avoid limited availability, the use of abattoir blood provides a possible alternative. However, abattoir blood might present elevated blood values and pre-activated platelets due to stressed and frightened animals as well as non-standardized and potentially blood-damaging collection methods, which possibly affect the test results.

To this end, we investigated the pre-activation state and the remaining activation potential of porcine abattoir blood in comparison to human donor blood.

Experimental Methods
Light transmission aggregometry (LTA) was used to evaluate the pre-activation state of human donor and porcine abattoir blood.

Human blood (n=30) was collected from healthy donors from the cubital vein without stowing (Ethical Committee reference number EK 033/18) and immediately anticoagulated with 3.2 % citrate. Porcine blood (n = 30) was collected at the abattoir from the jugular vein of the animals into a bottle prepared with 3.18 % sodium citrate in a ratio of 1:9. Platelet poor and platelet rich plasma (PPP and PRP, respectively) were obtained from the blood samples by centrifugation and PRP.

Light transmission aggregometry (LTA) was adjusted to a platelet concentration of 200 000 PLTs/µL – 250 000 PLTs/µL by mixing of PRP and PPP.

Light transmission aggregometry (LTA) was performed according to the Born method. Collagen I and adenosine diphosphate (ADP) were used as activators with three different concentrations each (ADP: 5 µM, 10 µM, 20 µM; collagen: 2.5 µg/mL, 5 µg/mL, 10 µg/mL).

Results and Discussion
The stimulation of porcine abattoir blood with either ADP or collagen showed considerable activation of platelets. This disproves the general expectation that abattoir blood is already maximally pre-activated and therefore gives evidence to our hypothesis that this type of blood is suitable for in-vitro thrombogenicity testing. However, there are some differences to human donor blood.

Especially after ADP activation, porcine platelets show a different activation profile than human platelets, particularly a lower maximum aggregation (Fig. 1) and a significantly higher disaggregation. The reduced maximum aggregation might be due to the pre-activation of the abattoir platelets. Disaggregation in the range of 59 % – 84 % shows that the platelet activation is not stable and thus reversible. The reversible ADP-activation is a porcine platelet

Page 1060 of 2028
characteristic, which is present in abattoir blood as well, indicating a normal platelet behavior in that special type of blood [1].

Collagen-induced platelet activation reveals more similarities of human and porcine blood and generally presents a higher degree of activation of porcine platelets compared to ADP stimulation. In terms of maximum aggregation (Figure 2), slope and lag phase, only single pairs present significant differences between the species, whereas the majority does not present significances. Only the disaggregation presents significantly higher values for the porcine blood than for human blood; however, values vary around 5 % in contrast to < 2 % in the human data. Collagen stimulation thus seems to trigger an irreversible platelet activation also for porcine abattoir blood that is stronger compared to the activation with ADP.

Drawing a general comparison between the platelet activation properties of human blood and porcine abattoir blood, our data proves that the abattoir blood still bears an activation potential between 60 % - 90 %, depending on the activator. Accordingly, abattoir blood seems to be suitable for in-vitro thrombogenicity and particularly platelet activation evaluation. Porcine characteristics, which particularly differ from the human blood behavior, are preserved and activation kinetics remain in a reasonable range.

**Conclusion**

Light transmission aggregometry was performed with human donor and porcine abattoir blood. ADP activation of porcine abattoir platelets revealed a reversible platelet activation that is typical for pig, whereas collagen activation resulted in a stable and irreversible platelet activation. Despite generally lower values of maximum aggregation in the abattoir blood, no severe differences or malfunctions were obvious for the abattoir blood, thus verifying the use of abattoir blood for in-vitro investigations. This result is an important finding, allowing for a wide range of in-vitro testing with an ethically acceptable and easily available blood type and still valid outcomes.

**Acknowledgement**
The authors thank Dr. Doris Keller from the University Medical Center RWTH Aachen University for human blood withdrawal.
Figure 2
Maximum aggregation after collagen stimulation for human (white) and porcine (grey) blood. Significant differences between species are marked with black horizontal bars, inter-species differences are marked with white and grey bars for human and porcine blood, respectively.

References
Immobilization of ECM proteins on silanized dental abutment surfaces through a crosslinker supports stable cellular adhesion of gingival cells

Alena L. Palkowitz1, Taskin Tuna2, Shaza Bishti3, Frederik Böke4, Nathalie Steinke5, Gerhard Müller-Newen6, Stefan Wolfart7, Horst Fischer8

1 RWTH Aachen University Hospital, Department of Dental Materials and Biomaterials Research, Aachen, DE; 2 RWTH Aachen University Hospital, Department of Prosthodontics and Biomaterials, Aachen, DE; 3 RWTH Aachen University Hospital, Department of Prosthodontics and Biomaterials, Aachen, DE; 4 RWTH Aachen University Hospital, Department of Dental Materials and Biomaterials Research, Aachen, DE; 5 RWTH Aachen University Hospital, Flow Cytometry Facility, Aachen, DE; 6 RWTH Aachen University Hospital, Institute of Biochemistry and Molecular Biology, Confocal Microscopy Facility, Aachen, DE; 7 RWTH Aachen University Hospital, Department of Prosthodontics and Biomaterials, Aachen, DE; 8 RWTH Aachen University Hospital, Department of Dental Materials and Biomaterials Research, Aachen, DE

Introduction
In order to guarantee long-term success of percutaneous components like dental implants, abutments and their superstructure, a stable attachment of the soft tissue to the material interface is of great relevance for the prevention of peri-implant diseases and tissue regression. To date, titanium and its alloys as well as high-performance oxide ceramics -like zirconia- representing clinically relevant materials for the mentioned application because of their advantageous biological, physical and esthetic properties. In dentistry, a major factor for the success of an implant-supported restoration apart from the osseointegration of the implant is the gingival adhesion on the abutment surface. A tight adhesion acts as a sealing and hinders bacteria and other pathogens from penetrating into the implant/bone interface. However, the mucosa around the implant abutment shows numerous differences from that around natural teeth, which result in a reduced sealing capacity of the gingiva against bacterial invasion. To overcome these limitations, here we propose a novel method to effectively bioactivate abutment materials surfaces through the covalent conjugation of ECM proteins in order to enable a stable soft tissue adhesion.

Experimental Methods
A silane monolayer was applied on relevant implant materials (Y-TZP and Ti6Al4V) to present various functional groups on the material interfaces. The successful application was verified via XPS, FTIR-ATR and AFM. Laminin and fibronectin were covalently bound to the silanized surfaces via specific crosslinkers. Successful coupling was proven by AuNP-antibody staining. Retained function of the ECM-proteins after surface coupling was shown in a centrifugation-assay. Mechanical exposure, acid and heat resistance on the modified specimens was verified via XPS, AFM and SEM.

Results and Discussion
Human gingival fibroblasts (HGFs) seeded onto the ECM-protein-coupled surface exhibited significantly higher adhesion (at 50xg) in comparison to non-functionalized controls. Confocal microscopy and SEM analysis revealed a much higher cell area and enhanced expression of pFAK-Y397 on ECM protein coated surfaces compared to those seeded on native Y-TZP and Ti6Al4V. The influence of saliva in terms of pFAK-Y397 expression was investigated additionally. Moreover, HGFs on ECM-protein coated surfaces showed a higher proliferation rate and exhibited an enhanced integrin expression.

Conclusion
We showed that the covalent conjugation of ECM proteins via crosslinking on the dental abutment materials Y-TZP and Ti6Al4V enables a strong adhesion of gingival fibroblasts. Therefore, the novel approach could improve the...
'sealing' against bacteria at this interface and thus holds promise to potentially reduce or prevent peri-implant diseases in clinical application.

Acknowledgement

We acknowledge the financial support of the German Research Foundation, DFG (grants 975/30-1 and WO 1576/6-1).
Liquid flow chambers for biomaterial degradation analysis - Flow simulation and in vitro testing of various liquids and flow velocities

Benjamin Kruppke, Hans-Peter Wiesmann

Technische Universität Dresden, Institute of Materials Science, Dresden, DE

Introduction
The application of degradable biomaterials, e.g. for hard tissue replacement, requires a precise knowledge of their degradation behavior in order to adapt them to the time course of the healing process by material selection, processing or scaffold geometry. The limited investigation possibilities with cell cultures and animal experiments make it of great interest to analyze the high number of biomaterials in advance with regard to their degradation. It would be desirable, obviously, to know the influences such as the exchange rate of the surrounding fluid (as a trigger for increased material dissolution), the effect of aggressive media (e.g. as a simulation of osteoclastic resorption) and the ion concentrations in the liquid (as an indication of the bioactivity of the materials).

Experimental Methods
To analyze the aforementioned parameters, three versions of a flow chamber were designed and manufactured consecutively. For this purpose, the CAD models were tested with a liquid flow simulation and two new design load factors were established for the materials. The utilization of liquid (UOL) and the shear homogeneity index (SHI) provide information about the effectiveness of the chamber designs.

To test the chamber designs in vitro, suitable degradable biomaterials were fabricated after the chambers were manufactured and tested in different fluids in the flow chambers. The materials are based on gelatin-modified calcium strontium phosphates, which have previously shown their different degradation rates in static and quasi-static regimes [1,2]. The liquids chosen were phosphate-buffered saline (PBS) and simulated body fluid as reference media and citric acid solution (pH 3) and PBS with lowered pH (pH 6.0) to mimic osteoclast resorption and wound acidification, respectively.

Results and Discussion
As a result of the flow chamber design with respect to simulation, an adaptation to an increasingly efficient utilization of the fluid can be seen. It was crucial not to design a perfusion reactor with high pressure differences, but to allow the specimens to be passed around by the liquid with priority. This is similar to a biomaterial used in a bone defect, since this is also the path with the supposedly lowest resistance for the tissue fluids in vivo. In addition, this approach can be used to get closer to the fact that the fluids, through their continuous exchange, always cause a refreshed concentration gradient for the degradation products of the substitute materials.

The adaptation of the chamber to the non-destructive monitoring of the degradation process with a µCT allows a detailed observation of the degradation process. It was shown, that even for different fluids, increased degradation occurs at higher previously calculated UOL and SHI. Furthermore, the use of aggressive fluids induced a degradation of up to >90% within only 7 days, while <20% occurred for the reference liquids. Despite this, the material influences can be clearly distinguished, making the degradation results much more meaningful for a late stage of material resorption. This corresponds to previously obtained findings of the material variants in animal experiments [3].

Conclusion
The study showed, that bone substitute material degradation can be analyzed very efficiently with in vitro flow chambers to reduce time-consuming and costly experiments with cell cultures or ethically questionable material testing in vivo. Testing of biomaterials in large numbers could thus initially be simplified by this type of analysis, in...
order to complete further material characterization in vitro and in vivo with a small pre-selected set of materials with precisely known degradation behavior.

Acknowledgement

Financial support from Deutsche Forschungsgemeinschaft DFG Collaborative Research Centre TRR 79/ SP M3 is gratefully acknowledged.

Chamber designs 1 and 3 with flow simulation

The fluid flow simulation for the two chamber designs demonstrates the improvement of homogeneous fluid transport based on the multicell chamber (left) and ending with the cascade chamber (right).

References


Aging effects on shape memory and tensile properties of high toughness polylactide composites for resorbable devices

Xabier Larrañaga, Jose-Ramon Sarasua, Ester Zuza

University of the Basque Country (EHU) and Polymat, Engineering faculty of Bilbao - Department of Metalurgy and Materials Science, Bilbao, ES

Introduction
Poly lactides are biodegradable polyesters obtained from renewable resources, widely investigated and used in the medical field. Due to their bioabsorbiability, tuneable mechanical properties and biodegradation rates, polylactides are exceptional materials for resorbable devices such as stents or orthopedic fixation anchorages [1]. Moreover, an additional property of the poly(L-lactide) (PLLA) is the shape memory effect (SME), a key feature for minimal invasive surgery [2].

However, PLLA particularly is featured by its mechanical brittleness. To overcome this drawback, previous works have been reported adding 10 wt.% of barium sulphate (BaSO₄) particles to the polylactide matrix in order to obtain a radiopaque composite with high toughness and ductility [3]. Nonetheless, the physical aging in PLLA takes an important role in the properties because they may vary along the time, due to its value of $T_g$ close to the room temperature. The effect of aging in the before mentioned composites has not been stated yet [4]. Consequently, this work consists in the analysis of physico-chemical properties, SME and mechanical behaviour of these high toughness composites within progressive aging times, paying attention in the effect of particle amount in the composites.

Experimental Methods
Commercial PLLA of molecular weight of 150,000 g/mol and BaSO₄ with a mean size of 1.45 µm was used.

Samples were prepared by melt blending using a Vertical DSM and injected in a dumbbell-shape mould. PLLA neat samples and PLLA composite samples with selected ascending particle quantities of 0.5, 1, 5 and 10 wt.% have been aged at 21 ± 2 ºC and 50 ± 5% relative humidity.

Tensile tests and SME were performed on 1 mm thick specimens in an Instron 5565 with a thermal chamber. Thermal transitions and aging were determined by means of differential scanning calorimetry on a DSC 200 (TA instruments).

Results and Discussion
DSC results depict aging effects, a glass transition temperature rise and an enthalpy relaxation signal. The presence of particles modifies the cold crystallization behaviour.

Figure 1 shows the stress-strain curves from which two set of values are obtained: toughness values and yielding energy values. Both are analysed as indicators of the aging effect on the mechanical behaviour.

Regarding the evolution of toughness with aging, the initial high toughness of composite specimens is affected. Samples with 0.5 and 1 wt.% of particles show a brittle behaviour after 10 aging days, while, those with 5 and 10 wt.% of particles remain tough for longer times. Therefore, high toughness PLLA composites are found to be sensitive to the enthalpy relaxation of chains, conditioned by the added particle proportion.

Concerning to the energy values measured, yielding energy values are higher for composite samples than those obtained for neat PLLA. With aging, energy absorption values rise and specimens with 0.5 and 1 wt.% load tend to reach higher absorption values unlike composites with 5 and 10 wt.% of particles, which retain lower yielding energy absorption values over time.

Page 1067 of 2028
Shape memory tests indicate how the addition of BaSO₄ particles and segmental relaxation during physical aging affects on the measurable parameters of programming and recovery stages. Therefore, properties can be tuned by adjusting the particle content in the matrix; yet, storage time of the material is a conditioning factor that must be taken into account.

**Conclusion**

Summarizing, this study comprehends PLLA and the PLLA/BaSO₄ composite as an evolving system over time. Based on the evidence of early aging of the neat polymer, this work focuses on the importance of the durability of the mechanical behaviour and SME of such high toughness composite materials oriented to design reliable bioresorbable devices.

**Acknowledgement**

The authors thank funding from the Basque Government Department of Education, University and Research (consolidated research groups GIC IT-927-16), Spanish Government MICINN (106236GB-I00/AEI/10.13039/501100011033), and Basque Government predoctoral grant for X.L.
Shape recovery stage of a PLLA + BaSO₄ sample, from programmed shape to complete recovery

References

Evaluation of \textit{In-situ} Intrahepatic Strain Distribution under Micro Deformation by Using Multiphoton Microscopy

\textbf{Yuna Nakano\textsuperscript{1}, Yusuke Morita\textsuperscript{2}, Koji Yamamoto\textsuperscript{2}}

\textsuperscript{1} Doshisha University, Graduate School of Life and Medical Sciences, Kyoto, JP; \textsuperscript{2} Doshisha University, Department of Biomedical Engineering, Kyoto, JP

\textbf{Introduction}

It is known that the mechanical properties of cell adhesion substrates can also affect the following cellular events, such as molecular synthesis, phenotypic expression and motility. For instance, changes in the elastic modulus of the artificial cell adhesion materials have an influence on the albumin production of liver cells\cite{1}. It is considered, however, that the mechanical properties of the micro-environment around the liver cell are uneven and anisotropic due to the collagen structure and density in the liver tissue, and those effects on cell activities have not been clarified yet. In this study, we focused on the micro-area mechanical property of liver tissue and evaluated the \textit{in-situ} strain distribution on the basis of the collagen image obtained by a multiphoton microscope (MPM).

\textbf{Experimental Methods}

\textbf{Materials} : In order to acquire the strain distribution generated in liver tissue, we used a chicken liver processed to a cylindrical shape (diameter:4 mm) with a biopsy punch, and the tissue with a 2\% agarose gel mold was installed in a lab-made apparatus capable of loading a micro deformation to the specimen. Before installing, the composite material was immersed in physiological saline for 2 hours, and to avoid the effect of Glisson’s capsule, 0.5-mm thickness of the liver specimen from the surface layer was removed with a medical blade.

\textbf{Analysis and evaluation of liver strain distribution} : Since the second harmonic generation signals can visualize the collagen fibers in living tissue without any fixing and staining, stacking images of the collagen before and after deformation were acquired by using MPM. A displacement of 0.2 mm was applied to the specimen by compressing both sides using stepping motors and the images taken at the center of the liver specimen (picture size: 442 μm × 442 μm) were analyzed using GOM Correlate (GOM), which is one of the strain analysis software using the digital image correlation method. After the equivalent strain distribution generated at the surface of the liver tissue was visualized using a color contour map, the mean collagen amount within each strain region was measured using gray scale value.

\textbf{Results and Discussion}

Figure 1 shows an image of strain distribution overlayed on the collagen image depicted with grayscale. It was found that the strain in the micro area changed with the collagen distribution (maximum magnification: 50 times). From this result, it was found that the various strains were widely distributed in liver tissue depending on the collagen distribution at the peripheral part of the cell and the strain gradient expressed in the tissue could vary within micro distance. Figure 2 shows the histogram of the mean gray value of each contour region calculated from the strain distribution image. It was found that small strain regions tended to show relatively high accumulations of collagen fibers even in micro area of liver tissue.

\textbf{Conclusion}

The intrahepatic strain distribution in the micro region was successfully obtained by using the collagen images before and after deformation. From the results of the strain distribution, it was suggested that the micro-area mechanical properties of live tissue can be affected by the collagen distribution.
Figure 1: Image of strain distribution overlayed on the collagen image, (Bar : 100 μm).

Figure 2: The histogram of the mean collagen amount in each strain using gray scale value.

References
**Introduction**

The aim of this study is to verify the interactions of different laser wavelengths on the titanium grade 4 and grade 5 surfaces commonly used in dentistry for the creation of implant screws for the replacement of dental missed elements; focusing attention on a new Q-Switch Nd:YAG laser device. To date, dental prosthesis on implants is considered the best solution for the rehabilitation of edentulous areas; this is demonstrated by the fact that around 5,000,000 dental implants are placed every year around the world. Hand in hand with the spread of implantology, there has also been a significant increase in cases of peri-implant disease, a multifactorial disease mainly of bacterial origin which, in the long run, can lead to implant loss. (1).

However, nowadays, it has not yet been possible to identify a truly predictable therapy for the treatment of these pathologies. Among the innovative technologies most used in the treatment of peri-implant mucositis and peri-implant disease, one of the most promising is certainly laser light. (2).

In fact, as already shown by publications in the literature, the use of laser devices is able not only to decontaminate the implant surface and the surrounding areas, but also to stimulate tissue repair, thus speeding up healing times. (3).

**Experimental Methods**

The research was carried out at the DICCA Department at University of Genoa. The two titanium metal surfaces were divided into 5 parts. (fig1). Of these, four were treated with different laser wavelengths and one was used as a control group. The samples were placed inside a bioreactor that faithfully reproduces the oral conditions specifically with the exact oral PH, temperature and humidity, in which they would be found during the interaction maneuvers with the laser lights. The wavelengths examined were: 810nm laser diode with 320um 1.5 w 50hz gated mode 20 sec optical fiber; Nd-yag 1064nm laser with 320um optical fiber 1 W 50Hz 20 sec 5 ms pulse; Erbium-Yag laser 2940 nm optical fiber 320um 1,2 W 30mj 40Hz 20 sec; Q-Switch Nd:YAG Laser 1064 nm with optical fiber 320um 1.2 W 50Hz 20 sec 3 ns pulse. Each of the four sections was treated by coming into contact with the surfaces, to best simulate situations of clinical management. This process was also recorded by a thermal-camera that made it possible to track the temperature variations that occurred on the titanium during exposure to laser light. To verify any damage and their extent on the surfaces, the samples were then analyzed by means of a scanning electron microscope (SEM).

**Results and Discussion**

The microscopic analyzes carried out have shown that the diode laser does not alter the surface of the titanium in the slightest, but during its use it causes an excessive temperature increase of about 25 °C.

Conversely, the Nd: YAG laser, although it causes a lower temperature increase than the previous laser (about 19 °C), greatly damages on the surfaces, creating craters and melting areas. The Er: YAG laser, on the other hand, being used under a constant water jet, does not cause any variation in the temperature of the titanium and only micro morphological alterations, however, not able to make the surface more subject to accumulation of bacterial biofilm. Finally, the Q-Switch Nd:YAG laser, in addition to not causing a rise in temperature above 5 °C, like the previous laser, does not cause surface alterations such as to be able to host bacterial cells.

**Conclusion**
Based on the results obtained, we can state that the most promising device to be used in laser-assisted peri-implant therapy may be the latest generation Q-Switch Nd:YAG laser. The latter, always having a wavelength of 1064 nm, has the same decontaminating and biostimulating properties of the other devices, but, being used with pulse emission mode in nano seconds, it never reaches high temperatures and consequently does not cause superficial alterations of the titanium structure. Nevertheless, these technologies require an excellent learning time on the part of the professional to be properly used in order to significantly improve the oral health of patients.

References

Bilateral double site (calvaria and mandibular) critical size bone defect model in rabbit for evaluation of craniofacial tissue regeneration

Sindhu Kotagudda Ranganath1, Matthias Schlund2,1, Pierre Marchandise3, Anne sophie Drucbert1, Nicolas Blanchemain1, Amit Chandrakar4, Katrina Moisley5, Matei Cirstea5, Paul Wieringa4, Feng Chai1

1 Univ. Lille, INSERM, CHU Lille, U1008-Controlled Drug Delivery Systems and Biomaterials, lille, FR; 2 Univ. Lille, INSERM, CHU Lille, Service de Chirurgie Maxillo-Faciale, 2 Avenue Oscar Lambret, lille, FR; 3 Univ. Lille, F-59000, Lille France, Univ. Littoral Côte d’Opale, F-62200, Boulogne-sur-Mer, France, CHU Lille, F-59000, Lille, France, ULR 4490 - MABLab - Adiposité Médullaire et Os, lille, FR; 4 Institute for Technology-Inspired Regenerative Medicine (MERLN), Maastricht, MERLN, Maastricht, NL; 5 The Electrospinning Company, Harwell campus, Didcot, GB

Introduction

Along with the development of innovative bone substitute materials, an appropriate animal model is essential to investigate in vivo biocompatibility, mechanical properties, degradation, and interaction with host tissue. Most existing preclinical models for evaluating the safety and bone-regeneration efficacy of the tested biomaterial are single-site defect model and vary drastically with anatomical locations. While the mechanism of bone healing often differs by anatomical location, owing to developmental, structural, and functional difference, which is particularly true for maxillofacial wounds (1-2). Taking this into account, the bone healing efficiency of a bone substitute shown at one anatomical defect location cannot ensure that at another. The novelty of this study was to develop a reproducible preclinical model of bilateral critical-sized defects (CSD) at two distinctly different anatomical locations (non-load bearing calvaria and load-bearing mandibular) in one rabbit. It makes each animal served as its own control subject, therefore, maximizing its statistical power for comparative testing by pairwise comparison. This bilateral double-site CSD model was validated by comparing the “gold standard” autograft to the sham (no graft) group. Subsequently, a newly developed 3D-Implant composite mesh material was implanted to assess the utility of this model for demonstrating the potential translational success of new bone substitute materials or tissue engineering constructs.

Experimental Methods

Twenty healthy adult male New Zealand rabbits, weighing 3.5–4 kg was randomly assigned to a sham or an autograft group (n=10 in each group), which were evenly subdivided into two observation periods (4-, and 12-week). The full-thickness cylindrical calvaria (10 mm diameter) defect on frontoparietal region and full-thickness mandibular (11 mm diameter, comprising removal of buccal cortex, tooth roots, and lingual cortex) defect on the body of the mandible were created bilaterally using low speed drilling with saline irrigation. The defect on one side was filled with autograft debris, and the other side was left empty. For further assessing its utility, a multilayer-stacked polycaprolactone/nano-hydroxyapatite composite (PCL/nHA) 3D-implant, fabricated by melt electrowriting was implanted on one side of the rabbits, and the other side was filled by autograft or left empty alternatively. At time points 4- and 12-week, the mandible and calvaria bone defect specimens were harvested and fixed in 10% neutral formalin buffer. Bone regeneration was assessed by microcomputed tomography (micro-CT) imaging and histological evaluation.

Results and Discussion

No clinical concerns were noted during the study, and no animals were euthanized before the experimental endpoints. All animals recovered within 5-6 days after surgery. Animals were euthanized at predetermined time points 4- and 12-week, no macroscopical sign of infection or necrosis was observed in any of the group: in the autograft group, the fragments of bone graft remained stable and integrated well with adjacent bone, and partial healing at 4w and almost complete healing at 12w were observed in both calvaria and mandible defect; while there was only a fibrous cap (associated with scattered ossified areas) at both 4w and 12w in the empty group.

Page 1074 of 2028
The percentage of bone volume ratio (BV/TV) by micro-CT showed that, in calvaria defect, BV/TV of the autograft group (39.52% ± 5.2% and 47.41% ± 5.4% at 4w and 12w, respectively) was statistically higher ($p<0.05$) than that of the empty group (15.20% ± 2.3% and 21.24% ± 3.17% at 4w and 12w, respectively) and 3D-implant group (15.7% ± 1.44% and 19.46 ± 2.5% at 4w and 12w, respectively); similarly in mandible defect, BV/TV of the autograft group (49.57% ± 6.6% and 41.72% ± 1.2% at 4w and 12w, respectively) was statistically higher ($p<0.05$) than the empty group (33.84% ± 2.3% and 26.24% ± 3.81% at 4w and 12w, respectively) and 3D-implant group (14.66% ± 1.7% and 30.42 ± 5.2% at 4w and 12w, respectively). Histological results were concordant with the micro-CT results. Thus, the untreated mandibular and calvaria defects did not heal without intervention (autograft) makes this model a well-defined and standardized CSD model, which is useful for evaluating the experimental 3D implants.

Conclusion
Based on the histological and micro-CT findings of the response of bilateral double site calvaria and mandible defects to autograft and sham, the developed model was validated as the CSD till 12w. For the first time, this study showed that the rabbit has a high tolerance for the studied bilateral double-site CSD model. The encouraging results of 3D-implant imply, though the results of animal models may not perfectly extrapolate to humans, this comparative rabbit model may be a useful tool for reliably assessing and comparing the efficacy of new tissue engineering constructs at two different anatomical locations in one rabbit.

Acknowledgement
The authors would like to thank the Platform Experimental Resources, D.H.U.R.E, University of Lille, for their support on animal studies and Mrs M.H Gevaert for technical help on histological analysis. It was supported by the cross-border INTERREG “North-West Europe” fund BONE 2014-2020.

References
Alginate di-Aldehyde-Induced Oxidative Stress in Endothelial Cells and Fibroblasts: Cell-specific Responses

Hatice Genc1, Jonas Hazur2, Emine Karakaya2, Barbara Dietel3, Faina Bider2, Jürgen Groll4, Christoph Alexiou1, Aldo R. Boccaccini2, Rainer Detsch2, Iwona Cicha1

1 University Hospital Erlangen, Section of Experimental Oncology and Nanomedicine (SEON), Else Kröner-Fresenius-Stiftung-Endowed Professorship for Nanomedicine, Department of Otorhinolaryngology, Head and Neck Surgery, Erlangen, DE; 2 Friedrich-Alexander-Universität Erlangen-Nürnberg, Institute of Biomaterials, Erlangen, DE; 3 University Hospital Erlangen, Department of Cardiology and Angiology, Erlangen, DE; 4 University Hospital Würzburg, Department of Functional Materials in Medicine and Dentistry, Würzburg, DE

Introduction
A hydrogel system based on oxidized alginate covalently crosslinked with gelatin has been utilized for different biofabrication approaches to design constructs, in which cell growth, proliferation and migration have been observed. However, cell–bioink interactions are not completely understood and the potential effects of free aldehyde groups on the living cells have not been investigated. In this study, alginate, alginate di-aldehyde (ADA) and ADA crosslinked with gelatin (ADA-GEL) were characterized via FTIR and NMR, and their effect on cell viability and cellular responses regarding on oxidative stress that caused by hydrogels were investigated in vitro.

Experimental Methods
Fourier-transform infrared spectroscopy (FTIR) and Solid state 13 C NMR spectroscopy were used for the characterization of hydrogels. Suitable oxidation degree was chose by live/dead cell viability assay that performed on NIH/3T3 and EA.hy926 cell lines. Oxidative stress caused by hydrogel systems was analyzed via Flow cytometry on primary endothelial and fibroblast cells. Proliferative state of cells determined with immunofluorescent staining of Ki67 protein.

Results and Discussion
With increasing oxidation degree of ADA, cell viability in NIH/3T3-fibroblasts and endothelial cell line EA.hy926 was reduced, with the strongest cytotoxicity observed after 72 h of culture in ADA with 26% degree of oxidation. This cytotoxicity was related to the presence of free aldehyde groups in ADA system and further investigations were performed to address this question (Fig. 1). Primary human cells, namely fibroblasts and endothelial cells (ECs) were grown in ADA and ADA-GEL hydrogels for 6h, 24h and 72h to investigate the molecular effects of oxidized hydrogels. Cell viability, mechanisms of cell death, intracellular reactive oxygen (ROS) and thiol level were measured using flow cytometry and proliferative state of cells was determined by immunofluorescent staining of Ki67 protein.

Conclusion
Taken together, we were able to show for the first time that two different cell types in contact with an oxidized alginate hydrogel respond with strikingly different reaction paths. We demonstrated that the free aldehyde groups of ADA trigger pathways of intracellular oxidative stress, which ultimately result in either necrotic cell death in cells with low antioxidant capacity, or recovery and renewed proliferation in cells with high antioxidant capacity. The interactions between this sensitive system of chemically active groups in ADA-GEL hydrogels and the cellular responses must be considered in order to design cell-friendly biofabrication approaches.\(^1\)

Acknowledgement

This study was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation, Project number 326998133) within the collaborative research center TRR225 (subproject A01, B02, B06). The authors thank Heike Kloos (Department of Cardiology and Angiology, University Hospital Erlangen) for help with primary endothelial cell isolation, Dr. Christina Janko (SEON, University Hospital Erlangen) for help with flow cytometry analyses and Christian Placht (NMR Department, Organic Chemistry I, FAU Erlangen-Nuernberg) for his support with NMR measurements.

---

**Figure 1**

Schematic summary of the study: alginate-di aldehyde hydrogel was prepared by NaIO4 treatment of pristine aldehyde and crosslinked with Ca2 solution. Free aldehyde groups of alginate-di aldehyde hydrogel induce intracellular reactive oxygen species production. Cell types with lower antioxidant capacity react with sudden necrotic death. Cell types with higher antioxidant capacity overcome the oxidative stress and regain their proliferative state.

---

**Figure 2**
Time-dependent comparison of cell viability in primary ECs and fibroblasts grown in ADA and ADA-GEL.

References
Different Effects of Grid Blasting on Mechanical Properties of Ceramic Coated Co-Cr Alloys

Christina Mouchtaridi¹, Marta Muñoz Hernández², Marta Multigner Domínguez², Maria D. Escalera Rodríguez², Juaquin Rams², Triantafyllos Papadopoulos¹, Ioannis Eleftheriadis¹

¹ National and Kapodistrian University of Athens, Dental Biomaterials, Athens, GR; ² Rey Juan Carlos University, Mechanical Engineering, Madrid, ES

Introduction
The purpose of this in vitro study was to determine the influence of grit blasting on microstructure and mechanical properties of cobalt chromium alloys coated with porcelain layer.

Experimental Methods
Thirty Co-Cr metal substrates were fabricated according to ISO 9693-1, by Direct Melting Laser Sintering. Ten of them were sandblasted with 50μm Zinc oxide nanopowder and another group of ten substrates were treated with 250μm. The third group of ten substrates were left as received. A commercially available feldspathic porcelain was placed on the 50μm and 250μm sandblasted substrates, and then the specimens were tested for metal-ceramic bond strength with the 3-point bend test, according to ISO 9693-1. The fractured specimens were observed with optical and scanning electron microscopy using electron dispersive spectroscopy to define the mode of failure. X-ray diffraction spectroscopy was conducted to determine changes in crystalline phases after fabrication and the 3-point bend test. Also, profilometry observations were conducted.

Results and Discussion
The differences in modulus of elasticity showed that the bigger the particles of the sandblasting are, the more elasticity the substrate reveals. The metal-ceramic bond strength for both of the groups was cohesive. The metallographic analysis of the as-received, the after porcelain firing, and the after 3-point bend test specimens revealed changes in microstructure. The crystallographic microstructure revealed that the patterns had minor changes among the groups. The profilometry, also indicated that the bigger the sandblasting particles were used, the smoother the surface was.

Conclusion
Sandblasting does not affect to the adhesion between the metal substrate and the ceramic layer. The study revealed that all of the techniques showed similar results. The modulus of elasticity and metal-ceramic bond strengths revealed that the bigger the particles of the sandblasting is the higher the mechanical properties are. The mode of failure was cohesive. The profilometry revealed differences among the three groups.

Acknowledgement
Special thanks to Mr Georgios Anastasiadis for his contribution.
Experimental investigation of the mechanical performances of biofabricated myco-materials

Chiara De Donno¹, Giulia Scalet¹, Antoni Gandia², Maria Garcia-Torreiro², Daniele Dondi¹, Dhanalakshmi Vadivel¹, Ferdinando Auricchio¹

¹ University of Pavia, DICAr, Pavia, IT; ² Mogu S.r.l., Inarzo (VA), IT

Introduction
Biofabrication is a disruptive manufacturing paradigm that utilises the metabolism of bio-organisms to produce complex living and non-living structures with advanced functionalities.

The use of fungal micro-organisms as cell factories in biofabrication is opening great possibilities for converting residues of agroindustrial value chains into renewable and compostable mycelium-based materials (myco-materials).

Such materials represent alternatives to a range of traditional materials/products, such as petroleum-based foams and plastics, textiles, and others [1]. Despite the number of publications on biofabricated myco-materials, in the literature there are few studies about their mechanical properties.

This work aims to experimental investigate the behavior of several categories of such materials.

Experimental Methods
Different types of chitin-based myco-materials produced by MOGU S.r.L are investigated (Figure 1). In particular, we perform a mechanical characterization through tensile and tear tests (Figure 2). Three standards referring to leathers are selected to conduct these tests. In particular, EN ISO 3376 is used for the determination of tensile strength and elongation at break, while EN ISO 3377-1 (single-edge tear) and EN ISO 3377-2 (double-edge tear) are used for the determination of tear load.

Results and Discussion
Tensile strength, elongation at break, Young's modulus, and tear loads are obtained from the performed tests. Results demonstrate that the properties of the analyzed myco-materials vary according to the production procedure, plastification, and post-treatments. The mechanical results obtained are satisfactory and promise a future full of possibilities for the use of chitin-based myco-materials in different applications. The examined materials are also pleasing to the eye and to the touch, similar to traditional petroleum-based leather-like materials. The color range obtainable in a natural way is varied and similar to the classic leather colors. This, combined with the mechanical properties found, makes, among other applications, the use of these materials in the textile industry of alternative leather very close.

Conclusion
All the achieved experimental results enrich the knowledge in the literature about the mechanical responses of myco-materials and are fundamental for providing guidelines on how to proceed in the design of targeted industrial products. Further investigations are essential to enable the full potential of mycelia to be used for a revolution in the world of alternative materials. The potential field of interest are multiple including automotive, packaging, insulation, wearable, building/construction, aerospace, and biomedical applications such as wound healing, bio-sensing, and drug delivery.

Acknowledgement
The authors acknowledge the project "MycO-advanced leather materials" no.2018-1765 by Fondazione Cariplo and Regione Lombardia.
Figure 1. Chitin-based myco-materials - MOGU Flexible Mycelium Materials.

Figure 2. Experimental tests for mechanical characterization: (a) tensile test, (b) and (c) two different type of tear tests.

References
Thin silica microsheets as a new biomaterial concept for Tissue Engineering applications

Marta M. Maciel1,2, João Borges2, Pavel A. Levkin3, Sónia G. Patrício2, Tiago R. Correia2, João F. Mano2

1 University of Minho, CEB- Centre of Biological Engineering, Braga, PT; 2 University of Aveiro, CICECO- Aveiro Institute of Materials, Aveiro, PT; 3 Karlsruhe Institute of Technology (KIT), Institute of Biological and Chemical Systems-Functional Molecular Systems(IBCS-FMS), Eggenstein-Leopoldshafen, DE

Introduction
In Tissue Engineering (TE), the engineering of highly bioactive materials with specific shape is extremely challenging when incorporating mammalian cells. Limiting factors such as materials processability, scaffold design and implantation into the host can result in functionality and survival restrictions for mammalian cells. To overcome these scenarios, efforts have been made, especially, using encapsulation-based systems to maintain cell integrity and functionality. Yet, most of them do not present adequate stiffness or specific cell binding domains. Microfabrication have been pointed out as a valuable method used to produce highly complex architectures with increased precision. For instance, inorganic materials have become an interesting source for developing new platforms due to properties like inertness, stiffness, easy functionalization and processability to produce materials with specific features and sizes. This work reports the high throughput fabrication of thin silica-based microsheets as a new concept of biomaterial platform to potentially guide cell fate in TE applications. Different geometries will be fabricated and compared to achieve the best shape for TE purposes.

Experimental Methods
The microarrays used on silica microsheets production were obtained by a protocol described by Feng and colleagues. (1) Silica-based microstructures were fabricated under acidic conditions as previously described (Figure 1). (2) Briefly, a gelatin (gel strength 300 g Bloom) solution was used to create a sacrificial layer to avoid silica interaction with the microarray surface. After gelation, a sol-gel method was performed by using a previously hydrolysed tetramethylorthosilicate (TMOS) (Sigma-Aldrich) solution that was applied under the gelatin layer and dried for 5 min at 37 ºC. To recover silica microstructures from the arrays, the glass slides were incubated at 60 ºC in a water bath until their detachment. The resulting microstructures were collected and characterized through different techniques (FTIR, XRD, SEM). A simulated body fluid assay was also carried out to investigate the mineralization capacity of the produced silica-based microsheets.

Results and Discussion
The results revealed the successfully manufacture of porous silica microstructures with defined shape and a thickness of 7mm (Figure 2). Moreover, microsheets bioactivity potential was confirmed by carrying out a simulated body fluid assay (Figure 2). Flower-like shape hydroxyapatite crystals were formed at the surface of the microsheets. In a near future, a Janus-like design (Figure 1) using different cell types is intended by functionalizing microsheets surfaces with suitable bioinstructive cues (e.g., antibodies, ions, peptides), providing the biochemical and biophysical cues to recruit the different living units in a controlled manner.

Conclusion
A novel and simple strategy for fabricating free-standing thin silica microstructures with well-defined geometry was reported. This enabling technology has a widespread potential not only due to silica properties but also due to the simplicity of processing. The possibility to control the sol-gel reaction allows the manufacture of other type of geometries or even use of other type of inorganic materials and hybrid systems. We envision that future studies will
enable to produce microparticles with high control over the microstructure, including mesopores with fixed geometries and sizes, and surface post-functionalization, that could find new applications in bottom-up Tissue Engineering area.

Acknowledgement

The authors would like to acknowledge the financial support from the European Research Council (ERC) for project ATLAS(ERC-2014-ADG-669858) and the Portuguese Foundation for Science and Technology (FCT) through the PhD grant PD/BD/139117/2018 (M. M. Maciel) and individual contract CEECIND/03202/2017 (J. Borges). This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the Foundation for Science and Technology/MCTES.

Figure 1
Scheme of the protocol to produce silica-based microstructures with defined geometries.

Figure 2
Microsheets characterization results: (A-D) SEM images of microsheet different geometries and respective EDS analysis; (E) SEM cross-section of silica-based microsheet with a thickness value around 7 µm; (F) SEM image obtained after SBF assay showing flower-like hydroxyapatite crystals formed at microsheets surface.

References

Strut spreading measurements as shape fidelity prediction tool

Stefan Schruefer, Dirk W. Schubert

Friedrich Alexander University Erlangen Nuremberg, Material Science / Polymer Physics and Processing, Erlangen, DE

Introduction
Shape fidelity is a key property, besides biological compatibility, for newly developed bioinks. Therefore, shape fidelity characterization methods evolved from comparative or binary (good/bad) methods towards more analytical approaches. Various researchers have tried more reliable and comparable data generation to reveal relevant correlations between material properties and the results of printing experiments. Common printability assets still rely on “one-picture” approaches and do not consider sufficient data to capture the visco-elastic nature of hydrogels during printing. Rheological properties of bioinks are often in the focus for predicting the printability of such materials. However, the key parameters for reliable predictions are yet to be revealed, which results from two main issues of classic rheological characterizations. First, many characterizations in the literature are not closely related to the real process conditions, which especially limits the information value for thermal responsive materials such as gelatin or collagen. A short guideline to improve the data quality is given in the scope of this presentation. Furthermore, the vast variety of potential information given by rheological measurements can mislead scientists during the evaluation process.

Experimental Methods
Therefore, we developed a novel approach to reveal rheological model parameters from commonly performed frequency sweeps. The evaluated traditional rheological equivalent circuits, consisting of springs and dashpots, can reduce the frequency dependent material properties to only two to four parameters, which include all necessary information to describe the viscoelastic nature of hydrogels and bioinks. During first evaluations, another major challenge did become evident. Many hydrogels and bioinks do show spreading behavior, which is not covered by common printability evaluation assets. Therefore, a novel set-up to evaluate the time-dependent strut diameter was developed. Fiber-filled alginate composites are used as model systems for tunable hydrogels. Therefore, we blended PH176 Alginate solutions of various concentrations (3 and 4 wt.%) with different degrees of fiber fillers. For this study, low aspect ratio poly-ε-caprolactone (PCL) fiber fragments and cellulose nanofibers are investigated. Furthermore, silicon oils of different viscosities (Wacker AK50 – AK5000) are analyzed in order to reveal artifact free correlations of rheological properties and spreading kinetics.

Results and Discussion
Rheological modelling revealed an augmented form of the burgers model as most suitable model system for all composite. Subsequent analysis of fiber-filled alginites revealed a clear correlation between process properties, namely material throughput and printing speed, rheological model parameters and the observed time-dependent strut spreading. The evaluation of silicon oil enabled detailed and artifact free modeling. The rheological properties of alginate composites altered by increasing the fiber infill of alginate composites, which also influenced the resulting rheological model parameters. The spreading behavior of composites can be drastically reduced by adding only 2 wt.% of cellulose nanofibers. Low amounts of PCL fiber fragments did not show a significant influence on the rheological and printing properties. Increasing fiber infill (>5 wt.%) also did reduce the spreading of the investigated hydrogel composites.

For further correlations between rheological properties and spreading characteristics Statistica, a statistical data evaluation software, is used. Here, a variety of possible parameters, ranging from the revealed model parameters to
traditional rheological parameters, such as the zero shear viscosity, and process characteristics are evaluated. The subsequent elimination of statistical non-relevant parameters did lead to two main influencing factors. Here, the ratio of material throughput and print speed as well as the viscosity of the Maxwell-dashpot (of the Burgers system) did emerge as key criteria for describing the spreading behavior of all composite systems. Those key criteria can potentially be used to predict the shape fidelity of bioinks from rheological measurements.

**Conclusion**
The here presented data did reveal a two distinct diameter-time-dependencies for hydrogel composites, that is not considered in conventional evaluations of printability. Therefore, an improved method for robust and analytical printability evaluations is established. Furthermore, a clear correlation of rheological measurements and printing results is revealed. The generalization of our findings is in progress.

**Acknowledgement**
Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Projektummer 326998133 – TRR 225 (subproject A07).
The biocompatibility of novel citric acid-based biomaterials modified with D-panthenol for vascular tissue engineering

Agata Flis¹, Martina Trávníčková², Filip Koper³, Lucie Bačáková², Tomasz Świergosz³, Wiktor Kasprzyk³, Elżbieta Pamuła¹

¹ AGH University of Science and Technology, Department of Biomaterials and Composites, Cracow, PL; ² Institute of Physiology of the Czech Academy of Sciences, Department of Biomaterials and Tissue Engineering, Prague, CZ; ³ Cracow University of Technology, Department of Physical Chemistry and Biotechnology, Cracow, PL

Introduction
Poly(alkylene citrates) (PACs) are novel biodegradable elastomers with potential application as substitutes of small blood vessels or as surface modifiers of the commercial Dacron® and ePTFE vascular prostheses. The advantages of PACs are non-toxic monomers and simple synthesis, which can be performed under mild conditions, as well as controllable mechanical properties and biodegradation kinetics. In addition, the PDC structure can be easily modified with D-panthenol to provide it with anti-oxidant properties. In this study, we report the results of two experiments on PACs modified with D-panthenol. In the first one, we used L929 cells to test cytocompatibility of 6-oxo-3,4-dihydro-2H,6H-pyrido[2,1-b][1,3]oxazine-8-carboxylic acid (ODPC), i.e. a product created during reaction of D-panthenol [AH1] with citric acid during polymerization, which is expected to be present in the modified PACs. In the second experiment, we tested PACs modified with D-panthenol in contact with human umbilical vein endothelial cells (HUVEC).

Experimental Methods
For isolation of pure ODPC, citric acid and D-panthenol at molar ratio of 1:1 were reacted (1 h, 180°C, 100 rpm), dissolved in methanol, separated by preparative liquid chromatography and freeze-dried. To test the potential cytotoxicity of ODPC, this compound was diluted in a DMEM medium in the concentrations of 1, 0.1, 0.01 and 0.001 mg/ml, and sterilized by filtration (0.22 μm syringe filter). L929 cells were seeded in 96-multiwell culture dishes at a concentration 5x10³ cells in 200 μl DMEM, and cultured for 24 h at 5% CO₂, 37°C. Next, the culture medium was aspirated and replaced by an ODPC solution with defined concentration or with a fresh medium (control sample). The viability of the cells was evaluated using live-dead staining (calcein-AM/propidium iodide based). The cell metabolic activity was measured using Alamar Blue assay (10% w/v resazurin sodium salt solution in phosphate-buffered saline). PACs were synthesised through polycondensation of citric acid with 1,6-hexamethylenediol or 1,8-octamethylenediol at molar ratio of 2:3. In brief, reagents were melted at 140°C for 40 min under stirring and subsequently purified, resulting in liquid prepolymers, in which D-panthenol at 0.4% or 0.8% concentration was added. Afterwards, the prepolymers aliquots were poured in 96-well plates and left for cross-linking for 8 days in a vacuum oven at 80°C, 200 mbar. Non-modified poly(1,6-hexamethylene citrate) (cPHC) and poly(1,8-octamethylene citrate) (cPOC) and those modified with D-panthenol (cPHC_P) and (cPOC_P) were sterilized with 70% ethanol and exposed to the UV light for 30 min. HUVEC in early passage, cultured in EGM-2 medium, were seeded on materials at a density of 5x10³ cells into 96-multiwell plates. After 24 h and 72 h, the cells were stained with hematoxylin/eosin and their morphology was assessed. The number of adhered cells, the cell spreading area and the cell aspect ratio were calculated.

Results and Discussion
In the first experiment involving L929 cells, the live-dead staining revealed almost all cells alive irrespectively of the ODPC concentration; the cell morphology was the same as in control conditions. Cells were distributed evenly and formed a monolayer after 72 h. The metabolic activity of cells in medium supplemented with ODPC in all studied concentrations was the same as in control conditions, what confirmed non-toxicity of ODPC. Higher D-panthenol
concentrations (4 and 8%) in PACs were found to impede the HUVEC adhesion and growth. Low concentrations of D-panthenol (0.4 and 0.8%) in cPHC_P and cPOC_P materials did not have a negative influence on the HUVEC morphology. The cells were less spread in comparison with their culture on TCPS, although they maintained their characteristic shape. Moreover, the cell viability and growth on cPOC were lower as compared to cPHC.

**Conclusion**
Tests with L929 cells showed that ODPC is non-toxic irrespectively of the concentration used. cPHC and cPOC were successfully synthesised and modified with D-panthenol. According to in vitro tests, cPHCs containing 0.4% and 0.8% of D-panthenol were found to be more compatible with HUVEC than cPOC. Thus cPHC seems to be prospective for vascular tissue engineering purposes. It will be further examined in terms of ability to support endothelial, smooth muscle and mesenchymal stem cell adhesion, proliferation and differentiation with the final goal to reconstruct small blood vessels.

**Acknowledgement**
This study was financed by National Science Center, Poland (2018/28/C/ST5/00461) and by the Program “Excellence Initiative – Research University” for the AGH University of Science and Technology. Further support was provided by the Ministry of Health of the Czech Republic (grant No. NV18-02-00422).

**References**
Improving osteogenic response of electrical discharge machined Ti6Al4V by plasma treatment

Deepak Rajendra Unune¹, Russell Goodall¹, Frederik Claeyssens¹, Gwendolen C. Reilly¹

¹ University of Sheffield, Material Science and Engineering, Sheffield, GB; ² University of Sheffield, Insigneo Institute of in silico Medicine, Sheffield, GB; ³ The LNM Institute of Information Technology, Department of Mechanical-Mechatronics Engineering, Jaipur, IN

Introduction
Ti6Al4V alloy usually need to undergo surface treatment to enable good bone growth and implant integration. Electrical discharge machining (EDM), owing to its simultaneous machining and surface modification ability, has emerged as a new trend for processing of biomaterials [1]. However, EDM treated surfaces are hydrophobic in nature which affect the osseointegration capabilities of the treated metal for implant applications. In this work, air plasma treatment is proposed to improve the hydrophilicity of EDM processed Ti6Al4V and to promote bone regeneration by supporting cell proliferation and differentiation.

Experimental Methods
Ti6Al4V plate was cut to prepare samples of 8mm X 8mm X 2mm size. Prepared sample were polished using Silicon Carbide papers from #400 to #1200 on a grinding machine and used for further treatments. Polished samples were used as control group. Two types of EDM treatments were performed including plain dielectric and hydroxyapatite mixed dielectric and samples were labelled as ‘EDM’ and ‘H-EDM’, respectively. EDM processing performed at a pulse current of 5 A and pulse-on-time of 15 µs. Air plasma was applied on EDM treated surfaces of with a power of 50 W and a pressure of 0.8 mbar for 60 s to improve cell attachment to the hydrophobic surfaces. The water contact angles were analysed using a drop shaper analyser with a 5 µL water droplet on all surfaces. The average surface roughness (Rₐ) of samples was evaluated using an optical focus variation microscope. Prepared samples were cultured using the immortalized bone marrow stromal cell line (Y201) [2]. To induce osteoblastic differentiation, osteogenesis induction media was added 24 h after seeding and changed over every 2-3 days. Resazurin reduction assays were performed to determine the appropriateness of treated samples for cell growth. Calcium deposition was evaluated through Alizarin Red S (ARS) staining analysis performed and the collagen deposition was evaluated using Sirius Red Stain Assay (SRS) assay on day 21 of cell culture.

Results and Discussion
Figure 1 (a) revealed generation of porous surface coating on Ti6Al4V, known to enable bone “ingrowth” into the implant and promoting a better bone-material connection. Plasma treatment significantly reduced the water contact angle of both EDM and H-EDM processed surfaces, thus inducing hydrophilicity post EDM processing (Fig.1(b)). H-EDM supported better Y201 cell growth and differentiation, as compared to EDM treatment, probably due to the coating containing Calcium and Phosphorous. Furthermore, plasma treated samples (EDMPT and H-EDMPT) supported enhanced metabolic activity of Y201 cells, in terms of Resazurin fluorescence (Fig. 1(c)), indicating a net increase in the viable cell number. Similarly, increased extracellular calcium deposition and collagen deposition was found for both EDMPT and H-EDMPT samples, as compared to EDM and h-EDM samples, respectively (Fig. 1(d,e)).

Conclusion
EDM is a promising technique for concurrent synthesis of an accurate shape of a biomaterial and provision of a favourable cell compatible surface for orthopaedic and dental applications. Hydroxyapatite containing dielectric EDM processing has shown better capacity for cell growth and osteogenic differentiation than that of pure dielectric based EDM. The proposed plasma treatment induced hydrophilicity and surface free energy to the implant surface post
EDM processing. Furthermore, plasma treatment has contributed to improved growth and osteogenesis of Y201 cells. Thus, plasma treatment can be exploited as an effective way to promote the formation of bone-like material on EDM treated implant surfaces.

Acknowledgement

This project was funded by the European Union’s Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreements No 836792 – EDiMplant and 777926 - NanoSurf.

![Figure 1: (a) Scanning electron microscopic images of treated surfaces; (b) Contact angle of treated samples.](image)

References


Influence of copper-substitution in calcium phosphate ceramics on endothelial cells

Arthur Brunel, Esther Pagès, Amandine Magnaudeix, Eric Champion

Université de Limoges, CNRS, Institut de Recherche sur Les Céramiques, UMR 7315, Limoges, FR

Introduction

Hydroxyapatite (HA) possesses a high biocompatibility making it an interesting candidate for bone graft substitutes. However, the use of HA scaffolds for the repair of large bone defects is hindered because of their inability to promote vascularization and blood vessel invasion within the porous network. Copper in its Cu(II) form is known to significantly stimulate endothelial cell (EC) proliferation with concentration as low as $10^{-5}$M (1). However, it can also induce cytotoxicity. The objectives of this study are to assess the biocompatibility of a novel copper-substituted hydroxyapatite (2) and to evaluate whether it promotes EC proliferation and/or activation.

Experimental Methods

Dense copper-substituted hydroxyapatite ceramics pellets containing 0.8 and 3.8 wt% of copper (Cu0.1HA: Ca$_{9.9}$Cu$_{0.1}$(PO$_4$)$_6$(OH)$_{1.9}$O$_{0.1}$ & Cu0.5HA: Ca$_{9.5}$Cu$_{0.5}$(PO$_4$)$_6$(OH)$_{1.5}$O$_{0.5}$, respectively) are seeded with C166 murine EC and cultured for up to 96h. EC viability is assayed using MTT metabolic assay or in situ calcein staining. Apoptotic cells are detected in immunofluorescence targeting cleaved-caspase 3. Proliferation is tested by incorporation and in situ detection of EdU. Proteins whose shift in expression is linked to EC function and activation such as MMP-9, VEGFR-2 or vWF are monitored by Western Blot. Wound healing assay is performed to evaluate migration and activation of EC. Finally, the release of ionic species in culture medium is characterized chemically by inductively coupled plasma.

Results and Discussion

Metabolic activity results indicate a good biocompatibility from the Cu0.1HA material with values comparable to the HA standard. On the other hand increased copper concentration in the Cu0.5HA material induces a loss of metabolic activity over time. Nonetheless, early results show similar cellular viability and no excess of mortality through apoptosis between the different materials. However, no adverse or positive effect on proliferation is found. Similarly MMP-9 enzyme levels are comparable between the tested materials. Further characterization are on the way to thoroughly assess their biological effects.

Conclusion

This novel copper-doped material shows comparable biocompatibility to the gold standard hydroxyapatite with the C166 EC.

Acknowledgement

This work was supported by institutional grants from the French Research National Agency, project CharaBioC (ANR-19-CE08-0003-01). The authors are grateful to Émeline Renaudie for assistance in hydroxyapatite synthesis.

References

Mechanical modeling of the porcine abdominal wall through a hyperelastic model.

Javier Ortiz-Ortiz, Georgina Carbajal-de-La-Torre, Marco A. Espinosa-Medina, Hilda Aguilar-Rodriguez, Ma. Lourdes Ballesteros-Almanza

Introduction
The abdominal muscles are responsible for biological functions such as breathing, containment, and protection of organs and tissues. The knowledge of the mechanical behavior of the abdominal wall is essential for the development of prostheses and techniques that generate favorable results for patients suffering from abdominal wall hernias. In the present work, an animal model of mechanical behavior in the transverse and perpendicular directions is proposed based on experimental data collected from porcine abdominal tissue due to the similarity concerning the human.

Experimental Methods
Abdominal wall muscle tissue samples were collected from pigs between 4 and 6 months of age. The samples were cut in the longitudinal and transverse directions to be analyzed in the CellScale® brand Univert® test machine. While the tests were being carried out, the samples were immersed in a saline solution to prevent dehydration. The data obtained were treated to obtain the stress-strain curves in each direction and in a next step to adjust the curves in the COMSOL Multiphysics® software using its optimization tool through the Levenberg-Marquardt least-squares algorithm. Various hyperelastic models were tested until the material model that best fits the experimental data is obtained.

Results and Discussion
The parameters of a hyperelastic model were obtained from experimental data for the longitudinal and transverse directions of the abdominal muscles of the pig, to obtain an animal model that is representative of the mechanical behavior of the human abdominal wall. Different mechanical behavior is observed in each direction, depending on the direction of the collagen fibers.

Conclusion
The generated model helps to describe the mechanical behavior of the abdominal wall in pigs, due to the similarity of the pig tissue concerning the human tissue, this model can be used in finite element analysis that generates results closer to the real behavior concerning to traditional mechanical behavior models.

Acknowledgement
The authors would like to thank financial support from the Conacyt Project CB-2014-243236.
2:30 p.m. – 4:00 p.m.

Poster floor

PS1-03 | Biomaterials for Tissue Engineering
3D printing strategy to design anisotropic and macroporous dense collagen hydrogels for tissue engineering applications

Marie Camman\textsuperscript{12}, Onnik Agbulut\textsuperscript{2}, Christophe Hélary\textsuperscript{1}

\textsuperscript{1} Sorbonne Université, CNRS, UMR 7574, Laboratoire de Chimie de la Matière Condensée de Paris, Paris, FR; \textsuperscript{2} Sorbonne Université, Institut de Biologie Paris-Seine (IBPS), CNRS UMR 8256, Inserm ERL U1164, Paris, FR

Introduction
Thanks to its biocompatibility and its biodegradability, collagen I is broadly used in tissue engineering to develop biomaterials such as dermal substitutes, scaffolds for neurons guidance or cardiac patches\cite{1}. Collagen based hydrogels, usually fabricated from low concentrated collagen solutions, are characterized by poor mechanical properties and low physical stability with a significant shrinkage by encapsulated cells\cite{2}. To circumvent these drawbacks, the addition of synthetic polymers or cross-linking are required to improve collagen hydrogel physicochemical properties\cite{3}. An original approach is to increase collagen concentration. Dense collagen hydrogels concentrated at 30 mg.mL\textsuperscript{-1} exhibit high mechanical properties, do not shrink and are stable over several weeks after in vivo implantation\cite{4}. However, their high collagen concentration limits cell infiltration and neovascularization from the host. In this study, dense macroporous collagen hydrogels have been shaped by 3D printing to better mimic extracellular matrix physical properties while promoting cell migration. In addition, intrinsic anisotropy of collagen fibrils has been generated by extrusion through the needle during printing within hydrogels to favor cell alignment, property required for axon growth or cardiomyocyte organotypic growth.

Experimental Methods
The 3D printing of dense collagen solutions (30 mg.mL\textsuperscript{-1}) allowed an easy hydrogel shaping and the alignment of collagen triple helices to form an anisotropic scaffold. The printing process by extrusion was performed within a gelation bath to set the collagen anisotropy quasi instantaneously and form fibrils. Besides, macropores were created using needles or a sacrificial matrix. This step created large macropores, appropriate for cell culture which will favor better cellular infiltration. The anisotropy of the final hydrogel was examined by polarized light and second harmonic generation microscopies. MicroCT was also performed to control and measure porosity. Finally, fluorescent light microscopy was performed to observe cells and their alignment on top of anisotropic collagen.

Results and Discussion
The first goal was to obtain anisotropic collagen gels. Combining a flat bottom extrusion needle and the suitable extrusion speed, collagen molecules aligned during the 3D printing process along the needle axis. However, the viscosity was too low to prevent relaxation. To tackle this issue, the collagen solution was printed within a buffer bath to trigger a rapid gelling. After the end of the 3D printing process, the gelling time was extended (from 24h to 7 days) using different gelling conditions. Ammonia vapors (30\% in aqueous solution) enabled the formation of collagen hydrogels with high mechanical properties (E=10 kPa) but without anisotropy. On the opposite, phosphate buffer 5X generated a gel with lower mechanical properties (E=3 kPa) but preserved collagen anisotropy. The anisotropy occurrence was observed by polarized light (Figure 1- A and B) and SHG microscopy (Figure 1- C). Gelling in a PBS 5X bath for two days followed by an incubation under ammonia vapors for one day was the optimal condition to generate anisotropy and high mechanical properties. To assess the impact of anisotropy on cells, fibroblasts were seeded on top of the printed hydrogels. Fibroblasts aligned along the collagen fibrils of anisotropic hydrogels following the axis of printing whereas they exhibited a random orientation on isotropic hydrogels only gelled by ammonia vapors (Figure 1- D and E). Beside anisotropy, the 3D construct requires a high porosity to allow cell colonization. Without 3D printing, there is no porosity within collagen hydrogels (Figure 2- A). Our novel 3D printing process generated an
intrinsic porosity of 20-40 µm in diameter between the different layers (Figure 2 - B). This porosity is suitable for vascularization or neuronal guidance. However, some tissues require a larger porosity to induce 3D organotypic cell growth. To increase the scaffold porosity, the addition of macroporous channels with sacrificial matrix printing or needles molding created larger channels ranging from 100 to 500 µm (Figure 2- C). These bigger channels were adequate for a 3D organotypic organization of cardiomyocytes. The porosity of the gels was controlled by microCT imaging and revealed well defined 300 µm pores with the needles process. In a heart tissue model, combining anisotropy and macroporosity is required to ensure a good conduction of the contraction and cell survival inside the gel.

**Conclusion**

These results demonstrate the performance of 3D printing of dense collagen solutions to generate elaborated biomaterials with macropores and anisotropy. Their characteristics can be tuned to suit for a specific field of applications. Small pores will be useful for neuronal guidance whereas larger pores will adequate to model bundles of muscle fibers.

**Acknowledgement**

This work received financial support from Sorbonne University – Program “Interfaces pour le vivant”.

---

Generation of anisotropy and effect on cell alignment

The range of porosity.

References
PS1-03-109

Biomimetic material for neuronal culture

Inês S. Pereira¹, Maria J. Lopez-Martinez¹,², Josep Samitier¹,²

¹ Institute for Bioengineering of Catalonia (IBEC), Institute for Bioengineering of Catalonia (IBEC), Barcelona, ES; ² Biomateriales y Nanomedicina (CIBER-BBN), Centro de Investigación Biomédica en Red en Bioingeniería, Madrid, ES; ³ University of Barcelona (UB), Department of Electronics and Biomedical Engineering, Barcelona, ES

Introduction

Brain modelling is mostly based on 2D in vitro or animal models but none offer the best solution to understand this organ and its related diseases. Recently, 3D in vitro models have been explored as they resemble more accurately physiological conditions. However, neuronal cultures face a challenge due to their high sensitivity to changes in their surroundings.

Experimental Methods

In this work we present a hydrogel composed of methacrylated gelatine and alginate for neural progenitor cell culture. Hyaluronic acid was added to this hydrogel composition to better mimic the native tissue. This material was characterized in terms of its Young Modulus and swelling capacity. Compatibility with neuronal cell culture and differentiation was also tested during 8 or 28 days in vitro.

Results and Discussion

Results showed that hydrogels with and without hyaluronic acid have good porosity, allowing nutrient and oxygen diffusion. They also present low Young Modulus, especially for hyaluronic acid formulation, rendering values similar to brain tissue. Comparing these new materials with Matrigel for 8 days, our formulations allowed neuronal proliferation and differentiation with high viability rates. After 28 days, the hydrogels in culture presented minimal degradation and cells showed projection growth, increased connectivity and expression of neuronal markers.

Conclusion

Overall, these results make these hydrogels a promising scaffold for induced pluripotent stem cells long-term culture and differentiation.

Maximum projection of immunostaining confocal images.

C17.2 cells after 28 days of differentiation embedded in (A) methacrylated gelatin and alginate or (B) methacrylated gelatin, alginate and hyaluronic acid.
PS1-03-111

Tailoring the physicochemical properties of tricalcium phosphate-based porous scaffolds

Joanna Czechowska, Szymon Skibiński, Ewelina Cichoń, Anna Ślósarczyk, Aneta Zima

AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Krakow, PL

Introduction
Implantable 3D scaffolds are widely applied for the restoration and reconstruction of bone defects. Recently, the focus is put towards biodegradable, macroporous templates with similarity to bone architecture. The most promising biomaterials applied for 3D scaffolds are tricalcium phosphates (α-TCP, β-TCP) due to their biodegradability and biocompatibility [1]. The production of scaffolds with favourable microstructure is one of the critical success factors in biomaterials development. The porosity parameters impact inter alia scaffolds permeability, cells infiltration, and angiogenesis [2].

Experimental Methods
In our study, the initial β-TCP powder was synthesized via the wet chemical method. Afterwards, three different methods of powder preparation were applied. Based on results from electrokinetic potential, specific surface area (BET) and grain size measurements by dynamic light scattering (DLS) the most favourable method of β-TCP powder preparation was chosen. Tricalcium phosphate-based scaffolds were prepared by a polymer sponge replication method. In order to tailor their physicochemical properties, different polyurethane matrices and various amounts of a solid phase (powder) per polyurethane sponge were used. Obtained materials were investigated by various techniques including powder X-ray diffraction, X-ray fluorescence, scanning electron microscopy, hydrostatic weighing and compression tests.

Results and Discussion
It was found that both the porosity and the mechanical properties of the scaffold depended on the kind of polymer sponge and the amount of β-TCP powder used. XRD analysis revealed that all materials composed of β-TCP 97 ± 1 wt% and α-TCP 3 ± 1 wt%. The microstructure of bioceramic scaffolds was characterised by a network of interconnected spherical pores with sizes between 100 to 1000 µm (Fig.1). Obtained materials possessed open porosity in the range of 56 to 70 vol% and compressive strength between 2.4 ± 0.7 and 4.8 ± 1.0 MPa. These results correspond to the porosity and compressive strength of spongy bone, which means they might act as potential scaffolds for bone tissue regeneration.

Conclusion
Developed biomaterials are promising candidates for bone tissue engineering. Further biological studies are needed.

Acknowledgement
Research funded by the National Centre for Research and Development, Poland, grant Technmatstrateg no. TECHMATSTRATEG2/407507/1/NCBR/2019 and the Faculty of Materials Science and Ceramics AGH UST - University of Science and Technology, Kraków, Poland, Project No. 16.16.160.557 (2021). JC has been partly supported by the EU Project POWR.03.03.00-IP.08-00-P13/18 - PROM NAWA
Fig. 1. 
The SEM microstructures of scaffolds with various porosity.

References

Development of the vibration stimulating culture system to evaluate cell activity under microscope

Yuki Masuda\textsuperscript{1}, Koji Yamamoto\textsuperscript{2}, Yusuke Morita\textsuperscript{2}

\textsuperscript{1} Doshisha University, Graduate School of Life and Medical Sciences, Kyoto, JP; \textsuperscript{2} Doshisha University, Department of Biomedical Engineering, Kyoto, JP

Introduction
Bone implants need to attach to bone in a short period after implantation. Mechanical stimulation with daily motion is involved in bone remodeling, and treatment with low-power high-frequency vibration and low-power ultrasonic vibration have been attempted in bone fracture treatment. Therefore, it is expected that vibration stimulation is effective to enhance bone formation at interface for early fixation between living bone and replacement implant. Vibration stimulation using a vibrating generator promoted cell proliferation and ALP activity of osteoblast-like cells \cite{1}. It is considered that vibration stimuli from outside of body propagates and affects to attached cells via surface of bone and implant. It is necessary to evaluate quantitatively evaluate effects of vibration stimulation on cell activity through adhesion surface to clarify the effect of vibration stimulation on bone formation. The purpose of this study was to develop the culture system that has vibration stimulation generated on culture surface and enable to evaluate effects of vibration stimulation on activity of MC3T3-E1 under a microscope.

Experimental Methods
The developed culture dish consisted of polypropylene chamber, PVDF as bottom surface and two ring-shaped electrodes (Fig.1(a)). PVDF was coated with PEDOT / PSS on both sides. Vibration stimuli was generated during cultivation by applying AC voltage to both sides of PVDF through ring-shaped electrodes. The culture area of the culture dish was 10 mm in diameter. MC3T3-E1 cells were cultured for on glass slide coated by PEDOT / PSS of 1.0, 1.5, 2.0 \% coating concentration to determine the concentration without toxicity. The glass slide without PEDOT/PSS was prepared as the control. After 1 and 3 days of cultivation, the number of adherent cells were evaluated by using MTT assay. Generated vibration stimulus with AC voltage were validated by measuring amplitude of vibrated culture surface with a laser doppler vibrometer. Applied frequencies were 3, 4, 5, 10, and 15 kHz and voltages were 2.5, 5, and 10 V\textsubscript{p-p}. Measuring points were center and points 2 mm and 4 mm from center. MC3T3-E1 cells were cultured for 12 hours in the culture dish, and then the cells were cultured for 12 hours under vibration stimulation with AC voltage of 15 kHz and 10 V\textsubscript{p-p}. MC3T3-E1 cells cultured for 24 hours without stimulation were prepared as the control. Expression of actin fibers and nuclei were observed with a fluorescence microscope to evaluate effects of vibration stimulation.

Results and Discussion
PEDOT / PSS showed low toxicity is not toxic, and number of attached cells increased with decreasing coating concentration of PEDOT / PSS. PEDOT / PSS concentration of 1\% was determined to coat both sides of PVDF. Figure 1 (b) shows the results of measurement of amplitude generated in the culture area under AC voltage of 15 Hz and 10 V\textsubscript{p-p}. Amplitudes at all measuring points showed sinusoidal wave form. The amplitude at the center was 0.185 \mu m, and amplitude value decreased toward the outer peripheral side of the culture chamber. Amplitude increased with increasing applied voltage and frequency. These results showed that vibration stimulation on culture surface can be controlled by the voltage and frequency. Figure 2 (a) and (b) show the fluorescence observation images. Development of actin fibers of MC3T3-E1 cells under by vibration stimulation was observed in comparison with the cells without stimulation. Vibration stimulation did not cause cell detaching from culture surface. It is expected that the developed culture system can evaluate effects of vibration stimulation on cell activity under an optical microscope.
Conclusion
We developed the culture system that enable to control vibration stimulation of the culture surface and evaluate cell activity under a microscope.

Figure 1
(a) Schematic drawing of the culture dish, (b) Amplitude of culture surface under AC voltage of 15 kHz and 10 Vp-p.

Figure 2
Fluorescent images of MC3T3-E1 cells cultured for 24 hours. (a) Control, (b) Stimulation, (Red: Actin, Blue: DAPI, ×200, bar: 25nm).

References
Magnetic bioactive glass-based 3D systems for bone cancer therapy and regeneration

Ricardo J.R. Matos1,3, João P. Borges1,3, Jorge C. Silva2,3, Paula I.P. Soares1,3

1 NOVA School of Science and Technology, Department of Materials Science, Caparica, PT; 2 NOVA School of Science and Technology, Department of Physics, Caparica, PT; 3 NOVA School of Science and Technology, CENIMAT|3N, Caparica, PT

Introduction
Malignant bone tumors are one of the main non-trauma factors resulting in critical size bone loss/defects. The subsequent regeneration of such defects still represents a clinical challenge [1]. Grafting procedures are considered the standard treatment for such bone defects. However, such implants in most cases result in unsuccessful repair of the defect. Moreover, after tumor extirpation, the local recurrence of the tumor cells is a major concern that requires further treatment as well. Therefore, a polymeric scaffold for bone regeneration that simultaneously kills residual tumor cells is of much benefit. Magnetic hyperthermia using superparamagnetic iron oxide nanoparticles (SPIONs) has emerged as a potential cancer treatment option since it is considered an effective treatment without adverse side effects [2]. On the other hand, mesoporous bioactive glasses (MBGs) dissolution products have demonstrated their effect on osteoblast cell gene expression and the potential effect on angiogenesis and neovascularization, which in turn promotes bone healing. Moreover, these systems can form an interfacial bond with bone tissue. We therefore propose a new concept for the treatment of bone cancer and the regeneration of bone defects: a polymeric scaffold produced by electrospinning containing MBG that combines magnetic hyperthermia therapy through the incorporation of the SPIONs into the scaffold [3].

Experimental Methods
The experimental work to produce the multifunctional scaffolds is divided into 3 parts: 1) production and characterization of SPIONs and MBGs individually; 2) Incorporation of these individual materials into a polyvinylpyrrolidone (PVP) solution; 3) Characterization of such composites. Herein we synthesized a sol-gel-derived MBG by templating with a block copolymer (pluronic F127) that acts as a structure-directing agent through an evaporation-induced self-assembly process [4]. MNPs were synthesized by chemical precipitation with a core diameter of about 10 nm. The electrospinning process was optimized through the design of experiments (DOE) method using the JMP software. Different electrospinning solutions of PVP/SPIONs (2, 5, and 8% of polymer's mass) and PVP/MBG (10, 28, 56, 83, 100% of polymer's mass) were prepared in order to obtain polymeric composites, which were then thermally crosslinked. Polymeric composite with both SPIONS and MBG added to the electrospinning solution was also prepared. The composites are under characterization in terms of heating ability, bioactivity, cytotoxicity, degradation rate, and swelling capability.

Results and Discussion
The produced MBG powder (Fig. 1 A, D) was morphologically characterized by N2 porosimetry (BET specific surface area of 232 m²·g⁻¹; BJH pore volume of 0.25 cm³·g⁻¹). The average pore diameter was about 5.9 nm as it's expected for a mesoporous bioactive glass. The mesoporous structure implies a higher specific surface area compared to non-mesoporous BAG (typically few m²·g⁻¹). Consequently, the mesoporosity implies a greater solubility of sol gel-derived BAG among other advantages such as enhanced protein adsorption [5]. Fig 1B and C represent the MBG after soaking 1 and 5 days in SBF solution, respectively. It's worth pointing out the morphological and chemical changes on the MBG surface less than 24h after soaking in SBF solution. The produced SPIONs (Fig. 1E) were magnetically characterized to assess and confirm their superparamagnetic
behavior. The composites have been produced by adding the MBG and SPIONs to the electrospinning solution. The SPIONs were also incorporated in the polymeric matrix by the adsorption method. In order to assess whether the materials keep their individual properties after blending, the individual materials were fully characterized. The PVP/MBG composites (Fig. 1F, G) were already characterized in terms of degradation ratio, swelling capability (Fig. 2A, B), and their bioactive behavior. Magnetic hyperthermia assays (Fig. 2C) were performed to evaluate the heating ability of SPIONs incorporated into PVP nanofibers (Fig. 1H, I). However, the composites produced by adding both MBG and SPIONs simultaneously to the electrospinning solution are currently under characterization.

**Conclusion**

PVP/SPIONs composites revealed their potential as possible candidates to perform magnetic hyperthermia therapy due to the heating ability of SPIONs within the membrane. Furthermore, more composite characterization is being done, particularly the bioactivity of PVP/MBG composites and the cytotoxic effect of both PVP/SPIONs and PVP/MBG composites. Lastly, the final PVP/MBG/SPIONs composite is being also characterized in order to assess whether the materials keep their individual properties allowing such composite to act as a multifunctional scaffold.

**Acknowledgement**

This work is funded by National Funds through FCT - Portuguese Foundation for Science and Technology, Reference UID/CTM/50025/ 2013 and FEDER funds through the COMPETE 2020 Program under the project number POCI-01-0145-FEDER-007688. R. Matos also acknowledges FCT for the PhD grant with reference SFRH/BD/140090/2018.

---

**Fig. 1**

SEM image of MBG before soaking in SBF solution (A), 1 day (B) and 5 days (C) after soaking in SBF solution; TEM images of MBG (D) and SPIONs (E); SEM image (F) and TEM image (G) of PVP/MBG composite; TEM image of PVP/SPIONs-DMSA (H) and PVP/SPIONs-AO (I) composites.
Degradation assay (A) and swelling assay (B) of PVP/MBG composites with different concentrations (% w/w) of MBG; Temperature variation generated by PVP/SPIONs composites (20 mg) during 10 min of an AC magnetic field application (300 Gauss; 418.5 kHz) (C).

References
A tubulogenesis assay to characterize the influence of hydroxyapatite-based ceramic properties on endothelial cells

Adeline Dumur¹,², Julie Usseglio¹,², Esther Pagès¹,², Emeline Renaudie¹, Joël Brie¹,², Eric Champion¹, Amandine Magnaudeix¹

¹ Limoges university, IRCER, Limoges, FR; ² Limoges university hospital center, maxillofacial surgery, Limoges, FR

Introduction
Vascularisation is mandatory for bone healing. Thus vascular endothelial cells actively participate in tissue repair through molecular communication with resident cells in the injured tissue. Hydroxyapatite-based ceramics (HA - Ca₁₀(PO₄)₆(OH)₂) are biocompatible and osteoconductive. They have been widely used as porous scaffolds in bone regenerative medicine since the 1980’s but an improvement of their ability to be vascularized is required to extend their applications.

Cells are sensitive to the chemical-physical properties of their environment modulating their activities. This influence depends on the cell type and may differ for endothelial and bone cells. A strategy to improve endothelial cell growth at the ceramics surface is to act on chemical and microarchitectural design of the scaffolds. Silicon has been described as acting positively on endothelial and bone cell activity¹. Microporosity, by increasing surface area and modulating its topography has an influence on the quality of bone cell colonization².

The first objective, was to investigate colonization of HA-based ceramics surface by endothelial cells, and their ability to form tubules, as a function of the composition (pure HA or silicon doped HA: SiHA) and microstructure (dense or microporous ceramics). This needed 3D conditions that were achieved by the set up of an expedient and efficient experimental protocol using fibrin-gels. With the aim of modelling the interaction between cells in bone tissue and taking account cell communication, coculture of pre-osteoblast and endothelial cells will be investigated in the same conditions, which required first to select an accurate ratio of the different cell types that constituted the second objective of this study.

Experimental Methods
Pellets of sintered HA and SiHA (Ca₁₀(PO₄)₅.₆(SiO₄)₀.₄(OH)₁.₆) were manufactured from well characterized powders³,⁴. Sintering temperature and duration allowed to obtain 2 different microstructures: dense and 25% microporous. An experimental method based on the use of a fibrin-based gel was developed to study the ability of cells to form vessels on the surface of materials: C166 endothelial cells were seeded either on top of a fibrin gel poured on HA ceramics or directly on ceramics surface before the addition of the fibrin gel, and cultured for 7 and 14 days. For the co-culture assays, pre-osteoblastic MC3T3-E1 cells and C166 endothelial cells were seeded on a fibrin-based gel at variable ratios: 2/1, 1/2, 1/1 for the same culture duration. After cell fixation and immunofluorescence staining, cells on ceramics were imaged. Computer-aided analysis of the images and 3D reconstructions allowed to evaluate the quality of tubulogenesis according to the materials properties.

Results and Discussion
Proliferation studies show the biocompatibility of the tested materials with a positive effect of Si on dense material. The fibrin-gel was effective to induce the formation of tubular structures on the surface of the 4 types of materials (dense and porous HA or SiHA) (Fig. 1). Importantly, the formation of tubules seems to differ according to the ceramics microstructure. The dense biomaterials appear to be more favourable to the formation of tubules than the porous ones. These vascular structures express VEGF2 and vWF. The influence of silicon addition is currently under investigation.
From cell co-cultures, organized tubular structures are obtained whereas there is only an unorganized cell layer in cultures with C166 cell type alone. The co-culture at a C166/MC3T3 ratio of 1/2 appears to be particularly interesting.

Conclusion
In this work an efficient method was set up to study the tubulogenesis on ceramics. The first results are promising with the formation of tubular structures on the biomaterial. Quantitative analysis of the tubules number, length and diameter are currently under progress to evaluate the influence of the materials properties. A predominant effect of endothelial cell/bone cell communication was also highlighted with a more favourable 2:1 bone cell/endothelial cell ratio.

Acknowledgement
This work was funded by institutional grants from the French Research National Agency, project CharaBioC (ANR-19-CE08-0003-01) and from LabEX SigmaLim (ANR-10-LABX-0074-01). The authors are grateful to Claire Carrion and the Confocal Imaging Platform of the University of Limoges - BISCEm.

Reference
Self-assembling Soft Peptide-Hyaluronan Hydrogels as 3D Environments for Stem Cell Culture

Yichen Yuan, Jayati Banerjee, Helena Azevedo

Queen Mary University of London, School of Engineering and Materials Science & Institute of Bioengineering, London, GB

Introduction
The extracellular matrix (ECM) of tissues contains multiple proteins and polysaccharides that provide much more than mechanical support for cells. Mimicking this intricate network has been a major goal in biomaterials engineering. Peptides are obvious choices to engineer synthetic ECMs as they can be designed to self-assemble into nanostructures with defined shape (e.g. nanofibers) and display desired functionality to control cell functions (e.g. cell adhesion, proliferation, differentiation). Hyaluronic acid or hyaluronan (HA) is an essential polysaccharide of the ECM mediating activities in cellular signalling, wound repair, morphogenesis and matrix organization [1]. As such, it has attracted large interest as starting biomaterial. However, to impart stability and improve functions of HA hydrogels, covalent and noncovalent crosslinking approaches have been exploited, requiring chemical modification of HA [2]. Using small positively charged peptide amphiphiles, the cross-link of unmodified HA could be triggered by self-assembly, where stable membranes and sacs were obtained [3]. Here we present the fabrication of novel 3D supramolecular hydrogels containing HA and rationally designed peptides. The gels are formed by self-assembly without requiring any chemical modification of HA, and their biomimetic composition offer the possibility to recapitulate stem cell niches.

Experimental Methods
Peptide-HA hydrogels were formed via self-assembly by casting aqueous solutions of rationally designed peptides on top of HA (2 MDa) solution. The micro-nanostructure of the gel was examined by scanning electron microscopy (SEM), and the mechanical properties evaluated by rheology (amplitude sweeps at frequency of 1 Hz). For 3D cell culture, hydrogels were first swollen in the culture media (DMEM with 10% FBS) and then human mesenchymal stem cells (hMSCs) were seeded inside the gel. The cell viability was assessed using the LIVE/DEAD assay and analysed by confocal microscopy.

Results and Discussion
Stable peptide-HA hydrogels were obtained which could be easily handled (Figure A). SEM examination revealed randomly entangled nanofibers forming the hydrogel network (Figure B-D). Rheology measurements showed elastic modulus $G'$ of 541±163 Pa and viscous modulus $G''$ of 251±50 Pa at 1% strain (within linear range) indicating a gel state. hMSCs encapsulated in the peptide-HA hydrogel have a good viability on day 3 (Figure E). Cells tended to aggregate and form sphere-like structures, which is different from the round-shaped morphology observed when entrapped into other supramolecular peptide-polymer hydrogels [4].

Conclusion
The excellent biocompatibility of the peptide-HA hydrogels, and their possibly tunable physical and biochemical properties, suggest hydrogel application as 3D artificial stem cell niches for differentiation or self-renewal.

Acknowledgement
Y. Yuan acknowledges China Scholarship Council for her PhD scholarship (no.201706630005).
The supramolecular peptide-HA hydrogel for 3D hMSC culture

References


3D printed hydrogel scaffolds for tissue engineering applications: an in-depth mechanical analysis as the key to success

Martina Meazzo¹, Fabrizio Barberis², Catherine Van Der Straeten¹, Peter Dubruel¹

¹ Ghent University, Ghent, BE; ² Genova University, Genova, IT

Introduction
Tissue engineering (TE) is known as a methodology that mimics the physiological microenvironment by combining biology, engineering and materials science to repair or replace damaged tissue. Incorporating the three elements of the tissue engineering triad (cells, biological factors as signals and scaffolds, figure 1) needs a good scaffolding technique [1]. Additive manufacturing techniques are the most common due to their potential to fabricate organized tissue constructs to repair or replace damaged or diseased human tissues and organs. The scaffold is expected to perform various functions, including the support of cell colonization, migration, growth and differentiation. Further, for their design the physicochemical properties, the morphology and the degradation kinetics need to be considered. External size and shape of the construct are of importance, particularly if it is customized for an individual patient. Besides the physical properties of a scaffold or matrix material (e.g. stiffness, strength, surface chemistry, degradation kinetics), the micro-architecture of the constructs is of great importance for the tissue formation process [2,3].

Experimental Methods
Starting from a patented polymer family (structure cannot be disclosed), 3D scaffolds were developed using an extrusion-based 3D printing device.
The printing parameters that were varied include the printing temperature, the printing speed, the pore dimension, the strut size and the printing pattern (0°/90°, 0°/45° etc). The scaffolds were subjected to an in-depth analysis including scaffold visualization through optical microscopy and a study of the mechanical properties using different techniques.

Before being mechanically tested, the efficiency of the UV-cross-linking process of the developed scaffolds was verified through sol-gel testing. Minimal gel-values of 80-90% are targeted as go/no go criterion for further testing.

Results and Discussion
Using our newly developed polymer class, reproducible scaffolds were obtained as exemplified in the following figure.
To realize this, the printing temperature and – speed required optimization. The optimal values were 57°C and 350 mm/s respectively. The gel fraction of all developed scaffolds exceeded 90%.
In a first part of the mechanical testing, the 3D printed scaffolds were subjected to mono-axial compression using Dynamic Modulus Analysis – DMA as non-destructive technique (NDT). The studies showed that the material revealed mechanical properties relevant for various TE applications. Indeed, our preliminary tests showed a clear and definite loss modulus (i.e. the dissipative part of the Young modulus) highlighting the viscous properties of the polymer and representing energy lost as heat or dissipated during one cyclic load.
As the produced scaffolds have a cylindrical shape and water-swollen dimensions of 7 mm in height and 15 mm of diameter, two pre-set displacements of 0.2 mm and 0.3 mm were investigated in DMA. The scaffolds Young modulus between the two pre-set displacements increased with 30% due to a more compacted structure and an increase of full-to-empty ratio of the scaffolds. The increase could also be noticed by the difference in loss modulus between the two pre-set displacements. Indeed, a higher applied load resulted in more contact points within the structure and therefore, in a greater dissipation. The results achieved during the NDT were then used to optimize the 3D printing
parameters. The results showed that the complex modulus of scaffolds with the 0°/45°/90°/135° printing pattern was higher than the pattern 0°/45° and 0°/90°, most likely due to its more isotropic structure (figure 2).

In a second stage of our testing campaign, the samples applied for NDT were subjected to destructive mechanical testing and the collected data were compared to detect both the stiffness and the energy dissipation features of the developed scaffolds. The results obtained from destructive compression static testing confirmed the DMA results: the highest Young modulus was obtained for the 0°/45°/90°/135° pattern.

When developing implant materials, sample storage is an important aspect as it determines, if possible and required, the to be applied storage conditions. To remove the small fraction of soluble material from the printed scaffolds, leaching was performed in water prior to removing the water by either freeze drying or desiccation. Very interestingly, despite DMA revealing no significant effect of the drying method on the mechanical properties, scaffold visualisation through optical microscopy indicated some damaged scaffold trabeculae (figure 2). The freeze-drying thus causes some internal stresses that lead to these effects.

**Conclusion**

An in-depth mechanical analysis revealed several aspects to be extremely important in developing 3D printed hydrogel scaffolds. These include optimization of the printing pattern and the drying methodology for the anticipated sample storage.

**Acknowledgement**

Internal funding UGent
Figure 2
Optical microscopy images of cylindrical scaffolds (enlargement 5X). On the top part, it is shown three different scaffolds with the same strut and porosity but with the three different printed patterns (0°/90°, 0°/45°, 0°/45°/90°/135°) analyzed.

On the bottom part, images of 0°/90° printed pattern samples after freeze-dryer on the left (showed discontinuities in the trabeculae) and after essicator on the right.

References
Development of porous 3D polysaccharide-based hydrogel with microchannels for vascularization

Chau Le Bao¹², Margaux Bourhoven¹³, Frédéric Chaubet¹², Didier Letourneur¹, Teresa Simón-Yarza¹

¹ INSERM U1148, Université de Paris, X Bichat Hospital, Paris, FR; ² Université Sorbonne Paris Nord, Galilée Institute, Villetaneuse, FR; ³ École Nationale Supérieure d’Arts et Métiers, Metz, FR

Introduction
The development of hydrogel-based biomaterials that can promote vascularization presents a great challenge in tissue engineering. 3D hydrogels (HGs) which can mimic soft tissue mechanical properties are promising candidates as scaffolds for tissue regeneration. Porous HGs are ideal for vascularization strategies as they facilitate nutrient and oxygen diffusion and enable cell migration¹. However, interconnected pores alone are not sufficient to promote anastomoses with host vasculature upon transplantation. Numerous studies have shown that the addition of channels inside a porous scaffold can facilitate cell growth and rapid vascularization, resulting in enhanced tissue formation¹. In this work, porous HGs with microvessel-like tubular structures were generated via two distinct strategies: i) mechanical removal and ii) dissolution of sacrificial components. Each method allows for the formation of microchannels with simple to complex designs and varying diameters of 100-500 µm.

Experimental Methods
Porous HGs were synthesized using a patented method as previously described². For HGs with microchannels, either the removable filaments (clinical-grade polypropylene filaments) or the sacrificial components (PVA or alginate gel) were positioned in between two spacers to guide channel formation and to control gel thickness. Finally, the gels were freeze dried following an established protocol³. The 3D prints were designed using Fusion 360 Autodesk software. PVA or PLA filaments were printed using Ultimaker S3 printer with nozzle diameter of 250-400 µm. Different tubular geometries and diameters were developed. Printing parameters such as layer height and infill speed were optimized for each design to ensure smooth prints with no deformation. To form alginate gel, alginic acid sodium salt 5% w/v and calcium chloride 5% w/v solutions were prepared by mixing each substance in miliQ water. The alginate solution was applied onto a 3D-printed PLA mold. Immediately afterwards, the template was immersed into CaCl₂ solution to crosslink the alginate gel. 3D printed PVA templates were dissolved in miliQ water and alginate templates were dissolved in EDTA 1M. Scanning electron microscopy was conducted to analyze the macro- and micro-structures of HGs, using the JEOL JSM-IT100 system under low vacuum conditions. Porosity and swelling studies were conducted as previously described⁴,⁵.

Results and Discussion
Macroscopically, the freeze-dried gels had pores that are visible with the naked eyes. SEM images confirmed the porous structure HGs where macropores were observed on the surface, while interconnected, micropores were present inside the HG. For HGs with simple, straight channel, hollow microchannel was revealed in the middle of the HG with pores existing inside the channel structure. Porosity measurements showed porosity values of 30-40%. Pore size was determined to be around 200 µm. Swelling ratio was 12.5±5. All channel formation methods produced HGs with hollow, smooth, and interconnected microchannels.

Conclusion
Mechanical removal method offers a facile setup and enables formation of linear channels with diameter as small as 100 µm. Sacrificial templates produced by fuse deposition modeling technology can generate channel-like structures with complex geometries, but also adds another level of complexity in the synthesis procedures.
Figure 1
Schematic illustration of hydrogel synthesis without channels (a) and with channels using two different strategies (b,c)

Figure 2
Different 3D designs of templates used to fabricate sacrificial components (PVA and alginate gel)

References
Tuneable fibrin scaffolds that enable on-demand secretome therapy

Christopher Lally, Abhay Pandit

NUI Galway, CÚRAM, Galway, IE

Introduction
Bone marrow stromal cells (BMSCs) exert short-range reparative effects in response to injury or disease, primarily via secreted signalling factors such as cytokines. The amount and nature of cytokine secretion are highly responsive to changes in the BMSC microenvironment. This inherent responsiveness allows for cytokine secretion to be experimentally tuned in-vitro using biomaterial scaffolds. This project aims to use fibrin, an endogenous hydrogel formed through blood coagulation, as a tuneable scaffold for BMSC implantation capable of enhancing therapeutic cytokine secretion while offsetting cell survival challenges. By varying cell density as well as fibrinogen and thrombin concentrations, this project aims to produce a fibrin-BMSC construct optimised for secretome therapy.

Experimental Methods
This study examined the effects of cell seeding density and gel formulation on the performance of a fibrin-BMSC construct intended for use in disease therapy. By combining three fibrinogen concentrations (6, 12, or 18 mg/ml) with three thrombin concentrations (2, 7.5 or 15 units/ml), nine distinct formulations were tested. 100 µl fibrin gels were prepared at a cell density of 0.1, 0.4 or 0.8 million cells/ml. All assays were performed on day three and day seven. Cell survival was measured by annexin V-FITC/propidium iodide flow cytometry. The growth and adaptation of the BMSCs throughout the fibrin gels were quantified using confocal microscopy and 3D image segmentation tools. The viscoelastic properties of the gels were compared to literature values for brain tissue via rheometry. Flow cytometry bead-based multiplex immunoassays (R&D Systems Luminex®/ Bio-Rad Bioplex®) were used to measure the secretion of cytokines and interleukins from fibrin-encapsulated BMSCs. Eighteen cytokines with potential therapeutic relevance in neurodegenerative disease and a mix of 11 pro- and anti-inflammatory interleukins were quantified.

Results and Discussion
Annexin V FITC/propidium iodide flow cytometry confirmed cell survival above 75% for all gel formulations on day three and seven. The BMSCs appeared to be adapting successfully to the 3D fibrin environment when visualised by confocal microscopy. In all gel formulations, cells grew larger and adopted a spread, fibroblastic morphology over time. The rheological analysis demonstrated that the fibrin gels had viscoelastic properties similar to published values for brain tissue. The gels also did not display significant swelling over 48 hours post-formation. Multiplex immunoassay demonstrated that cytokines and interleukins involved in angiogenesis, inflammation, CNS signalling events and regeneration were secreted by encapsulated BMSCs (Fig. 1). A mixed-model ANOVA with Tukey’s correction for multiple comparisons revealed that variations in fibrinogen and thrombin contributed significantly to differences in cytokine secretion (Table 1).

Conclusion
As cells appear to thrive in all formulations tested, it can be concluded that the system supports a broad range of tuneability without compromising cell viability. The soft, non-swelling nature of the fibrin gels minimises secondary injury risk in their intended role as an in-situ forming, space-filling hydrogel for brain applications. These results imply that a tuneable fibrin scaffold could be a useful means of delivering tunable BMSC-derived cytokine therapy to the neurodegenerative brain.

Acknowledgement
This work has emanated from research supported by a research grant from Science Foundation Ireland (SFI), funded under the European Regional Development Fund through Grant number 13/RC/2073_P2, and National University of Ireland, Galway Hardiman Scholarship. The authors acknowledge the facilities and scientific and technical assistance of the Centre for Microscopy & Imaging and Flow Cytometry Core Facilities at the National University of Ireland Galway.

Cytokine Secretion by Day and Gel Formulation

Figure 1. Multiplex immunoassay at day three and day seven detected angiogenic and neuroprotective cytokines secreted by BMSCs encapsulated in nine different fibrin gel formulations – 6, 12, or 18 mg/ml fibrinogen with 2, 7.5 or 15 units/ml thrombin. Presented here are the results from the highest cell seeding density, 0.8 million cells/ml. Results drawn from duplicate measurements of pooled triplicate samples.
Percent contribution of each factor to observed variance in cytokine secretion

Table 1. Mixed-model ANOVA with Tukey’s multiple comparison test showed how much of the variation in quantity detected for each analyte was accounted for by the fibrinogen variable, thrombin variable, or the interaction between both variables.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Percent Contribution to Observed Variance</th>
<th>Day 3</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fibrinogen</td>
<td>Thrombin</td>
<td>Interaction</td>
</tr>
<tr>
<td>Angiogenin</td>
<td>66.5</td>
<td>13.57</td>
<td>19.76</td>
</tr>
<tr>
<td>Angiopoietin</td>
<td>27.71</td>
<td>25.45</td>
<td>42.26</td>
</tr>
<tr>
<td>IL-6</td>
<td>4.108</td>
<td>83.74</td>
<td>11.55</td>
</tr>
<tr>
<td>IL-8</td>
<td>51.27</td>
<td>36.33</td>
<td>12.15</td>
</tr>
<tr>
<td>VEGF</td>
<td>4.376</td>
<td>39.02</td>
<td>53.28</td>
</tr>
<tr>
<td>FGF2</td>
<td>7.742</td>
<td>52.71</td>
<td>39.49</td>
</tr>
<tr>
<td>BDNF</td>
<td>56.15</td>
<td>11.15</td>
<td>26.83</td>
</tr>
<tr>
<td>GDNF</td>
<td>49.94</td>
<td>26.28</td>
<td>21.42</td>
</tr>
</tbody>
</table>

P-value: ns>0.05, *<0.05, **<0.01, ***<0.001, ****<0.0001

Table 1. Mixed-model ANOVA with Tukey’s multiple comparison test showed how much of the variation in quantity detected for each analyte was accounted for by the fibrinogen variable, thrombin variable, or the interaction between both variables.

References

Melt-Electrowritten Scaffolds with Ultrasmall Superparamagnetic Iron Oxide Nanoparticles as Contrast Agent for Non-Invasive Magnetic Resonance Imaging

Kilian M.A. Mueller¹, Geoffrey J. Topping², Sebastian P. Schwaminger³, Younzh Zou¹, Diana M. Rojas-González¹, Elena De-Juan-Pardo⁴, Sonja Berensmeier³, Franz Schilling², Petra Mela¹

¹ Technical University of Munich, Chair of Medical Materials and Implants, Department of Mechanical Engineering, Garching, DE; ² Technical University of Munich, Department of Nuclear Medicine, School of Medicine, Klinikum rechts der Isar, Munich, DE; ³ Technical University of Munich, Bioseparation Engineering Group, Department of Mechanical Engineering, Garching, DE; ⁴ The University of Western Australia, Translational 3D Printing Laboratory for Advanced Tissue Engineering, Harry Perkins Institute of Medical Research, Perth, AU

Introduction
Melt electrowriting (MEW) is an advanced fiber-forming technology for additive manufacturing of micro-structured scaffolds for tissue engineering. The translation of such constructs to the patient requires the capability to visualize them upon implantation with clinically accepted methods such as magnetic resonance imaging (MRI). To this end, this work presents the modification of polycaprolactone (PCL) scaffolds with ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles as contrast agents to render them visualizable by MRI.

Experimental Methods
USPIOs were synthesized with a primary particle size of 10 nm. USPIO/PCL composites of different weight percentages (0.1, 0.2, and 0.3 % w/w) were produced. The composites were melt-electrowritten at 75 °C through a 23 G needle onto a grounded collector with pressurized air at 2 bar applied to the print head, a voltage of +5 kV at the needle and a working distance of 3.1 mm. Scaffold architecture and fiber diameter (n = 3, i =10) were assessed by SEM. The composites’ mechanical properties were investigated by tensile testing (n = 10). Their cytocompatibility was tested with a cytotoxicity assay (n = 3) and cell adhesion was verified via fluorescence microscopy. MRI of the scaffolds was performed with a 7 T scanner and a 10 mm inner diameter solenoid radiofrequency coil.

Results and Discussion
Melt electrowriting of the composites resulted in regular and highly ordered micro-structured 3D scaffolds for all USPIO concentrations, without any electrical instabilities. USPIO incorporation caused small to no decrease in ultimate tensile strength and Young's modulus. USPIO/PCL composites were cytocompatible at all concentrations. In MRI, pure PCL showed no contrast with agar-agar in R1, R2, or R2* relaxation rate maps. However, with USPIO-labeled PCL, the contrast in R2 and R2* relaxation maps strongly increased. Hence, the composite scaffolds were sensitively detected in vitro using T2- and T2*-weighted MRI.

Conclusion
This work contributes to the material library for MEW by successfully incorporating USPIOs into PCL - the gold standard polymer for MEW. Our findings open the possibility of using MRI to effectively visualize melt-electrowritten scaffolds with a clinically accepted non-invasive monitoring option.
2:30 p.m. – 4:00 p.m.

Poster floor

PS1-04 | Biomaterials for Orthopedic Applications
**PS1-04-131**

**Ti/Ta composite hydrogel scaffolds for bone tissue engineering**

**Luis García-Fernández**¹,², Hector García-Robledo³, Basilio de la Torre³, Blanca Vázquez-Lasa¹,²

¹ Institute of Polymer Science and Technology (ICTP-CSIC), Biomaterials group, Madrid, ES; ² Centro de Investigacion Biomédica en Red, Bioingeniería, Biomateriales y Biomedicina (CIBER-BBN), Madrid, ES; ³ Hospital Ramón y Cajal, Madrid, ES

**Introduction**

The regeneration of bone tissue continues to be an important challenge in orthopaedic surgery and traumatology. Critically sized bone defects need to be filled with a bridging material, in combination with suitable cells and molecules, to promote the regeneration of bone tissue[1]. Different biomaterials have been used as graft materials or bone substitutes. In this work, natural polymers like Chitosan (Ch) and Arabic Gum (G) were chosen due to their good biological and biochemical properties to prepare a composite scaffold reinforced with either Ti or Ta microparticles due to their good osteoinductive capacity [2]. Recent studies showed that osteoinduction on Ta implant is significantly higher than in Ti implants [3]. In this work, we propose to evaluate the biocompatibility and osteoinduction capacity of Ti/Ta loaded scaffolds.

**Experimental Methods**

**Synthesis of Ti/Ta composite hydrogel scaffolds**

Ch chloride aqueous solution (1%wt) was mixed with G (Ch:G = 80:20) and the corresponding Ti or Ta microparticles (Ti, size < 44 µm; Ta, size < 45 µm). The system was crosslinked with oxidized dextran (1 wt % respect to polysaccharides) at 37 °C for 1 h under stirring. The mixture was poured in multiwell plates and left to dry. Finally, the scaffolds were washed with a NaOH solution (0.1 M) and PBS (pH = 7.4) until neutral pH.

**Release assays**

Release of metal was studied by immersion of samples in PBS at 37°C. At different periods of time aliquots of the release medium were taken and analysed. Quantitative determination of Ti and Ta aliquots was performed by inductively coupled plasma optical emission spectrometry (ICP-OES).

**Cytotoxicity analysis**

Composite scaffolds were submerged in 5 mL of culture medium and placed on a shaker at 37 °C. The medium was removed after different time periods (2, 7, 14 and 21 days) and the toxicity of the extracts was tested on Human Mesenchymal Stem Cells (hMSCs). Cell viability was analysed with the MTT reactive.

**Cell adhesion and proliferation assay**

Composite scaffolds were placed in a 48-well plate and hMSCs were seeded on them and incubated for 24 h. The medium was removed after different time periods (1, 7 and 14 days) and cell adhesion and proliferation on the samples were quantified using Alamar Blue reactive.

**Alizarin red assay**

Calcium deposition on the scaffold surfaces was evaluated by Alizarin red assay after 21 days of incubation. For this experiment, hMSCs were seeded on the scaffolds for 14 days. Ca depositions were analyzed using Alizarin Red reactive.

**Results and Discussion**

SEM micrographs of representative composite scaffolds showed smooth and uniform surface for Ch80G20 scaffolds and the presence of aggregates of Ti and Ta for the corresponding metal loaded samples (Figure 1a). Release of metals from scaffolds was very scarce (Figure 1b). In fact, it was almost negligible for either Ti or Ta that means that the majority of metallic nanoparticles remained adhered to the surface of the scaffolds.
Any synthesized scaffold did not present cytotoxicity (Figure 2a and 2b) giving values of cell viability higher than 80% in all samples, and even for those containing Ta in the highest proportion, cell viability values around 100% or superior were observed. Cell adhesion and proliferation behavior is shown in Figure 2c. Cell proliferation increased over time being higher for composite samples than for the Ch80G20 control. The presence of Ti or Ta improved the proliferation of hMSC on the surface specially for samples with (Ch/G):metal ratio 1:3 (Figure 4a). In the case of Ti samples, the decrease on cell proliferation with the concentration of Ti could be related to the increase of toxicity at 21 days (Figure 2a). Alizarin Red results (Figure 2d) showed a remarkable increase on Ca depositions with the increase of Ta concentration respect to that observed in Ti containing samples. This indicates that the presence of Ta in the composite scaffold improved the osteoconductivity of the sample.

**Conclusion**

Composites scaffold based on polysaccharides and Ti or Ta microparticles are proposed as composite systems with promising characteristics for bone defect regeneration. Specially scaffolds containing Ta can be considered a good option due to their remarkable osteoconductive properties.

**Acknowledgement**

This work was supported by the Ministry of Science, Innovation and Universities (Spain) (MAT2017-2017-84277-R), Instituto Salud Carlos III (ISCIII)-Fondo Europeo de Desarrollo Regional (FEDER), Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN). L. García-Fernández and B. Vázquez-Lasa are members of the SusPlast platform from CSIC.
Figure 2
Cell viability (%) of hMSCs cultured with extracts of Ch80G20-Ti (a) and Ch80G20-Ta (b) scaffolds. (c) Cell proliferation values of hMSCs seeded on the composite scaffolds surfaces. (d) Relative Alizarin Red content on hMSCs cultured on the surface of different composite scaffolds. Results are expressed as mean value ± SD (n=6). Significant differences are compared with Ch80G20 at same times (* p<0.05; ** p<0.01; *** p<0.001).

References
PS1-04-133

Effects of the synthesis conditions on the hydrolysis of α-tricalcium phosphate to octacalcium phosphate

Ilijana Kovrlija1, Clara Barbut1, Janis Locs1,2, Dagnija Loca1,2

1 Riga Technical University, Rudolfs Cimdins Riga Biomaterials Innovation and Development Centre, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga, LV; 2 Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, LV

Introduction

Being one of the members of calcium orthophosphates, octacalcium phosphate (OCP, Ca₈(HPO₄)₂(PO₄)₂x5H₂O) is presumed to be the precursor during the crystallization of hydroxyapatite (HAp), where the interlayered structure made of an apatite layer and a hydrated layer plays an important role. The similarity of the structure gives several benefits to OCP. Moreover, the OCP’s idiosyncrasy to be the first one to induce the bone tissue appearance made it indispensable to research it even further. The most common way to synthesize OCP is through precipitation route or through hydrolysis of α-tricalcium phosphate (α-TCP) and brushite. Even though α-TCP is often used as a precursor, the reaction can be influenced by the number of factors such as temperature, pH and time. This can lead to the formation of different CaP phases, thus the aim of the current study was to examine the influence of different parameters on the hydrolysis of α-TCP to OCP, and subsequently, to perform the scale up of the reaction.

Experimental Methods

OCP was synthesized by hydrolysis of α-TCP powder. In the first set of experiments, the synthesis in open and closed system were performed, where at least five independent trials were performed for each approach. 100 mg of α-TCP were deposited into 50 mL of 0.0016 M H₃PO₄ solution at room temperature, under constant stirring, with an initial pH of 6. In the second set of experiments, the synthesis using the same starting reagents and process parameters was scaled up tenfold. Reaction time was 24 h and 72 h, respectively. The obtained products were centrifuged for 2 min at 3000 rpm, washed with deionized water and dried overnight at 37 °C. X-ray powder diffraction (XRD) and Fourier transform infrared–attenuated total reflectance (FTIR-ATR) were employed to verify the composition of all the obtained samples, while the Specific Surface Area (SSA) was determined by Nitrogen Adsorption following the calculations applying Brunauer–Emmet–Teller method (BET).

Results and Discussion

Crystallographic structures of the synthesized materials were confirmed by the XRD patterns. Based on the OCP reference pattern (ICDD PDF 00-026-1056), all of the obtained samples exhibited the position and shape of characteristic maxima which corresponded to the structure of OCP (reflection at around 4.7, 9.8 and 32 degrees 2θ). The FTIR absorption spectra correlated with the findings from XRD, where typical attributes of OCP were observed in all the sample spectra (absorption bands characteristic of ν3 PO₄²⁻ stretching vibration in the regions 1075–1017 cm⁻¹, two sharp bands of ν4 PO₄³⁻ at 600 and 560 cm⁻¹ and ν1 PO₄³⁻ at 962 cm⁻¹, additionally bands at 1120, 916, and 860 cm⁻¹ due to HPO₄²⁻ stretching were present). The specific surface area quantified with BET method was around 70 m²/g.

Conclusion

The presented research provides evidence that OCP has been successfully produced by the direct hydrolysis of α-TCP powder, both in the closed system and with the exposure to the surrounding atmosphere. In order to perform the 10-fold upscaling and to obtain a ten times higher amount of the final product with only OCP phase present, extending the time of hydrolysis to 72h was necessary.

Acknowledgement

Page 1122 of 2028
This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860462.

References
Thiol-ene photo-click hydrogels to stimulate osteoregeneration through recapitulation of the osteoid microenvironment

Laurens Parmentier¹, Peter Dubruel¹, Sandra Van Vlierberghe¹,²

¹ Ghent University, Polymer Chemistry and Biomaterials Group – Centre of Macromolecular Chemistry (CMaC) – Department of Organic and Macromolecular Chemistry, Ghent, BE; ² Vrije Universiteit Brussel and Flanders Make, Brussels Photonics – Department of Applied Physics and Photonics, Brussels, BE

Introduction

The most common causes for bone defects include trauma, disease, tumour removal and surgical interventions. When a bone defect exceeds the regenerative capabilities of bone tissue, a critical defect arises in which bone is unable to heal itself through bone remodelling so surgical intervention becomes required. Four million surgeries are performed annually to treat bone defects using either bone grafts or synthetic substitutes rendering it the second most transplanted tissue worldwide [1].

Given the drawbacks associated with auto- and allografts, synthetic scaffolds mimicking the composition, architecture and properties of the native extracellular matrix are gaining increasing interest. These biophysical cues are hereby converted into biochemical cues through mechanosensitive pathways that regulate cell behaviour [2]. Therefore, the aim is to develop a biomimetic hydrogel mimicking the bone osteoid. Natural polymers have the advantage that they are characterized by biodegradability and intrinsic biological signalling pathways. Considering the composition of the osteoid extracellular matrix, collagen type I is a popular candidate to be used in bone scaffold materials. However, collagen carries the risk of exhibiting an immune response. Therefore, gelatin, derived from denatured collagen, is of special interest since it resolves this issue while still maintaining the cell-interactive properties of collagen. Gelatin however suffers from its upper critical solution temperature [UCST] behavior that leads to dissolution at 37°C. To overcome this, gelatin can be modified with photo-crosslinkable groups [Figure 1].

Experimental Methods

Thiol-ene step growth photo-crosslinking was selected to enable superior control, homogeneous network formation and lower radical concentrations compared to traditional chain growth systems [3]. More specifically, norbornene-functionalised, aminated gelatin systems were combined with thiolated gelatin functioning as cell-interactive crosslinker thereby forming networks with mechanical properties capable of triggering osteogenesis. The modification degree of these derivatives was characterised through nuclear magnetic resonance spectroscopy and an orthophthalic dialdehyde assay. Furthermore, in-situ photo-rheology, swelling and gel fraction experiments, differential scanning calorimetry, collagenase degradation tests and high resolution magic angle spinning nuclear magnetic resonance spectroscopy were used to characterize the obtained networks. Furthermore, cell viability and adipose-derived stem cell osteogenesis were evaluated in vitro through Live/Dead staining, the production of alkaline phosphatase, the stimulation of collagen type I production and the presence of calcium deposits.

Results and Discussion

In order to obtain thiol-ene networks in the osteogenic range [25-40 kPa], the carboxylic acid groups present in gelatin type B were partially substituted with amine groups through addition of either diaminoethane [red] or -pentane [blue] [Figure 1A] [4]. A substantially higher degree of norbornene substitution could be obtained thanks to this amination compared to the traditional reaction exploiting gelatin type B [Figure 1B] [5]. The thiol-ene networks were benchmarked against the chain growth system characteristic for gelatin-methacrylamide [GelMA] [Figure 1C]. The presence of a higher amount of amine groups resulted in a significantly higher [Figure 2A, #] compressive modulus.
compared with conventional norbornene-functionalised gelatins [Figure 2A, green]. A further significant increase was observed when the alkyl chain of the amination reagent was longer. Being situated in the osteogenic range, 7.5 w/v% GelMA and 10 w/v% GeINBNB-GelSH [red] were further evaluated in terms of physico-chemical properties. Chemical crosslinking was the main mechanical contribution for the GeINBNB-GelSH network with a drastically reduced amount of physical crosslinks [by a factor of 300 compared to GelMA] paving the way towards processing of these hydrogels at room temperature [Figure 2B-2G]. Upon modification, the degradation properties of the newly modified gelatin were preserved [Figure 2H]. The preliminary cell viability data indicated an elongated morphology and excellent biocompatibility for the GeINBNB-GelSH networks [Figure 2I, scale bar: 500 µm].

**Conclusion**

Overall, the results showed that highly-controlled biocompatible networks could be created which could stimulate osteogenesis based on favourable mechanical properties and retained biodegradability despite the chemical crosslinking applied.

**Acknowledgement**

The authors would like to acknowledge the financial support from the Research Foundation Flanders (FWO80227) and Interreg 2Seas 3DMed.
Bio-instructive Collagen-Magnesium Nanocomposite Scaffolds to Manipulate Bone Metabolism and Promote Bone Repair

Silvia S. Paiva1,2, Fergal O'Brien1,2,3, Ciara Murphy1,2

1 Royal College of Surgeons in Ireland, Dept. of Anatomy and Regenerative Medicine, Dublin, IE; 2 Advanced Materials and BioEngineering Centre, Dublin, IE; 3 Trinity Centre for Biomedical Engineering, Trinity College Dublin, Dublin, IE

Introduction

The native bone tissue holds a staggering capacity for self-regeneration owing to the metabolically dynamic process of bone remodelling. Bone remodelling is mediated through the synchronism of bone resorption (catabolism) by osteoclasts and bone formation (anabolism) by osteoblasts1. Imbalances in the bone remodelling cycle represent an underlying cause of metabolic bone diseases such as osteoporosis, and present a major risk for implant loosening due to excessive resorption activities, which hampers the correct regeneration of bone post-injury. Current biomaterials strategies to repair bone fractures often focus solely in targeting anabolism or suppressing catabolism, which may increase the risk for impaired fracture healing. In alternative, the incorporation of bioactive ions such as Magnesium (Mg2+) into biomaterials has gained remarkable attention due to their ability to stimulate suitable biological responses necessary for the repair of bone defects, in a safe and cost-effective manner2. Herein, we focus on investigating a combined versatile pro-anabolic and anti-catabolic effect of Mg2+ in modulating bone cell behaviour, to develop an engineered biomimetic bio-instructive biomaterial scaffold structurally designed to enhance bone formation while impeding uncontrolled osteoclast resorption activities to facilitate better bone regeneration and promote repair.

Experimental Methods

In vitro 2-D model: to determine the effect of Mg2+ on osteoblasts, MC3T3-E1 cells were cultured in osteogenic media exposed to increasing concentrations of MgCl2 ranging from 0–25mM, and their proliferation, gene expression, and mineralisation was assessed over 28 days. To determine the effect of Mg2+ on osteoclast progenitor cells, RAW 264.7 cells were stimulated with 20ng/mL of RANKL, and exposed to the same range of increasing concentrations of MgCl2. Multinucleated and TRAP-positive cells were labelled as osteoclast-like cells and the ability to form osteoclasts in the presence of increasing concentrations of MgCl2 was evaluated for 14 days.

In vitro 3-D model: we determined that 10mM and 25mM MgCl2 presented favourable osteogenic properties while simultaneously inhibiting osteoclast formation in 2-D, thus these concentrations were utilised in doping pro-anabolic hydroxyapatite nanoparticles (nHAp). Collagen-Mg2+ porous-scaffolds were prepared by incorporating Mg2+-doped nanohydroxyapatite (10Mg nHAp and 25Mg nHAp) into a bovine type I fibril collagen slurry, and obtained through a freeze-drying method previously optimised in our group3. Scaffolds were assessed via FTIR, SEM, water contact angle, and mechanical testing to determine the effect of Mg2+ on both physical and chemical properties. Scaffolds were cultured separately with MC3T3-E1 and RAW 264.7 cells to investigate the behaviour of bone cells in response to Mg2+, and assessed for cell viability using Live/Dead assay, proliferation, migration via histochemical characterisation, and gene expression.

Results and Discussion

In vitro 2-D study: we reported an increase in cell proliferation over time for all Mg2+-treatments in both MC3T3-E1 and RANKL-induced RAW 264.7 cells. All Mg2+-treated groups presented improved expression of alkaline phosphatase activity, with 25mM MgCl2 exhibiting superior mineral deposition when compared to positive control (osteogenic media) group (Fig. 1A). We observed a dose-responsive inhibition in the formation of TRAP-positive...
multinucleated osteoclast-like cells with increasing concentration of Mg$^{2+}$ (Fig. 1B), and elevated extracellular Mg$^{2+}$ significantly downregulating the expression of the osteoclast-specific markers TRAP and Cathepsin K.

In vitro 3-D study: we successfully developed a range of highly porous collagen composite scaffolds: Collagen as a control (Coll), Coll-hydroxyapatite (nHAp), 10Mg nHAp and 25Mg nHAp. Our developed scaffolds were able to induce the proliferation of bone cells over 14 days and cell cytompatibility was also confirmed through live/dead imaging on seeded scaffolds. Scaffolds containing 25Mg nHAp showed a significant increase in MC3T3-E1 cells proliferation, and decrease in RANKL-induced RAW 264.7 cell proliferation at day 14 relative to Coll. Histological characterisation of developed scaffolds confirmed the migration of RANKL-induced RAW 264.7 cells from day 5 in Coll and nHAp scaffolds. However, Mg-modified scaffolds presented significantly less migrated cells, suggesting an inhibitory chemotactic effect induced by the presence of Mg$^{2+}$ within the scaffolds. Mg-modified scaffolds reduced the expression of osteoclast-related genes over time, demonstrating an effect in delaying osteoclast differentiation.

Conclusion
Our research to date has demonstrated the potential of elevated extracellular Mg$^{2+}$ to concurrently enhance osteogenesis while inhibiting osteoclastogenesis in vitro in both 2D and 3D models, potentially introducing new strategies in the development of bio-instructive biomaterials to repair metabolically compromised bone fractures.

Acknowledgement
This study has received financial support of Science Foundation Ireland, SFI/AMBER (17/RC-PhD/3477).

Fig. 1
In vitro 2D effect of Mg$^{2+}$ on A) MC3T3-E1 cells calcium deposition, and B) RAW 264.7 cells formation of TRAP+-multinucleated cells

Fig. 2
A) Scanning Electron Microscopy micrographs of collagen composite scaffolds showing highly porous microstructure of developed scaffolds, and B) NFATc1 and Cathepsin K gene expression of RANKL-stimulated RAW 264.7 cells showing reduced expression in Mg-modified scaffolds.

References
Electrical Stimulation of Osteoblast-Like Saos-2 Cells using electroless Palladium-Coated Polymer Scaffolds

Oriol Careta¹, Asier Salicio-Paz², Eva Pellicer³, Elena Ibáñez¹, Jordina Fornell³, Eva García-Lecina², Jordi Sort³,⁴, Carme Nogués¹

¹ Universitat Autònoma de Barcelona, Departament de Biologia Cel·lular, Fisiologia i Immunologia, Bellaterra (Cerdanyola del Vallès), ES; ² CIDETEC, Basque Research and Technology Alliance (BRTA), Donostia-San Sebastián, ES; ³ Universitat Autònoma de Barcelona, Departament de Física, Bellaterra (Cerdanyola del Vallès), ES; ⁴ Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, ES

Introduction

The use of three-dimensional porous scaffolds offers various advantages over conventional treatments for bone tissue engineering, but they must fulfil some criteria as (i) an open and interconnected pore network to promote the formation of new tissue, (ii) vascularization in vivo, (iii) good biocompatibility (iv) mechanical properties similar to those of the replaced tissue (v) resistance to corrosion and (vi) ease of fabrication. Even though ceramics are the most widely studied materials, their limited degree of elasticity hampers their use in bone regeneration applications. Polymers, also considered as potential biomaterials for scaffolds, suffer from some drawbacks, such as an excessively low elastic modulus and, in some cases, poor corrosion resistance. On the other hand, biocompatible metallic scaffolds present mechanical properties more similar to those of the human bone and can be used as electrodes with different electric field intensities (or voltages) for electric stimulation (ES). Metals typically suffer from corrosion, which leads to ions release into the medium. For this reason, the use of alloying elements which release non-cytotoxic ions upon dissolution is of utmost importance. The application of an external electric field has been widely studied and successfully used in clinical practice to stimulate bone healing and it has also been shown that ES modifies osteoblast activities including adhesion, proliferation, calcium nodule formation, gene expression, protein synthesis, and bone formation markers.

Experimental Methods

In the present work we have used a palladium-coated polymeric scaffold, generated by electroless deposition, as a bipolar electrode to electrically stimulate human osteoblast-like Saos-2 cells. Osteoblasts were grown on four different conditions: untreated glass coverslip, electrically stimulated glass coverslip, untreated foam and electrically stimulated foam. Foams were individually inserted into 24-well cell culture plates and Saos-2 cells were seeded on top and cultured in DMEM with 10% FBS under standard conditions or ES. Under ES, cells were exposed daily to an arbitrary sinusoid function of 2.5 V amplitude and 200 Hz frequency for 1 h along 28 days. Osteoblasts proliferation was evaluated by Alamar Blue at days 3, 7, 14 and 28. Cell adhesion and morphology were evaluated by scanning electron microscopy and actin staining at 7, 14 and 28 days. Differentiation was assessed at 1, 7, 14 and 28 days by determining the expression profile of six osteoblast genes (alkaline phosphatase, osteocalcin, bone sialoprotein, type I collagen, osteonectin and osteopontin) and by mineralization assay at days 7, 14 and 21.

Results and Discussion

Cells grown on palladium-coated polyurethane foams under ES presented higher proliferation than cells grown on foams without ES for up to 14 days. Growth was also higher in control cells, stimulated or not. In addition, cells grown in foams in both conditions were well adhered, with a flat appearance and a typical actin cytoskeleton distribution. After 28 days in culture, cells under ES appeared rounded and not well adhered, a sign of cell death onset, while cells without ES were filling the entire structure. Cell grown on glass coverslips were also rounded and not well
adhered after 28 days under ES. When looking at osteoblast differentiation, ES seems to enhance the expression of early expressed genes.

**Conclusion**
The results suggest that palladium-coated polyurethane foams may be good candidates for osteoblast scaffolds and also demonstrate that ES enhances osteoblast proliferation at least up to 14 days, while upregulating expression genes related to extracellular matrix formation.

**Acknowledgement**
The work was supported by MINECO (MAT2017-86357-C3-1-R and MAT2017-86357-C3-3-R)
Properties of premixed calcium phosphate cements based on α-tricalcium phosphate

Zilgma Irbe1,2, Dagnija Loca1,2

1 Riga Technical University, Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga, LV; 2 Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, LV

Introduction

Calcium phosphate bone cements are materials used mainly to augment bone and promote the regeneration of the bone tissue. Calcium phosphate cements set at body temperature, so they can be easily given additional functionality, such as delivery of biologically active substances to the defect site. As these materials set upon application, their application is time sensitive. One way to improve the ease handling of calcium phosphate cements is to formulate premixed compositions. Calcium phosphate cements should set rapidly after application without washout of cement particles [1, 2]. Fast conversion of starting materials [2] and mechanical strength are also considered advantageous [3].

Previously, we established that cement additives that provide low initial pH values in the cement paste can ensure very rapid setting. In this study we investigated the properties of premixed cementing calcium phosphate pastes based on α-tricalcium phosphate (α-TCP) and glycerol with additives providing low initial paste pH. The setting reactions in these pastes start upon implantation as the inert paste liquid (glycerol) is gradually displaced by water from the surrounding fluids. Soluble sodium or potassium phosphate salts were used as additives. We evaluated setting, phase composition, and anti-washout properties of these premixed pastes.

Experimental Methods

α-TCP powder was obtained by high temperature synthesis at 1400°C from calcium carbonate and dicalcium phosphate (molar ratio 1:2), followed by milling to reduce particle size. α-TCP was mixed with glycerol and additives (monosodium phosphate or monopotassium phosphate) to obtain a workable paste. Molar ratios of α-TCP:additive were 10:1, 10:2 or 10:4. Phase composition of the set cements was determined using XRD in conjunction Rietveld refinement method. Anti-washout characteristics were evaluated visually and by monitoring pH and soluble substance content of the surrounding aqueous liquid.

Results and Discussion

All investigated cement pastes set very rapidly; a hard, set layer formed immediately (< 1 min) on the surfaces in contact with water. This layer served as a barrier, and the inner mass set slowly as the water diffused through the initial set layer. The thickness of the set layer for the tested compositions was 1.8-2.5 mm after 2.5 h of contact with aqueous surrounding liquid, and it did not correlate with the amount of additive used in the paste. For pastes with α-TCP:additive ratio of 10:1, some washout occurred, and it was more pronounced in the case of monopotassium phosphate. For the pastes with α-TCP:additive ratio of 10:2 and 10:4, no washout was observed.

The use of additives did not substantially reduce the pH of the surrounding aqueous liquid. The pH values next to the setting cement were near neutral: 6.3-6.7 in the case of monosodium phosphate additive and 6.4-7.0 in the case of monopotassium phosphate additive. Larger amount of additive used resulted in slightly higher pH values. The conversion of α-TCP to hydroxyapatite (see Fig. “Conversion of starting material to hydroxyapatite”) was also affected. It was delayed if higher amount of additive was used. Two days after contact with water, the set cements contained 62-86% of hydroxyapatite and the residual unconverted α-TCP. The use of higher amount of additive resulted in slower hydroxyapatite formation.
Conclusion
We recommend using monosodium phosphate or monopotassium phosphate to ensure setting and cohesion of premixed calcium phosphate cement pastes based on α-TCP. Although complete hydrolysis of α-TCP is slower, if the used amount of additive is increased, the setting is not delayed. Investigated compositions in both cases have acceptable anti-washout properties if sufficient amount of additive is used. The pH of the aqueous liquid surrounding setting cement was not reduced below pH 6.

Acknowledgement
This work has been supported by the European Regional Development Fund within the Activity 1.1.1.2 “Post-doctoral Research Aid” of the Specific Aid Objective 1.1.1 “To increase the research and innovative capacity of scientific institutions of Latvia and the ability to attract external financing, investing in human resources and infrastructure” of the Operational Programme “Growth and Employment” (No.1.1.1.2/VIAA/4/20/640) The authors acknowledge financial support from the European Union’s Horizon 2020 research and innovation programme under the grant agreement No 857287.

References
Composite & hybrid materials based on bioactive glass, toward optimized substitutes for bone regeneration

Amel Houaoui¹,², Jonathan Massera¹, Michel Boissière², Emmanuel Pauthe²

¹ Tampere University, Laboratory of Biomaterials and Tissue Engineering, Faculty of Medicine and Health Technology, Tampere, FI; ² CY Cergy Paris Université, Biomaterials for Health Research Group, ERRMECe, Equipe de recherche sur les Relations Matrice Extracellulaire-Cellules (EA1391), Institut des matériaux I-MAT (FD4122), CY Tech, Neuville-sur-Oise, FR

Introduction
Most of the bone fractures are simple and common traumas usually repaired by osteogenesis. However, some complex and critical size defects, due to specific traumatic or pathophysiological context, cannot be repaired without a substitute that serves as a mechanical support and/or as bone filling to assist in the bone regeneration. Such bone substitute materials must present “osteo-properties”: from osteocompatibility to osteocompetency. Bioactive glass (BAG) has the particularity of releasing calcium and phosphate ions which precipitate and form a reactive apatite layer, considered to be the first indication of bioactivity [1]. Inspired by bone, the combination of BAG with organic matrices would provide innovative materials for bone bioengineering. Here we present the development of composite and hybrid materials based on bioactive glass for bone bioengineering. Two strategies were explored, i) a composite material based on Poly (Lactic Acid) (PLA) and bioactive glass and ii) a hybrid material based on gelatin and bioactive glass. The first system finds more applications as mechanical support, whereas the second is more pertinent to address the need of filling bone defects. In this study, two BAGs were compared: 1) the 13-93 a known, FDA approved composition and 2) the 13-93B20 where the silica was partially replaced with boron, in view of accelerating the glass dissolution kinetics and increase the glass conversion to hydroxyapatite.

Experimental Methods
Each system contains 70% of organic matrix (PLA or gelatin) and 30% of BAG (13-93 or 13-93B20 particles, in weight %). The composites were processed by twin screw co-extrusion and the hybrids were synthetized by sol-gel transition. The bioactivity in vitro (related to the precipitation of an apatite layer at the materials’ surface when immersed in aqueous solution) of both types of devices was studied in Simulated Body Fluid (SBF) for up to two weeks. The ion release was quantified by Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) and the materials were analyzed by Scanning Electron Microscopy coupled with Energy Dispersive X-ray (SEM-EDX). Cell experimentations were done to assess the cytocompatibility of the different devices and show their ability to promote a commitment toward an osteoblastic lineage.

Results and Discussion
The characterization of the composites based on PLA and 13-93 or 13-93B20 showed that boron allows to modulate the dissolution rate of the BAG in the composites, leading to a faster dissolution. In SBF, the [P] and [Ca] concentration decreases overtime, suggesting the precipitation of a reactive layer. The reactive layer was further confirmed to be apatite. The bioactivity, in vitro, is more important with the 13-93B20 BAG. Myoblastic cells culture on the composites exhibits a decrease of myosin and increase of osteopontin. This indicates that the myoblastic cells in presence of BAG evolve toward an osteoblastic lineage (Figure 1).

The second strategy is focused on the elaboration of hybrids based on gelatin and 13-93 or 13-93B20 using a
bifunctional coupling agent, the 3-glycidoxypropyltrimethoxysilane (GPTMS). The amount of GPTMS was optimized and covalent links between the gelatin and the BAG are evident as the gels appear self-supported at biological temperature. The characterization of these materials showed that the 13-93B20 BAG allowed to accelerate the dissolution and to increase the hybrids bioactivity. The cytocompatibility of the hybrids has been demonstrated using MC3T3-E1 pre-osteoblastic cells (Figure 2).

Conclusion
The results show that the composition of the BAG is a key property towards controlling the materials properties as well as the cellular response. Boron inclusion in the formulation allows a smart tailoring of the dissolution rate of the BAG. These tailor-made materials have a real potential for mechanical support, bioresorbability, and bone regeneration via the induction of suitable cellular behaviors favored by the BAGs dissolution by-products, bringing an osteo-competent and osteo-stimulating dimension to the implant.

Acknowledgement
The authors would like to acknowledge Remy Agniel and Lamia El Guermah for technical assistance and expertise, the Imaging and Analysis Platform at CY Cergy Paris Université as well as the Institute for Advanced Studies (IAS) for technical and financial support.

References
Evaluation of liquid phase influence on properties of $\alpha$-TCP-based biomicroconcrete-type bone substitutes

Piotr Pańtak, Ewelina Cichoń, Szymon Skibiński, Joanna Czechowska, Aneta Zima, Anna Ślósarczyk

AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Kraków, PL

Introduction
Due to the increased demand for bone tissue substitutes caused by a rapidly aging society, civilization diseases or bone injuries many biomaterial studies are conducted around the world. The main efforts are focused on the optimization of the utility parameters of those biomaterials (such as mechanical strength or surgical handiness). Among many commercially available materials for bone tissue substitution, biomicroconcretes are distinguished by specific characteristic due to their unique composition, which is inspired by classical concretes, where aggregates in the form of granules provide material with satisfactory mechanical properties. Biomicroconcretes based on calcium phosphates are self-setting, bioactive materials for bone tissue engineering with high surgical-handiness. The aims of this study were to develop and obtain novel biomaterials for bone substitution and to recognise the influence of the liquid phase composition on the physicochemical properties of obtained biomicroconcretes.

Experimental Methods
The solid phase of the investigated materials composed of: 60 wt% of highly-reactive $\alpha$-TCP powder and 40 wt% of hybrid hydroxyapatite-chitosan (HAp/CTS) granules. Mixtures of 2 wt% of disodium phosphate (Na$_2$HPO$_4$) and 5 wt% citrus pectin aqueous solutions in various ratios (0:100, 25:75, 50:50, 75:25, 100:0 wt%) were used as a liquid phase of the developed biomicroconcretes. Obtained materials were characterized by several methods: setting times measurements (Gilmore needles according to the C266-08 ASTM standard), phase composition studies (D2 Phaser diffractometer, Bruker), compressive strength tests (Instron 3345), chemical stability and bioactivity in vitro studies (H198129 Combo, Hanna). Samples microstructure observations were performed (PhenomPure, Thermo Fisher Scientific).

Results and Discussion
In this study, the effect of liquid phase composition on the properties of biomicroconcretes was investigated. Studies have shown that obtained bone substitute materials were characterised by unique physicochemical properties due to different liquid phase combinations and dual setting system originated from hydrolysis of $\alpha$-TCP to non-stoichiometric hydroxyapatite and interactions between polycationic chitosan in hybrid granules and polyanionic pectin in liquid phase. The study reviled that liquid phase in the form of pectin solution prevented the biomicroconcrete from set. However, the rest of materials were characterised by self-setting properties due to the presence of disodium phosphate. Nevertheless, the addition of pectin solution led to achieve fully-injectable materials, which was impossible for biomicroconcretes where the liquid phase was only disodium phosphate.

Conclusion
The innovative materials such as obtained and developed in this study biomicroconcretes based on highly-reactive $\alpha$-TCP powder and hybrid HAp/CTS granules combine beneficial properties of bioceramics and biopolymers and can be prospectively injectable and self-setting materials for bone cavity fillings. They also can act as an alternative to commercially used sintered ceramic implants of predefined shape and size. Surgically handy and self-setting materials are potential candidates for bone tissue engineering, supporting its regeneration.

Acknowledgement
Research funded by the Faculty of Materials Science and Ceramics AGH UST - University of Science and Technology, Kraków, Poland, Project No. 16.16.160.557 (2021). Supported by the National Science Centre, Poland Grant No. 2017/27/B/ST8/01173.
Artificial extracellular matrix components and impaired bone metabolism

Sabine Schulze\textsuperscript{1,2}, Christin Neuber\textsuperscript{3}, Jens Pietzsch\textsuperscript{3}, Stefan Rammelt\textsuperscript{1}

\textsuperscript{1} University Hospital Dresden, University Center for Orthopedics and Trauma Surgery, Dresden, DE; \textsuperscript{2} TU Dresden, Medical Faculty, Centre for Translational Bone, Joint and Soft Tissue Research, Dresden, DE; \textsuperscript{3} Helmholtz Zentrum Dresden Rossendorf, Institute of Radiopharmaceutical Cancer Research, Department Radiopharmaceutical and Chemical Biology, Dresden, DE

Introduction
Diabetes mellitus is a huge socio-economic burden primarily for western societies. More than 9\% of the World’s population suffer from this systemic disease that not only affects glucose metabolism but can also be related to vision impairment, cardiovascular disease, kidney damage, foot ulcer, nerve damage and impaired bone metabolism. Especially the last two secondary diseases result in complicated fragmentation of the foot bones. Due to limited healing capacity that is also associated with diabetes mellitus, fracture treatment is complicated and healing takes longer than in individuals with healthy bone metabolism.

In diabetes patients, altered markers of bone turn-over were detected. For instance, the reduction of collagen, decreased mineral content and reduced osteocalcin synthesis may contribute to low bone mass and, therefore, increased fracture risk [1].

Experimental Methods
During reconstructive foot and ankle surgery, bone fragments and blood samples were obtained from patients who suffered either from diabetic neuropathy or from posttraumatic osteoarthritis. Osteoblasts were isolated by outgrowth from bone fragments. Monocytes were obtained from patient’s blood and differentiated into osteoclasts. After treatment with artificial extracellular matrix components (sGAG), alterations in osteogenic differentiation were investigated by qPCR. Osteoclastogenesis with and without sGAG was analysed by using a quantitative resorption assay [5]. The expression of osteoblast- and osteoclast-specific genes was determined by qPCR.

Results and Discussion
Osteoblasts showed increased gene expression of osteogenic genes after sGAG treatment. Especially osteoblasts from diabetes patients were affected by sGAG, which demonstrates increased gene expression of the osteocalcin and the alkaline phosphatase genes compared to the healthy control group. Although gene expression of osteoclastic genes was enhanced by sGAG, the osteoclast-specific resorption of calcium phosphate was significantly decreased in diabetes patients. The secretion of interleukin-6 (IL-6) was increased in osteoclast culture after sGAG treatment. Additionally, the cells exhibited morphological characteristics of osteoclasts such as multinuclearity and actin ring formation.

Conclusion
Artificial extracellular matrix components cause increased expression of osteogenic genes, especially in diabetic patients. On the other hand, they inhibit bone resorption by inhibiting osteoclast resorption. Since the expression of osteoclast-specific genes is not decreased, a post-translational effect of sGAG on osteoclasts is considered.

Acknowledgement
The Deutsche Forschungsgemeinschaft (DFG; German Research Foundation, grant number 59397982) funded this work.

Page 1138 of 2028
References
[2] Hempel U et al. Artificial extracellular matrices with oversulfated glycosaminoglycan derivatives promote the
[4] Förster Y et al. The influence of different artificial extracellular matrix implant coatings on the
A novel highly porous type I/type II collagen-based scaffold as simple and prospective “off the shelf” approach to enhanced cartilage repair

Claudio Intini1,2, Mark Lemoine1,2, John P. Gleeson1,3, Fergal J. O’Brien1,2

1 Royal College of Surgeons (RCSI), Department of Anatomy/Tissue Engineering Research Group (TERG), Dublin, IE; 2 Royal College of Surgeons (RCSI) & Trinity College Dublin (TCD), Advanced Materials and Bioengineering Research (AMBER) Centre, Dublin, IE; 3 Dublin College University (DCU), Fraunhofer Project Centre for Embedded Bioanalytical Systems, Dublin, IE

Introduction

A major challenge in cartilage tissue engineering (TE) is to develop scaffolds capable of providing an improved instructive biomimetic response, to drive effective mesenchymal stem cells (MSCs) differentiation and robust de novo tissue formation while retaining the essential mechanical properties of the implant [1]. The composition of biomimetic porous scaffold-based biomaterials can play a fundamental role in TE by providing essential three-dimensional (3D) cues for infiltrating cellular populations, such as MSCs, and subsequently directing the type of the engineered tissue [2]. Biomimetic implants have shown some potential to guide with good success MSCs chondrogenesis, and subsequent cartilage-like tissue formation [1]. In particular, type I collagen-hyaluronate (CI-HyA) scaffolds have demonstrated significant chondrogenic regenerative capacity preclinically and clinically [3,4]. However, we propose that the chondrogenic potential of these biomimetic implants can be further improved for enhanced and longer lasting cartilage repair. Therefore, the aim of this work was to investigate the incorporation of type II collagen (CII), a potential chondro-inductive cartilage extra cellular matrix (ECM) component, within collagen-based scaffolds and to assess its role in directing an improved cartilage-like tissue formation [5].

Experimental Methods

Novel highly porous collagen-based scaffold were manufactured by incorporating type II collagen (CII) and/or hyaluronic acid (HyA) within a type I collagen (CI) framework using a freeze-drying (lyophilisation) method previously described and regularly used in our research group [3]. Following lyophilisation, the porous scaffolds were dehydrothermally (DHT) and chemically crosslinked. The architectural, morphological and mechanical properties of cell-free scaffolds were investigated. Scaffolds were subsequently cultured with rat-derived bone-marrow MSCs in vitro under chondrogenic conditions for 28 days. The effect of matrix components, CII and HyA, on cellular viability, growth and migration within the scaffold and their capability to direct an effective MSCs chondrogenic response, that consequently results in enhanced cartilage-like tissue formation in vitro was assessed.

Results and Discussion

Type II collagen (CII) was successfully incorporated into type I collagen (CI) and/or hyaluronic acid (HyA) scaffold biomaterials with no impact on the mechanical properties. The newly developed CI/II and CI/II-HyA scaffolds exhibited highly porous interconnected structures with 99% porosity and increased pore size compared to the CI and CI-HyA scaffolds (Fig. 1). All four scaffold variants demonstrated early cartilaginous matrix deposition but the incorporation of CII in combination with HyA allowed CI/II-HyA scaffolds to accumulate increased quantities of sulphated glycosaminoglycans (sGAG) deposition per scaffold compared to the other scaffold variants at day 28 (p≤0.0001) (Fig. 2A). In addition, the incorporation of CII, in combination with HyA, allowed CI/II-HyA scaffolds to achieve more homogeneous sGAG distribution throughout the matrix compared to the other scaffold variants as shown by safranin-O staining. Gene expression analysis revealed potential for CI/II-HyA scaffolds to further improve chondrogenesis, with no signs of progression to hypertrophy or endochondral bone formation.

Conclusion
The combination of CII and HyA resulted in the development of a scaffold with potent chondrogenic benefits, with no impact on the essential architectural and mechanical properties of the scaffold. It is speculated that the biochemical nature of these components plays a significant role in directing improved cellular growth and cartilage-like deposition/distribution. In summary, the incorporation of CII into CI-HyA scaffolds has showed promise to improve cartilage-like tissue formation and has offered a simple and prospective “off the shelf” approach to enhanced and more robust cartilage repair.

Acknowledgement

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under Marie Sklodowska-Curie grant agreement No 721432.

![Fig 1. CII incorporation increased the pore size of highly interconnected 99% porous scaffolds. Representative histological images of cell-free scaffolds stained with toluidine blue. Scale bar represents 100 µm length (A). The mean pore size (B) and percentage porosity (C) of cell-free scaffolds were determined. Data presented as mean ± SD, n=3. * denotes p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001 and **** = p ≤ 0.0001.](image1)

![Fig 2. CII incorporation in combination with HyA increased sGAG quantity per scaffold at day 28. Overall sGAG per scaffold (A) and sGAG normalised to DNA content (B) respectively were determined after 28 days in incomplete chondrogenic medium (ICM) culture, and after 14 and 28 days in complete chondrogenic medium (CCM) culture. Data presented as mean ± SD, n=3. * denotes p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001 and **** = p ≤ 0.0001.](image2)

References


Modelling adsorption of proteins and cells on biomimetic hydroxyapatite

Abdul Raouf Atif¹, Ugis Lacis², Maria Tenje¹, Shervin Bagheri², Gemma Mestres¹

¹ Uppsala University, Uppsala, SE; ² KTH Royal Institute of Technology, Stockholm, SE

Introduction
Calcium-deficient hydroxyapatite (CDHA), a biomaterial similar to the inorganic bone matrix, can be used in non-load bearing areas to promote bone regeneration. Upon implantation, CDHA is exposed to blood, leading to serum protein deposition on the surface and enabling cell attachment via membrane-bound receptors. In cell culture studies, biomaterials are often pre-incubated in serum supplemented medium to mimic this process. In this work, to study the extent the protein layer assists in cell adhesion, a Langmuir isotherm-based model for protein and cell adhesion kinetics was utilized.

Experimental Methods
CDHA was prepared through a cementitious reaction. \( \alpha \)-tricalcium phosphate powder was mixed with 2.5% \( \text{Na}_2\text{HPO}_4 \) in a liquid-to-powder ratio of 0.65 ml/g. The cement paste was cast into discs (\( \phi = 6 \text{ mm}, h = 2 \text{ mm} \)) and then set in 0.9% \( \text{NaCl} \) for 10 days, thus completing the transformation into CDHA.

To analyze protein adsorption/desorption, a two-step experiment was performed. First, CDHA discs were incubated in culture medium supplemented with 10 mg/ml bovine serum albumin (BSA) for 3 days. Subsequently, the CDHA discs were transferred to wells containing protein-free medium and further incubated for 3 days. At set intervals along both incubation periods, medium aliquots were taken and protein content was quantified using the bicinchoninic acid assay. Modelling of adsorption and desorption was performed using a diffusion equation and the Langmuir isotherm to describe time-dependent interactions between the medium and surface concentrations:

\[
\frac{D \partial c}{\partial z} = -k_a(c_s - c_m)c - k_d c_s
\]

Here, \( c \) and \( c_s \) are bulk and surface concentrations respectively, \( c_s^m \) is the maximal surface concentration, \( D \) is the diffusion coefficient, \( z \) is the depth of the medium, \( k_a \) is the adsorption coefficient and \( k_d \) is the desorption coefficient.

Cell adhesion was evaluated over time for serum pre-incubated CDHA samples. To evaluate the influence of proteins adsorbed on the surface, pristine discs (pristine-CDHA) and discs pre-incubated for 48 h (pre-incubated-CDHA) in supplemented medium (10 v/v% FBS, 1 v/v% penicillin/streptomycin) were used. MC3T3-E1 pre-osteoblasts were stained with CMFDA dye and seeded on CDHA discs at a density of 3,500 cells/cm². Samples were imaged at 0.5, 1, 2, 4 and 6 h and subsequently counted using Cellprofiler (ver 3.1.5) software. As previously, a model inspired by the Langmuir isotherm was constructed for cell adhesion, now neglecting the detachment term as MC3T3-E1 cells are adherent.

Results and Discussion
CDHA displayed strong BSA adsorption, with 50% of total protein concentration adsorbed after 72 h (Fig. 1A). As for BSA desorption, a burst release occurred over the first 2 h of incubation, which then gradually subsided over time (Fig. 1B). Only 18% of the total BSA content desorbed after 3 days, indicating a majority remained on the HA surface. The model appeared to satisfactorily fit the data, capturing the experimental trends well and approaching a plateau state which indicates BSA exchange reached equilibrium (Fig. 1A, B).

The number of adhered cells increased in the first hour of culture, before stabilizing for the rest of the culture period (Fig. 2). Pristine-CDHA and pre-incubated-CDHA displayed similar cell counts over time (Fig. 2). This behavior was captured by the model within close fit of the experimental data, thus indicating the validity of the assumptions taken. Morphology-wise, cells cultured on pre-incubated-CDHA appeared more spread compared to cells grown on pristine-
CDHA (Fig.2), which correlated with the greater protein content adsorbed on CDHA after a 48 h incubation in supplemented medium (Fig.1A).

**Conclusion**

To summarize, this work represents a conceptual study to evaluate the use of computational modelling for the analysis of protein and cell adhesion on CDHA surfaces. Modelling allowed capture of the relation between protein and cellular behavior observed *in vitro*, thus solidifying its potential as an additional form of analysis in the biomaterial evaluation pipeline. This model is envisioned as a steppingstone towards insightful numerical models of more complex cell-fluid systems.

![Figure 1. A) Adsorption and B) desorption of BSA on biomimetic HA surface.](image)

The solid line corresponds to the experimental data and the dashed trace corresponds to the model. * indicates statistical difference against starting medium conditions, specifically against BSA-rich medium for the adsorption experiment and BSA-free medium for the desorption experiment (p < 0.05). Identical letters between individual time-points indicate no statistical significance (p < 0.05).

![Figure 2. Osteoblast cell density on A) pristine-CDHA and B) pre-incubated CDHA.](image)

The individual time-points correspond to the experimental data and the dashed line corresponds to model. Cell morphology is displayed at 6 h of culture.
Evaluating the bioactivities of 3D-printed hydroxyapatite-reinforced polyetheretherketone biocomposite for orthopedic and dental applications

Xingting Han¹,², Jibao Zheng³, Chuncheng Yang³, Dichen Li³, Stefanie Krajewski², Frank Rupp²

¹ Peking University, Department of Prosthodontics, Peking University School and Hospital of Stomatology, Beijing, CN; ² Tübingen University, University Hospital Tübingen, Section Medical Materials Science and Technology, Tübingen, DE; ³ Xi'an Jiaotong University, State Key Laboratory for Manufacturing System Engineering, School of Mechanical Engineering, Xi'an, CN

Introduction
In the last few decades, polyetheretherketone (PEEK) has been used as a bone reconstruction material in orthopedic and dental applications due to its good biocompatibility, chemical resistance, low density, radiolucency, and especially for the mechanical properties resembling human bone [1]. Recently the development in 3D printing technologies has made it possible to prepare customized PEEK implants[2]. However, the unmodified PEEK surface is bioinert, which impedes tissue osseointegration and hampers its clinical adoption. One possible approach to improve PEEK’s bioactivity is to prepare composites by incorporation bioactive materials into PEEK substrates, such as hydroxyapatite (HA). Some previous studies have analyzed the bioactivities of HA-PEEK composites and found that after incorporation of HA, in vitro cell adhesion, proliferation, osteogenic mineralization, as well as in vivo bone contact and new bone formation improved significantly [3]. However, most previous studies used traditional manufacturing methods to prepare PEEK composites, e.g., injection-molding. Our former research indicated that 3D printed PEEK surface demonstrated specific printing structures with enlarged surface roughness and area, which revealed increased bioactivities of PEEK material compared with the polished surfaces[4]. Until now, studies about the bioactivities of 3D printed HA-PEEK, to the best of our knowledge, are still lacking.

Experimental Methods
In this study, 10%, 20%, 30%, 40% HA-PEEK, and pure PEEK disk samples (Φ = 14 × 2 mm³) were manufactured by a fused filament fabrication (FFF) 3D printer, the printing parameters were consistent with our previous research[5]. After preparation, the sample surface microstructure, wettability, and roughness were analyzed by SEM, drop shape analyzer, and profilometry devices, respectively. SAOS-2 osteoblast cell line was used for testing the in vitro bioactivities of PEEK and HA-PEEK composites. Cell adhesion on sample surfaces was measured after culturing for 1 d by live/dead fluorescent staining. Cell metabolic activity and proliferation were evaluated after incubation for 1 d, 3 d, and 5 d using cell counting kit-8 (CCK-8) assay. The osteogenic differentiation of SAOS-2 osteoblasts was determined by alkaline phosphatase (ALP) activity and alizarin red staining (ARS) after 5 d and 21 d incubation.

Results and Discussion
Fig. 1 indicates HA-PEEK and pure PEEK surface characterization, in vitro cell adhesion, metabolic activity, and proliferation results. The SEM result demonstrated that the pure PEEK samples indicated a relatively flat surface with clear FFF printing structures, while the HA-PEEK composite surfaces displayed a rougher and more porous appearance. Surface wettability and roughness results indicated that there was no significant difference between pure PEEK and HA-PEEK groups, and a similar tendency could also be detected in cell adhesion and proliferation test. Cell metabolic activity demonstrated that 3D printed HA-PEEK and PEEK samples were not toxic, which indicated that the FFF 3D printing process of PEEK and its composites would not generate toxic effects. The similar results between HA-PEEK and PEEK samples in surface characteristics, cell adhesion, and proliferation can be
explained by the FFF 3D manufacturing methods. After printing, the HA particles were wrapped by PEEK substrate and the bioactive effect of HA particles could not reveal in the early stage of cell culture.

Fig. 2 indicates SAOS-2 osteogenic mineralization of ALP activity and ARS tests. After 5 d incubation, the ALP activity of SAOS-2 in the 40% HA-PEEK group was significantly higher than other groups ($p < 0.001$). The ARS result revealed that compared with pure PEEK samples, the calcium phosphate attached to HA-PEEK composite surfaces was significantly higher, especially for the 40% HA-PEEK composites ($p < 0.001$). The optical microscope images of HA-PEEK also indicated stronger stained bone-like nodules with lighter red color than pure PEEK samples. The result indicated that as the incubation time prolonged, the osteoinductive effect of HA particles was revealed, especially for the increase in mineralized nodules formation.

**Conclusion**

1. After the incorporation of HA particles, a more porous surface could be obtained, but a similar surface roughness and wettability were maintained.
2. FFF printing procedure would not introduce or produce toxic substances for manufacturing HA-PEEK composites.
3. The cell adhesion and proliferation between HA-PEEK and pure PEEK were similar, but the osteogenic differentiation was increased significantly for HA-PEEK composites, especially for the 40% HA-PEEK. Therefore, the FFF 3D printed 40% HA-PEEK is regarded a promising material for orthopedic and dental implants.

**Acknowledgement**

The Forschungsgemeinschaft Dental, Germany (Grant 01/2019) and National Nature Science Foundation of China (Grant 51835010) are gratefully acknowledged for the financial support for this research. The authors would like to thank Ernst Schweizer and Evi Kimmerle-Müller for assistance in the SEM analysis and biological tests.
Fig. 2 Osteogenic differentiation of SAOS-2 osteoblasts on HA-PEEK and pure PEEK samples. Different concentrations of HA/PEEK (10%, 20%, 30%, 40%) and pure PEEK ALP activity and alizarin red staining tests after 5 and 21 d cell incubation respectively. The data is represented in means ± standard deviations, ** p < 0.01, *** p < 0.001.

References
hMSCs response to polymer-calcium phosphate composite scaffolds containing zinc produced using additive manufacturing

Martyna Nikodym1,2, Jiaping Li1,2, David Koper1,2,3, Elizabeth Rosado Balmayor1, Lorenzo Moroni2, Pamela Habibovic1

1 Maastricht University, Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, NL; 2 Maastricht University, Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, NL; 3 Maastricht University Medical Center, Department of Cranio-Maxillofacial Surgery, Maastricht, NL

Introduction
Currently available options for treating critical-size cranio-maxillofacial bone defects involve the use of either autologous bone or, alternatively, a bioinert bone graft substitute. However, the use of autologous bone can result in complications including donor site morbidity, while bioinert materials lack osteointegration ability and have a high risk of implant failure (1). We propose the use of a novel bioactive composite with high ceramic content composed of poly(ethyleneoxide terephthalate)/poly(butylene terephthalate) (1000PEOT70PBT30, PolyActive, PA) and 50% beta-tricalcium phosphate (β-TCP) with the addition of zinc (Zn) in a form of a coating of the TCP ceramic. Several inorganic ions have been shown to affect biochemical functions important for different aspects of bone regeneration (2). Although Zn is found in the bone only in trace amounts, it plays an essential role in its metabolism by stimulating bone formation by osteoblasts and inhibiting osteoclasts differentiation (3). Therefore, we hypothesise that the addition of zinc to the β-TCP will result in enhanced osteogenic properties.

Experimental Methods
Additive manufactured porous 3D scaffolds composed of 1000PEOT70PBT30, 1000PEOT70PBT30 and β-TCP in 1:1 ratio, 1000PEOT70PBT30-β-TCP composites with addition of 15% or 45% of Zn in a form of TCP coating were sterilised and seeded with bone marrow-derived human mesenchymal stromal cells (hMSCs) and cultured in either basic or mineralization medium. DNA content, alkaline phosphatase (ALP) activity and extracellular matrix (ECM) formation were assessed on days 3, 7, 14 and 28.

Results and Discussion
Additive manufactured 3D composite scaffolds used in this study contained 50% of β-TCP, which is among the highest content of ceramic in a composite with a polymer achieved so far, without the need for the use of solvents. Regarding the in vitro assessment of the osteogenic properties of the porous 3D scaffolds, hMSCs cultured on all types of scaffolds proliferated and showed ALP activity. Higher cell growth and ALP production were observed on scaffolds containing β-TCP and β-TCP with the addition of zinc than on 1000PEOT70PBT30 scaffolds. Furthermore, extracellular matrix formation was observed on all scaffolds except for 1000PEOT70PBT30 scaffolds.

Conclusion
The osteogenic properties of tested scaffolds will be further investigated by evaluation of cell differentiation at mRNA level by qPCR analysis for a set of specific osteogenic markers.

References
**PS1-04-159**

**Fabrication and characterization of a novel composite Collagen-Magnesium and Silicon doped Hydroxyapatite scaffolds for bone tissue regeneration**

_Paola Nitti_¹, Sanosh K. Padmanabhan¹, Eleonora Stanca², Luisa Siculella², Antonio Licciulli¹, Christian Demitri¹

¹ University of Salento, Department of Engineering for Innovation, Lecce, IT; ² University of Salento, Department of Biological and Environmental Sciences and Technologies, Lecce, IT

**Introduction**

The bioceramic scaffold composed of pure hydroxyapatite (HA) and doped HA with osteoinductive ions like magnesium (Mg) or silicon (Si) are widely used for bone tissue regeneration. Many works have reported the codoping with both ions in the crystal structure of HA [1], whereas in this work we doped Mg²⁺ and SiO₄⁻⁴ ions separately during powder synthesis and made composite scaffolds, using polymer sponge replica method, by mixing the doped HA powders. Subsequently, to increase the bioactivity, these scaffolds were impregnated with a collagen matrix and freeze-dried, using a new approach that led to the formation of open pores on composite scaffolds surface. The bioceramic scaffolds and composite scaffolds impregnated with Collagen were evaluated for physical, mechanical, biodegradation and biocompatible properties.

**Experimental Methods**

Pure and substituted (Mg and Si) HA were synthesized by aqueous precipitation reaction. Slurries with powders previously calcinated (HA-Mg/HA-Si powder weight ratio kept 50/50), PVA 1%wt and Dolapix were prepared and used to impregnated polyurethane sponges [2]. The impregnated scaffolds were dried and sintered. The ceramic scaffolds obtained were impregnated with collagen type I slurry (0.5 % w/v). The innovative freeze-drying approach (scaffolds were placed on ice plates, immersed in cold distilled water ~ 0°C, cooled to −20°C and then lyophilized) and DHT crosslinking (121°C for 24h under vacuum) were carried out. The ceramic powders and scaffolds were analyzed by TEM and XRD. For all were evaluated the morphology (SEM) mechanical properties, biostability in Tris-HCl, pH 7.4 up 28 days and citocompatibility using Human Bone Marrow-Derived Mesenchymal Stem Cells (BMSC).

**Results and Discussion**

The XRD analysis on powders and scaffolds after calcination and sintering processes revealed the presence a secondary β-TCP in HA-Mg and HA-MgSi, absent in the other ones. All ceramic scaffolds presented Mg and Si ions homogeneously distributed (XRF analysis), porosity >90% and pore size ranging between 200- 850 μm. TEM analysis and microstructural analysis (SEM) of powders and scaffolds HA-Si showed littler grain size compared to the others ones. This decrease seems positively influence the mechanical properties of ceramic scaffolds. Indeed, HA-Si and HA-MgSi scaffolds revealed better compression trends compared to HA and HA-Mg. This trend was observed also after 28 days of soaking in TRIS, despite the overall decrease observed for all scaffolds. To increase the bioactivity, the bioceramic scaffolds are impregnated with collagen using a new freeze-drying approach studied firstly in this work. SEM analysis showed scaffolds with more open surface porosity compared to tradition freeze-drying. This new approach avoids the formation of collagen skin layer, thanks the formation of ice crystals on the scaffolds surface that lead to have open pores on the outer layer of the scaffold. The mechanical tests on bioactive scaffolds shown the same trend observed for ceramic ones but with a considerable decrease of stress at failure (σmax), probably due to the poor compressive strength of type I collagen fibrils. The HA-Si_Coll and HA-MgSi_Coll scaffolds showed better mechanical resistance before and after immersion in physiological solution. In particular, for both scaffolds after a week of soaking, the loss of the collagen component from the scaffolds made the ceramic...
component prevail, thus leading to an increase in $\sigma_{\text{max}}$ with values similar to those observed for the corresponding ceramic ones. Cells proliferation experiment showed that all scaffolds where biocompatible and composite scaffold (HA-MgSi_Coll) is more effective for cells adhesion and proliferation compared to all other scaffolds.

**Conclusion**

In this study, bioactive scaffolds with a composite structure made with a new ceramic mix (HA-MgSi) and collagen were fabricated successfully using a new freeze-drying approach. Firstly, bioceramic scaffolds produced with the new blend of HA doped were fabricated. These scaffolds showed improved mechanical properties also up 28 days of soaking in physiological conditions and good biocompatibility compared to HA, HA-Mg and HA-Si scaffolds. In addition, to improve the bioactivity, the optimized HA-MgSi scaffolds were impregnated with collagen using a new freeze-drying approach that allow having open pores on surface. The presence of collagen and open pores have in fact promotes better cells proliferation than corresponded bioceramic scaffolds.

References


**PS1-04-163**

**Doubled Crosslinked Sodium Alginate-Graphene Oxide Hydrogels for Cartilage Substitution**

Carolina M. Costa¹, Andreia S. Oliveira¹,²,³, Célio G. Figueiredo-Pina²,⁴,⁵, Ana P. Serro¹,²

¹ Centro de Química Estrutural (CQE), Instituto Superior Técnico, Universidade de Lisboa, Lisbon, PT; ² Centro de Investigação Interdisciplinar Egas Moniz (CiiEM), Instituto Universitário Egas Moniz, Caparica, PT; ³ Instituto de Engenharia Mecânica (IDMEC), Instituto Superior Técnico, Universidade de Lisboa, Lisbon, PT; ⁴ CDP2T and Department of Mechanical Engineering, Setúbal School of Technology, Instituto Politécnico de Setúbal, Setúbal, PT; ⁵ CeFEMA, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, PT

**Introduction**

The articular cartilage is of utmost importance in the musculoskeletal system due to its almost frictionless surface and biomechanical properties. Hydrogels have been studied as potential cartilage substitution for their resemblance to the extracellular matrix. In particular, hydrogels based on sodium alginate (SA) are promising candidates due to the biocompatibility of this natural polysaccharide, moisture retention properties and processing flexibility, since it can be crosslinked through ionic, covalent, or physical bonds [1]. Nevertheless, the uncontrollable degradation in saline solutions of ionic crosslinked SA and the poor mechanical strength of covalent crosslinked SA hinder their application in the tissue engineering field [2]. On the other hand, graphene oxide (GO) has gained research thrust in many areas, including biomaterials for their outstanding mechanical strength, lightweight and high surface-to-volume ratio [3]. This study aimed to develop a material with suitable mechanical and tribological properties for cartilage substitution, through a double step crosslinking and incorporation of graphene oxide within the network.

**Experimental Methods**

Alginic acid sodium salt from brown algae with medium viscosity (MW = 100 000 - 200 000 g/mol) and low viscosity (MW < 100 000 g/mol) were dissolved in DD water at 50 ºC to prepare 5% wt. SA solutions. Methacrylic modification of both solutions took place at RT for 72 h. Thereafter, solutions were vacuum filtered, and the methacrylate-alginate (MA) product was dissolved in DD water to prepare three solutions with 0.05% (w/v) photoinitiator: 10% wt. with MA of medium viscosity (10M), 10% wt. with a SA:MA ratio of 50%/50% (10M 50%) and 16% wt. MA of low viscosity (16L). Those solutions were poured in petri dishes and exposed to UV light (254 nm) for 4 hours. After covalent crosslinking, the resulting hydrogels were immersed in 1 M CaCl₂ aqueous solution for one day. SA-GO hydrogels were prepared with 0.1%, 0.2% and 0.3% (w/v) of GO in 10M solutions. 10M, 10M 50% and 16L hydrogels were characterised regarding their degradation in phosphate buffered saline solution (PBS) for 72 h at 37ºC, equilibrium water content (EW₃), mechanical performance (assessed through unconfined and hydrated compression tests at a strain rate of 0.1 mm/s at RT of as-prepared hydrogel and after 72 h in PBS. The effect of the presence of GO on the mechanical properties and tribological behaviour of the material was also evaluated. Tribological tests were performed in a pin-on-disc equipment using pins of porcine knee cartilage as countersurface (reciprocating sliding at 4 mm/s with a load of 20 N, in either PBS or human synovial fluid lubricating media).

**Results and Discussion**

A chemical modification of the hydroxyl groups of SA’s backbone enabled covalent bonding by UV exposure with further ionic bonding of the carboxyl groups. 10M, 10M 50% and 16L presented an EWC of 85-89%, similar to natural articular cartilage. The degradation of hydrogels was assessed through the weight loss quantification after immersing the dried samples in PBS for 72 h. 10M, 10M 50% and 16L had a weight loss after 72 h of 4% ± 1.67, 28% ± 2.12, and 19% ± 2.31, respectively. 10M was selected to carry out mechanical and tribological testing. Compressive modulus of 10M were evaluated before (0.65 MPa ± 0.27) and after immersion in PBS (0.57 MPa ± 0.23), falling in
the range of values reported in the literature for the articular cartilage and presenting minimal degradation after 72 h. Addition of GO improved the mechanical properties, as expected. Regarding the tribological performance, the mean friction coefficient decreased as GO content increased. Smaller friction coefficients were obtained in synovial fluid compared with PBS solution. SA-GO composite hydrogels revealed a low friction coefficient, mimicking the cartilage-on-cartilage contact.

Conclusion

In summary, a dual step crosslink proved to be a suitable approach to produce SA hydrogels for load-bearing cartilage substitution. Degradability and mechanical properties can be tailored by varying covalent and ionic bonding density. The presence of GO, even in small amounts, lowered the friction coefficient of composite hydrogels.

Acknowledgement

The authors acknowledge to Fundação para a Ciência e a Tecnologia for the financial support (grant numbers: PD/BD/128140/2016 [A.S. Oliveira], PTDC/CTM-CTM/29593/2017 [CartHeal], UIDB/00100/2020 [CQE], UIDB/50022/2020 [IDMEC/LAETA], and UIDB/04585/2020 [CiiEM]).

References

Influence of scaffold geometry on BMP-2 incorporation and in vivo bone regeneration

Charlotte Garot¹, Julie Vial²,³, Paul Machillot¹, Adeline Decambron²,³, Delphine Logeart-Avramoglou²,³, Julien Vollaire⁴,⁵, Véronique Josserand⁴,⁵, Emeline Maurice²,³, Véronique Viateau²,³, Mathieu Manassero²,³, Georges Bettega⁴,⁶, Catherine Picart¹

¹ CEA, CNRS, Université Grenoble Alpes, ERL5000 BRM, Grenoble, FR; ² Université de Paris, CNRS, INSERM, B3OA, Paris, FR; ³ Ecole Nationale Vétérinaire d’Alfort, B3OA, Maisons-Alfort, FR; ⁴ INSERM, U1209 Institut Albert Bonniot, Grenoble, FR; ⁵ Université Grenoble Alpes, Institut Albert Bonniot, Grenoble, FR; ⁶ Centre Hospitalier Annecy-Genevois, Service de chirurgie maxillo-faciale, Epagny Metz-Tessy, FR

Introduction
Critical-size bone defects are unable to heal by themselves during the patient’s lifetime. Currently, autologous bone graft is the gold standard solution to treat such defects, but it is associated with some limitation such as limited availability, high donor-site morbidity, and inconsistency of repair in very large bone defect [1]. To tackle these drawbacks, synthetic bone grafts called scaffolds are currently being developed. These scaffolds should be biocompatible, preferably biodegradable and bioresorbable, osteoconductive, osteoinductive, and have physico-chemical properties optimizing bone regeneration. A critical parameter to obtain efficient scaffolds is their internal geometry. It has been shown that an efficient scaffold should be porous [2]. However, the ideal pore size and shape have not been evidenced yet, since they depend on many parameters and could be different depending on the site of implantation. The objective of this study was to investigate the influence of scaffold geometry on bone morphogenetic protein 2 (BMP-2) incorporation on the scaffold and on in vivo bone regeneration in a sheep metatarsal critical-size bone defect.

Experimental Methods
Three different scaffold geometries were selected for the in vivo regeneration of a long-segmental critical-size bone defect in a sheep metatarsal bone based on a literature study, previous results, and a design of experiments optimizing the geometries in terms of physico-chemical properties, mechanical properties, and ability to coat them with a biomimetic film previously developed by the authors [3]. Each scaffold (cylinder 25mm high and 14mm in diameter) was implanted in a 25-mm long segmental metatarsal defect in sheep subsequently stabilized by plate osteosynthesis (Figure 1). The first scaffold geometry, called Lines, had cubic pores with a pore size of about 880µm and a central cylinder hole of 5mm in diameter (Figure 1A). The second geometry, called Gyroid, was a triply-periodic minimal surface design with a pore size of about 1mm and a central cylinder hole of 5mm in diameter (Figure 1B). Finally, the third geometry, called Double Lines, was a mix of the two first geometries: the outer of the cylinder had cubic pores with a pore size of about 1.2mm while its center (7mm in diameter) had a gyroid pore shape with a pore size of about 2mm (Figure 1C).

Scaffolds made of medical-grade poly(L-lactic) acid were 3D printed by fused deposition modeling and coated with a polyelectrolyte film as previously described [4]. BMP-2 was then loaded in the film at a targeted surface dose of 9.2 µg/cm² of film for each geometry. The scaffolds surfaces (so the film surfaces) were assessed by micro computed tomography (µCT) scans (Figure 1): 68 cm² for the geometry Lines, 44 cm² for the geometry Gyroid and 54 cm² for the geometry Double Lines. The amount of BMP-2 effectively incorporated in the film was then quantified using a UV/Vis spectrophotometer by first measuring the concentration of the loading solution prior incubation and then measuring the concentration of this same solution after incubation. Two scaffolds of each geometry were implanted in six sheep, each sheep receiving one implant. X-ray radiographs were acquired right after the implantation and...
every month until the end of the study at four months. Then, the metatarsal bone was explanted and bone formation in the bone defect area filled with scaffolds was assessed by µCT and histology.

**Results and Discussion**

BMP-2 quantification showed that, despite the fact that all scaffolds were loaded with the same surface dose, the incorporated dose was different depending on the scaffold geometry. Indeed, the geometry Lines incorporated less BMP-2 than the other geometries (4.4 ± 1.3 µg/cm²) and the geometry Gyroid incorporated more BMP-2 than the other geometries (7.7 ± 0.9 µg/cm²). The geometry Double Lines incorporated 7.5 ± 2.8 µg of BMP-2 per cm² of film. Surprisingly, scaffolds had incorporated similar total amounts of BMP-2 (about 350 µg/implant), their effective surface being different. These results suggest that the geometry of scaffolds has an influence on BMP-2 incorporation in the biomimetic film. Interestingly, X-ray radiographs showed that despite its lowest incorporated BMP-2 surface dose, the geometry Lines presented the fastest and strongest bone formation, followed by the geometry Gyroid. The geometry Double Lines did not allow bone repair (Figure 2). As the total amount of BMP-2 was similar in every scaffolds, this suggests that the scaffold geometry influenced bone regeneration kinetics and the amount of new bone formed.

**Conclusion**

Further investigation has to be led on the influence of pore size and shape on BMP-2 incorporation and *in vivo* bone regeneration but this preliminary study opens perspective to optimize the scaffold geometry in view of long bone repair. The optimization of pore size and shape along with the optimization of the BMP-2 dose could lead to efficient implants with reduced fabrication costs and reduced secondary effects.

**Acknowledgement**

We thank the Agence Nationale de la Recherche (ANR) for the funding (ANR-18-CE17-0016, OBOE). CP is a senior member of the Institut Universitaire de France whose support is acknowledged.
Figure 2

Representative X-ray radiographs of scaffolds implanted in sheep metatarsus after 0, 1, 2, and 3 months.

References


Injectable Bone Substitute based on Demineralized Bone Matrix, using a Polymeric Viscoelastic System as a Carrier

Daniela Medrano David1, Aura M. Lopera Echavarría1, Yesid D.J. Montoya Goez1, Edison Duque Caballero5, Jaime Duque Ramírez4, Pedronel Araque Marin2,3, Martha E. Londoño López1, COLCIENCIAS

1 EIA University, Research group GIBEC, Envigado, CO; 2 EIA University, Research and Innovation Group in Chemical Formulations, Envigado, CO; 3 CECOLTEC, Research Group, Medellin, CO; 4 Foundation, Tissue Bank, Medellin, CO

Introduction

The occurrence of bone-related disorders and diseases has increased drastically over recent years worldwide. Autologous bone grafting remains the gold standard for bone tissue repair due to their histocompatibility, no immunogenicity, capacity to promote osteoinduction and osteoconduction. Despite the many advantages of bone grafting, there exist issues such as secondary damage, significant donor site injury and morbidity, deformity, scarring, in addition to surgical risks such as bleeding, inflammation, and high cost [1]. Therefore, science has now taken on the challenge of developing new materials to be used for bone regeneration, such as Demineralized Bone Matrix (DBM) which has been widely used as a bone implant due to its osteoinduction and bioactivity [2]. However, the use of DBM is limited, due it is a particulate material, is difficult to manage and implant accurately, in addition, these particles are susceptible to migrate to other sites through the bloodstream. The main reason is DBM is commonly incorporated into a variety of carriers. An injectable scaffold composed of an inorganic and organic phase presents advantages when compared to bone grafts or pre-formed scaffolds such as the ability of the injectable scaffold to flow and fill the bone cavity or defect [3]. This project aims to develop a DBM carrier with such viscoelastic properties to obtain an injectable bone substitute (IBS). The developed DBM carrier consisted of a PVA/glycerol network cross-linked with borax and reinforced with calcium carbonate as a pH neutralizer and source of calcium.

Experimental Methods

The preparation of the material is based on mixing in a ratio of approx. 1/7 a liquid phase (PVA/glycerol/water) and a solid phase (DBM/CaCO3). Two samples were tested, where the concentration of PVA was varied. F1 represents the formulation with 2% (w/w) of PVA in the liquid phase and F2 corresponds to 4% (w/w). The mixture from the two phases resulted in a putty-like consistency, moldable and homogeneous. The final IBS samples were added to a syringe to facilitate its application. The physicochemical characterization was determined using X-ray diffraction (XRD) and Scanning electron microscopy (SEM). The force required to extrude the IBS from a syringe was determined using an Instron 3345 with a 5000 N load cell and at a speed of 5 mm/min. Injectability was determined using Eq. (1).

$$\text{Injectability} \% = \left( \frac{\text{mass extruded from the staying}}{\text{total mass before injection}} \right) \times 100\% \quad \text{Eq. (1)}$$

Degradation was calculated by gravimetric technique for 28 days. The bioactivity of IBS after immersion in SBF (simulated body fluid) for 14 days was evaluated by (SEM-EDX). Cytotoxicity in SAOS-2 was indirectly assessed by the MTT assay and cell proliferation using the Alamar Blue kit for 72 h.

Results and Discussion

The results of the injectability test are summarized in Table 1. Neves et al. indicate that for a bone substitute to be considered injectable, the force to extrude it through a syringe must be less than 100 N [4]. Under this criterion, it is concluded that F1 is an injectable formulation and whose injectability is greater than 90%. The results obtained for F2 suggest that when the concentration of PVA is doubled, the resistance to flow increases and makes injectability...
more difficult. Because of this, the research continued to characterize only F1. Using XRD, the demineralized bone matrix was found to be a highly amorphous material. The developed IBS showed an amorphous content greater than 80%, this can facilitate the adhesion of osteoblasts and influences the reabsorption processes [5]. The degree of crystallinity (approx. 14.9%) is attributed to the semi-crystalline nature of PVA. The degradation results showed a stable material, at 28 days only about 18% of its mass was degraded. After 14 days of immersion of the IBS in SBF, the formation of calcium phosphates was detected on the surface, with a Ca/P ratio of 1.3 approx. (Figure 1.B). This shows that when DBM is conveyed in the developed carrier, its bioactivity is not affected. This demonstrates the biomineralization capacity of the material, which is an essential factor in promoting the osteogenic capacity of bone-binding for bone repair materials. The MTT results demonstrated the non-cytotoxicity of the created IBS. The proliferation test showed that at 72 hours of incubation, the cell viability of the osteoblasts treated with IBS doubled with respect to the untreated cells. This proliferative effect in osteoblasts is promising for further investigation. Table 1. Injectability results

<table>
<thead>
<tr>
<th>F</th>
<th>Max. compression load (N)</th>
<th>Injectability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50.7 ± 2</td>
<td>93 ± 1.2</td>
</tr>
<tr>
<td>2</td>
<td>193 ± 28.7</td>
<td>59.3 ± 18.8</td>
</tr>
</tbody>
</table>

Conclusion
A promising material was developed to be an injectable bone substitute, with the characteristics that the DBM offers. Their injectability, stability, bioactivity, non-cytotoxicity, and cell proliferation in osteoblasts were tested.

Acknowledgement
The authors would like to thank EIA University, COLCIENCIAS, and Tissue Bank Foundation for making this research possible.

Figure 1. A) IBS before and B) IBS after SBF immersion
Figure 2. Cell proliferation results.
* $p < 0.05$, ** $p < 0.01$ and *** $p \leq 0.001$ compared to the control. Error bars represent ± standard deviation.

References
Surface characterization of dental ceramics before and after thermal cycling and immersion in artificial gastric acid

Panagiotis Pandoleon, Katia Sarafidou, Eleana Kontonasaki

Aristotle University of Thessaloniki, Department of Dentistry, Thessaloniki, GR

Introduction
Nowadays there is a plethora of all-ceramic materials available in the dental market. Their different composition may result in different biological and mechanical behavior. Furthermore, temperature and pH alterations in the oral cavity may affect the biological properties and surface stability of dental ceramics. The aim of the present study was to investigate the impact of thermal artificial aging and exposure to artificial gastric acid on the surface roughness of monolithic zirconia (MZ) and zirconium lithium silicate (ZLS) ceramics. The null hypothesis was that surface roughness will not be affected after each aging treatment.

Experimental Methods
The following materials were included in the in vitro testing: Katana High Translucent - Kuraray (MZ) and Celtra Duo - Densply Sirona (ZLS). Twenty specimens were fabricated from each material in the following dimensions 10x10x1.5mm. Half of the specimens in each group underwent thermal artificial cycling, whilst the other half of them were immersed in artificial gastric acid. Thermal cycling was performed in distilled water. A total of 10,000 thermal cycles were performed between 37°C – 55°C – 37°C – 5°C, with a dwell time at each temperature of 15 seconds representing 1-year clinical time [1]. The gastric acid solution consisted of 5% hydrochloric acid (pH = 2). Specimens were immersed and placed in an incubator at 37 °C for 91 hours, equivalent to 1 year of exposure to HCl in a patient with bulimia [2]. Surface characterization was performed before and after thermal cycling and exposure to gastric acid through X-Ray Diffraction Analysis-XRD and roughness was measured with a 3D optical profilometer. The main roughness parameters Sa, Sz, Sp, Sv, and Sku were recorded, and data were statistically analyzed by one-way ANOVA with the level of significance set at p<0.05.

Results and Discussion
Initial mean values of surface roughness was 0.34 (±0.1) μm for MZ and 0.33 (±0.06) μm for ZLS without presenting any statistically significant difference between the two materials. After thermal cycling, mean surface roughness (Sa) was moderately affected by both treatments (p>0.05) accepting the null hypothesis. After immersion in artificial gastric acid all roughness parameters of MZ were slightly decreased, however the differences were not statistically significant. Similarly, non-significant differences were found for the roughness of ZLS ceramics. Ahmed et al. [3] resulted in similar conclusions, regarding milled ZLS ceramics and MZ, that presented non-significant alterations in roughness’ mean values after immersion in gastric acid with pH=1.2 for 96 hours. However, al-Thobity et al. [4] reported significant increase in surface roughness of MZ after prolonged in vitro aging of 168h in an acidic environment with pH=2.2. XRD analysis revealed the presence of cubic and tetragonal zirconia on the surface of MZ ceramics, without remarkable alterations after both thermal cycling and acidic storage. However, different crystalline phases of lithium silicates (Li4(SiO4), Li2(SiO3), Li2SiO3) were observed before and after both treatments. Qualitative analysis after in-vitro testing revealed that thermal cycling and immersion in gastric acid affected the surface micromorphology of ZLS to a greater extent compared with MZ.

Conclusion
Under the limitations of this in vitro study, it can be concluded that both dental ceramics are not affected by thermal cycling or storage in artificial gastric acid, at least for the specific time evaluated. However, slight morphological changes can occur under the thermal and pH fluctuations in the oral cavity which may accelerate the degradation of...
the materials and thus should be taken into consideration with respect to the biological long-term behavior of dental ceramics. Further long-term in vitro and in vivo studies are essential to validate these in vitro outcomes.

Acknowledgement
This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postdoctoral Researchers - 2nd Cycle" (MIS-5033021), implemented by the State Scholarships Foundation (IKY).

![Graphical representation of ZLS and MZ specimens before and after aging procedures.](image)

**Figure 1.** Representative optical profilometer images of ZLS and MZ specimens before and after aging procedures.

References
PS1-04-173

Animal bone matrices purification based on modified supercritical CO2 fluid processes: immunological characterisations and bone tissular regeneration capacities

Solène Rota1,2, Ludovic Sicard3, Caroline Gorin3, Catherine Chaussain3, Michel Boissière1, Raphaël Bardonnet2, Emmanuel Pauthe1

1 CY Cergy-Paris Université, ERRMECe laboratory, International House of Research, Neuville-sur-Oise, FR; 2 Biobank, Lieusaint, FR; 3 Laboratory URP2496 Orofacial Pathologies, Imaging and Biotherapies, Paris, FR

Introduction
The craniofacial area is prone to trauma or pathologies, often resulting in large bone damages, that cannot heal spontaneously. Their reconstruction is a real challenge and the gold standard surgical technique remains human bone grafting (autologous and allogenic). The increasing demand for bone grafting and the relative availability of autologous or allogenic materials are important driving forces of bone replacement research [1]. In this context, the use of alternative xenogeneic materials to repair human bone defects is a promising approach, with their natural structure and properties close to human bone. However, the risk of rejection due to immunogenic molecules – such as the alpha-Gal epitope [2] – is a major concern in animal bone transplantation to the human.

Experimental Methods
To avoid immunogenicity without compromising biological and mechanical properties of the xenogeneic bone matrix, bones were treated with the Supercrit® process. It is based on the delipidation of bone tissue by supercritical carbon dioxide, combined with a chemical oxidation of the residual proteins [3]. Supercrit® is already approved on human bone, showing complete viral inactivation and good preservation of bone properties [4]. The interest of transposing it to animal bone tissues is patent.

Results and Discussion
Our in vitro results show the efficiency of the Supercrit® process on animal bone. However, remaining traces of alpha-Gal epitope are evident on the treated bone, suggesting the need to improve the purification process. An optimized process, called Supercrit +, has been developed. It results in an important reduction of the quantity of solvent used, and therefore diminishes the potential toxicity of the chemicals on bone integrity and quality. In vivo studies are currently in progress, the first one to analyze the tolerance of the treated biomaterials in subcutaneous mice ectopic sites; and the other one to assess their osteogenic capacity on bone regeneration in rat calvaria bone defect.

Conclusion
In vitro bone characterizations are conducted to complete our in vivo data. By comparing thermogravimetric analyses, scanning electron microscopy (SEM) images, and the biochemical composition of bones, the effectiveness of the Supercrit + process on animal bone has been demonstrated, with the added benefit of using less solvent.

References
Physiologically Engineered Scaffolds for Restoring Large Bone Defects

Dina Abdulaziz¹, Neelam Iqbal¹, Peter Giannoudis², Animesh Jha¹

¹ University of Leeds, School of Chemical & Process Engineering, Leeds, GB; ² University of Leeds, Academic Department of Trauma and Orthopaedic, School of Medicine, Leeds, GB

Introduction
Bone is a complex multiphasic tissue with significant metabolic and regenerative properties, essential for physiological and anatomical functions, which, are disrupted when the tissue is damaged. Restoring large bone defects is one of the most critical challenges in orthopaedics because of the time needed for healing such fractured injuries. The “ideal” scaffold’s material must have the following four properties collectively to avoid missed union or non-union in a damaged bone: a) osteogenic properties for inducing and maintaining the neo-osteogenesis. b) appropriate scaffold structure for supporting the circulation of blood and nutrients. c) promote angiogenesis and d) biomechanical compatibility comparable to that of natural bone to withstand the body's load. If anyone of these properties is missing the healing of bone is compromised [1,2]. The above four properties in a bone structure are controlled within the cortex, cancellous structures, and medullar canal. In the present work, we discuss the design and fabrication of a two-layered scaffold that embodies the properties b), c), and d) in a physiological analogue of a natural bone. The scaffold provides mechanical stability, biomechanical stimulation of bone growth and tissue regenerative characteristics via increased vascularisation for improved diffusion of nutrients and oxygen to cells.

Experimental Methods
Layer-1 material was prepared by mixing different ratios of titanium powder with 10% iron-doped brushite mineral. Porosity within the cortical structure was controlled by adjusting the ratio of mineral and alloy phase. The powder pressed pellets were sintered in Argon atmosphere at 850 °C, 1000 °C and 1150 °C for 2 h. For the fabrication of cancellous Layer-2, 6 g of chitosan flakes was dissolved in a 2 (v/v)% aqueous acetic acid solution under continuous mixing for 24 h, after which different quantities of brushite minerals (i.e. 20, 30, 40 and 50 (w/v)%%) were added. The mixtures were left to dry until the formation of a compact membrane and frozen at -80 °C for 24 h, then freeze-dried for 24 h. The structure of the scaffold is shown in figure 1 (a) and (b).

Results and Discussion
Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) techniques verified the synthesis of brushite mineral and the phase changes in the metal part. XRD confirmed that titanium samples sintered in an inert atmosphere were not oxidised regardless of the sintering temperature. While the heated iron-doped brushite (DPCS-Fe) has been transformed into β-pyrophosphate, known for its osteoconductive properties and controllable degradation rate. For both Layer-1 and Layer-2 materials, the porous structures were confirmed via SEM characterisation as depicted in figure 1 (d) and (e). The elastic modulus values obtained for the Layer-1 materials ranged between 10 - 40 GPa based on the level of porosity, brushite ratio and sintering temperature. These values are comparable with that of human cortical bone (2-18 GPa), which means they can minimise the stress-shielding effect. Also, they showed suitable yield strengths (~125 MPa) to resist permanent shape change under Loading. Contact and Extract Cytotoxicity Assay verified that Layer-1 and Layer-2 materials do not present any toxicological risks. Figure 1 (c) displays the confocal microscopy image of Alexa Fluor-488 and Dapi fluorescent stained cells on the Layer-1 porous titanium samples. Osteoblasts were able to grow and proliferate on both synthesised materials, as shown in figure 2.

Conclusion
This work investigated the fabrication of two-layered synthetic bone scaffolds which, can provide the appropriate mechanical support and biological response during bone restoring. The synthesised materials were characterised, mechanically and biologically tested. Results of both Layer-1 and Layer-2 biomaterials indicate comparable Young Modulus to cortical and cancellous bone, respectively. The scaffold materials do not present any toxicological risks, while the cell attachment and proliferation results for up to 7 days were promising. The confocal microscopy results confirmed osteoblast growth and proliferation throughout the Layer-1 and Layer-2 biomaterials.

Acknowledgement
Authors would like to thank Royal Commission for the Exhibition of 1851, Glass Technology Services and University of Leeds for the financial and technical support.

Figure 1:
(a) Layer-1 of the scaffold (synthetic cortical bone), (b) Layer-2 (synthetic cancellous bone) precipitated on layer-1, (c) Confocal microscopy image (scale bar: 50 μm) of osteoblast G292 cell line proliferated onto the surface of Layer-1 biomaterial after seeding for 3 days. The green colour represents actin, and the blue colour represents nuclei. (d) SEM image of Layer-1 sample with pore sizes ranging between 50 μm and 350 μm, and (e) Layer-2 sample with pore sizes ranging between 20 μm and 90 μm.

Figure 2:
(a) Osteoblast cell proliferation on Layer-1 biomaterials and (b) Layer-2 biomaterials evaluated using PicoGreen assay. The error bars are equivalent to standard deviation, and the * represents statistical significance where p < 0.05.
References

2:30 p.m. – 4:00 p.m.

Poster floor

PS1-05 | Biomaterials Development
Analysis of the recovered biopolymers-bioactive glass with gold nanoparticles after bone implantation

Klara Magyari¹,³, Alexandra Dreanca²,¹, Marian Taulescu², Milica Todea⁴,¹, Tamas Gyulavari³, Lucian Baia¹,⁵

¹ Babes-Bolyai University, ICI-BNS, Cluj-Napoca, RO; ² University of Agricultural Science and Veterinary Medicine, Faculty of Veterinary Medicine, Cluj-Napoca, RO; ³ University of Szeged, Department of Applied and Environmental Chemistry, Szeged, HU; ⁴ Iuliu Hațieganu University of Medicine and Pharmacy, Faculty of Medicine, Cluj-Napoca, RO; ⁵ Babes-Bolyai University, Faculty of Physics, Cluj-Napoca, RO

Introduction

The ideal scaffold can simulate the natural mechanism of regeneration from the human body; thus, the ideal bone scaffold has the following requirements: excellent bioactivity, good biocompatibility, osteoconductivity, osteoinductivity, simulate angiogenesis, relevant structural-mechanical properties and biodegradable properties [1]. A promising approach to solve several mentioned issues is the use of composites with polymer, ceramics, metals, cells and growth factors [2]. Excellent bioactivity could be given by bioactive glasses (BG) that can bind with bone in the living organism. BG with gold nanoparticles (AuNPs) content can stimulate keratinocytes cell proliferation [3]. In our recent study was demonstrated that alginate-pullulan-bioactive glass with spherical gold nanoparticles (Alg-Pll-BGAuSP) are promising materials for soft and bone engineering endeavors [4]. Continuing this study, the composites were implanted in bone defect in Wistar rats for 8 weeks, after that the remained materials were recovered. For the control β-tricalcium phosphate-hydroxyapatite (Alg-Pll-βTCP/HA) was used after introducing it in an alginate-pullulan composite.

The aim of this study was to understand the formation of apatite layer on materials surface implanted into the bone defect (Figure 1). This will be useful in the future for designing materials for applications in medicine.

Experimental Methods

The Alg-Pll-AuSP and Alg-Pll-βTCP/HA composites were obtained by cross-linking method. In vivo implantations were carried out in Wistar rats. A bone defect was created into the distal part of the femoral diaphysis. The implanted materials were recovered after 8 weeks and were analyzed via X-ray diffraction (XRD, Shimadzu XRD 6000), FT-IR spectroscopy (Jasco 6200), scanning electron microscopy (SEM, Hitachi S-4700 Type II ) and X-ray Photoemission Spectroscopy (XPS, SPECS PHOIBOS 150 MCD system ). For comparison the samples were immersed in simulated body fluid (SBF, pH 7.4) at 37°C for 8 weeks.

Results and Discussion

The XRD pattern of the recovered composite, like that immersed in SBF, shows the diffraction pattern of apatite. The FT-IR spectra of the recovered material, beside the specific vibration bands of apatite, presents the vibrations of the proteins and amino acids: amide I (1650 cm⁻¹), amide II (1550 cm⁻¹) and amide III (1300 cm⁻¹), proving the attachment of proteins on the material surface. The presence of the proteins and apatite layer on the samples surface also has been proven by XPS spectroscopy. The presence of the apatite layer on the composites’ surface is also confirmed by the SEM micrographs both for the recovered materials and for immersed materials in SBF. In the case of recovered materials, the granulation of apatite is smaller than after SBF immersion due to the presence of proteins.

Conclusion

The composites of alginate-pullulan–bioactive glass with gold nanoparticles and alginate-pullulan-hydroxyapatite/tricalcium phosphate were successfully synthesized by cross-linking method and implanted in the rat at the distal part of the femoral diaphysis. The surface of the recovered composites was covered by an apatite layer,
as a result of the immersion of the sample’s surface in SBF, this behavior indicating that the in vitro bioactivity assay is a good and inexpensive method for the evaluation of the materials bioactivity.

Acknowledgement

This work was supported by a grant of Ministry of Research and Innovation, CNCS - UEFISCDI, project number PN-III-P1-1.1-TE-2019-1138, within PNCDI III and by UNKP-20-5-SZTE-657 New National Excellence Program of the Ministry for Innovation and Technology. K. Magyari acknowledge the support of the János Bolyai Research Scholarship of the Hungarian Academy of Science.

References

Supercritical sterilization for the processing of sensitive biomaterials

Clara López-Iglesias¹, Víctor Santos-Rosales¹, Beatriz Magariños², Carlos A. García-González¹

¹ Universidade de Santiago de Compostela, Department of Pharmacology, Pharmacy and Pharmaceutical Technology, I+D Farma Group (GI-1645), Faculty of Pharmacy, Santiago de Compostela, ES; ² Universidade de Santiago de Compostela, Department of Microbiology and Parasitology, Faculty of Biology and Center for Biological Research of the University of Santiago de Compostela (CiBUS), Santiago de Compostela, ES

Introduction
Sterility is defined as the absence of viable microorganisms and is a fundamental requirement for medical devices to minimize the risk of infection after implantation [1]. Some materials can be sensitive to standard sterilization techniques, such as steam, dry heat, UV light, ethylene oxide or gamma irradiation, and suffer important damage in their structure. This scenario is particularly challenging for the sterilization of biomaterials, including polymeric scaffolds.

Supercritical CO₂ (scCO₂) is a recognized antimicrobial agent effective against vegetative forms of a wide range of microorganisms [2]. By the incorporation of additives, such as or (H₂O₂, EtOH, …) the sterilization of, spores i.e. resistance forms, can be also achieved. Overall, the mild conditions of temperature and pressure required for scCO₂ sterilization opens up the processability of biomaterials with complex structures.

In this work, the efficacy of different supercritical sterilization methods was studied against not only Gram(+) and Gram(-) vegetative bacteria, but also bacterial endospores. In addition, the developed sterilization protocol was tested for the processing of polymeric aerogels of different sources (alginate, chitosan, starch and pectin) in terms of physicochemical and structural stability.

Experimental Methods
Staphylococcus aureus and Pseudomonas aeruginosa were selected as model Gram(+) and Gram(-) bacteria, respectively.

Liquid suspension of the bacteria (10⁸ CFU/mL) were loaded in a high-pressure autoclave (NovaGenesis, NovaSterilis Inc., Ithaca, NY, USA), which was heated to the desired temperature and pressurized with CO₂. After a certain contact time, the system was depressurized at a constant flow rate until atmospheric pressure.

The samples were recovered and seeded in tryptic soy agar (TSA) plates for colony forming units (CFU) quantification. The parameters of the sterilization trials are summarized in Table 1. The optimal conditions of pressure, temperature, duration of the process and depressurization rates were obtained.

For the sterilization of bacterial endospores, a commercial suspension of Bacillus pumillus was employed. The bacterial suspension was inoculated in a 1 cm² polymeric porous membrane, which was introduced into thermally sealed sterilization sachets in triplicate. The sterilization procedure was carried out in the NovaGenesis equipment, and afterwards the samples were incubated for 24 h in TSB. Conditions for the sterilization procedures are also summarized in Table 1.

To evaluate the stability of biopolymeric aerogels to the sterilization process, the aerogels were subjected to the same conditions and evaluated in terms of textural properties using nitrogen adsorption-desorption tests, and morphology using scanning electron microscopy and image analysis.

Results and Discussion
A sterilization process for vegetative S. aureus and P. aeruginosa was optimized at 100 bars of pressure, 40 °C of temperature, 30 min of duration and 5 bar/min of depressurization velocity without using any additives, achieving a logR of 8 both for Gram(+) and Gram(-) bacteria (Table 1). Attempts to reduce the sterilization time to 10 min resulted...
in reduction in the bacterial growth, but did not achieve complete bacterial inhibition. At these conditions, the process was more efficient for *P. aeruginosa* (logR = 6) than for *S. aureus* (logR = 7). Gram(+) bacteria are more resistant to scCO₂ sterilization due to lower diffusion of CO₂ through the peptidoglycan membrane [3].

Regarding the endospore sterilization, the process was optimized at 100 bar, 39 ºC, 30 min of duration and a depressurization rate of 17 bar/min, achieving complete endospore inhibition when including 300 ppm H₂O₂ as an additive (Table 1).

Regarding the stability of the aerogels under the supercritical sterilization conditions, the textural and morphological properties were maintained for both the optimized procedures.

**Conclusion**

Optimized sterilization procedures of short duration and mild conditions of P and T were obtained both for vegetative bacteria and bacterial endospores. The process did not affect the structural properties of biopolymeric porous materials and is therefore compatible with the sterilization of these structures.

**Acknowledgement**

This research was funded by Xunta de Galicia [ED431F 2016/010, ED431C 2020/17], MCIUN [RTI2018-094131-A-I00], Agrupación Estratégica de Materiales [AeMAT-BIOMEDCO2, ED431E 2018/08], Consellería de Sanidade, Servizo Galego de Saúde, Axencia de Coñecemento e Saúde (ACIS, CT850A-G), Agencia Estatal de Investigación [AEI] and FEDER funds. V. Santos-Rosales acknowledges to Xunta de Galicia (Consellería de Cultura, Educación e Ordenación Universitaria) for a predoctoral research fellowship [ED481A-2018/014]. C.A. García-González acknowledges to MINECO for a Ramón y Cajal Fellowship [RYC2014-15239].

<table>
<thead>
<tr>
<th>Biopolymer</th>
<th>Pressure (P)</th>
<th>Temperature (T)</th>
<th>Concentration of H₂O₂</th>
<th>Duration of the Process</th>
<th>Sterility assurance level (SAL)</th>
<th>Logarithmic reduction (logR)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>60</td>
<td>20</td>
<td>0</td>
<td>60</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>100</td>
<td>39</td>
<td>300</td>
<td>30</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><em>B. pumillus</em></td>
<td>120</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>140</td>
<td>20</td>
<td>0</td>
<td>140</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 1. Supercritical sterilization conditions and sterility levels achieved

Conditions of pressure (P), temperature (T), concentration of H₂O₂, duration of the process and depressurization rates used for the sterilization of vegetative Gram(+) and Gram(-) bacteria and *B. pumillus* endospore suspension. Logarithmic reduction (logR) and Sterility assurance level (SAL) achieved for each sterilization test.

References


Nanocarrier loaded pollen microcapsules as ocular delivery platform

Sandra Robla¹, Jose Manuel Ageitos¹, Rubén Varela-Calviño², Ignacio Alcalde³, Jesús Merayo-Lloves³, Noemi Csaba¹

¹ University of Santiago de Compostela, Department of Pharmacy, Pharmacology and Pharmaceutical Technology/Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), Santiago de Compostela, ES; ² University of Santiago de Compostela, Department of Biochemistry and Molecular Biology, Santiago de Compostela, ES; ³ University of Oviedo, Fernández-Vega University Institute, Ophthalmological Research Foundation, Oviedo, ES

Introduction
Topical administration constitutes a challenge in the design of ocular drug delivery strategies. Despite its easy accessibility, the eye is protected by extraordinary anatomical and physiological barriers which strongly limit drug residence time and, therefore, the efficacy of drug delivery systems. In this work, we present a biomimetic strategy to overcome these limitations by using purified pollen as a specific tool for enhancing mucointeraction of drug nanocarriers.

Experimental Methods
We designed a multi-stage delivery platform based on echinate Helianthus annuus pollen grains. Purification of this natural platform was performed by sequential washing steps to remove the pollenkitt and internal components. Morphology and composition were analyzed by analytical and microscopical techniques. Processed hollow pollen microcapsules (HPMs) were loaded with model nanoparticles of different properties (i.e. size, surface charge) using freeze-drying and vacuum-driven methods. Loading efficiency was analyzed by fluorimetry. Considering the results obtained with commercial nanoparticles, biodegradable protamine nanocapsules were developed, physically characterized, and loaded into Helianthus annuus HPMs. Evaluation of the initial immune response to pollen allergens of loaded and non-loaded HPMs platforms was performed in vitro, using immature dendritic cells obtained from patient blood. In vivo histological staining of the different sections of the rat eye was accomplished with different ocular lymphocytes and dendritic cells markers to evaluate activation after pollen exposition. Biocompatibility and physical irritation effects were evaluated upon ocular administration to rat eye in vivo. Additional analysis of epithelial damage was carried out in vitro by means of measuring the transepithelial resistance (TEER) on an artificial 3D cornea model of human sclerocorneal limbus epithelium (HCLEC).

Results and Discussion
Purified HPMs prepared from Helianthus annuus kept the same external 3D structure as intact pollen grain but were free of internal compounds. Among all the prototypes, non-biodegradable model nanoparticles (200 nm) reached the highest loading efficiency (around 85%), so that we designed biodegradable polymeric nanocapsules (NCs) with similar size and surface charge characteristics. NCs were fluorescently labelled with DiD to subsequently study their localization in the pollen and eye structures. Studies in ocular environment showed that protamine NCs were stable, and no relevant signs of aggregation were observed during 24h in Simulated Lacrimal Fluid at 37°C in suspension, and upon reconstitution after freeze-drying. As next step, HPMs were combined with fluorescently labelled protamine nanocapsules, using several pollen/NCs ratios and loading procedures, achieving up to 66% association efficiency rates (nanocarriers associated/total of nanocarriers) and NCs loading capacity (mass of loaded nanocarriers/mass of pollen grain loaded with nanocarriers) of 40% at ratio 1:1 and vacuum conditions. The presence of pollen grains loaded with fluorescent nanocarriers in the cornea and conjunctiva was confirmed. Released nanocarriers could be detected in conjunctiva 30 minutes after ocular administration by fluorescence microscopy examination. The immune cell recognition assay showed that the instillation of HPMs did Page 1170 of 2028
not cause greater cell activation than that observed in non-treated eyes, which is an indicator of an optimal purification procedure able to avoid allergenicity when used via ocular route. Moreover, regarding ocular biocompatibility and tolerability, no signs of hyperemia, anormal mucus/tear secretion or animal discomfort were detected in vivo by in-situ macroscopic observation even 6 hours after pollen administration. Finally, the 3D corneal HCLEC tissue model showed that no epithelial rupture took place, showing preserved integrity over 3 h of exposure to pollen.

Conclusion

The developed purification procedure could produce hollow and resistant pollen derived microcapsules with a high internal and external surface which make them ideal candidates for the association with nanostructures, such as protamine nanocapsules. Their biocompatibility and tolerance, allergen-free composition, as well as their mucoadhesion properties make this 3D microneedle-like platform an efficient strategy to overcome the biological and mechanical barriers of ocular drug delivery.

Acknowledgement

Xunta de Galicia (Centro singular de investigación de Galicia accreditation 2019-2022), European Union (European Regional Development Fund - ERDF), and Ministerio de Ciencia e Innovación, Gobierno de España – FEDER (SAF2016-79230-R and ERC2018-092841).

References

Advanced modification of alginate dialdehyde-gelatine composite bioinks with bioactive inorganic fillers for 3D bioprinting approaches

Susanne Heid¹, Kevin Becker¹, Jiwon Byun¹, Hsuan-Heng Lu¹, Aldo R. Boccaccini¹

¹ Friedrich-Alexander University, Institute of Biomaterials, Erlangen, DE; ² Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, DE

Introduction
Recent years have seen tremendous advances in the field of biocompatible composite hydrogel-based biomaterials and their use in 3D biofabrication [1]. Encapsulating cells into such hydrogels has become an important approach for the reproduction of customized tissues in terms of healing or the replacement of damaged human tissues and cartilage. A favorable material that can be used for 3D bioprinting is sodium alginate due to its comparatively low cost, tunable properties and similarity to the extracellular matrix. In order to overcome its poor cell attachment it can be oxidized to alginate dialdehyde (ADA) and crosslinked afterwards with RGD-containing ligands for example with gelatin (ADA-GEL). Not only cell viability and proliferation but also the printability of hydrogels, which is defined by shape fidelity and printing resolution, is a crucial factor in the design of application-specific bioinks.

The aim of this project was to evaluate the 3D printability and cell compatibility of a composite bioink consisting of pharmaceutical grade alginate, gelatine and incorporated inorganic fillers with respect to 3D bioprinting approaches.

Experimental Methods
In this study, composite bioinks containing bioactive inorganic fillers were developed. Therefore, filler amounts between 0 and 0.5 wt% were incorporated into ADA-GEL hydrogels having different ratios. Degradation studies, mechanical testing, cell encapsulation of fibroblast cells and bioprinting took place. Moreover, the rheological properties of the composite bioinks were assessed. 3D printing studies were performed to analyze the smallest possible resolution and a printing window in dependence of the composition and the effect of the single components.

Results and Discussion
Hydrogel-based bioinks need to reconcile sufficient mechanical properties and a good shape stability of bioprinted 3D constructs with high cell viabilities and functionalities post-printing. As it is challenging to attain such issues with pristine ADA-GEL bioinks, a bioactive ADA-GEL composite hydrogel for 3D bioprinting was designed by incorporating different amounts of BIF like calcium silicates on the basis of research outcomes by Leite et al. [2]. The material composition was optimized and the printing resolution, strand homogeneity and shape fidelity were evaluated under the effect of gelation time resulting in a predictable printing window. We observed that both printing resolution and accuracy depend on material’s as well as printing parameters that have to be critically analyzed for every hydrogel bioink. It could be clearly shown that there was an optimal amount of incorporated BIFs in ADA-GEL and that the bioink reveals temperature-time dependent gelation kinetics due to gelatine incorporation. Furthermore, bioactive properties of BIF and their release of bivalent calcium ions can strengthen the polymer network of the composite hydrogel over time leading to enhanced viscoelastic properties and a better stability after printing. Chemical and mechanical properties of the composite bioink were examined, too. Furthermore, cell viability and cellular behavior during incubation as well as the effect of the inorganic fillers on these issues were studied using fibroblast cells proving the cytocompatibility of the ADA-GEL-BIF composite bioink for 3D bioprinting and its use in regenerative medicine.

Conclusion
Composite bioinks composed of ADA-GEL and bioactive inorganic fillers were developed and their suitability for 3D bioprinting was shown. Particle filler concentrations up to 0.1 wt% could enhance the printability and post-printing...
shape stability, whereas higher concentrations led to non-uniform ink deposition which lowered the printing resolution as well as printability. In further studies, the long-term evaluation of printed constructs with fibroblast and muscle cells will be assessed.

Acknowledgement

Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project number 326998133 – TRR 225 (subproject B03).

References


Bioresorbable Electrospun Sol-Gel-derived Membranes to Prevent Post-surgical Adhesions without Shrinkage Effects during the Inflammatory Phase

Bastian Christ¹, Tobias Weigel¹, Christina Ziemann², Maximilian Oppmann¹, Susann Dehmel², Martin Engelke², Jörn Probst¹

¹ Fraunhofer ISC, Translational Center Regenerative Therapies TCL-RT, Würzburg, DE; ² Fraunhofer ITEM, Hannover, DE

Introduction
Post-surgical adhesions are frequent complications after soft tissue surgeries at bowel or uterus. They often result in severe pain, reoperation, infertility or bowel obstruction.[1] Unfortunately, anti-inflammatory and immunosuppressive drug therapy is not sufficient to reduce development of adhesions, but absorbable barrier membranes seem to be more effective. Such membranes act by direct separation of wounded and healthy tissue layers during the inflammatory phase of tissue healing and prevent migration of cells through the barrier. Electrospun membranes of biodegradable polymers might represent promising new adhesion barrier materials.[2, 3] As organic electrospun membranes are known to show shrinkages under physiological conditions, which causes shear stress during the healing process, the authors decided to develop an electrospun sol-gel-based inorganic barrier membrane that remains dimensionally stable for at least 7 days and is then fully resorbable within a few weeks.

Experimental Methods
Hydrolysis and condensation of tetraethylorthosilicate resulted in a sol that was spun to a fibrous membrane by electrospinning. The electrospun membrane itself was characterized by scanning electron microscopy (SEM) and infrared spectroscopy (FTIR). The resorption process of the membrane was monitored gravimetrically, accompanied by SEM imaging. All resorption products were identified by gas chromatography, ICP-analysis and photometric assays to finally quantify soluble silicic acids. Cell culture experiments were performed with primary human dermal fibroblasts (hdf) and L-929 murine fibroblasts to evaluate both cytotoxicity and the genotoxic potential of the material [WST-1-assay, cell number, lactate dehydrogenase (LDH) release and comet assay]. A cell migration experiment was performed on a membrane pre-degraded for 7 days and potentially infiltration of the material by stained cells was visualized via confocal microscopy.

Results and Discussion
The obtained electrospun silica fiber membrane with a thickness of 200 µm consisted of fibers with a diameter of about 1 µm and reveals a microstructured surface. A gravimetrical resorption study showed full material resorption in an excess of phosphate buffered saline and o-silicic acid was identified as the only resorption product. SEM pictures of the resorbed fiber membranes indicated an open mesh structure even during material resorption, allowing nutrition and metabolite transport through the membrane. In parallel, cell culture experiments with direct material contact showed undisturbed hdf proliferation and form stability of the membrane for 4 weeks (see figure). Cyto- and genotoxicity testing of the fibrous materials demonstrated neither a toxic or genotoxic effect when tested in direct contact nor when tested in form of o-silicic acid saturated cell culture media. In a final experiment, the silica membrane was pre-degraded over a period of 7 days – equivalent to the relevant inflammatory phase causing tissue adhesions – and subsequently seeded with murine fibroblasts (L-929). After a cell culture period of 7 days, cells were live/dead-stained and growth of cells into the membrane was monitored by confocal microscopy. Living cells were detected on the entire membrane surface and the function of a barrier was given, since no cell migration was observed.

Conclusion
Page 1174 of 2028
The developed electrospun silica membrane, which fully resorbs into o-silicic acid, showed excellent properties for the use as a physical adhesion barrier against post-operative adhesions. Due to its form stability over at least 4 weeks, wound healing will not be affected by shear stresses based on material shrinkage effects. Additionally, cyto- and genotoxicity testing verified biocompatibility of the fibrous material and a barrier function of the membrane was proven in a cell colonization experiment for 14 days under physiologic conditions.

**Acknowledgement**

The authors thank Miranda Rothenburger-Glaubitt for the synthesis of the spinning solution.

**References**


Tailoring dermal models using collagen type I from various animal sources

Maria Crespo-Cuadrado, Gema Dura, Ana Ferreira-Duarte, Kenny Dalgarno, Peter Hanson, Mark Birch-Machin, Philippe Mondon, Bhaven Chavan

School of Engineering, Newcastle University, Newcastle-upon-Tyne, GB; School of Chemistry, Newcastle University, Newcastle-upon-Tyne, GB; Mental Health, Dementia and Neurodegeneration, Translation and Clinical Research Institute, Newcastle University, Newcastle-upon-Tyne, GB; Dermatological Sciences, Translational and Clinical Research Institute, Newcastle University, Newcastle-upon-Tyne, GB; Sederma SAS, Le Perray en Yvelines, FR; Croda Europe Ltd, Snaith, East Yorkshire, GB

Introduction
Collagen type I is the most abundant extracellular-matrix components in the human body. This natural fibrillar protein provides structural support for cells and stimulates their proliferation. The different assembly and distribution of collagen fibres define the mechanical properties of tissues and organs such as skin, cornea, bones or tendons. For these reasons, collagen type I has become a widely used material for reproducing tissue models in research. Despite diverse animal sources and extraction methods having been used through the years to obtain collagen type I, they are often utilised indistinctively, and little is known on the impact of their origins on the properties of in vitro models. In this study, we explored how collagen's physicochemical properties are affected by their sources and how they ultimately influence cell behaviour.

Experimental Methods
The properties of two of the most commonly used animal sources of collagen, rat and bovine, were compared with human collagen type I properties. The effect of the collagen isolation method was assessed by comparing acid-soluble telocollagen and pepsin-extracted atelocollagen. Collagen solutions were characterised by Fourier-Transform Infrared Spectroscopy (FTIR) to determine the chemical differences between collagen sources and extraction methods. Rheological measurements were performed to study the fibrillogenesis and mechanical properties of collagen gels. Additionally, collagen fibre morphology and distribution were characterised by Scanning Electron Microscopy (SEM). To evaluate cell behaviour, 50000 fibroblasts were encapsulated per gel and samples were fixed after 1, 3 and 7 days. Proliferation was assessed by quantifying dsDNA content, whereas fibroblast morphology and distribution were studied by staining the samples with rhodamine-phalloidin and DAPI.

Results and Discussion
Although essential amide groups related to native collagen type I were present in all collagens, additional chemical bonds were found in the collagens depending on their source. These differences were observed to affect collagen's gelation, fibre morphology, and subsequently, their mechanical properties. In bovine telo-collagen, thick fibres originated by the entanglement and alignment of fibrils with different thickness resulted in stiffer collagen gels. The heterogeneity and complexity of these structures increased the time required to form the gels. In contrast, bovine atelocollagen and human collagen presented thinner and more aligned fibre networks with higher porosity, while rat collagen showed spherical bundles of thin fibres, which reduced their gelation time and final stiffness. Fibroblast attachment, morphology and proliferation were also affected by the selection of collagen source and extraction methods. Bovine collagen demonstrated to promote higher cell adhesion and proliferation than collagen from other sources.

Conclusion
The possibility to tailor key properties of collagens such as fibre morphology, gelation profile, and cellular behaviour by selecting specific collagen sources opens the door to the improvement of the reliability of *in vitro* tissue models and their production processes.

**Acknowledgement**

This project is funded by the EPSRC through the Centre of Doctoral Training in Additive Manufacturing and 3D Printing (EP/L01534X/1), CRODA International plc. and Newcastle University.
Antibacterial nanocoatings from biogenic marine sources

Gabriela Graziani¹, Giuseppe Falini², Daniele Ghezzi¹, Enrico Sassoni³, Martina Cappelletti⁴, Giorgia Borciani¹, Marco Boi¹, Nicola Baldini¹,⁵

¹ IRCSS Istituto Ortopedico Rizzoli, Rizzoli RIT - Research, Innovation and Technology, Bologna, IT; ² University of Bologna, Department of Chemistry “Giacomo Ciamician”, Bologna, IT; ³ University of Bologna, Department of Civil, Chemical, Environmental and Materials Engineering, Bologna, IT; ⁴ University of Bologna, Department of Pharmacy and Biotechnology, Bologna, IT; ⁵ University of Bologna, Department of Biomedical and Neuromotor Sciences, Bologna, IT

Introduction
Calcium phosphates (CaP)-based biomaterials are widely used in orthopaedics and dentistry, in the form of bone substitutes and coatings, to promote osseointegration and regeneration of native bone [1]. Together with promoting integration, implantable materials shall discourage infection, which is frequent, hard to eradicate and can have severe complications [2]. To this aim, thin films (thickness below 1 micron) having high adhesion to the substrate and a nanostructured surface texture are desired [3]. In addition, a tunable composition is required to resemble as closely as possible the composition of mineralized tissues and/or to intentionally substitute ions having possible therapeutic functions. However, the mineral phase of bone, enamel and dentine is a very complex material, a multi-substituted apatite containing several foreign ions in variable concentrations [4,5]. Each trace ion has an impact on the characteristics of the apatitic lattice, its solubility and crystallinity, and on its interactions with the host. In addition, some ions (such as zinc and fluorine) have specific therapeutic functions, including a concentration-dependent capability to inhibit microbial proliferation [4,5]. Reproducing the composition of mineralized tissues by synthetic routes, to date, is not possible. For this reason, the use of biogenic sources is receiving increasing attention [5]. Here, we propose new antibacterial bone- and enamel-like coatings, obtained by a natural fluorapatite deriving from a seashell, Lingula Anatina, for application in orthopaedics and dentistry. Different from the majority of seashells, which are composed of ion-substituted calcium carbonates, lingula consists of a rhythmic alternation of organic and mineralized layers, the latter being a carbonate-substituted fluorapatite.

Experimental Methods
Using lingula shell, nanostructured thin films are manufactured, obtained by Ionized Jet Deposition (IJD), directly by using the shell as a deposition target (Figure 1a). Morphology (FEG-SEM) and composition (ICP, EDS, FT-IR) of the nanostructured coatings are assessed (figure 1), to investigate the capability of IJD to preserve the main phase and the substitutions that are present in the shell. Then, their biocompatibility is tested on fibroblasts (Alamar blue) and their antibacterial efficacy is tested against Gram-positive and Gram-negative bacterial strains (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis), in terms of reduction of bacterial viability and substrate adhesion.

Results and Discussion
Results show that lingula is composed by a carbonated fluorine- doped apatite, also containing traces of zinc, magnesium, strontium and manganese and by chitin, allowing a significant antibacterial activity. Once deposited by Ionized Jet Deposition, nanostructured films are obtained, composed by nanoscale aggregates having the same composition of the inorganic phase of lingula (Figure 1b,c). This indicates that IJD permits a good conservation of the target stoichiometry, avoiding the formation of secondary products and/or a poor conservation of ion doping, characteristic of many plasma-assisted techniques. Coatings cause a high reduction of viability on all the tested strains, and a moderate effect on E. coli. The effect is dose-dependent causing the delay/impairment of bacterial
growth when supplied at low concentration and exerting a toxic/killing effect towards bacteria when added at high concentration.

Conclusion

Results show that nanostructured antibacterial and biomimetic nanostructured films can be obtained by Ionized Jet Deposition of lingula seashell. The obtained coatings are promising for application in orthopaedics and dentistry.

Acknowledgement

Dr. Gabriela Graziani acknowledges funding from the project Starting Grant SG-2018-12367059, financed by the Italian Ministry of Health (BANDO RICERCA FINALIZZATA 2018).

IOR Authors acknowledge funding the “Italian Ministry of Economy and Finance” (5 per mille for Scientific Research).

Ionized Jet Deposition of Lingula shell

a) Deposition scheme, b) morphology (FEG-SEM) and c) EDS composition

References

Tribological performance of leucite reinforced with zirconia for dental applications

Ana C. Branco1,2,3, Tiago Santos4, Mário Polido3, Rogério Colaço5, Ana P. Serro1,3, Célio G. Figueiredo-Pina2,3,6

1 Instituto Superior Técnico, CQE, Lisbon, PT; 2 Instituto Politécnico de Setúbal, CDP2T, Setúbal, PT; 3 Instituto Universitário Egas Moniz, CiiEM, Monte da Caparica, PT; 4 HiTEC, Porto Salvo, PT; 5 Instituto Superior Técnico, IDMEC, Lisbon, PT; 6 Instituto Superior Técnico, CeFEMA, Lisbon, PT

Introduction
Due to the attractive aesthetic properties, ceramic materials are the ultimate choice to repair/replace damaged/lost dental tissues. Zirconia is one of the most used ceramic material for dental restorations, mainly due to its toughness [1]. However, zirconia frameworks need to be coated with a glass veneer to improve their optical properties [2]. One of the major problems of these type of coatings arises from their inability to support the high loads during biting and mastication processes, leading to chipping of the glass veneer [3]. Furthermore, the wear induced on the antagonist dental surface, mainly due to microfracture based wear mechanisms, is also an issue. Vitroceramics, like leucite and lithium disilicate, constitute an alternative to produce monolithic dental structures without the need of a surface coating, due to their excellent optical properties. However, due to their low toughness, they usually lead to fracture and extensive wear of the antagonist teeth and the prosthetic material itself [4], which limits their application to the anterior part of the dental arcade, where the mechanical requirements are lower. Thus, the aim of this work is to obtain leucite-based glass ceramics with improved tribological performance through the reinforcement with zirconia nanoparticles.

Experimental Methods
Samples were produced by mixing leucite and zirconia powders (25 wt % tetragonal zirconia stabilized with 3 mol % of yttria (3YTZP)), by unidirectional compression applying a pressure of 75 MPa. Samples of 100% leucite and 100% zirconia were also produced using the same procedure for comparison purposes. Samples containing leucite were sintered at 1000°C for 6h, while 100% zirconia was submitted to 1500°C for 2h. The mechanical properties of the materials (hardness and toughness) were characterized, as well as their tribological behaviour against natural human cusps in artificial saliva, using a chewing simulator (360000 cycles, which corresponds approximately to 1.5 years of in vivo chewing). The wear mechanisms were accessed by scanning electron microscopy (SEM) and chemical analysis was performed by energy dispersive spectroscopy (EDS). The wear coefficient of the ceramic samples was determined from the volume loss obtained from profilometer images, while for cusps it was calculated based on 3D scans performed before and after the wear tests.

Results and Discussion
A wear coefficient reduction of 18 times was found for the composite when compared to 100% Leucite (Figure 1A). The presence of zirconia in the composite material led to a mild abrasion wear mechanism (Figure 2B), in contrast to that observed for 100% Leucite, where severe wear was observed, mainly due to fracture followed by spalling of large quantities of material (Figure 2A). Regarding dental cusps, the results pointed out that the composite material led to a dramatic reduction of dental wear when compared to 100% Leucite, being very similar to that observed for 100% zirconia (Figure 1B). SEM analysis showed that 100% Leucite induced abrasion, delamination and fracture on the dental surfaces, with adhered dental particles and also particles rich in SiO2 and Al2O3 (leucite wear debris) (Figure 2C). For the cusps tested against the composite material, abrasion was the predominant wear mechanism with some adhered dental particles (Figure 2D).
Conclusion
Overall, the results obtained with the studied composite are promising, since the wear of the antagonist teeth is much lower than that observed with 100% Leucite, being close to the one obtained with 100% zirconia.

Acknowledgement
To Fundação para a Ciência e a Tecnologia (FCT) for funding through the unit projects UIDB/00100/2020 (CQE), UIDB/04585/2020 (CiiEM), UID/CTM/04540/2020 (CeFEMA) and UIDB/50022/2020 (IDMEC/LAETA), and for the PhD grant of A.C. Branco (SFRH/BD/145423/2019).

References
PS1-05-193

Bacterial Cellulose as Cell Substrate and Mesh to Reinforce Body Tissues

Anna Roig, Irene Anton-Sales, Soledad Roig-Sanchez, Anna Laromaine

Institut de Ciència de Materials de Barcelona (ICMAB-CSIC), Campus UAB, 08193 Bellaterra, ES

Introduction
Bacterial cellulose (BC) is an animal and donor-free natural polymer presenting some inherent characteristics that are highly regarded in the healthcare sector - purity, biocompatibility, mechanical resistance, thermal stability and a fibrilar microstructure similar to that of the collagen extra-cellular matter.

Experimental Methods
BC hydrogels were synthesized by Komagataeibacter xylinus grown on Hestrin-Schramm medium. K. xylinus cultures were culture for 3 d under static conditions at 30 °C to obtain BC fleeces that were sterilized by autoclaving. Extensive biomaterial characterization encompassing mechanical properties, microstructure and rugosity was undertaken.

Results and Discussion
Here, we will present technical data supporting the use of BC films as cryopreservable cell culture substrates for fibroblasts [1] and as cell support for limbal stem cells with the final objective to treat injured cornea (Figure 1A) [2,3]. Besides, we will also report on the use of BC as a mesh for hernioplasty gathered after the in vivo implantation of BC films in rabbits for 21 days (Figure 1B).

Conclusion
BC substrates present excellent biocompatibility and provide a self-standing and easy to manipulate mechanical substrate for the cells. It supports the formation of new daughter cells for fibroblast while additionally retaining the progenitor phenotype in the case of limbal stem cells. Regarding the use of BC as tissue reinforcement, the in vivo study revealed that BC exhibits favourable surgical features and excellent accommodation to the implantation site. Besides, mild adhesion scores involving low percentages of the implant's area together with excellent integration on the peri-implant zone could be demonstrated.

Acknowledgement

Figure 1.
Bacterial nanocellulose films: A) limbal stem cell (LSC) support and B) mesh for hernioplasty.
References
Fibroblast viability and orientation on BaTiO3 functionalized zirconia.

Laura K. Tiainen¹, Oscar Carvalho¹, Michael Gasik³, Filipe S. Silva¹, Beatriz Ferreira Fernandes²

¹ University of Minho, Mechanical Engineering Department, Guimaraes, PT; ² Universidade de Lisboa, Faculdade de Medicina Dentária, Lisboa, PT; ³ Aalto University, Department of Chemical and Metallurgical Engineering, Espoo, FI

Introduction
A successful dental implant relies on successful osseointegration and soft-tissue integration. In the present study, we investigated human gingival fibroblast (HGF) viability and orientation at poled and non-poled barium titanate functionalised yttria stabilised zirconia (BT/YSZ) and yttria stabilised zirconia (YSZ). Cell viability was evaluated at 1, 3 and 7 days with resazurin based method. To study adhesion, morphology and orientation SEM observation was done at 24h of cultivation. Fibroblasts attached well on all samples and their viability was mostly comparable to the control. However, a decrease in viability was observed in non-polarized BT/YSZ samples. Orientation was analysed from SEM backscattered electron images. The orientation of non-poled composite was different to poled composite and YSZ.

Experimental Methods
Composite samples with 5 wt.% BT in YSZ were prepared through press-sinter route (1380°C, 2h, 5"/min) from commercial 3 mol% Y2O3 stabilised zirconia powder (TZ–3YSB–E, Tosoh, Japan) and barium titanate (Ticon P., FerroR, USA). The sintered disks were ground to final dimensions ±0.02 mm with a resin bonded diamond grinding disk (Struers, MD-Piano 120) and polished (P4000 SiC, Ra<0.5um). Possible polishing induced monoclinic phase was retransformed to tetragonal by heat treatment of 2 h at 600 °C in air. Contact poling was carried out in silicone oil bath under DC 2 kV/mm electric field at 130 °C for 30 min followed with field cooling. Reference samples of YSZ were processed parallel to the composites.

Immortalized Human Gingival Fibroblasts (HGF; Applied Biological Materials Inc., Richmond, BC, Canada) are primary cells conditionally immortalized through hTERT gene. Cells were cultured at 37 °C in an atmosphere of 5% CO₂ and 100% humidity in culture medium composed of Dulbecco's Modified Eagle's Medium-DMEM (Biowhittaker, Lonza, Walksville, USA) supplemented with 10% Fetal bovine serum (Biowest, Nuaillé, France) in 75 cm² flasks (Corning, Corning NY, USA) until reaching 80% confluence. Cells were detached using trypsin-EDTA (Lonza, Veners, Belgium), centrifuged at 777 rpm and re-suspended at a density of 1 x 10⁴ cells/well and cultured at 37°C for all biological assays. All experiments were conducted using a fourth passage.

Cell viability was evaluated at 1, 3 and 7 days using a commercial resazurin-based method, CellTiter-Blue (Promega, Madison, WI, USA) according to the manufacturer protocol. The conversion rate was measured as fluorescence intensity in arbitrary fluorescence units (AU) after 1, 3, and 7 days of culture. Fluorescence intensity was detected at excitation/emission wavelengths of 560/590nm using a fluorescence spectrometer (VICTOR NivoTM HH3500, PerkinElmer., Pontyclun, UK).

For fluorescence staining, discs cultured with fibroblasts were removed after 1 day of incubation. Cytoskeleton staining was evaluated by Phalloidin reagent (Phalloidin FITC Reagent - ab235137,Abcam, Amestardam, Netherlands) and staining of cell nucleic acid, using Propidium Iodide (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany, P4864-10ML), both according to manufacturers’ instructions. Images were obtained using fluorescent microscope (Olympus-BX50, filters: Ex / Em = 493/517 nm WEB - Phalloidin; Ex / Em = 535 / 617nm WG - Propidium Iodide).
Results were presented as mean ± standard deviation (SD). Group comparisons were tested using Anova (Tukey’s post-hoc) using appropriate statistical software and significance was set at $p<0.05$. The orientation of the fibroblasts on YSZ, 5%BT/YSZ poled and non-poled 5%BT/YSZ was studied by an image analysis from the BSE/SEM micrographs. The study utilized an algorithm described by Kazantseva et al. [1]. Prior the analysis the micrographs were converted to 8 bit greyscale images by applying threshold to cells only (Li filter). Noise Despekle filter was used for noise reduction.

**Results and Discussion**

Cell density on the surfaces of different samples appeared to be very similar and differences in viability were small. Non-poled samples had however, decreased viability and changes in their orientation.

**Conclusion**

The findings may give a new possibility to enhance fibroblast responses and control the formation of fibrous encapsulation without any pharmaceuticals. The control over fibroblast adhesion can hence improve results in root coverage and improve designing transgingival parts of dental implants.
Cell viability was evaluated at 1, 3 and 7 days using a commercial resazurin-based method - Cell-Titer Blue (Promega, Madison, WI, USA) according to the manufacturer protocol. The conversion rate was measured as fluorescence intensity in arbitrary fluorescence units (AU) after 1, 3, 7, and 14 days of culture. Fluorescence intensity was detected at excitation/emission wavelengths of 560/590nm using a fluorescence spectrometer (VICTOR NivoTM HH3500, PerkinElmer, Pontyclun, Reino Unido).

References
PS1-05-197

Green route fabrication approaches of lignin nanoparticles from different technical lignins: a comparison study

Patrícia Figueiredo¹, Maarit Lahtinen¹, Melissa Agustin¹, Kirsi Mikkonen¹.²

¹ University of Helsinki, Department of Food and Nutrition/Faculty of Agriculture and Forestry, Helsinki, FI; ² University of Helsinki, Helsinki Institute of Sustainability Science (HELSUS), Helsinki, FI

Introduction

Lignins derived from plant and wood sources have gained increased consideration from the research community because of their promising benefits, such as low production cost, antimicrobial properties and eco-friendly characteristics, such as biocompatibility and biodegradability.¹ Lignin is composed of three units, found in varying proportion according to the source of the lignocellulosic biomass (i.e., softwood, hardwood or grass), which can affect the physicochemical properties of the resulting technical lignins.¹ Despite the high abundance of lignin in nature, only ~ 2% of lignin is employed as a starting material for low value applications, mainly limited by its highly variable and complex molecular structure. However, the fabrication of lignin nanostructures can overcome these limitations, offering structural and morphological control of the lignin polymer, while enabling the typically water insoluble lignin to form stable colloidal suspensions in aqueous media. Therefore, the production of lignin nanoparticles (LNPs) has greatly increased their potential for high value applications in many fields.² However, the homogeneity and morphology of LNPs varies depending on the lignin grade and preparation method, and a systematic comparison is lacking. In this study, we aimed to find a green fabrication method with a distinct solvent fractionation of lignin to prepare LNPs using three different technical lignins as starting polymers: BLN birch lignin (hardwood, BB), alkali Protobind 1000 (grass, PB), and Kraft LignoBoost (softwood, LB).

Experimental Methods

Here, we systematically compared three anti-solvent precipitation approaches; 70% ethanol,³ acetone/water (3:1),⁴ and NaOH⁵ as the lignin solvent, and water/aqueous HCl as the anti-solvent (Figure 1). The resulting nanostructures were further characterized using dynamic light scattering (DLS), transmission electron microscopy (TEM), Fourier-transform infrared (FTIR) and nuclear magnetic resonance (NMR) spectroscopy. In addition, their phenolic content was quantified using UV spectroscopy and compared with conventional ³¹P NMR. Furthermore, the stability of LNPs at different ionic strength and pH was evaluated in order to select the best approach to obtain stable, spherical and homogeneous LNPs from the three technical lignins.

Results and Discussion

In this study, the molar mass of the technical lignins used in our study (LB > BB > PB) was shown to be inversely proportional to the hydrodynamic diameter of the LNPs (LB-LNPs < BB-LNPs < PB-LNPs). Additionally, all methods allowed production of homogeneous and monodispersed LNPs, in particular the acetone/water (3:1), with a negative surface charge. The LNP morphology was confirmed using TEM, in which the LNPs prepared with acid precipitation method appeared to be like clusters or aggregates of very small particles, while the 70% ethanol and acetone/water (3:1) led to the formation of quasi-spherical or spherical LNPs. The FTIR spectra of the raw lignins was compared with the respective LNPs, no substantial differences were observed. However, the ¹H NMR spectra suggested that the acid precipitation approach induced structural changes after fractionation of the raw lignins during the preparation of LNPs. Regarding the phenolic content determination, the UV-Vis approaches followed similar trends to the values measured with ³¹P NMR, but gave slightly different values compared to the ³¹P NMR measurement. Nevertheless, the UV-Vis represents a simple, reproducible, and low-cost approach to quantify the phenolic content when similar
types of samples are compared. Finally, the produced LNPs are stable at low to moderate ionic strength and over a pH ranging from 4 to 8, in particular the LNPs prepared with the acetone/water (3:1) method.

**Conclusion**

Overall, our results revealed that the acetone/water (3:1) mixture was the most effective solvent tested to obtain homogenous, small and spherical LNPs from the three technical lignins. Moreover, these LNPs showed an improved stability at different ionic strengths and a wider pH range compared to the other preparation methods, which can greatly increase their application in many fields, such as pharmaceutical and food sciences.

**Acknowledgement**

The authors acknowledge the financial support from the European Research Council. The authors also thank the Electron Microscopy Unit, University of Helsinki, for providing the necessary laboratory facilities and assistance. The facilities and expertise of the HiLIFE NMR unit at the University of Helsinki, a member of Instruct-ERIC Centre Finland, FINStruct, and Biocenter Finland are gratefully acknowledged.

![Figure 1. Overview of the different approaches for the LNP preparation used in this study: acid precipitation (blue arrows), 70% ethanol (yellow arrows) and acetone/water 3:1 (green arrows).](image)

**References**


Investigation of the shrinkage phenomena during the solvent exchange step for the production of biopolymer-based aerogels

Anja Hajnal, Pavel Gurikov

Hamburg University of Technology, Institute of Thermal Separation Processes, Hamburg, DE

Introduction

Biopolymer aerogels are ultra-light porous materials that have gained interest for research, development and application in the last two decades. Their biocompatibility and biodegradability makes them especially attractive for applications in life sciences such as drug delivery, tissue engineering, food and cosmetics.

To produce biopolymer-based aerogels, a gel first needs to be formed in an aqueous solution. Due to the low solubility of water in supercritical CO$_2$ (drying fluid) at moderate pressures, it must be exchanged for a solvent that is miscible with water and supercritical CO$_2$ and suitable for life science applications (solvent exchange). This step is followed by the supercritical drying, in which the solvent in the pores is extracted by means of compressed CO$_2$.

During both solvent exchange and supercritical drying a gel volume reduction has been observed, which leads to significant changes in the gel pore structure and final aerogel properties. The gel shrinkage is mainly attributed to the interactions of gel matrix and solvent and its extent is specific to a biopolymer/solvent system. For solvent exchange step, the change of gel volume is dependent on the solvent exchange path and can be significantly reduced when gels are sequentially submitted into water/solvent solutions with smaller concentration gradients (stepwise solvent exchange). In order to make biopolymer-based aerogels suitable for commercial applications, it is necessary to find a balance between the processing costs and final material properties. To achieve this, basic principles that lead to changes in the gel matrix during solvent exchange and supercritical drying need to be understood. In this work, the focus was on experimental investigation of the shrinkage of biopolymer-based hydrogels during solvent exchange.

Experimental Methods

The gel volume change was quantified by photographing the gels in an optically accessible container using a digital camera and analyzing the moving boundary of the gel sample by means of an edge detection script. Since the shrinkage of the gel is countered by the stiffness of its matrix, the evolution of compressive strength on its path from hydrogel to alcogel was also measured for different biopolymer/ethanol systems. To study the effect of polymer/solvent interactions on shrinkage, agar gels were subjected to solvent exchange in ethanol, acetone and acetonitrile.

Results and Discussion

As the solvent exchange progresses and the shrinkage takes place, the compression strength of gels increases. This increase in gel stiffness was proportional to the decrease in volumetric yield, implying that the increase in gel strength counteracts the shrinkage. Young's moduli of alcogels increased with increasing biopolymer concentration, indicating that the final stiffness of the gel can potentially be correlated with its shrinkage in a master curve relating Young's modulus and alcogel biopolymer concentration. The influence of the polymer/solvent interactions on shrinkage was evaluated through the solubility parameters of the biopolymer and respective solvents.

Conclusion

The outcome of the work is a description of the shrinkage phenomena which encompasses both thermodynamic system properties and gel mechanics. It is achieved by deeper understanding of an interconnected influence of polymer-solvent interaction and gel matrix strength on the changes of gel volume.

Acknowledgement
Work carried out in the framework of the COST Action “Advanced Engineering and Research on aeroGels for Environment and Life Sciences” (AERoGELS, ref. CA18125) funded by the European Commission. Financial support by the Deutsche Forschungsgemeinschaft is also gratefully acknowledged (DFG GU 1842/3-1).

References
Graphene nanoplatelets as an economical alternative in the reinforcement of PMMA bone cements

Eva Paz, Yolanda Ballesteros, Sara López de Armentia, Juan Carlos del Real

Universidad Pontificia Comillas, Institute for research in technology/Mechanical engineering department, Madrid, ES

Introduction
The reinforcing effect of G and GO has attracted great interest during recent decades, giving rise to nanocomposites with enhanced mechanical properties [1,2]. In previous studies, it was demonstrated that the incorporation of well-dispersed Graphene (G) and Graphene oxide (GO) can be a promising solution in augmenting the mechanical performance of PMMA bone cement (BC) in an attempt to enhance the long-term survival of the cemented orthopaedic implants [3]. It has been demonstrated that the presence of G and GO within a polymer matrix produces a deviation and detention of crack fronts during their propagation, increasing the required energy for failure [4]. In addition, it has been proven that these nanoparticles do not significantly influence the thermal properties and biocompatibility of BC, potentially allowing its clinical progression.

One of the most important constraints in the large-scale production of nanocomposite applications is that the industrial production of G and GO currently is limited by two main aspects: the scalability of the chemical production process and the cost. A wide variety of cheaper and easier-to-produce graphene derivatives have emerged as the case of graphene nanoplatelets (GnPs).

GnPs exhibit exciting properties such as light weight, high aspect ratio, electrical and thermal conductivity, mechanical toughness, low cost, and planar structure. As such, they are attractive options to replace different nanostructured fillers [5]. GnPs are cheaper than G and GO; and are comparable in modifying the mechanical properties of polymers. The objective of this study is to make a comparison of the effect that the addition of GnPs has on the mechanical performance of bone cements, compared to the use of other carbon-based nanoparticles such as G and GO.

Experimental Methods
The BC used in this study was a two-phase non-comercial bone cement. BC without filler (Control), with G (Avanzare Nanotechnology, Spain), GO and GnPs (Avanzare Nanotechnology, Spain) at different levels of loadings (0.1, 0.05, 0.025 and 0.01 wt.%) were prepared. In each case, the nanoparticle powder was dispersed into the liquid phase of the BC using ultrasonication.

The bending strength and fracture toughness of each kind of cement was studied. A four-point bending load arrangement was used to determine bending properties in accordance with ISO 5833 [36]. The fracture toughness was determined according to the standard [38]. Single edge notch bend specimens (SENB) were used to calculate the fracture toughness (Kc). The tests were conducted using a Universal Testing Machine IBTH/500 (Ibertest, Madrid, Spain) using a load cell of 5 kN, which operated at a crosshead speed of 5 mm/min. A total of three batches were tested for each cement composition with a minimum of five samples per batch. Finally, the fracture surface was evaluated using an Olympus DSX1000 digital microscope.

Results and Discussion
As each type of nanoparticle has a different density, the optimum level of loading in each case could be different. Consequently, the effect of the level of loading has been studied. In all cases, it was observed that when the loading decreased, there was an increase in fracture toughness. Figure 1 shows the average fracture toughness in the case
of GnPs for the different load levels. As can be seen, the best results were obtained for the low loading (0.01 wt.%), in this case, the fracture toughness was 20% higher than for 0.1 wt.%.

The effect of the different nanofillers at the optimum obtained level of loading (0.01 wt.%) were compared. In Figure 2 it is represented the fracture toughness of the samples with 0.01 wt. % of the different nanoparticles. It can be observed that in all cases the fracture toughness was improved in comparison with the control. In the case of the GO and GnPs, these significantly increased the fracture properties (p<0.001) by 30% and 40% respectively. This result demonstrated that the addition of GnPs is even better that the use of G and GO at this level of loading.

The bending tests were carried out at two different levels load, 0.1wt.% and 0.01wt.% and in both cases the resultant trend was similar to that obtained in the fracture tests- the addition of low loadings produced better results, and the improvement was significantly higher in the case of GnPs than G and GO.

The analysis of the fracture surfaces showed that fewer agglomerations of nanoparticles were found in the case of low loading, which could explain the obtained results. The better reinforcement effect observed in the GnPs could be related with a lower tendency of these to form agglomerates, as they are less exfoliated and are more stable, and with the better dispersability of them within the polymeric matrix.

Conclusion
The use of GnP as a reinforcing agent for BC produces very promising results with significant improvements in fracture and bending properties. Compared to G and GO, GnPs has been shown even better results, being a very interesting alternative with a lower cost.

Figure 1
Fracture toughness of the PMMA bone cement reinforced with GnPs at different levels of loading (0.1, 0.05, 0.025 and 0.01 wt.%). The variation in comparison with the control cement also indicated. ** meaning a p-value < 0.001.

Figure 2
Fracture toughness of the different PMMA bone cement with the different nanofillers (G, GO and GnPs) with 0.01 wt.% of load. The variation in comparison with the control cement also indicated. ** meaning a p-value < 0.001.

References
PS1-06 | Ceramic Biomaterials
Silver- and/or titanium-doped calcium phosphate highly porous bioceramic with antibacterial activity

Liga Stipniece\textsuperscript{1,3}, Ingus Skadins\textsuperscript{2}, Dagnija Loca\textsuperscript{1,3}

\textsuperscript{1} Riga Technical University, Institute of General Chemical Engineering, Riga, LV; \textsuperscript{2} Riga Stradins University, Department of Biology and Microbiology, Riga, LV; \textsuperscript{3} Baltic Biomaterials Centre of Excellence, Riga, LV

Introduction
Regeneration and reconstruction of bone defects is significant challenge in modern medicine and often requires surgery where biomaterials are used. About 10% of these procedures are at risk of bacterial infections \cite{1}. Due to the possible systemic toxicity and growing emergence of antibiotic-resistance caused by systematic use of antibiotics methods are being sought to ensure local delivery of antibacterial agents at the site of implantation. The use of alternative antibacterial agents such as bacteriophages, bacteriophins and inorganic agents (Ag, Cu, Zn, Ti compounds, etc.) with far lower susceptibility to induce resistance than common antibiotics has resurge recently \cite{2}. Broadening of the spectrum of calcium phosphate (CaP) materials by modification with metal ions to impart advanced biological and physicochemical properties has been observed. Attaining antibacterial properties is one of the aims and the most common dopants studied in this regard are Ag(I), Cu(II), Zn(II), Co(II) etc. \cite{3}. Combining Ag(I) with Ti(IV) is a potential way to promote bone-implant integration, with reduced bacteria\textsuperscript{2} infection and enhanced bioactivity \cite{4, 5}.

The aim of this study was to impart antibacterial properties to highly porous CaP bioceramics, which could be used in orthopaedic surgery, by doping them with Ag(I) and Ti(IV) ions, and to evaluate the synergy and cumulative effects of dopants.

Experimental Methods
Method of producing highly porous Ag- and/or Ti-doped CaP bioceramic scaffolds compromised following steps:(i) preparation of porous bioceramic scaffolds (the substrates), through PU foam replica method, (ii) aqueous precipitation of Ag- and/or Ti-doped CaP (hydroxyapatite (HAp) and apatitic-tricalcium phosphate), (iii) surface modification of the substrates by depositing the Ag- and/or Ti-doped CaP layer by vacuum-assisted impregnation.

Prepared bioceramic scaffolds were analyzed for microstructure and composition using common analytical techniques - SEM, XRD, FT-IR, EDX etc.

Minimum inhibitory concentration (MIC) of the Ag- and/or Ti-doped CaP against gram-positive \textit{S. aureus} (ATCC 25923) was determined by microdilution method. The antibacterial properties of Ag- and/or Ti-doped CaP bioceramic scaffolds were determined by the broth dilution method, which involves inoculating a diluted bacterial suspension into TSB agar medium and incubating at 37 °C. A suspension of \textit{S. aureus} bacterial reference culture ATCC 25923 (10\textsuperscript{8} CFU/mL) was used.

\textit{In vitro} bioactivity of Ag- and/or Ti-doped CaP bioceramic scaffolds in semi-dynamic conditions in the SBF was evaluated. The dynamics of the decrease of Ca ions concentration in the SBF after exposure to the bioceramic scaffolds was performed using an automated titration station and Ca-ion-selective electrode. The formation of a biomimetic apatite layer on the scaffolds surface was assessed using SEM.

Results and Discussion
The obtained bioceramic scaffolds had an open porous structure and highly interconnected pores (Fig. 1). A porosity of 92 ± 2%, pore sizes in a range from 50 to 450 µm. According to FT-IR and XRD analyses the bioceramic scaffolds were composed of biocompatible phases. No impurities form the raw materials were detected at least at the detection
limits of the instruments. No significant differences in positions and intensities of the characteristic XRD diffraction peaks and FT-IR absorbance bands were observed among the compounds of various compositions. EDX results confirmed the presence of Ag and Ti in the synthesis products. The content of Ag in the samples was higher than planned, while Ti was close to the planned. However, it was observed that with increasing calcinations temperature, the Ag content decreases. Thus, for sintering the coating 900 °C was chosen to maintain Ag concentration as close as possible to the non-calcined CaP. The MIC values for all Ag- and/or Ti-doped CaPs were 10 mg / mL, except for Ag-doped and Ag- and Ti-co-doped, which showed MICs of 5 and 2.5 mg / mL, respectively. This also coincides with the results of antibacterial evaluation of Ag- and/or Ti-doped CaP bioceramic substrates. Namely, the respective bioceramic substrates showed the highest antibacterial activity. All scaffolds, regardless of composition, showed in vitro bioactivity.

**Conclusion**
The combination of polymeric foam replica and vacuum-assisted impregnation technologies is suitable for preparation of the Ag- and/or Ti-doped CaP bioceramic scaffolds with highly interconnected porous architecture. The physical properties, as well as a phase and chemical purity are sufficient to provide a suitable initial three-dimensional support for cells to grow into it and make a new bone and allows the scaffolds produced to be used for osteoconductive bone implants, tissue engineering implants. Antibacterial activity of the scaffolds depends on the phase composition which affects concentration of antibacterial ions released into the surrounding environment.

**Acknowledgement**
This work has been supported by the European Regional Development Fund within the Activity 1.1.1.2 “Post-doctoral Research Aid” of the Specific Aid Objective 1.1.1 “To increase the research and innovative capacity of scientific institutions of Latvia and the ability to attract external financing, investing in human resources and infrastructure” of the Operational Programme “Growth and Employment” (No. 1.1.1.2/VIAA/2/18/339).

Fig. 1. SEM micrograph of the porous bioceramic

References

New pyrophosphate-stabilized amorphous calcium carbonate doped with bioactive ions: toward bone substitute materials?

Marion Merle, Jérémy Soulié, Christian Rey, Prescillia Lagarrigue, Christèle Combes

Institut National Polytechnique de Toulouse, CIRIMAT, Toulouse, FR

Introduction
Calcium carbonate is the most abundant biominer [1] and is often used as a biomaterial due to its excellent biocompatibility [2]. Among all polymorphs, amorphous calcium carbonate (ACC) is the least stable and the most soluble, leading to a high reactivity. Various ACC syntheses are detailed in the literature. They are mainly based on the same strategy that aims to avoid crystallisation either with kinetic parameters (such as low temperature) or with additives. Among these latter, the use of pyrophosphate as an additive is poorly documented. Such material should promote bone regeneration through the presence of carbonate, calcium and pyrophosphate [3] and allow the modulation of biodegradation rate in vivo by varying the pyrophosphate amount in the ACC matrix. The purposes of this study are to understand the influence of the pyrophosphate (from 0 to 10 %mol. with respect to carbonate content) and cation doping rates (up to 10 %mol. with respect to total cation content) on the nature, structure and multi-scale organization of the resulting calcium carbonate. Moreover, the feasibility to process these doped-ACCs in biomimetic bone cement and as macroporous composite scaffold by freeze-casting will be demonstrated.

Experimental Methods
ACC powders were synthesised by double decomposition in aqueous solution, with various pyrophosphate content and fully characterised (XRD, Raman and FTIR spectroscopy, SEM and FEG-SEM, TEM, TGA, chemical titrations and SAXS).

Results and Discussion
We determined the pyrophosphate content threshold required for avoiding the crystallisation of calcium carbonate. Above this threshold, no diffraction peaks was detected by XRD whereas some calcite formed for lower pyrophosphate content. The average particles sizes (∼ 50 nm) evaluated by SEM and FEG-SEM were quite homogeneous between the different ACC powders synthesised, and SAXS studies highlighted a nano-based hierarchical organization. This structuration is predominant as reactivity is correlated to the internal surface. Finally, calcium, carbonate (chemical titration) and water (TGA) contents were demonstrated to be correlated to the pyrophosphate content. Beyond these fundamental aspects, additional biological properties can be conferred to these materials by doping with bioactive ions such as Cu²⁺, Zn²⁺ or Sr²⁺ that could enhance angiogenesis, osteoblastic activity and/or show anti-inflammatory effects [4]. We revealed that these doped ACC can integrate high contents of bivalent ions (M²⁺/cations ratio up to 10 %mol.) via similar synthesis method and without subsequent crystallisation. All together these results are important to control the reactivity and consequently bioactivity of pyrophosphate-stabilized cation-doped ACCs in view to their use as potential bone substitute materials. These doped-ACCs can be processed not only as self-supported biomaterial, but also included in a bone mineral cement formulation or freeze-casted as a porous scaffold. We demonstrated the feasibility of in situ ACC/alginate suspension freeze-casting to obtain a composite scaffold with promising physicochemical and mechanical properties and in particular adequate macroporous anisotropic porosity mimicking bone structure (with a porosity diameter ∼ 100 - 500 µm) [5] while preserving the amorphous structure of the constitutive powder.

Conclusion
These whole results are opening new perspectives of bioactive materials with tunable properties and adaptative shape for several bone substitution applications.

Page 1198 of 2028
Acknowledgement
The authors thank the Occitanie Region (BioPhORM project n°19008740/ALDOCT-000734) for supporting this research work.

References
Yttria Stabilized Zirconia/Clay Composites for Dental Applications

Yiğithan Tufan¹, Jongee Park², Abdullah Öztürk¹, Batur Ercan¹,³

¹ Middle East Technical University, Metallurgical and Materials Engineering, Ankara, TR; ² Atılım University, Metallurgical and Materials Engineering, Ankara, TR; ³ Middle East Technical University, BIOMATEN, Center of Excellence in Biomaterials and Tissue Engineering, Ankara, TR

Introduction

Among dental bioceramics, yttria stabilized zirconia (YSZ) is gaining increased use due to its superior mechanical properties and aesthetic concerns [1]. It is available in the market especially for the fabrication of crowns, inlays, dental implant abutments and fixed partial dentures [2]. Clay based minerals, being one of the most common ceramic raw materials, is also used for various medical purposes including antacids, gastrointestinal protectors and drug delivery modifiers [3]. Recent studies revealed that clay minerals are beneficial for osteoblast adhesion, proliferation and osteogenic differentiation [4]. For instance, enhanced osteoblast proliferation was reported after the incorporation of montmorillonite in chitosan-poly(glycolic acid) films [4]. In addition, enhanced osteogenic response of preosteoblast cell line MC3T3 was obtained with the addition of laponite in several polymeric matrices including poly(ethylene oxide), poly(ethylene) glycol and GelMA [4]. Therefore, in this study, clay was incorporated in YSZ to enhance its mechanical and biological properties for dental applications.

Experimental Methods

The starting powder was 3 mol% yttria stabilized tetragonal ZrO₂ (3Y-Z, AMS) with an average particle size of 150 nm. Nanoclay additive (avg. 800nm) was used as-received form. Various amounts of Nanoclay (0, 0.5, 1, 2, and 4 wt%) were added into YSZ using mechanical ball milling. Each batch was wet-milled in a zirconia jar at 350 rpm for 3 h. The prepared powders were then dried overnight and then uniaxially pressed under at pressure of 15 MPa to form the disc-shaped compacts. After that, all discs were sintered at 1450 °C for 2 h. The metabolic activities of osteoblasts (hFOB, ATCC CRL-11372) on YSZ/clay samples were investigated using MTT assay. A FEI Nova Nano SEM 430 microscope was used to image sample surfaces and osteoblasts. Actin filaments and nuclei of the cells were stained with F-actin Staining Kit – Red Fluorescence (abcam ab112127) and DAPI solutions, respectively. Once staining was completed, cellular images were captured with a Leica DM2500 microscope.

Results and Discussion

Backscattered SEM images of YSZ having 0 and 4% clay were given in Figure 1a and 1b, respectively. SEM images revealed that there was no pore left in the microstructure of both YSZ and YSZ/clay composites (the presence of clay is apparent in Figure 1b as darker regions) indicating the efficacy of sintering. Hardness values of YSZ samples decreased with the incorporation of clay. Indentation fracture toughness measurements of samples showed that YSZ/4% clay has the highest fracture toughness value compared to other YSZ samples having lower clay contents. Surface hydrophobicities of YSZ samples decreased with the addition of clay. This was correlated with the changes in surface chemistry of samples (no significant difference was found in the surface roughness values of samples).

The effect of clay on the biological properties of YSZ/clay composites were assessed using osteoblasts. Enhanced osteoblast proliferation and spreading was achieved on the YSZ/4% clay composites compared to YSZ samples having 0% clay. SEM images of osteoblasts at 3 days on YSZ and YSZ/4% clay composites were given in Figure 1c and 1d, respectively. Better spreading of osteoblasts were apparent on the YSZ/4% clay composites compared to YSZ sample. This was further confirmed with confocal laser micrographs at 3 days (Figure 1e and 1f) for YSZ and YSZ/4% clay, respectively. Briefly, these results indicated that YSZ/4% clay composites may provide prolonged...
osseointegration between the implant and surrounding bone tissue making them a possible alternative implant material for dental applications.

**Conclusion**

YSZ/clay composites were developed for dental applications. With the addition of clay, increased fracture toughness and decreased hydrophobicity was obtained compared to YSZ having 0% clay. Also, enhanced osteoblast proliferation and spreading on YSZ/4% clay composites were achieved indicating their potential as an alternative dental implant material.

**Acknowledgement**

We would like to thank the BIOMATEN-METU Center of Excellence in Biomaterials and Tissue Engineering for their support in biological characterizations. Special thanks to Dr. Menekşe Ermiş for her help in confocal laser microscopy.

![Figure 1](attachment:image.png)

(a) Backscattered SEM images of a) YSZ and b) YSZ/4% clay, (c-d) SEM images of osteoblasts at 3 days on c) YSZ and d) YSZ/4% clay and (e-f) Actin (red) and nuclei (green) stained images of osteoblasts at 3 days cultured on e) YSZ and f) YSZ/4% clay samples.

**References**


PS1-06-209

Color stability of different aesthetic restorative materials: an in vitro study

Luca Solimei¹, Nicola De Angelis¹, Abdulkhaleq M. Garaghoolee¹, Mohamed T. El Halawani²

¹ University of Genova, Genova, IT; ² University of Alexandria, Cairo, EG

Introduction
A wide spectrum experimental campaign has been set up in order to study the capability of different categories of materials to maintain their colors in different common beverages of human feedings. This specific feature is a very important asset in commercial products and is a kpi (key performance indicator) to define overall expected quality. In details, the study is to evaluate the color stability of different dental veneer restorative materials (feldspathic ceramic, hybrid ceramic, zirconia reinforced lithium silicate glass ceramic, and composite resin) after exposure to commonly consumed beverages used as staining solutions (coffee, black tea, and red wine).

Experimental campaign was conducted at DISC (Dipartimento di Scienze Chirurgiche e Diagnostiche integrate ) and analyzed by the research Team of University of Genova medical Doctors.

Experimental Methods
Forty five specimens (n=15 each test material) were prepared into rectangular slices with fixed dimensions (10 x 12 x 2.5 mm) by milling commercial material disc and polishing the obtained samples with specific dental use polishing rubber bur (MEISINGER 9613P-900-220 SIL.). Machinable feldspathic ceramic (FC), Zirconia reinforced lithium silicate glass ceramic (LS) and a hybrid ceramic (HC), were milled using CAD/CAM procedures. Fifteen specimens of microparticle composite resin (MPC) were manually prepared by utilizing a silicone mold with the same diameters and thickness. All specimens were divided randomly into 3 subgroups (n=5) and , according to the most known testing protocol available in literature, were immersed in staining solutions (coffee, black tea, and red wine) for a period of 72 hours. A colorimetric evaluation according to the CIE-Lab system was performed for each specimen using a spectrophotometer before and after the immersion process.

Statistical analysis was done using Two-way ANOVA and One-way ANOVA to compare between the different study groups, and a Post Hoc test (Tukey) for pairwise comparisons.

Results and Discussion
The restorative material type was found to play a statistically significant role in color change (p<0.001) while there was no statistically significant difference among the staining beverages in color change (p= 0.113).

Conclusion
All the staining solutions used in the present study may cause color change in the restorative materials tested. All tested ceramic materials had better color stability compared to composite resin.

Acknowledgement
VITA Zahnfabrik H. Rauter GmbH & Co. KG - Bad Säckingen, Germany
Material Specimens
All the material specimens, divided in 4 groups 15 each one.

Beverages
The different beverages

References
Self-assembling fibers inside a calcium phosphate bone cement

Maria Francesca Di Filippo1, Demetra Giuri1, Milena Fini2, Melania Maglio2, Gregorio Marchiori2, Paola Torricelli2, Stefania Pagani2, Claudia Tomasini1, Silvia Panzavolta1

1 University of Bologna, Department of Chemistry ‘G. Ciamician’, Bologna, IT; 2 IRCCS Istituto Ortopedico Rizzoli, Bologna, IT

Introduction
Calcium phosphate bone cements (CPCs) are biocompatible, bioactive and osteogenic systems which can be molded into bone defects and implant sites and then harden in situ, mimicking the mineral phase of native bone. However, their mechanical properties are far from those of bone, not only in terms of strength, but especially in terms of toughness, ductility and fatigue resistance [1]. The incorporation of fibers into a brittle cement matrix has been proven to increase the fracture toughness of the composite as well as the tensile and flexural strength by the crack arresting processes. In fact, fiber reinforcement has been extensively explored even in the field of hydraulic cements and concretes for civil engineering and building applications [1,2]. Natural fibers and man-made fibers have been used for this purpose [1], but, on the best of our knowledge, they have always been introduced inside the pasty material after their synthesis.

In order to obtain a better cohesion between fibers and cement paste thus improving the mechanical performances, in this work we demonstrate the feasibility of forming self-assembling fibers in just one step during cement setting. Fibers were obtained by the introduction of a low-molecular-weight gelator (MW<1000Da) able to form supramolecular structures stabilized by weak interactions. Addition of proper amount of Ca2+ ions promotes fibers assembling inside the cement paste thus producing a composite matrix where the fibers are strictly embedded [3]. Our gelator is composed of Boc-L-DOPA(Obn)2–OH, which chelates Ca2+ ions in order to arrange in fibers.

Experimental Methods
The cement powders are composed of a gelatin/α-TCP mix and CaHPO4·2H2O, synthesized as described in a previous article [4]. The liquid phase is made by an aqueous solution of the gelator at two different concentrations: 2 and 5% wt. The gelator was dissolved in ultra-pure water with 1.3 equivalents of NaOH and sonicated. CaCl2 was added as a trigger to the gelator solution, which was mixed with the cement powders to obtain a paste of workable consistency. Different amounts of Ca2+ and different powder to liquid ratio were evaluated. The obtained pastes were compacted for 1 min inside Teflon molds with a dynamometer. Then, cements were demolded and put in phosphate buffer pH 7.4 at 37°C for different periods of time in order to ensure cement hardening. Barium sulfate was added to the cement composition as radiopacifying agent. Mechanical properties under compression and flexural properties were evaluated, as well as porosity studies by means of MicroCT analyses. Rheological measurements and morphological investigations by mean of SEM were also performed. The compositions were optimized in order to obtain injectable cements. Eventually, in vitro biocompatibility with osteoblast-like cells MG63 and qPCR gene expression were performed to ensure the biocompatibility of the materials.

Results and Discussion
XRD patterns indicate that the cements loaded with 2% and 5% wt of gelator were totally converted after 7 days of soaking, suggesting that these amounts did not interfere with the hardening reaction. SEM images reveal the presence of the fibers, which cover the pores surfaces and are embedded into the cement’s matrix, thus demonstrating a good interaction with it (Fig 1). Also the toughness of the materials are positively affected by the presence of the gel into cement composition. Time sweep measurements before and after breaking the gel (by shaking the vial, as in the preparation of cements) demonstrated that the hydrogel is thyrotrophic. Mechanical tests
confirmed that the gelator addition to the cements enhances their mechanical strength, especially when tested in bending, and increases their work of fracture. MicroCT analyses showed no significant variation on the porosity of the composite materials, but the presence of pores with higher diameter (>50um) was observed. Moreover, biological assays ensured the biocompatibility of the materials and their ability to express the main gene markers that are necessary for bone formation.

**Conclusion**

In this work we demonstrated that this approach could represents a new, simple and effective method to obtain cements reinforced with self-assembled fibers in just a single step. The formation of fibers during the hardening reaction provides structural and mechanical support to the material without interfering with their porosity. The obtained fiber-reinforced CPC is biocompatible and able to promote the deposition of extracellular matrix.

![Figure 1. Fibers of Boc-L-DOPA(Obn):-OH self-assembled within the cement.](image)

**References**


Marginal integrity of cubic zirconia inlay-retained fixed partial dentures

Luca Solimei¹, Mohamed T. El Halawani², Nicola De Angelis¹, Alberto Lagazzo¹, Fabrizio Barberis¹

¹ University of Genova, Genova, IT; ² University of Alexandria, Alexandria, EG

Introduction
“Katana”, cubic Zirconia firstly introduced on the market by Kuraray Noritake Dental Inc., Tokyo, Japan has been introduced as a new restorative material for Inlay-retained fixed partial dentures (IRFPDs), replacing missing dentition. Various studies already exist on Zirconia on literature have been conducted on the flexural strength of such restorations; however, there is a lack of evidence on their marginal integrity i.e. the capability of the material to guarantee along the time the same mechanical and functional performances.

Evaluating the marginal integrity and the overall material behaviour of three different designs of IRFPD using monolithic translucent zirconia, “Katana”.

Experimental Methods
Thirty IRFPDs divided into the 3 groups, 10 each one, were fabricated by the medical/engineering Team of University of Genova using a translucent monolithic zirconia (Katana STML, Super Translucent Multi Layered). The three groups were created with specific design in order to sort out any possible project limit.

Group 1 received an inlay cavity preparation including a proximal box and a 2 mm deep occlusal extension, Group 2 received an inlay cavity preparation including a proximal box and a 1.5 mm deep occlusal extension, and Group 3 received only a proximal box cavity preparation without an occlusal extension.

The restorations were fabricated and cemented using a dual cure resin cement (Panavia V5 system) while all the samples underwent to cyclic loading with a force of 50 N. The load was applied on the central fossa of the pontic using a metal rod with a spherical tip. Each sample was subjected to 1,200,000 cycles at a frequency of 2 Hz and thermocycled at 5°C to 55°C to 5°C for 5,000 thermal cycles to simulate 5 years of function inside the oral cavity.

In a bath of laboratory-prepared 2% methylene blue solution at 37° C for 24 h,dye penetration test were performed on the specimens, and the values of marginal penetration were analyzed under a stereomicroscope.

Results and Discussion
During the whole 5-year aging process, no specimens showed signs of cracking, fracture or loss of retention in any of the restorations. There was a significant difference between the marginal leakages values obtained in the 3 groups of the study (H=10.208, p<0.05). Comparing the groups of the study showed significant difference in marginal leakage between Group 1 and Group 3 (p<0.05).

The group with the smaller design and consequent thicknesses of the residual wall had reduced performance.

Conclusion
The closure margins of inlay-retained fixed partial dentures IRFPD of Group 1 and Group 2 has maintained accuracy, shape and dimensional stability without color changing after aging accordingly to recommended bonding protocols for zirconia. Inlay cavity design including a proximal box and an occlusal extension exhibited better marginal stability than proximal box without any occlusal extension, included in Group 3, this design is not recommended, further studies have to be conducted.

Acknowledgement
Kuraray Noritake Dental Inc., Tokyo, Japan
CAVITY DESIGN
THE TWO DENTAL ELEMENTS WITH THE PREPARED CAVITY

Inlay-retained fixed partial dentures (IRFPDs)
THE Inlay-retained fixed partial dentures (IRFPDs) CEMENTEDON THE DENTAL ELEMENTS

References
**Introduction**

Zirconia oxide (ZrO$_2$) nanoparticles are widely used as filler particles in dentistry because of their biocompatibility and antibacterial properties$^1$. Up to date, the production of commercial monoclinic ZrO$_2$ nanoparticles could be achieved by high-temperature synthesis. Using a simple and versatile sol-gel method, yttria stabilized zirconia (YSZ) nanoparticles could be synthesized at low temperatures. Such nano YSZ with tetragonal form could be a promising alternative commercial ZrO$_2$ for dental material applications because of better mechanical characteristics and stability. So far, the biological responses to sol-gel derived nano YSZ and their dependence on sintering temperature have not been investigated. The aim of the current research is to produce YSZ nanoparticles by sol-gel method at three different temperatures and to assess their biocompatibility and oxidative stress with primary human gingival fibroblasts.

**Experimental Methods**

YSZ nanopowders were synthesized using Pechini sol-gel method$^2$. The dried gel was sintered at three different temperatures: 800, 1000, and 1200°C for 2 hours. Then, the morphology and composition of the obtained powders was investigated by Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray Analysis (EDX) and Transmission electron microscopy (TEM). For evaluation of biocompatibility MTT assay with primary gingival fibroblasts (HGF) with dilutions of nanoparticles at different concentrations (0, 1, 0.5 and 0.25 μg/mL) was performed. Optical density was determined spectrophotometrically at a wavelength of 545 nm and a reference filter of 630 nm after 24 and 72 h of cell incubation. For the detection of intracellular reactive oxygen species (ROS) levels, cell culture medium from the wells cultured for 24 and 72h was collected and we employed the cell-permeable ROS-sensitive probe 2,7-dichlorodihydrofluorescein diacetate (CM-H2DCFDA) which fluoresces at 520 nm (λex = 480 nm) upon oxidation.

**Results and Discussion**

SEM analysis (Fig. 1) showed fine submicron-sized (~10-15nm) particles with uniform spherical morphology in YSZ800 specimens. With increase of sintering temperature to 1000°C, grains become bigger in size (30-50nm) and have low agglomerated microstructure. Finally, specimens sintered at 1200°C seem more agglomerated with polyhedral morphology and particle size more than 100nm. EDX analysis detected Zr content in the range of 43.89-53.43 wt%, O content in range of 26.08-32.14 wt%, Y=2.67-3.87 wt%, and minor traces of Hf. TEM analysis of YSZ1000 revealed that the nanoparticles were uniform, well-defined and had cubic morphology. The electron pattern of crystals corresponded to tetragonal zirconia, with traces of cubic.

MTT assay of the tested specimens (Fig. 2) showed that YZS1000 and YZS1200 presented a biocompatible biological behaviour as compared to positive control. The lowest cell viability was found in the highest concentrations
of nanoparticles (0.5 μg/ml) in all groups. The lowest cell proliferation was recorded in YZS800 group at 72h, suggesting its mildly toxic behaviour. YSZ1000 and YSZ1200 nanoparticles show free radical scavenging activity, which was not dependent on NP concentrations. Higher antioxidant activity of YSZ800 nanoparticles might be due to the smaller particle size3.

Conclusion
The results of the present study suggest that the sol-gel method is an effective alternative to traditional high-temperature synthesis techniques for the stabilization of the tetragonal zirconia at room temperature. With the increase of sintering temperature nano YSZ increases in size and becomes agglomerated. The obtained nanoparticles possess good antioxidant potential and good biocompatibility and are applicable in dental biomaterials.

Acknowledgement
The authors would like to acknowledge the Operational Program “Human Resources Development, Education, and Lifelong Learning 2014-2020” in the context of the project “Development of zirconia adhesion cements with stabilized zirconia nanoparticles: physicochemical properties and bond strength under aging conditions” grant number MIS5047876, co-financed by Greece and European Union (EUROPEAN SOCIAL FUND-ESF) for providing financial support to this project. Also the authors want to thank As. Professor E. Lymperaki for providing the facilities for ROS analysis.

References
2:30 p.m. – 4:00 p.m.

Poster floor

PS1-07 | Other Biomaterials Applications
Chitosan – Rosmarinic Acid conjugates for wound healing applications

Miguel Huerta-Madroñal1, Javier Caro-León2, Eva Espinosa-Cano1,3, Maria Rosa Aguilar1,3, Blanca Vazquez-Lasa1,3

1 Institute of Polymer Science and Technology, Biomaterials group, Madrid, ES; 2 Centro de Investigación en Alimentación y Desarrollo, Grupo de investigación en biopolímeros, Sonora, MX; 3 Networking Biomedical Research Centre in Bioengineering, Biomaterials and Nanomedicine, Madrid, ES

Introduction

Rosmarinic acid (RA) an ubiquitous phenolic compound, is a very attractive candidate for wound healing purposes because of its antioxidant, anti-inflammatory, and photoprotective functions [1-3]. However, its poor bioavailability due to high instability, inefficient permeability through biological barriers and poor water solubility, hamper its therapeutic outcome. In this context, synthesis of polymer conjugates is presented as an alternative to enlarge its skin applications. This work describes the synthesis of novel water-soluble chitosan-rosmarinic acid (CSRA) conjugates with emphasis on their physicochemical characterization, antioxidant and biological properties.

Experimental Methods

Synthesis and characterization of CSRA conjugates. RA was conjugated to CS backbone by carbodiimide coupling using EDC as a crosslinker, at pH 5.0 and room temperature avoiding light exposure and varying CS:EDC:RA molar ratios. Chemical structure was studied by ATR-FTIR, 1H-NMR and UV (325 nm) spectroscopies, and thermal properties by TGA. Release kinetics of thin films of CSRA samples obtained by solvent evaporation were studied in PBS 7.4 at 37°C. Radical scavenger activity (RSA) of conjugates was determined by the DPPH method and compared to that of free RA sample.

In vitro cytotoxicity. Alamar Blue Reagent was used to determine cell viability of human fibroblasts (FBH), human keratinocytes (HEK) and macrophages RAW 264.7 in presence of film lixiviates.

Anti-inflammatory activity. Nitric oxide (NO) release on lipopolysaccharide (LPS) activated RAW 264.7 macrophages was determined using Griess Reagent to investigate the anti-inflammatory capacity of film lixiviates. Percentage of NO release was compared to non-activated and LPS-stimulated macrophages.

Photoprotective capacity. Cell viability and reactive oxygen species (ROS) assays were conducted to assess the photoprotective capacity of CSRA lixiviates in UVB-irradiated HFB, HEK and RAW 264.7 cells. ROS production was measured with the H2DCF-DA probe.

Antibacterial activity. Bactericidal properties of CSRA lixiviates collected in bacterial medium were investigated against E. coli and S. epidermidis. Percentages of bacteria growth inhibition of lixiviates were compared to those of bacteria in growth media or in presence of Gentamicin or Ampicillin, respectively. Also free RA and lixiviates from unmodified CS films were studied for comparative purposes.

Results and Discussion

CSRA ATR-FTIR and 1H-NMR spectra and thermal properties confirmed RA conjugation into chitosan backbone of derivatives. Composition of conjugates was determined by UV spectroscopy. Percentage of RA conjugation was calculated for all chitosan derivatives which were named as CS-XRA, where X denotes the effective percentage of RA conjugated to chitosan polysaccharide rings (i.e. CS-10RA, CS-5RA, CS-0.8RA and CS-0.4RA). The RA:CSRA ratio (ug:mg) was 173.5, 99, 17.5 and 8, respectively. Release kinetics of CSRA conjugate films showed a first initial moderate burst release and a further controlled release pattern approaching to a plateau of catechol release was observed for all derivatives. Antioxidant capacity of CSRA samples was initially studied versus concentration and compared to that of free RA. Rosmarinic acid showed a half-maximal effective concentration (EC50) of 9.6 µg/mL. However, for the conjugates, the higher the RA content, the higher the ability to donate protons and thus, the higher the antioxidant capacity (Figure 1). Interestingly, RA immobilization into the chitosan backbone improves threefold
its antioxidant effect, as free rosmarinic acid presents an EC$_{50}$ value around 10 µg/mL while CSRA EC$_{50}$ concentrations correspond to 2.8-3.7 µg/mL of free RA. **Biological evaluation.** Lixiviates from CS-5RA and CS-10RA films were toxic for all three cell lines whereas those from the CS-0.4RA and CS-0.8RA samples were not. Biocompatible CSRA lixiviates reduced NO production in LPS-activated RAW 264.7 below 40% compared to positive control. Results also showed similar anti-inflammatory capacities of lixiviates respect to free RA, and greater NO suppression with higher grafting of RA. CS-0.8RA and CS-0.4RA lixiviates increased cell viability and attenuated UVB-induced ROS effects after irradiation with respect to positive control (Figure 2). Based on the results, we attribute the photoprotective ability to the capacity of attenuation of intracellular ROS upon UVB irradiation. CS-0.8RA and CS-0.4RA had higher growth inhibition values in both types of bacteria when compared to native chitosan, however, non-significant differences were observed in the case of *S. epidermidis*. Nevertheless, in the case of *E. coli*, both CSRA showed a two-fold growth inhibition capacity when compared to free RA.

**Conclusion**
This work describes for the first time, the synthesis and characterization of novel water-soluble CSRA conjugates. Their promising biological antioxidant, anti-inflammatory and photoprotective properties envision their potential application in chronic wound healing.

**Acknowledgement**
This work was supported by MICINN (Spain) (MAT2017-2017-84277-R, and PRE2018-083873 M. Huerta’s scholarship). M.R. Aguilar and B. Vázquez-Lasa are members of the SusPlast platform from CSIC.
Figure 2
Intracellular reactive oxygen species production of HFB, HEK and RAW 264.7 upon UVB irradiation.

References
Graphene oxide- and magnetic nanoparticles-enriched gelatin meshes, potential substitutes for peripheral nerve regeneration

Aida Selaru¹, Elena Olaret², Alexandra E. Dobranici¹, Izabela Stancu², Marieta Costache¹,³, Sorina Dinescu¹,³

¹ University of Bucharest, Department of Biochemistry and Molecular Biology, Bucharest, RO; ² University Politehnica of Bucharest, Advanced Polymer Materials Group, Bucharest, RO; ³ University of Bucharest, Research Institute of the University of Bucharest (ICUB), Bucharest, RO

Introduction
Advanced scaffold fabrication methods have made it possible to generate smooth tissue substitutes enhanced with nano-components which have been tremendously explored in the field of peripheral nerve regeneration (PNR). In this context, our studies are based on exploring the potential of gelatin electrospun meshes enhanced with graphene oxide (GO) and Fe₃O₄ nano-particles (MNPs) for supporting one of the slowest regeneration processes in the mammalian body, namely PNR.

Experimental Methods
Nano-composite fibrous substrates were fabricated by electrospinning technique in a controlled environment. The electrospinning precursors were prepared by dissolution of fish gelatin into GO, MNPs suspensions and mixtures of both (GO-MNPs) with different concentrations (0% w/v, 0.5% w/v and 1% w/v) of nano-structures. Morpho-structural characteristics of the fibrous substrates were observed through optical microscopy (OM) and atomic force microscopy (AFM). The influence of nanostructure presence on substrates' wettability was investigated through contact angle measurements (CAM). In addition, attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectrometry was performed. Next, these materials were put in contact with murine neuronal progenitor cells and murine Schwann cells in order to assess their cytocompatibility against the main cell types found in peripheral nerves. During one week of in vitro cell culture, the biosystems were tested for their biocompatibility by MTT, LDH and Live/Dead assays.

Results and Discussion
Overall, both cell types interacted with the composites in a biofriendly manner, namely cells spread out evenly, proliferated and populated the scaffolds, proportionally to the content in nanospecies. All composites displayed a low rate of toxicity. Moreover, the materials enriched with GO and MNPs presented significantly better cell response and proliferation rate compared to the gelatin control.

Conclusion
Based on the results, GO and MNPs-enriched meshes presented biocompatibility characteristics and can be further used for in-depth studies and PNR applications.

Acknowledgement
This work was supported by PN-III-P1-1.1-TE-2019-1191/MAGNIFICENT grant.
**PS1-07-221**

**pH-responsive, wet-spun coaxial microfibers loaded with Ala-Ala-Pro-Val peptide for prospective chronic wound care**

**Catarina S. Miranda**, Ana Francisca G. Silva, Marta Teixeira, Silvia M.M.A. Pereira-Lima, Susana P.G. Costa, Natália C. Homem, Helena P. Felgueiras

1 University of Minho, Centre for Textile Science and Technology (2C2T), Guimarães, PT; 2 University of Minho, Centre of Chemistry, Guimarães, PT

**Introduction**

Chronic wounds (CW) are a worldwide concern, affecting a vast portion of the population, and compromising the health and quality of life of patients. The tetrapeptide Ala-Ala-Pro-Val (AAPV) has the ability to inhibit the activity of the enzyme human neutrophil elastase (HNE), which levels, in case of excessive inflammatory processes, are abnormally high. Incorporation of peptides within polymeric structures (e.g. coaxial fibers) is very attractive to protect the payload from the surrounding environment and allow its controlled release for a sustained action. To this end, we proposed to engineer coaxial wet-spun fibrous structures loaded with the AAPV peptide. This system was designed to serve as a new delivery platform capable of a controlled and stepwise release of its content following pH-trigger, that not only fights infections but, most importantly, restores local enzymatic activity to normal levels. The outer layer (sheath) of the microfibers was made from blends of sodium alginate (SA) and gelatin (GN) combined with the N-carboxymethyl chitosan (NCMC), a chemically modified version of chitosan, responsive to basic pH (characteristic of CW) and endowed with antimicrobial action. Polymers were selected based on their biocompatibility, biodegradability and spinnability. To the authors knowledge, this is the first report on coaxial wet-spun systems loaded with AAPV for CW care.

**Experimental Methods**

AAPV peptide was synthesized by microwave-assisted solid-phase peptide synthesis[1], and characterized by 1D/2D nuclear magnetic resonance spectroscopy, Fourier-transformed infrared spectroscopy (FTIR) and UV-visible absorption (UV-VIS)/fluorescent spectroscopy. HNE evaluation was carried out by combining AAPV with elastase and evaluating their inhibitory effects via absorbance differences (UV-VIS). NCMC was produced using in house deacetylated chitosan (100-300 kDa, acetyl content determined by FTIR)[2]. The NCMC synthesis was performed in a chloroacetic acid/distilled water (dH$_2$O) mixture[3] and its degree of substitution was determined using the potentiometric titration method[4]. NCMC minimum inhibitory concentration (MIC) was defined by the broth microdilution method, in order to assess its antimicrobial potential against *Pseudomonas aeruginosa*.

SA (alginic acid sodium salt from brown algae, ≥ 2,000cP) and GN (~300 Bloom, Type A, Porcine skin) were prepared at 2% w/v and 1% w/v (in dH$_2$O), respectively, while NCMC was prepared at 2xMIC (in NaOH-containing solution). GN and NCMC were added to the SA solution under slow stirring in a proportion of 70:20:10 v/v, respectively[5]. The wet-spinning set-up was composed of two syringe pumps (NE-1600/NE-300, NewEra Pump Systems) connected to a coaxial spinneret. AAPV prepared at optimal elastase inhibition concentration was injected through the innermost port of the spinneret at a low flow rate (0.1-0.5 mL/min), to ensure the core was covered by the sheath layer (SA/GN/NCMC, flow rate of 0.5-1.0 mL/min). A calcium chloride (CaCl$_2$) solution (2 wt%, 21°C) was applied as coagulation bath. Microfibers were characterized by brightfield microscopy and FTIR. After production, coaxial fibers were incubated in physiological, acidic and basic pH buffers for 1, 2, 4, 6, 24 and 48 h to evaluate the AAPV release profile and NCMC pH-trigger dissolution, and monitored by UV-VIS. The antibacterial activity of the AAPV-loaded fibers against *P. aeruginosa* bacteria was assessed by time-kill kinetics studies for equal periods to the release. HNE inhibitory testing was again conducted, to establish the AAPV efficiency after loading.
Results and Discussion
AAPV peptide was successfully synthesized, and its purity confirmed. The peptide was seen to inhibit elastase, reducing its activity. NCMC was acquired from deacetylated chitosan and successfully transformed in NCMC, responsive to basic pH. NCMC was found effective against P. aeruginosa. To the contrary, AAPV did not inhibit the action of this bacterium. Co-axial fibers were produced from the three-polymer blend, with a smooth morphology and a hollow interior. Controlled release of AAPV from within the fibers was only accomplished in basic pH, revealing the stability of the fibers in acidic and physiological media. Time-kill kinetics tests proved the effective action of NCMC in inhibiting the growth of P. aeruginosa, as being released from the sheath of the fibers. AAPV was also seen to sustain its anti-elastase performance, guaranteeing optimal enzyme levels.

Conclusion
In the end, the potential of the engineered coaxial fibers to serve as controlled release platforms for AAPV was demonstrated. With this investigation a step further was taken in establishing wet-spun constructs for drug delivery in CW care.

Acknowledgement
This research received funding from the Portuguese Foundation for Science and Technology (FCT) under the scope of the projects PTDC/CTM-TEX/28074/2017 and UID/CTM/00264/2021. CSM also acknowledges FCT for PhD grant (2020.08547.BD).

References
Injectable hydrogel based on chitosan/polycyclodextrin/cinnamaldehyde for the local treatment of diabetic foot ulcers

Henry Chijcheapaza-Flores¹, Maria José Garcia-Fernandez¹, Mickael Maton¹, Jean-Noël Staelens², Nicolas Tabary², Frédéric Cazaux², Feng Chai¹, Bernard Martel², Nicolas Blanchemain¹

¹ Univ. Lille, INSERM, CHU Lille, U1008 - Controlled Drug Delivery Systems and Biomaterials, F-59000 Lille, FR; ² Univ. Lille, CNRS, INRAE, Centrale Lille, UMR 8207 – UMET – Unité Matériaux et transformations, F-59000 Lille, FR

Introduction
Diabetic foot ulcers are considered as a frequent complication in diabetic patients. It has been classified as a severe infection that could lead to bone infections also known as osteomyelitis. The wound healing process has become more and more difficult to manage due to poor blood circulation, sclerotic tissues, and antibiotic resistance. Traditional treatments are based on debridement, wound dressing, and systematic or intravenous antibiotic administration but even under treatment, it leads to amputations in 25% of patients (1). The most recent studies, introduce hydrogels as a potential candidate for wound healing management because of their properties as a drug delivery system and also their capacity to promote debridement and rehydration. A previous work presented by Flores C. et al (2017)(2) shows the interesting viscoelastic properties of a hydrogel based on chitosan (CHT) and poly β cyclodextrin (PCD). This project has been focused on the development of CHT/PCD hydrogels combined with a natural antimicrobial molecule, Cinnamaldehyde (CN). Since CN (obtained from Cinnamon essential oil) is considered as an efficient antimicrobial compound (3), it is a promising candidate for the local treatment of diabetic foot ulcers.

Experimental Methods
Firstly, CHT (deacetylation degree: 76% and molecular weight: 256 kDa) and PCD (molecular weight: 21 kDa and degree of substitution: 56%) were milled to obtain particles of <125 µm. Then, the first step consisted of hydrogel pre-formulation, CHT and PCD powders were co-milled at different concentrations, and the powder was suspended in distilled water and acidified with Lactic Acid (patent FR 3038318). The hydrogel formation was assessed by inversed-vial test: 1 mL of hydrogel was injected in a vial, and the resistance to flowing was evaluated. Later, hydrogel cohesion and structure stability were assessed by hydrogel injection in phosphate-buffered saline (PBS, pH 7.4). Thereafter, PCD-CN complexation was studied by the solubility phase diagram (4) and Nuclear Magnetic resonance spectroscopy (NMR H1 and ROESY). At a second stage, CN was added in the aqueous phase before the suspension of CHT/PCD powder. New CHT/PCD/CN formulation was as well evaluated and characterized by rheology (viscoelastic properties, recovery, and viscosity). CN release study was performed under dynamic conditions with USP4 apparatus (Sotax®, 37°C, 5 mL/min) and quantification was performed using a UPLC-DAD (Nexera, LC-2040C 3D Plus, Shimadzu, Japan) with diode array detector (DAD) at 287 nm. The bacterial kinetic reduction of hydrogels was proved by kill time test with strains of S. aureus and E. coli according to JIS Z 2801. Finally, the hydrogel cytocompatibility was assessed using a pre-osteoblast cell line (MC3T3-E1) according to the ISO 10993-5.

Results and Discussion
Inversed-vial test and hydrogel injection in PBS proved the hydrogel formation as well as their stability in PBS. On the other hand, Cyclodextrin (CD) has been widely used as an excipient to form inclusion complexes for drug delivery and to improve the solubility profile of molecules with low solubility. In this context, the solubility phase diagram proposed proved a complexation constant of 119.028 M⁻¹ between CN-PCD cavity and NMR spectroscopy confirmed...
the inclusion of CN aromatic group into the β-CD cavity. Once CN was added at a concentration of 1% w/w, the study of the storage modulus (G') and loss modulus (G'') by rheology confirmed the hydrogel formation. Therefore, viscosity and recovery assay proved the shear-thinning character and structure recovery after stress (>75%) respectively. These features will influence the facility for injection as well as the restructuration of the hydrogel after injection. The release and microbiological test confirmed a CN release of 5 mg/g of hydrogel and a 6 log reduction of S Aureus and E. Coli after 15 hours. Finally, 98% of cell viability was obtained in the in vitro cytotoxicity by AlamarBlue® test.

Conclusion
After hydrogel evaluation, a new promising hydrogel with interesting rheological properties and in vitro activity for the treatment of diabetic foot ulcers has been obtained. Nevertheless, further studies are needed to prove its efficacy in an in vivo model.

Acknowledgement
This project had the funding of the MOBLILEX scholarship

References
Skin hydration via electrospun polyimide patches with oil: *in vivo* experiments compared with numerical model

**Ewa A. Sroczyk**¹, Marek Jaszczur², Urszula Stachewicz³

¹ AGH University of Science and Technology, Faculty of Metals Engineering and Industrial Computer Science, Krakow, PL; ² AGH University of Science and Technology, Faculty of Fuels and Energy, Krakow, PL

**Introduction**

Electrospinning is an excellent method to produce polymer fibers and membranes, which are characterized by high surface area to volume or mass ratio. Moreover, they are characterized by high porosity. These features are beneficial for biomedical applications such as skin patches. In our study we focused on atopic and allergic skin. Atopic dermatitis is the most common inflammatory skin disease, it affects up to 20% of children worldwide, and significantly decreases the patient’s quality-of-life [1,2]. Both diseases require continuous skin care. Long-term hydration is a one of supporting therapies, which can be achieved by applying skin patches. The purpose of our study was to develop a numerical model of skin and hydrating electrospun patches.

**Experimental Methods**

In our study we selected polyimide (PI) polymer, because is biocompatible, rigid, thermally stable and resistant to most chemical reagents and oils. We produced PI membrane with electrospinning method and imaged the membrane with scanning electron microscope (SEM). Then we evaluated mean fiber diameter by measuring 100 diameters of randomly chosen fibers using ImageJ J1.53v.

Atopic skin is deficient in gamma linoleic acid (GLA), therefore we selected a natural oil which is one of the richest in GLA – blackcurrant seed oil [3]. The obtained PI membrane pores were filled with the oil. Then, such patches were applied on human skin. We used corneometer for skin hydration evaluation before and after patch application. The PI membrane numerical model was developed from focused ion beam – scanning electron microscope images with COMSOL Multiphysics software.

**Results and Discussion**

We electrospun PI as presented in **Figure 1a**. The PI membrane pores can be filled with beneficial components which release in a controlled manner and hydrate the atopic or allergic skin. Obtained fibers have average diameter of 0.50 ± 0.07 µm, pore size in range 0.40 – 3.45 µm with median value 1.56 µm. The membrane porosity is 95.6% and contact angle 134 ± 2° which indicated hydrophobic character.

The skin hydration significantly increased after patch application – the longer patch was applied, the more hydrated skin. Additionally, the numerical simulation of GLA diffusive transport from oil in patch to the skin model was performed as showed in **Figure 1b, c**.

Combining the excellent membrane features, which are stability, high porosity and stretchability, with beneficial blackcurrant seed oil composition, we designed patches for long-term skin hydration. These patches were tested *in vivo*, by assessing the skin hydration before and after patch application. The PI patches were designed for long-term oil release, and the simulation showed the increase of GLA passing from oil to the skin. The model confirms that the GLA transport is directly related to, and responsible for, the increase of skin hydration.

**Conclusion**

Compatibility of experiments and numerical results showed the constructed model may be applied for individually tailored dermatologic therapy. The obtained patches have a great potential in other skin applications. The prepared skin model is a first base for further developments toward other skin disorders.

**Acknowledgement**

Page 1219 of 2028
This study was conducted within Nanofiber-based sponges for atopic skin treatment project, carried out within the First TEAM programme of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund, project no POIR.04.04.00-4571/17-00.

Figure 1.
(a) SEM micrograph of PI electrospun fibers; (b, c) GLA concentration distribution over 6 h in the skin (lower layer in the model) and PI membranes loaded with oil (upper layer in the model), where the black dots are cross-sections of PI nanofibers. skin-oil means the interphase boundary between skin and PI membrane.

References
Polymeric nanoparticles by design to target cancer

Gianluca Ciardelli, Clara Mattu, Giulia Brachi, Luca Menichetti, Alessandra Flori, Paolo Armanetti, Elia Ranzato, Simona Martinotti, Monica Boffito, Susanna Sartori

1 Politecnico di Torino, Turin, IT; 2 Imperial College, London, GB; 3 CNR-IFC, Pisa, IT; 4 Università del Piemonte Orientale, Alessandria, IT; 5 Fondazione Gabriele Monasterio-CNR, Pisa, IT

Introduction
Cancer nanomedicine has the potential to enhance efficacy and reduce off-target effects of chemotherapy. Nanosized drug delivery systems (called nanomedicines) have been shown to passively accumulate in certain tumors through the Enhanced Permeability and Retention (EPR) effect and to selectively interact with specific receptors on cancer cells, upon proper surface-functionalization. Polymeric nanoparticles (NPs) are appealing tools, since polymers can be largely modified to comply with the requirement of high loading, extended release, and targeting capacity. Polyurethanes (PURs) are particularly attractive in this endeavor by virtue of their tunable chemistry, biocompatibility, and ease of both, surface and bulk, functionalization. Here, we showed that proper selection of PUR building blocks can be exploited for surface-coupling of targeting ligands as well as to modulate the loading and release of single and multiple chemotherapeutics.

Experimental Methods
Different PURs were obtained by a two-step synthesis procedure and their hydrophilic/hydrophobic balance was modulated by selecting different ratios between the hydrophobic poly(ε-caprolactone) (PCL) diol and the hydrophilic poly(ethyleneglicole) (PEG). N-BOC-serinol, which bears pendant BOC-protected amino functionalities was inserted as chain extender. NPs were prepared and fully characterized in terms of size, polydispersity (PDI), zeta potential, and morphology. Active tumor targeting was achieved by surface-modification with the monoclonal antibody Herceptin (HER), which targets the HER2 receptor overexpressed by many cancer cells, including breast and colon cancer. A BOC de-protection protocol was first optimized to expose functional groups post NPs preparation, and HER was covalently attached via EDC/NHS-mediated coupling.

In another experiment, core shell PUR NPs were designed to possess different PUR cores (i.e., cores with a different hydrophilic/hydrophobic balance) and a phospholipid outer shell. In details, three polyurethanes obtained by varying the percentage of PCL from 100% to 70% were obtained and named PU100, PU80, and PU70. Two different chemotherapeutics, namely Doxorubicin and Docetaxel were co-loaded inside the PUR cores together with iron oxide NPs for imaging purpose. Cyanine 7 was attached to the surface of the NPs for dual fluorescent/photoacoustic detection. Loading efficiency of all agents, biodistribution, and tumor targeting ability were assessed both in vitro and in vivo.

Results and Discussion
Functional groups on the surface were successfully exploited for modification with HER. HER-NPs were able to selectively target HER-2 over-expressing cells in vitro. Core-shell PUR NPs were able to co-load multiple payloads with high loading efficiency (FIG. 1A), which was particularly relevant in mildly hydrophilic PURs. Moreover, contrast agents for MRI and photoacoustic imaging were also inserted in the particles, obtaining an efficient imaging tool in all tested modalities (MRI, Photoacoustic, and fluorescence (FIG 1B, 1C). When administered i.v. to mice, all NPs possessed a similar biodistribution profile, which was not affected by the composition of the PUR core. Moreover,
NPs resulted high in vivo tumor accumulation in a breast cancer flank tumor model (4T1 cells), where they were able to transport both drugs with a significantly higher efficacy as compared to non-encapsulated drugs.

**Conclusion**

We demonstrated that PURs are excellent biomaterials to design nanomedicines, as they offer high loading capacity of single and multiple payloads, extended release, cell targeting ability and high in vitro internalization.

![Figure 1](image-url).

**Figure 1.** A) loading efficiency of Doxorubicin and Docetaxel in different PUR cores. B) MRI contrast enhancement achieved with the different PUR cores. C) Photoacoustic imaging in ex-vivo phantoms. Figure adapted from [4].

**References**


Fiber diameter as determining factor for oil spreading and release from the electrospun skin patches

Zuzanna J. Krysiak, Urszula Stachewicz

AGH University of Science and Technology, Faculty of Metals Engineering and Industrial Computer Science, Cracow, PL

Introduction
Atopic dermatitis is widely spread skin disease affecting people regardless the age. Skin moisture is highly decreased due to transepidermal water loss. Patches, which can increase hydration in long term application are required. Electrospun fibers are reported to be applicable as membrane loaded with natural oils, beneficial for eczema [1].

Experimental Methods
Nano and micro poly (vinyl butyral-co-vinyl alcohol-co-vinyl acetate) (PVB) fibers were electrospun with low and high molecular weight polymer, respectively. Both membranes show high porosity, over 90% and high surface to volume ratio. Those properties are beneficial in a dermal drug delivery system [2]. Nano and micro PVB fibers were elongated uniaxially with tensile testing equipment. Evening primrose oil spreading was observed on both random and elongated fibers. Moreover, two different ways of oil application was analyzed, pipetting and spray brush. Biocompatible PVB membranes were examined for secretion of proinflammatory cytokines. For this purpose, human keratinocytes (HaCaT) were seeded on nano and micro fibers and cell culture medium was used as a substrate for enzyme-linked immunosorbent assay (ELISA). Obtained results, showed possibility of controlling oil behavior on the fibers by changing their geometry. Furthermore, we performed skin hydration test with evening primrose oil applied on the electrospun membranes.

Results and Discussion
The shape of oil droplet was ellipsoid for elongated fibers and circular for random. Oil area was greater in the case of nanofibers after 20 minutes. Oil spreading observation indicate that, oil pathway was through the fibers, regardless their orientation. Oil application by pipetting was selected as more efficient in comparison to the spray brush. Skin hydration test with nanofibers showed a great advantage over microfibers. Nanofibers were more attached to the skin, while microfibers were sticking out of the skin.

Conclusion
This study shows that evening primrose oil behavior was influenced by the diameter of fibers and alignment. Obtained results are promising for application electrospun PVB membranes as patches for skin hydration.

Acknowledgement
This research was part of the “Nanofiber-based sponges for atopic skin treatment” project carried out within the First TEAM program of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund, project No POIR.04.04.00–00–4571/17-00.

References
Fabrication of chitosan-based nanofibre scaffolds as potential wound dressings

Nguyen D. Tien¹, Tianxiang Geng¹, Catherine A. Heyward¹, Jonny J. Blaker¹,², Janne E. Reseland¹, S. Petter Lyngstadaas¹, Håvard J. Haugen¹

¹ University of Oslo, Department of Biomaterials, Institute of Clinical Dentistry, Oslo, NO; ² The University of Manchester, Department of Materials and Henry Royce Institute, Manchester, GB

Introduction
Chitosan is a sustainable biopolymer that has remarkable intrinsic properties, including non-toxic, antibacterial, and biodegradable [1]. Among the different physical forms of chitosan (e.g., powder, film, foam, and gel), chitosan-based nanofibres have drawn much attention due to their unique characteristics, such as larger surface-area-to-volume ratio and appropriate mechanical strength when blended with other polymers [2]. In this work, chitosan-based nanofibrous scaffolds for new wound dressings were fabricated via solution blow spinning (SBS) [3]. This wound dressing should mimic some structural aspects of the skin’s extracellular matrix, which could improve conditions for cell attachment, proliferation and growth, and thus promote skin tissue repair and regeneration with shortened healing time [4].

Experimental Methods
Chitosan powders with different degree of deacetylation (DDA, 57 – 97%) were prepared in 4 wt.% solutions in 0.5M acetic acid and mixed with 4 wt.% poly(ethylene oxide) (PEO) aqueous solutions at different weight ratios to obtain chitosan/PEO blend solutions. All solutions were spun into nanofibres using SBS. The obtained nanofibres were characterized by various techniques including SEM, DSC, TGA, FTIR, confocal fluorescent microscope, and mechanical testing. In vitro studies were conducted by culturing human fibroblasts onto the chitosan nanofibre scaffolds.

Results and Discussion
Processing neat chitosan into nanofibres is challenging due to its cationic nature and high crystallinity, therefore, PEO, an FDA-approved polymer for safe use in several medical applications, is introduced to improve the spinnability of chitosan solutions. Indeed, uniform fibre structures with submicrometer-sized fibres were successfully obtained in all compositions (Figure 1). When the PEO content in the blend increases, the fibre diameter becomes large, as well as wider fibre diameter distribution. Chitosan nanofibres containing 10 - 50 wt.% PEO produce an ultrafine fibre with near defect-free fibre network (i.e., no large droplet was seen as in case of 98/02 (98 wt.% chitosan, 2 wt.% PEO) and 95/05 (95 wt.% chitosan, 5 wt.% PEO) compositions). As a result, the 90/10 composition was selected for in vitro studies due to its nanofibrous-structural integrity, while the amount of chitosan was maximized.

Due to the fact that PEO is water-soluble, this polymer was removed from the chitosan/PEO blended nanofibres prior to cell culture. This step requires neutralization in order to prevent the chitosan fibres losing their fibrous structures. Neutralization and the total removal of PEO were completed by immersing the membrane in aqueous 1M potassium carbonate solution for 3 h. The removal of PEO was verified by FTIR and NMR analyses. Stable chitosan fibre membranes were obtained without significant loss of their nanofibrous structures. In vitro tests were performed by culturing human fibroblasts onto chitosan nanofibre scaffolds (using cell crowns) for 14 days. Attachment and organization of cells on the fibrous network can be seen in Figure 2. The cell bodies of fibroblasts were stretched and attached in a three-dimensional network of chitosan nanofibres.

Conclusion
Chitosan nanofibres with submicrometer-sized fibres were successfully fabricated using SBS. The chitosan nanofibrous structure promoted cell adhesion, showing normal cell morphology and spreading, indicating appropriate cell viability for the nanofibres. The fabricated nanofibres show promise as potential skin graft substitutes.

Acknowledgement
This work was funded by the Research Council of Norwegian (RCN) Nano2021 grant number 287991.

Figure 1
SEM images of chitosan/PEO nanofibres at different blended compositions.

Figure 2
(A) Confocal image shows the cell nucleus (magenta) and their membranes (red) in the matrix of nanofibres (green) and (B) SEM image shows the cells seeding on the nanofibrous network.

References
Neuronal Networks in a Fiber-Reinforced Hyaluronic Acid-Based Matrix

Dieter Janzen1, Ezgi Bakirci2, Julia Hauptstein3, Leonard Forster2, Jörg Teßmar2, Torsten Blunk3, Paul D. Dalton2,4, Carmen Villmann1

1 University Hospital Würzburg, Institute of Clinical Neurobiology, Würzburg, DE; 2 University Hospital Würzburg, Department for Functional Materials in Medicine and Dentistry, Würzburg, DE; 3 University Hospital Würzburg, Department of Trauma, Hand, Plastic and Reconstructive Surgery, Würzburg, DE; 4 University of Oregon, Phil and Penny Knight Campus for Accelerating Scientific Impact, Eugene, US

Introduction
Three-dimensional in vitro cell culture models are becoming essential to replicate the in vivo. Such 3D in vitro models use matrices to encapsulate cells, thus creating an extracellular environment that ideally should resemble mechanical and biological properties experienced within native tissue. For the central nervous system, creating such model systems with primary neurons and glia can be especially challenging, as weak hydrogels mimicking the softness of the brain are difficult to handle. Incorporation of reinforcing fibers created by using additive manufacturing (3D printing) technologies can improve mechanical properties of soft hydrogels and enable proper handling. One of these technologies, melt electrowriting (MEW), allows creation of small-scale, organized structures that don’t occupy significant volumes of the matrix. Recently, we successfully demonstrated high viability, network formation, and network function for cortical neurons in fiber-reinforced Matrigel. [1]

Experimental Methods
In this study, melt-electrowritten poly(ɛ-caprolactone) (PCL) fiber scaffolds were used to reinforce thiolated hyaluronic acid (HA-SH). Hyaluronic acid is one of the major components of the extracellular matrix in the brain. Cortical neurons and astrocytes were seeded into these 3D matrix composites and studied for their viability, network formation, network function, and migration.

Results and Discussion
Cortical neurons showed low viability and almost no network formation when cultured in HA-SH without supplements. However, co-culture with astrocytes seeded as a monolayer below the 3D fiber-reinforced HA-SH significantly increased viability. It is hypothesized that the secreted extracellular matrix proteins or cytokines from the astrocytes elicit the higher survival rate in the HA-SH matrix. Neuronal network formation followed by immunocytochemical stainings and characterization of the network firing activity is under investigation. Furthermore, astrocytes migrated into the hydrogel in close proximity to the cortical neurons.

Conclusion
In summary, we successfully cultured cortical cells in a fiber-reinforced hyaluronic acid-based matrix. This 3D in vitro model represents a powerful basis allowing customization of matrix formulation and study of cell-cell interactions under normal and disease conditions.

Acknowledgement
Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project number 326998133 – TRR 225 (subproject B01).

References
Development of CaZn-releasing platforms for wound healing applications

Celia Ximenes-Carballo¹, Soledad Pérez-Amodio¹,²,³, Oscar Castano¹,²,⁴, Elisabeth Engel¹,²,³

¹ Institute for Bioengineering of Catalonia (IBEC), Barcelona, ES; ² CIBER en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, ES; ³ Technical University of Catalonia (UPC), Department of Materials Science, EEBE, Barcelona, ES; ⁴ University of Barcelona (UB), Electronics and Biomedical Engineering, Barcelona, ES

Introduction
Chronic wounds represent a major burden in human society: just in the US, there are about 6.5 million of patients, and this number is expected to increase due the upward trend of aging population, incidence of diabetes and obesity. In Europe, one out of five hospitalized patients suffer from a pressure ulcer. Moreover, the costs associated with its extensive care treatments are high; about 25 billion dollars are spent annually in the US.¹ These costs can be reduced by appropriate diagnose and treatment. Thus, research has focused on the development of new wound healing devices. However, a device that enables fast-effective closure, low cost, and scalability is still missing.

Ions such as calcium (Ca²⁺) and zinc (Zn²⁺) are essential for skin homeostasis. Calcium regulates a plethora of skin vital functions, such as platelet aggregation or epidermal stratification. We have shown that calcium-releasing platforms such as calcium phosphate nanoparticles (NPs) stimulate in vitro and in vivo wound healing.²,³ On the other hand, the skin contains about 5% of total body zinc content, and deficiencies on this ion are associated with impaired wound healing and roughened skin. Zinc’s antimicrobial properties have been recently suggested,⁴,⁵ making this ion promising for its application on wound dressings.

This work aims to develop an ion releasing platform based on nanocomposites for local and sustained calcium and zinc release at the wound site, to achieve wound closure and provide antimicrobial protection.

Experimental Methods
Submicrometric particles incorporating different amounts of Zn²⁺ and Ca²⁺ ions were combined by wet methods. Then, they were thermally treated in order to regulate their degradability. Ion release was analyzed by colorimetric methods. pH was measured with a pH-Meter. Particle size was determined by Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM). Particle composition was studied using X-Ray Diffraction and Energy Dispersive X-Ray Spectroscopy (SEM-EDX). Direct and indirect particle toxicity was assessed in vitro in human dermal fibroblasts (hDFs) using the MTT assay. Particles were incorporated in collagen scaffolds in order to perform in-situ ion release.

Results and Discussion
Calcium and zinc were successfully incorporated into the particles. Two different compositions (CZ1 and CZ2) where Zn²⁺ and Ca²⁺ were combined successfully. Thermal treatment of the particles diminishes degradation due to its increase in phase crystallinity. Particles showed a rounded morphology, with submicronic sizes. Backscattering images show that zinc and calcium are homogeneously mixed. In fact, they seem to be formed of Ca(OH)₂ with the presence of Zn impurities, as suggested by XRD data. Ion release was sustained for up to 1 month (assay endpoint). Particles increased pH at the first 24h and then returned at physiological levels. The lower the Zn content, the lower the toxicity. The concentration of particles had to be in both cases lower than 1 mg/mL to avoid cytotoxicity. Particles were encapsulated in collagen hydrogels to achieve particle immobilization (local delivery) of the ions at the wound bed.

Conclusion
Ion releasing platforms were successfully produced. Their composition, size, and ion release profiles indicate their potential use in soft tissue applications such as wound healing therapies. On-going research to test their wound healing potential includes cell migration, MMP regulation, proangiogenic factor production, and collagen synthesis.

Acknowledgement
This work was supported by the Spanish Ministry of Science and Education and the EuroNanomed3 program through the project nAngioDerm.

References
PS1-07-237

Characterising the Physical Properties of Silicone Oil Tamponades Containing Polymer Based Additives for Ocular Drug Delivery

Alys E. Davies¹, Stephanie Edwards², Helen Cauldbeck²,¹, Steve Ranna²,³, Tom McDonald², Victoria Kearns¹

¹ University of Liverpool, Eye and Vision Science, Liverpool, GB; ² University of Liverpool, Chemistry, Liverpool, GB; ³ University of Liverpool, Materials Innovation Factory, Liverpool, GB

Introduction
Silicone oil tamponades are used as a treatment in retinal detachments and help prevent the formation of unwanted wound healing responses associated with diseases such as diabetic retinopathy and proliferative vitreoretinopathy (PVR). Drug releasing tamponades have the ability to reduce the incidence of PVR. This study aimed to characterise clinically relevant physical properties of novel silicone oil (SiO) based tamponades with the addition of polymer based additives¹,² to control drug release.

Experimental Methods
Reversible addition-fragmentation chain-transfer (RAFT) polymerisation was used to polymerise poly(dimethylsiloxane)methacrylate (PDMSMA) of varying molecular weights, as either homopolymers or copolymers with oligo(ethylene glycol)methacrylate (OEGMA) and blended 10 v/v% in technical and purified Siluron¹⁰⁰⁰ and Siluronxtra (Fluoron GmbH). Emulsification behaviour was tested by measuring volume of emulsion following exposure to shear in a 1:1 ratio of oil:foetal bovine serum. Viscosity was measured using ARES G2 controlled-strain rheometer and 50mm diameter 1˚ cone geometry. Light transmittance was measured using UV-Vis spectrometry over the visible light spectrum and cytotoxicity testing was conducted on a human retinal pigmented epithelium cell line, ARPE-19, to ISO10093 standard.

Results and Discussion
Emulsification studies highlighted differences in behaviour between technical and purified SiO grades. Shorter chain polymers increased emulsion volume in technical grade SiO¹⁰⁰⁰ (29.8% to 36.8%) but not purified; conversely, longer polymer chains showed opposite behaviour. In both technical and purified SiOxtra, emulsion volumes increased with polymer additives but not beyond the clinically-relevant threshold of purified SiO¹⁰⁰⁰. In the rheology studies, the presence of polymers had little difference on the purified oils. The inclusion of polymer additives also showed no detriment to the shear thinning properties of SiOxtra. Light transmittance remained above 80% in all oils. The effect of long term exposure to the modified oils on proliferating cells appeared to be inconclusive. For metabolic activity, no statistically significant differences were observed in the purified oils compared with the negative control however changes in cell number varied across the blends and showed no obvious trend. For stable monolayers, no statistically significant differences were observed in either metabolic activity or cell number when cells were cultured with the oils for extended periods.

Conclusion
Inclusion of PDMS based additives does not appear to impede clinically relevant physical characteristics of SiO tamponades. Siluronxtra is a promising base oil to develop a new, drug releasing tamponade which could provide a novel treatment to reduce the complications of retinal detachment treatment, however, caution must be taken when using technical grade materials during product development as behaviour can differ from that of purified grade materials.

Acknowledgement
Fluoron GmbH for supplying the silicone oils and financial support.

Page 1229 of 2028
EPSRC Grant number: EP/R024839/1

References

[1] Cauldbeck, H et al., 2016, ‘Controlling drug release from non-aqueous environments: Moderating delivery from ocular silicone oil drug reservoirs to combat proliferative vitreoretinopathy’, Journal of Controlled Release, 244, 41-51

Design and characterization of an in vitro 3D liver lobule model

Matteo Pitton¹, Giorgio Dallera¹, Nicola Contessi Negrini², Silvia Fare¹,³

¹ Politecnico di Milano, Dept. of Chemistry, Materials and Chemical Engineering, Milan, IT; ² Imperial College, Dept. of Bioengineering, Faculty of Engineering, London, GB; ³ National Interuniversity Consortium of Materials Science and Technology, Florence, IT

Introduction
The development of 3D in vitro models allows overcoming relevant drawbacks associated with 2D in vitro cultures and in vivo animal models, thanks to the closer reproduction of the in vivo conditions and maintenance of the stability in long-term cultures [1]. In order to fully replicate in vitro the complex architecture of the liver for the realization of a functional hepatic model, an accurate intrinsic vascularization is crucial for hepatocytes function, such as detoxification of drugs, and for the creation of the physiological oxygen gradient. The aim of this work is to design and develop a 3D in vitro liver model closely mimicking the architecture of the hepatic parenchyma and the complex vascularization of the hepatic lobules to investigate the main physiological mechanisms occurring both in the healthy and pathological liver.

Experimental Methods
Gelatin methacryloyl (GelMA) hydrogels (10% w/v) were prepared by pouring the GelMA solution in a specifically designed PDMS mold and subsequently by photo-crosslinking under UV rays (Irgacure 2959). PVA sacrificial structures were embedded before the photocrosslinking to assemble the final model. PVA was selected as the material for the sacrificial structure, carefully designed in order to mimic the radial arrangement of the hepatic sinusoids in the liver lobules. The PVA structure was printed, after processing optimization, via FDM. The dissolution of the PVA structure was investigated by stereomicroscope and perfusion tests were performed to verify the expected formation of patent channels inside the GelMA hydrogel. The final hexagonal model was investigated by analyzing the physical (degradation test in physiological-like solution, T = 37°C, up to 6 weeks), mechanical (compression cyclic test, T = 37°C, strain ramp = 2.5% min⁻¹ down to 30% and 5% min⁻¹ up to 1%) and in vitro biological (in vitro cytocompatibility tests up to 14 days, L929 cell line) properties.

Results and Discussion
The realized model is composed of a hexagonal GelMA hydrogel, resembling the structure of the hepatic lobule, in which the 3D printed PVA sacrificial structure, mimicking the vascular network of the hepatic sinusoids, is embedded. By microscope observation and perfusion test, the patency of the obtained channels was verified (Figure 1). Stability tests up to 6 weeks showed an initially expected degradation of the uncrosslinked gelatin chains, followed by the swelling plateau (after 72 h). The assembled GelMA model maintained its physical stability up to six-week weight variation analysis and therefore appears suitable for in vitro modeling. Compressive mechanical tests (Figure 2a) exhibited a compressive elastic modulus comparable to GelMA hydrogels, either with and without embedded PVA sacrificial structure (p > 0.05). On the other hand, a significant difference in stiffness (p<0.01) and in the maximum stress reached (p<0.05) was evidenced. This decrease in the mechanical properties could be due to the presence of the open channel network, after PVA dissolution, which allows the aqueous solution to penetrate more easily between the meshes of the hydrogel. This reduction in GelMA_PVA formulation is not a disadvantage for the in vitro model, as it is able to mimic the mechanical characteristics of the liver. In fact, the liver physiological values of elastic modulus [2-4] do not show differences compared to the values of the GelMA_PVA structure developed here (Figure 2b).

Preliminary results obtained by AlamarBlue assay showed that cell metabolism in the GelMA model increases...
considerably in time, reaching values closer to those achieved by cells grown on TCPS (Figure 2c). This result indicates that the proposed model adequately supports cell adhesion, spreading, and proliferation resulting. Live/Dead staining qualitatively showed that L929 cell viability increased at each considered time point for cells encapsulated in the upper face of GelMA samples and for those in the cross-section (Figure 2c).

**Conclusion**

Despite the open challenges still present to produce an accurate scalable in vitro model of the liver, due to its very complex architectural and vascular features, an attempt to mimic the lobule structure was here described and characterized. The obtained results confirm that the GelMA model, characterized by the presence of patent channels obtained by PVA sacrificial structures, is suitable to successfully replicate the compressive mechanical characteristics of native hepatic tissue as well as good cell viability in the structure.

**Figure 1**

Preparation of the crosslinked GelMA hydrogel embedding the PVA sacrificial structure; after the dissolution of PVA, channels are observable as well as their patency.

**Figure 2**

a) Mechanical parameters obtained in compressive mode for GelMA hydrogel without (GelMA_only and with the open channel network (GelMA_PVA). *: p<0.05, ** p<0.01; b) comparison among the elastic modulus values for GelMA_PVA and the ones for human (Yeh et al, [2]), rat (Mattei et al, [3]), and porcine (Ogawa et al, [4]) livers; c) AlamarBlue assay: cell viability for GelMA structure and control (TCPS) up to 14 days of culturing; d) Live/dead staining: comparison between cell viability in the upper face and the cross-section of the GelMA_PVA model up to 14 days after seeding.

**References**

Ultra-thin ELR membranes for wound healing applications

Diana J. Gusano, Lubinda M. Mbundi, Miguel G. Pérez, José Carlos R. Cabello

Universidad de Valladolid, GIR BIOFORGE-CIBER-BBN, Valladolid, ES

Introduction
Dermal damage through trauma or diseases that affects the basement membrane is characterised by delayed healing, contractures and scarring. In this regard, biomaterials are very promising for building scaffolds for therapeutic purposes, simulating most of the properties of these tissues, such as mechanical ones, thus making them ideal supports for skin regeneration. Among novel strategies for wound healing is the development of natural polymer based ultra-thin membranes [1]. Here we report an in vitro evaluation of the efficacy of an elastin-like recombinamer based ultra-thin membrane for wound healing applications.

Experimental Methods
For the purpose of this work, two biodegradable and cell-adhesive ELRs were obtained by genetic engineering techniques, assessed by agarose gel electrophoresis, and expression in Escherichia coli bacteria, whereas its purification was performed through Inverse Temperature Cycling (ITC) as described elsewhere [2]. Then, ultra-thin membrane were produced by the catalyst-free click-chemistry based crosslinking of these two biodegradable ELRs [3] at an interface of two immiscible liquids (aqueous and organic solvents). After that, they were washed through water, acetone, methanol and collected in PBS followed by UV exposure. The ability to support the viability and activities of skin cells was determined in vitro using human foreskin fibroblasts (HFF1) and human keratinocytes (HaCat). Cells were immunostained for markers for differentiation, material-cell and cell-cell interaction and structural protein.

Results and Discussion
The novel ELR-based ultra-thin membranes could support cell viability and activities in vitro for at least 21 days in culture. The high porosity of the scaffold supported media diffusion, a characteristic that was vital for maintaining the integrity of the fibroblast and keratinocytes. Indeed, markers indicative of established cell-cell and cell-matrix interactions were observed. Moreover, cells demonstrated matrix remodeling through the production of ECM features such as collagens (I and IV), laminin and fibronectin, which are relevant for wound healing.

Conclusion
This work demonstrates the successful production of biocompatible ELR-membranes with tunable physicochemical properties such as shape, thickness, porosity, diffusion coefficient and biofunctional moieties. The ease of production and derivatisation, as well as stability in culture and robust mechanical properties suitable for handling make this novel ELR-based membrane an ideal candidate for use in the development of bioactive dermal substitutes (i.e. basement membrane) for wound healing and other tissue engineering applications.

Acknowledgement
The authors are grateful for the funding from the Spanish Government (MAT2016-78903-R, RTI2018-096320-B-C22, PID2019-110709RB-100, RED2018-102417-T), Junta de Castilla y León (VA317P18, Infrared2018-UVA06), Interreg V España Portugal POCTEP (0624_2IQBIONEURO_6_E) and Centro en Red de Medicina Reg. y Terapia Celular de Castilla y León.

References
In vitro studies of dual-responsive composites for simultaneous hyperthermia and chemotherapy

Filipe V. Almeida, Adriana Gonçalves, João P. Borges, Paula I. Soares

Universidade Nova Lisboa, CENIMAT/ i3N, Caparica, PT

Introduction
Nanotechnology-based approaches to perturb tumour’s biology and deliver therapies are gaining momentum due to their nanoscale-related properties. Iron oxide nanoparticles \( \text{Fe}_3\text{O}_4 \)-NP respond to high-frequency alternated magnetic field (HFAFM) by promoting heating. Recently, \( \text{Fe}_3\text{O}_4 \)-NP coated with oleic acid (OA NP) and dimercapto succinic acid (DMSA NP) were incorporated into thermo-responsive microgels (MG) made of poly (N-isopropyl acrylamide, PNIPAAm), which in turn were incorporated into polyvinylpyrrolidone (PVP) nanofibers. The material properties of NP/MG/PVP were thoroughly characterized recently [1], although their biological role is not yet characterized.

Experimental Methods
Here, we focus in studying the impact of PVA/MG/NP in controlling adhesion, proliferation and cell death. PVA was used, instead of PVP as previously, given their ability to form thinner fibers, which has the potential to outperform PVP functionally (i.e. heating capacity and drug releasing). To assess adhesion, we performed immunostaining analysis of focal adhesion (FA) protein vinculin and filamentous actin in different tumor cells lines (melanoma cells, WM983b, osteosarcoma, SaOs) and compared with non-malignant cells (adult dermal fibroblasts). To assess proliferation, resazurin, a colorimetric dye that measures cell viability was used, as well as 3D laser scanning confocal microscopy. Cell death was quantified by combining resazurin measurements and imaging of fluorescently probes, namely live/dead dyes (calcein-AM and Ethidium homodimer-1) and immunostaining with apoptosis markers (cleaved caspase-3).

Results and Discussion
Across the different designs (OA vs DMSA, 8% vs 10% of NP, with/without MG), PVA/MG/NP displayed similar rate of cellular adhesion and the FA staining did not change. FA were more elongated in cell seeded onto PVP fibers when compared to collagen type I coated glass. These demonstrate that neither NP nor MG altered the affinity of cells to the PVA fibers. Next, we analysed the proliferation within a period of 14 days, being the most dramatic changes observed amongst controls. This data suggests that the incorporation of MG and/or NP do not alter cellular responses in comparison with PVA alone. Currently, we are investigating the full potential of PVA/MG/NP to deliver chemotherapeutic agents in 3D cultures of tumor cells. Preliminary data shows that doxorubicin loaded NP/MG promote cell death, as measured by live/dead assay and cleaved-caspase-3 immunostaining. In the early future, we will incorporate doxorubicin loaded NP/MG into PVA fibers and analyse their ability to promote cell death in response to HFAFM.

Conclusion
We demonstrated that PVA provide good polymeric scaffold to incorporate both MG and NP without overall biocompatibility and bioactivity. Future experiments will focus in exploring the dual-responsiveness of magnetic nanocomposite, which will ultimately demonstrate its potential as novel cancer nanotheranostics.

Acknowledgement
This work is funded by FEDER funds through the COMPETE 2020 Program and National Funds through FCT—Portuguese Foundation for Science and Technology under the project POCI—01- 0145-FEDER-007688 (Reference UID/CTM/50025) and PTDC/CTMCTM/30623/2017 (DREaMM).
References

PS1-07-245

Moxifloxacin imprinted silicone-based hydrogels for sustained ocular release

Diana Silva¹, Nadia Toffoletto¹, Herminio C. de Sousa², Maria Helena Gil², Carmen Alvarez-Lorenzo³, Madalena Salema Oom⁴, Benilde Saramago¹, Ana Paula Serro¹,4

¹ Instituto Superior Técnico, University of Lisbon, CQE, Lisbon, PT; ² University of Coimbra, CIEPQPF, Chemical Engineering Department, Coimbra, PT; ³ Universidade de Santiago de Compostela, Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, I+D Farma (GI-1645), Facultad de Farmacia and Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, ES; 4 Instituto Universitário Egas Moniz, CIIEM, Caparica, PT

Introduction
The use of drug-loaded ophthalmic lenses has been regarded as an adequate solution to overcome the main disadvantages of conventional topical ocular treatments. In fact, therapeutic soft contact lenses (SCLs) allow achieving a higher bioavailability of the drug in the eye, minimize the drug wastage and side effects, and avoid the frequent and regular instillation of eyedrops, which may compromise the patients’ compliance with the treatments. To obtain efficient devices, several strategies have been attempted. Imprinting the hydrogels with drug molecules that create cavities in the polymeric matrix with the specific shape/binding sites is often used to increase the drug uptake capacity of the materials and prolong the drug release. In this work, imprinted silicone-based hydrogels were designed for the loading of the antibiotic drug moxifloxacin hydrochloride (MXF) and their relevant properties to be used as therapeutic SCLs was evaluated.

Experimental Methods
Four different silicone-based hydrogel formulations were prepared:

1. TRIS: A silicone-based hydrogel with the composition TRIS/NVP/HEMA 40:40:20 w/w (where TRIS refers to 3-tris(trimethylsilyloxy)silylpropyl 2-methylprop-2-enolate, NVP to N-vinyl pyrrolidone and HEMA to 2-hydroxyethyl methacrylate) obtained by thermal polymerization at 60°C for 24 h,
2. TRIS/AA: An alternative composition where acrylic acid (AA), a functional monomer that may establish potential interactions with MXF, was added at a concentration of 300 mM,
3. TRIS+D: A molecular imprinted silicone-based hydrogel with the same composition as 1, where MXF was used as template drug (added in the concentration 3 mM),
4. TRIS/AA+D: A molecular imprinted silicon-based hydrogel containing AA (same composition as 2), using also MXF as template drug (same concentration as 3).

After extensive washing, all hydrogels were loaded with the drug by soaking in MFX solution (5 mg/mL) at room temperature for 72 h. Release kinetics was investigated under sink conditions in simulated lacrymal fluid (SLF, pH=7.4) at 36°C with agitation (180 rpm). Quantification of the drug loaded and released was done by absorbance measurements at λ=290 nm. Transmittance, refractive index, swelling, wettability, ionic permeability and mechanical properties were evaluated. The most promising imprinted hydrogel was further studied in terms of antibacterial activity against S. aureus and S. epidermidis, submitted to the chorioallantoic membrane test (HET-CAM test) for potential ocular irritation, and to cytotoxicity tests according to ISO standard 10993-5 using NIH/3T3 fibroblasts. The MXF release from TRIS/AA+D was carried out in dynamic conditions, using a homemade microfluidic cell, that simulates the lacrymal fluid turnover. The obtained profiles were compared to the minimal inhibitory concentrations (MICs) range for S. aureus and S. epidermidis to estimate the efficiency of the devices.

Results and Discussion
All hydrogels showed adequate physical properties for contact lenses manufacture. TRIS+DD released a higher amount of drug than TRIS, but TRIS/AA showed a very significant increase in the amount of MXF loaded and released (Figure 1). Imprinting and adding AA functional monomers seemed to have a synergistic effect: TRIS/AA+D hydrogels hosted a larger amount of MXF and released the drug for a longer period. MXF (pH=7.2) is predominantly on its zwitterionic form (pK_{a1}=6.3 and pK_{a2}=9.3). With the carboxylic moiety at C-3 position ionized and the basic moiety at C-7 position, protonated MXF can form reversible electrostatic interactions with negatively charged AA. Moreover, hydrogen bonds may be established between MXF with available non-protonated amino and hydroxyl groups in TRIS and HEMA, respectively. The HET-CAM test revealed no ocular irritation potential, as no lysis, hemorrhage or coagulation was detected. The antibacterial activity of MXF was confirmed by the presence of inhibition halos for both bacteria. No toxicity was observed has cell viability level was well above 70%. Release studies carried out in dynamic conditions showed that MXF concentration remains above the MXF MICs for at least 10 days for TRIS/AA+D.

**Conclusion**

In summary, the MXF-imprinted hydrogels bearing AA demonstrated proper MXF release kinetics and promising properties to produce therapeutic SCLs.

**Acknowledgement**

This work was developed in the frame of the STEReoSTRAT Project - ‘Sterilization of clinically active hydrogels: looking for efficient strategies’, PTDC/CTM-BIO/3640/2014 (2016-2019) and is funded by Fundação para a Ciência e a Tecnologia - FCT.

![Figure 1. Cumulative MXF release profile from the studied silicon-based hydrogels.](image)
**In vitro and in vivo performance of intraocular lenses double loaded with moxifloxacin and ketorolac for the prevention of endophthalmitis after cataract surgery**

Ana Topete¹, Nadia Toffoletto¹, Renata A. Amaral², Jorge A. Saraiva², Junmei Tang³, Xiaoxu Ding³, Quankui Lin³, Ana Paula Serro¹, Benilde Saramago¹

¹ Instituto Superior Técnico, University of Lisbon, CQE, Lisbon, PT; ² University of Aveiro, QOPNA & LAQV-REQUIMTE, Department of Chemistry, Aveiro, PT; ³ Wenzhou Medical University, School of Ophthalmology & Optometry, Eye Hospital, Wenzhou, CN; ⁴ Instituto Universitário Egas Moniz, CIIEM, Caparica, PT

**Introduction**

Cataracts are the leading cause of blindness worldwide. The only effective treatment is surgery that involves the removal of the cloudy natural lens and the implantation of an intraocular lens (IOL). In the post-operative period, complications such as endophthalmitis can occur. To prevent this, eye drops of antibiotics and anti-inflammatories are administered with a frequent posology: antibiotics for 1-2 weeks and anti-inflammatories for 2-4 weeks. However, this drug delivery method has several drawbacks: it involves significant drug losses (>95%), leads to undesirable side effects, and demands frequent applications, which may result in poor patient compliance. An alternative to overcome these problems may be the use of IOLs as platforms for drug vehiculation. In this work, the possibility of using drug-loaded IOLs to ensure the simultaneous release of an antibiotic and an anti-inflammatory is explored both in **in vitro** and **in vivo** tests.

**Experimental Methods**

Acrylic IOLs were double loaded with the antibiotic moxifloxacin (MXF) and with the anti-inflammatory ketorolac (KTL) by soaking in a solution containing both drugs, for 2 weeks at 60°C. The IOLs were sterilized by high hydrostatic pressure combined with moderate/high temperature (600 MPa, 10 min, 70°C). The lenses were characterized regarding swelling and optical properties. **In vitro** drug release experiments were carried out in PBS in sink conditions (36°C, 180 RPM). The antibacterial activity of the release solution was tested against two of the most common microorganisms present in the ocular medium: *Staphylococcus aureus* and *Staphylococcus epidermidis*. The minimal inhibitory activity (MIC) of MXF against these two bacteria was determined. **In vivo** experiments were done with Japanese white rabbits. Sterile drug-loaded IOLs were implanted into the right eye of 5 rabbits (experimental group). Sterile blank IOLs were implanted into the right eye of another 4 rabbits (control group). In this group, the usual post-cataract prophylaxis therapy was applied: Vigamox (MXF) and Acular (KTL) eye drops were instilled in the right eyes after the surgery, 4 times a day. Vigamox was administered until the day 7 and Acular until the day 14. For both groups, the ocular inflammation was evaluated by slit lamp examinations on days 1, 3, 7, 14 and 21. Aliquots of aqueous humor were collected on the days 2, 7, 14 and 21 for the experimental group and on the days 2, 7 and 14 for the control group and the drug concentration there present was obtained by LC-MS. On day 21, the rabbits were humanely euthanized and the eye balls removed for histological analysis.

**Results and Discussion**

The loading of the drugs led to an increase in the swelling capacity of the lenses, but the transmittance in the region 500-700 nm was maintained (>90%). **In vitro** drug release experiments demonstrated that MXF and KTL were released in a controlled manner for 26 days. No loss of MXF activity was detected against both bacteria after the release. In the **in vivo** tests, the slit-lamp examinations revealed that after 7 days no inflammation was present on the eyes of the rabbits. The concentration of MXF and KTL in the aqueous humor was always higher for the experimental group than for the control group. Also, the concentration of MXF in the aqueous humor of the experimental group
was above the determined MIC (2 µg/mL for both bacteria) until 14 days, and the concentration of KTL was higher than the half maximal inhibitory concentration (IC-50) of cyclooxygenase 1 and 2 (enzymes responsible for inflammation) for almost 21 days. Histological analysis revealed good biocompatibility of the drug-loaded IOLs concerning the cornea and the iris.

**Conclusion**
The IOLs double loaded with MXF and KTL and sterilized by HHP revealed to be promising devices to replace the usual eye-drop therapy for prophylaxis of post-cataract surgery endophthalmitis.

**Acknowledgement**
The authors acknowledge funding from Fundação para a Ciência e Tecnologia (FCT) [projects UID/QUI/00100/2013, PTDC/CTM-BIO/3640/2014 and FCT UID/QUI/00062/2019)] and support from PhysIOL for providing the IOLs.
PS1-07-251

Influence of Amino Acids on the Mechanism and Kinetics of Pt Microelectrode Corrosion

Arne K. Schierz1,2, Kristina Kebel1, Peter Behrens1,2

1 Leibniz University Hanover, Institute of Inorganic Chemistry, Hannover, DE; 2 Cluster of Excellence 'Hearing4All', Hannover, DE

Introduction

Platinum (Pt) is used for microelectrodes implanted into the human body e.g. as Cochlear implant (CI). Contact to human tissue and simultaneous electrical stimulation induces corrosion of the Pt microelectrode with correlating Pt dissolution, degeneration of neurons and finally loss of function.1,2 Different mechanisms of Pt corrosion have been published linked to different faradaic reactions occurring at the interface between the Pt surface and the electrolyte, namely oxidation reactions like the oxygen evolution reaction (OER) or chlorine formation (when the electrolyte contains chloride) as well as reduction reactions like the hydrogen evolution reaction (HER) or the formation of Pt-H bonds.3 Also, different electrolyte additives were investigated with regard to their influence on Pt corrosion, like amino acids, especially cysteine.4 In this work we have investigated Pt corrosion mechanisms and the possible suppression by additives using the rotating disc electrode (RDE) as a means to obtain deeper insight into the reactions. RDEs are commonly used for the determination of faradaic reactions and electrode kinetics. The RDE accomplishes this through a strong convective flow towards the electrode caused by the rotation, resulting in a homogenous flow rate of reagents towards the electrode surface during the electrochemical measurements.5

Experimental Methods

To investigate corrosive reactions at the Pt interface a RDE (Model: 636A; Princeton Applied Research) with a Pt disc electrode (Diameter: 5 mm), an Ag/AgCl reference electrode and a Pt mesh counter electrode were used. The electrolyte and the measuring chamber were purged with O2. The electrochemical operations were carried with a potentiostat (Model: PMC-1000; Princeton Applied Research). Cyclic voltammetry (CV) was conducted in a voltage range from 0.35 V to 0.5 V vs. Ag/AgCl with different scan rates (1 cycle each), a hold of 10 s at the vertex and without rotation. Potentiostatic electrochemical impedance spectroscopy (EIS) was performed at open circuit voltage (OCV) and an amplitude of 10 mV without rotation. To investigate reductive faradaic reactions, linear scan voltammetries (LSV) were measured in a voltage range of 0.4 to -1.6 V vs. Ag/AgCl (10 mV/s) with different rotation frequencies. For oxidizing faradaic reaction a voltage range of 0.4 to 2.1 V vs. Ag/AgCl (5 mV/s) at a constant rotation frequency of 1600 RPM was used. The electrolyte was PBS (ROTI®Cell, sterile, Carl Roth), used pure and with addition of L-methionine (≥ 98.5%, Fisher Scientific), L-cysteine (≥ 98%, Alfa Aesar), L-lysine (98%, Alfa Aesar), L-histidine (98+%, Alfa Aesar), L-phenylalanine (cell culture reagent, Alfa Aesar), L-alanine (cell culture reagent, Alfa Aesar), L-cystine (cell culture reagent, Alfa Aesar), L-thyrosin (cell culture reagent, Alfa Aesar), L-methionine sulfone (98+%, Alfa Aesar) or L-tryptophan (cell culture reagent, Alfa Aesar) at a concentration of 600 µmol/L.

Results and Discussion

LSVs in the reductive potential range lead to a constant current density with higher values for a higher rotation rate, which can be linked to a depletion of reactant during the measurement. Also the determination of half-wave potentials (E1/2) suggests a suppression of reductive faradaic reactions for different additives in comparison to pure PBS. LSVs for anodic currents likewise suggest a reduced Pt corrosion by additives. Here we used the potential at a current density of 10 mA/cm² (Ej=10) to determine the overpotential for different amino acids. Instead of a constant current density only a steep increase in current density was obtained suggesting an oxidation of the solvent. The presence
of cysteine and methionine leads to small oxidative currents before the steep increase which were linked to the oxidation of these additives. This presumption was confirmed by carrying out measurements with the corresponding oxidation products cystine and methionine sulfone.

**Conclusion**

Electrochemical characterization of a Pt disc electrode with an RDE setup showed a good sensitivity towards reductive and oxidative faradaic reactions. LSV showed a diffusion-limited faradaic reaction in the reductive potential range, which confirms the oxygen reduction reaction (ORR), while showing no diffusion limitation in the oxidative regime suggesting the oxidation of the aqueous solution to oxygen (OER). The suppression of corrosion was confirmed for different additives and also partially an oxidation of this additives. Further investigations as an possible implant modification for Pt microelectrodes with suppressed Pt corrosion and therefore a longer life cycle are needed.

**Acknowledgement**

This work was funded by the DFG under Germany's Excellence Strategy – EXC 2177/1 - Project ID 390895286.
Linear scan voltammetry of Cysteine and Cystine confirming the oxidation of Cysteine at the Pt electrode during the oxidation scan.

References


Development of 3D combined bioactive tissue matrices and evaluation of myogenic cell activities on these matrices under electrical stimulation

**Nergis Z. Renkler¹, Edanur Selam¹, Seyda Gokyer², Emre Ergene², Meric Goker², Sedat Odabas³, Pinar Yilgor Huri², Kadriye Tuzlakoglu¹**

¹ Yalova University, Polymer Engineering, Yalova, TR; ² Ankara University, Biomedical Engineering, Ankara, TR; ³ Ankara University, Chemistry, Ankara, TR

**Introduction**

Skeletal muscle tissue has a high regenerative capacity, unlike many tissues, due to the satellite cells in its structure which become active as a result of injury and differentiate into myoblasts. However, in case of large damage, or in chronic muscle diseases such as muscular dystrophy, in which muscle cells are irreversibly damaged, the natural biological environment of the cells is also damaged. In this case, the self-renewal capacity of the muscle tissue remains insufficient. Cell-based therapies and different methods including natural or synthetic grafts have been proposed for the repair of damage. However, these treatments with a single functional component are often inadequate to repair complex tissue damage. In this study, a spiral form 3-dimensional (3D) matrices with electrical conductivity were developed for fast and effective treatment of major injuries to skeletal muscles. These structures were supposed to act as a carrier for muscle cells and at the same time stimulate the growth and differentiation of these cells and accelerate the formation of 3-dimensional muscle tissue.

**Experimental Methods**

The abdominal rectus muscle was decellularized using enzymatic and chemical methods. The amounts of DNA and GAGs in the samples were analyzed to determine the efficiency of the process and whether the protein structure is preserved or not. To produce a conductive oriented nanofiber membrane, a mixture of polyaniline (PANI) and polycaprolactone (PCL) polymer solutions was electrospun onto a rotating drum collector. The morphology of the nanofibers was determined using scanning electron microscopy (SEM). The electrical conductivity of the membranes was measured with a multimeter and to determine the chemical structure, FTIR-ATR analysis was performed. For 3D spiral matrices, a viscous solution of DCM was poured on the nanofiber membrane and rolled into a spiral form, and lyophilized. A tensile test and in vitro degradation studies were also conducted in PBS under physiological conditions. To evaluate the interactions of 3D matrices with skeletal muscle cells, myoblast cells (C2C12) were seeded on both surfaces of plane DCM/PANI/PCL matrices and matrices/cell constructs were analyzed using the Alamar Blue assay and Phalloidin/DAPI staining at 1, 7, and 14th days of the culture. To further assess the effect of electrical properties of 3D nanofiber matrices on cell viability, morphology and myotube formation, electrical stimulation was applied during cell culture studies. Immunofluorescence staining with anti-troponin (TNNT) and anti-desmin was performed to determine the muscle-specific marker expression of cells in tissue matrices after electrical stimulation.

**Results and Discussion**

The results revealed that more than 75% of DNA reduction and 90% of GAG and protein protection were obtained after decellularization. Nanofiber membranes have been successfully produced from a PCL/PANI polymer blend and the spiral form structures were also formed with good integration between the components as presented in SEM micrographs. The electrical conductivity of the PCL/PANI nanofiber membrane was calculated as between 3x10⁻⁹-2.5x10⁻⁹ S/cm. The presence of both components, PANI and PCL, on the surface of nanofiber membranes were
confirmed by FTIR-ATR spectra. The results from degradation studies revealed that the weight of 3D combined matrices was higher than PCL/PANI nanofibrous mats after 90 days.

Regarding cell culture with myoblast cells, it was observed that the cells on the nanofibrous surface continued to survive and proliferate until the 14th day, while the cell viability on the DCM surface decreased between the 7th and 14th days. Supporting the results of the Alamar Blue cell viability test, it was determined with DAPI/Phalloidin staining that the nanofibrous surfaces of the matrices showed well spread on the surfaces. Tube formation and alignment in the direction of electrical stimulation were observed in the cells (E+). Tube formations were observed in patches in samples without electrical stimulation (E0), but the orientation did not occur (Fig1). According to the immunofluorescent staining, TNNT and Desmin markers were observed in both groups (E0, E+). More intense anti-TNNT and anti-Desmin were observed in samples with electrical stimulation (E+).

**Conclusion**

The results obtained from the present work indicate that spiral shape combined structures possess favorable properties to serve as a matrix for the regeneration of damaged skeletal muscle tissue. In vitro cell culture tests, and cell behavior under electrical stimulation have also successfully demonstrated that these 3D combined tissue matrices are suitable for the application of muscle tissue defects.

**Acknowledgement**

We gratefully acknowledge the support of The Scientific and Technological Research Council of Turkey (TUBITAK) through project 118M610.
BSA hydrogels for EPR spin-labeled anticancer drug delivery

Dura Nakarada¹, Ana Vesković¹, Anatolie Dobrov², Vladimir B. Arion², Ana Popović Bijelić¹

¹ University of Belgrade, Faculty of Physical Chemistry, Belgrade, RS; ² University of Vienna, Institute of Inorganic Chemistry, Vienna, AT

Introduction
Due to its intrinsic physiological function to bind and transport various endogenous and exogenous substances, including hydrophobic drugs, the protein serum albumin (SA) has been shown to be an excellent choice of polymer for the design of biocompatible hydrogels for controlled and targeted drug release. SA increases drug solubility in blood plasma, prolongs in vivo half-life, thereby increasing the therapeutic efficacy of the drug. Several types of SA hydrogels obtained by heat/pH induced conformational changes, disulfide linking, or by the addition of cross-linkers, have been prepared to date, from bovine and human SA (BSA, HSA), with the aim to synthesize robust hydrogels with retained physiological functions of the protein. The objective of this study was to investigate drug release kinetics from an anticancer drug-depot SA hydrogel, ultimately intended for molecular imaging of drug treatment response.

Experimental Methods
The spin-labeled cytotoxic ligand (HL) was synthesized as reported previously [1]. The hydrogels were prepared from BSA (Sigma). Electron paramagnetic resonance spectroscopy (EPR) was performed on a Bruker Biospin Elexsys II E540 EPR spectrometer. The molecular docking (MD) simulations were performed using Autodock software.

Results and Discussion
The binding and release of a highly cytotoxic modified paullone ligand bearing a TEMPO free-radical (HL) to BSA was investigated in solution, and in the thermally-synthesized hydrogel by EPR. Namely, drug binding to SA may be monitored by EPR when the drugs are spin-labeled with an "EPR-active" moiety, such as TEMPO. It has been previously shown that HL, which belongs to a class of indolo[3,2-d]benzazepines, besides inhibiting cyclin-dependent kinase and glycogen synthase kinase-3, exhibits high antiproliferative activity in human cancer cell lines, also targeting human R2 ribonucleotide reductase protein. The results show that HL binds to BSA in solution (Fig 1a), and that the binding is not affected by the heat-induced conformational change of the protein during hydrogel synthesis. Based on the EPR spectral parameters, it appears that HL is more or less immobilized. By comparison with the EPR spectrum of TEMPO incubated with BSA (Fig 1b), it is concluded that the binding of HL to BSA does not occur through the free radical moiety, but rather through the paullone backbone. Furthermore, HL is not released from the hydrogel during dialysis in NaCl physiological solution at room temperature for 48 h. After 72 h, only the strongly bound HL is detected in the hydrogel. This suggests that the SA hydrogel is a suitable drug-depot, since the water uptake during the 48 h swelling process leads only to the weakly bound ligand displacement. Additional ligand release is likely to be determined by the rate of the in vivo gel biodegradation, which certainly may be fine-tuned by varying the physicochemical properties of the hydrogel. The binding was confirmed by MD simulations which showed that HL binds to one BSA site with high affinity (Fig 1c).

Conclusion
This work shows that EPR is suitable for SA hydrogel drug release studies, with some advantages over the typically used methods (ultracentrifugation, UV/vis spectrophotometry, fluorescence, Raman spectroscopy), such as the requirement of extremely small volumes of protein (20 μl), sensitivity to minor changes in the spin-label environment (protein conformational changes), nanomolar detection limit, as well as time efficiency. The described methodology,
involving EPR spin-labeling and MD simulations, may give insight into the extent of drug binding and the affinity of the drug for SA, which is of essential importance for controlled drug release assessment.

**Acknowledgement**

This research was supported by the Science Fund of the Republic of Serbia, PROMIS, #6062285, PHYCAT. The EPR measurements were performed on the EPR spectrometer obtained by the Ministry of Science, Education and Technological Development of RS, project #III41005.

---

**Spin-labeled anticancer drug binding to BSA**

Figure 1. EPR spectra of a) HL, and b) TEMPO in water (green), and bound to 30 wt% BSA (black). Experimental parameters: microwave frequency 9.85 GHz, microwave power 10 mW, modulation frequency 100 kHz, modulation amplitude 1 G; c) 3D representation of HL interaction with BSA (PDB ID: 4or0). Starting geometry of HL was constructed using Avogadro software and further treated by energy minimization using density functional theory until reaching all positive Hessian matrix eigenvalues. Intermolecular interactions were simulated using Autodock.

---

**References**

Photosynthetic Biomaterials Show Safe and Effective Wound Healing for the Treatment of Full-Thickness Skin Defects in Human Patients.

Rocio Corrales-Orovio\textsuperscript{1,2}, Miguel Obaid\textsuperscript{3}, Juan P. Camacho\textsuperscript{3}, Marianne Brenet\textsuperscript{1}, Felipe Carvajal\textsuperscript{1}, Ximena Martorell\textsuperscript{4}, Consuelo Werner\textsuperscript{5}, Valeska Simón\textsuperscript{6}, Juan Varas\textsuperscript{7}, Wilfredo Calderón\textsuperscript{3,8}, Christian D. Guzmán\textsuperscript{9}, Maria R. Bono\textsuperscript{6}, Sebastián San Martín\textsuperscript{7}, Antonio Eblen-Zajjur\textsuperscript{1}, José T. Egaña\textsuperscript{1}

\textsuperscript{1} Pontificia Universidad Católica de Chile, Institute for Biological and Medical Engineering, Schools of Engineering, Santiago, CL; \textsuperscript{2} Ludwig Maximilian University of Munich, Division of Hand, Plastic and Aesthetic Surgery, University Hospital, Munich, DE; \textsuperscript{3} Hospital del Salvador, Department of Plastic Surgery, Santiago, CL; \textsuperscript{4} Hospital del Salvador, Critical Care Unit, Santiago, CL; \textsuperscript{5} Clínica Alemana, Department of Nursing, Santiago, CL; \textsuperscript{6} Universidad de Chile, Department of Biology, Faculty of Science, Santiago, CL; \textsuperscript{7} Universidad de Valparaíso, Biomedical Research Center, School of Medicine, Valparaíso, CL; \textsuperscript{8} Universidad de Chile, School of Medicine, Faculty of Medicine, Santiago, CL; \textsuperscript{9} Sky-Walkers SpA, Andes Scientific Instruments, Santiago, CL

Introduction

Oxygen is produced by photosynthetic organisms such as plants and cyanobacteria, and is the key molecule for aerobic metabolism. Several fundamental processes including mitochondrial respiration and reactive oxygen species production rely on the local availability of oxygen. Unlike other vital metabolites, such as calcium or glucose, the absence of oxygen reservoirs in the body generates an imperative need for external oxygen supply. Hence, humans have evolved sophisticated mechanisms to capture and further distribute oxygen from the environment into each cell of the body. As hypoxia is the leading cause for wound healing impairment, we have proposed that the use of photosynthetic biomaterials could contribute to tissue regeneration by increasing the local oxygen tension in wounds\textsuperscript{1–4}, an auspicious concept that has been recently confirmed by an independent group\textsuperscript{5}. However, the local and systemic response to photosynthetic cells has not yet been studied in humans, representing a major gap for the translation of photosynthetic therapies into clinical practice.

Experimental Methods

In this work, commercially available collagen scaffolds containing high concentrations of microalgae were implanted in 8 patients with full-thickness skin defects and covered with an illumination dressing (ClinicalTrials.gov identifier: NCT03960164). 21 days after implantation, a partial thickness skin autograft was used to cover the scaffold. Local and systemic response to the implanted scaffold was analyzed for up to 90 days, by means of hematological and biochemical profiles, concentration of plasma cytokines and immune cells in peripheral blood, histological and immunohistochemical analysis of biopsies, clinical aspect of the wound and patient self-evaluation.

Results and Discussion

After implanting photosynthetic scaffolds in 8 patients, macroscopic analysis of all wounds did not show signs of inflammation, such as edema or erythema, at any time point. Similarly, no clinical signs of adverse effects were detected by means of laboratory blood analysis. Additionally, complete integration between the photosynthetic scaffold and the wounds were observed. Hence, in this work we demonstrate that photosynthetic microalgae can be safely implanted in human patients, allowing key regenerative processes such as cell migration, ECM deposition, and vascularization. These results could be extremely relevant for the translation of photosynthetic therapies into clinics, but further studies need to be done in order to confirm this in a larger population of patients and against appropriate controls, such as standard of care.

Conclusion

This study represents the first-in-human trial to evaluate the safety of implanting photosynthetic scaffolds for dermal regeneration in full-thickness skin wounds.
These results will significantly help to translate photosynthetic biomaterials and therapies into clinical settings, and will contribute to the understanding of potential symbiotic relationships between humans and photosynthetic cells. This novel concept is intriguing and could have enormous translational applications, with an impact far beyond tissue engineering and regeneration.

Acknowledgement

We thank the study volunteers for their participation in this study, the staff of the Plastic Surgery Dept. at Hospital del Salvador for assistance in the execution of this clinical trial, and Ivo Carrasco for performing histological processing of the skin biopsies at Universidad de Valparaíso. We are grateful to P. García, C. Zumarán and T. González for the microbiological testing of the scaffolds, to D. Ncuñir for the graphical art, and M. McLuckie and M. J. Sikorski for the critical review of this manuscript. This work was supported by CORFO Portafolio I+D grant 18PID98887, FONDECYT 1200280, FONDECYT 1191438 and FONDEQUIP/EQM 140016.

Photosynthetic scaffold implantation

All patients presented full-thickness wounds prior to scaffold implantation. The wound bed was cleaned and photosynthetic scaffolds were then placed directly over the wound and trimmed as needed. Surgical sutures were used to fix the scaffolds among them and to the wound edges. Then, a transparent polydimethylsiloxane membrane was used to cover the scaffold and act as a draining system, which was further secured with a negative pressure wound therapy (NPWT) dressing. Finally, a light device specially designed for this study was placed on top providing specific and controlled illumination.

References

2:30 p.m. – 4:00 p.m.

Poster floor

PS1-08 | Hydrogels and Smart Biomaterials
Photopatterning Stress Relaxation into Hydrogels for Corneal Regenerative Applications

Antonio Feliciano, Rhiannon Grant, Stefan Giselbrecht, Matthew Baker
Maastricht University, MERLN, Maastricht, NL

Introduction
Via mechanotransduction, corneal keratocytes have demonstrated the ability to migrate to areas of optimal stiffness by durotaxis. Altering stiffness can upregulate actin stress fiber production and focal adhesions, as well as alter keratocytes phenotypic markers. Stiffness however, is a static mechanical property and does not truly capture the dynamic environment of the host tissue. An alternative approach is to introduce a dynamic mechanical property, stress relaxation. Stress relaxation has been shown to influence cell behavior including migration, proliferation and differentiation. Using light activated chemistry, we grafted side groups via an ultraviolet-initiated reaction. Grafted alginate hydrogels have been shown to enhance relaxation and cell spreading, and promote osteogenic differentiation in mesenchymal stromal cells. We hypothesized that stress relaxation could be photopatterned into the hydrogels using a light-activated grafting approach and subsequently influence cell spreading and proliferation. We manufactured fast and slow relaxing gels, and assessed their impact on human primary corneal keratocytes adhesion and behavior.

Experimental Methods
Alginate was functionalized in order to allow photopatterning. Photopatternability was assessed via fluorescent image analysis on model reactions. The resulting grafted polymers were characterized by $^1$H-NMR and GPC. Mechanical properties (stiffness and relaxation) of hydrogels were characterized by rheological frequency sweeps and relaxation tests. Human primary corneal keratocytes were cultured on the hydrogels for 7 days and assessed for viability and phenotypic marker expression.

Results and Discussion
Results demonstrate controlled photopatterningability of the hydrogels with good feature reconstruction. A light activated reaction allowed us to introduce regions of localized stress relaxation. While stiffness measurements remained similar (~1000 Pa) after calcium crosslinking throughout the gel series, the stress relaxation ($\tau^{1/2}$) of these gels spanned a range from 21s to 3455s. The corneal keratocytes were found to exhibit differences in adhesion between the slow and fast relaxing hydrogels and regions.

Conclusion
Photopatterned hydrogels can be used to assess cellular response to stress relaxation. This study highlights the ability to determine optimal stress relaxation for corneal cells specifically but can be customized for other cell types. While stiffness is known to affect many areas of cell biology, with such a 3D configurable system, we begin to elucidate how dynamic material properties play a role in mechanotransduction and ultimately tissue regeneration. Furthermore, the spatiotemporal control of relaxation through photopatterning can be useful for modeling disease or regeneration in different viscoelastic microenvironments.
Photopatterning Increases PEG density and Faster Relaxation

References
Unravelling controlled release via structure of responsive nanocomposites for cell culture applications

Danielle Winning, Jacek Wychowaniec, Dermot Brougham

University College Dublin, School of Chemistry, Dublin, IE

Introduction

‘Smart’ polymers can respond to external stimuli such as temperature, pH and magnetic fields through reversible chemical/physical changes in their structure [1]. Thermoresponsive polymers, and microgels of these exhibit phase transitions on increasing temperature. In particular, thermoresponsive microgels have potential applications as components of composites for use in cell culture controlled release applications, once they respond to temperature changes in the range of 37 - 46 °C. Poly(N-isopropylacrylamide) (pNIPAM) is one of the most extensively studied thermoresponsive polymers, exhibiting lower critical behaviour with a phase transition c.32 °C [2], which limits its applicability for such applications. On the other hand, poly(N-isopropylmethacrylamide) (pNIPMAM) is structurally similar to pNIPAM, but has a reported LCST in the range 38 – 44 °C [3]. Some of the key aspects for controlling thermally activated diffusion of loaded molecules and biocompatibility is microgel size and structure. Surfactants such as Sodium Dodecyl Sulfate (SDS) can be exploited in polymer synthesis to provide good size control and monodispersity. Crosslinking can be carried out in situ or post synthesis, typically to increase the mechanical strength, reduce the degradability of the polymer network and allow for efficient swelling/deswelling in the loading/release of encapsulated molecules [4], [5]. Here we have incorporated thermoresponsive microgels formed from pNIPMAM into a non-responsive three-dimensional gelatine-based hydrogel matrix allowing thermally triggered release of dye/drug molecules for use in thermally responsive release applications in cell culture.

Experimental Methods

Polymer microgels were synthesized by free-radical polymerisation of the monomer N-isopropylmethacrylamide (NIPMAM) in the presence of a crosslinker, N,N’-Methylenebis(acrylamide) (BIS) and surfactant, Sodium Dodecyl Sulfate (SDS). Polymerisation was initiated using the radical initiator Potassium Persulfate (KPS). Microgels were characterised using Dynamic Light Scattering (DLS) and rheological analysis (Figure 1). Release studies of Methylene Blue (MB) and Cisplastin were carried out on pNIPMAM loaded gelatine-based hydrogels using Ultraviolet-visible Spectroscopy (UV-Vis) at 37 °C and at 45 °C, a temperature above the phase transition, to mimic suitable biological conditions. The release above LCST was also measured using a pulsed approach i.e., by subjecting the nanocomposite to variations between 37 °C and 45 °C, to mimic ‘pulsed’ release on demand. Finally, trigger of dyes was also demonstrated using magnetically driven hyperthermia approach in combination with magnetically responsive materials.

Results and Discussion

By controlling the synthetic conditions, we were able to synthesise pNIPMAM microgels with precise hydrodynamic diameters and low polydispersity (<0.2) in the range 75 to 2100 nm, and defined LCST across the range of 39 to 47 °C. The study design and outcomes will be described in detail in the presentation. We have additionally identified the maximum crosslinker content appropriate for use in biomedical applications so that the phase transition will theoretically not result in temperature-induced damage to cells, experimental assessment of this is underway. The outcomes include control over both the swollen size and the degree of deswelling of the thermoresponsive component at the phase transition. In the study presented we will describe the connection between these attributes and the extent of release of selected biologically relevant molecules at 45 °C whilst minimising release at 37 °C.
Conclusion
For the first time, we have elucidated the link between synthetic conditions and the obtained structures for pNIPMAM microgels using rheological measurements and small-angle x-ray scattering. Through the tailoring of the phase transition, size and deswelling properties of pNIPMAM microgels we have gained controlled release within the cell culture appropriate temperature range of 37 to 45 °C. Various possibilities of controlled release will be demonstrated, with both typical cumulative release over time, as well as pulsed release ‘on demand’ by precise external and remote spatiotemporal control over the temperature. In particular, demonstration of magneto-thermal pulsed release will be demonstrated. These findings establish the applicability of these microgels as thermo-responsive nanocomposite components in advanced cell culture applications, which will be discussed in the presentation.

Acknowledgement
The authors acknowledge support from Science Foundation Ireland (16/IA/4584 and 13/IA/1840).

References
Dynamic covalent crosslinked multi-compartmentalized hydrogel matrix

Clio Siebenmorgen, Guangyue Zu, Patrick van Rijn
UMCG, Biomedical Engineering, Groningen, NL

Introduction
Extensive research has been done to create a three-dimensional network that mimics the native cellular environment in order to encapsulate cells in vitro.\[1\]
Especially, hydrogels which are swollen polymer networks are a promising candidate. These networks are biocompatible, have a high water content, and show tunable chemical and physical properties. Hereby, well-defined microenvironments can be generated, which match the mechanical properties of the desired tissue. In order to successfully mimic the corresponding environment, a dynamic system is required, which is able to adapt the cell environment by e.g. incorporating biologically relevant molecules and allowing a triggered response to external stimuli.\[2]\[3\]
The aim of this project is the development of a hydrogel-based matrix using dynamic covalent chemistry. Making use of amine containing and ketone containing NIPAM nanogels, spontaneous matrix formation is facilitated in a reversible manner with tunable hydrogel-capsule hybrid structures in terms of physicochemical properties. Ultimately, this nanogel-based matrix can be used to mimic native cellular environments in order to encapsulate and expand cells, requiring a micro/macro-porosity, which was considered in the systems development.

Experimental Methods
The matrix formation is based on an imine formation of ketone functionalized and amine functionalized NIPAM hydrogels. Hollow structures are being formed on the interface of two immiscible solvents based on a Pickering emulsion. A schematic overview is depicted in Figure 1. Hereby, chloroform containing ketone hydrogel is tip sonicated with an amine hydrogel aqueous phase. The resulting matrix is separated for imaging and its pH responsiveness, and stability is being investigated.

Results and Discussion
Upon tip sonication of amine and ketone functionalized microgels, the matrix formation was investigated using fluorescence microscopy. Hereby, Nile Red in CHCl\(_3\) containing ketone functionalized hydrogels and H\(_2\)O containing Nile Blue labelled amine functionalized hydrogels were imaged. The network consists of capsules with CHCl\(_3\) as their inner core. The corresponding matrix shows capsules in sizes of several microns up to 100 μm. Formation of a dynamic covalent crosslinked nanogel matrix based on imine bonds, allows the incorporation of a pH responsive system, as imine bonds only remain stable under neutral conditions.\[4\] We analyzed the stability of the matrix under pH 4, 7 and 10. Figure 2b displays the instability of the matrix in basic and acidic conditions after 20 h. Whereas, the matrix remained stable under neutral conditions, proofing the formation of imine bonds between amine and ketone functionalized hydrogels.

Conclusion
In this work we present the successful formation of an external stimuli responsive dynamic covalent crosslinked nanogel-based matrix based on imine bonds. To successfully encapsulate and culture cells, further investigations on the physical properties, such as stability upon oil removal and stiffness of the matrix need to be investigated in order to mimic the desired cell niches.
Schematic overview of dynamic covalent crosslinked hydrogel
Dynamic covalent crosslinked hydrogel matrix based on ketone functionalized and amine functionalized NIPAM hydrogels forming imine bonds at the solvent interface upon tip sonication

pH dependant stability of imine crosslinked nanogel matrix
Hydrogel matrix at pH 4, 7 and 10; (a) t = 0; (b) t = 20 h

References
Citric acid crosslinked methylcellulose hydrogels for cell sheet engineering

Lorenzo Bonetti¹, Luigi De Nardo¹,², Silvia Farè¹,²

¹ Politecnico di Milano, Department of Chemistry, Material and Chemical Engineering, Milan, IT; ² National Interuniversity Consortium of Materials Science and Technology (INSTM), Florence, IT

Introduction
Methylcellulose (MC) hydrogels have been described as noteworthy materials in the field of cell sheet engineering (CSE) since they allow to control cell attachment/detachment on their surface by means of a temperature trigger [1]. However, their reduced water-stability and mechanical properties may limit the breadth of their possible applications [2]. Citric acid (CA)-crosslinked MC hydrogels have been recently reported to possess superior physical and mechanical performances, also preserving their thermo-responsive behavior [3]. This work aims at exploiting the possibility to use CA crosslinked MC-based hydrogels to obtain cell sheets (CSs). The crosslinked MC substrates were characterized from a mechanical point of view, in vitro tested, and the obtained CSs were studied to assess their regenerative potential.

Experimental Methods
MC hydrogels (8 % w/v MC in 50 mM Na₂SO₄[3,4]) were crosslinked by adding CA (1, 3, 5 % wCA/wMC) to the hydrogel solutions, followed by oven-drying and thermal treatment (T = 165, 177.5, 190 °C) [4]. Following a Design of Experiment approach, three crosslinked MC hydrogels, MC-L, MC-M, MC-H (low, medium, and high crosslinked, respectively), were obtained. Non-crosslinked MC hydrogels were used as control. All the specimens were tested, investigating their tensile mechanical and in vitro biological characteristics. Indirect cytotoxicity was assessed culturing L929 murine fibroblasts in contact with 24, 48, and 120 h extracts for 24 h. CSs were harvested 48 or 120 h after L929 cells seeding on the MC substrates, simply by lowering the temperature to 4 °C. The detached CSs were characterized by immunofluorescence and image analysis (ImageJ, NIH). Lastly, the CSs regenerative potential was assessed by evaluating their adhesion and proliferation capability after their transfer to a new tissue-culture substrate.

Results and Discussion
CA crosslinking effectively modulated the mechanical properties (i.e., Young’s Modulus, E) of MC hydrogels. The E values were found in the range 5 kPa - 3.5 MPa, increasing the crosslinking degree (Figure 1A). Cell viability around 100 % (Figure 1B) was observed for each specimen (MC, MC-L, MC-M, MC-H), suggesting that the CA amounts used to achieve crosslinking did not cause any in vitro cytotoxic effect on L929 cells. CSs were then harvested simply by lowering the temperature at 4 °C. Intact CSs were detached from MC, MC-L, and MC-M hydrogels. Conversely, CSs harvesting was not feasible from MC-H hydrogels, confirming previous results which disclosed that a high crosslinking degree leads to the loss of MC hydrogels thermo-responsiveness [4]. Fluorescence images of the detached CSs (Figure 2A) revealed the presence of widespread actin filaments in the cytoskeleton, contributing to cell-cell junctions in the obtained CSs. For all the detached CSs, cell count was found to increase (p < 0.05) between 48 and 120 h of cell culture (Figure 2B). No significant differences (p > 0.05) in terms of cell count were observed among the CSs obtained for the differently crosslinked samples (MC, MC-L, and MC-M). Adhesion and proliferation assay on a new tissue-culture multiwell plate revealed that the CSs started to adhere to the new substrate shortly (~ 20 min) after their transfer. Optical microscopy observation 72 h after CSs transfer revealed that L929 cells migrated from the CS to the bottom of the new well (Figure 2C), indicating the potentiality of the obtained CSs for tissue regeneration.
Conclusion
This work reports for the first time the use of crosslinked MC substrates in CSE. CSs were successfully detached from the surface of mild crosslinked MC hydrogels (MC-L and MC-M). Conversely, high crosslinked MC hydrogels (MC-H) did not exhibit the expected thermo-responsive character, preventing CSs harvesting. The obtained CSs displayed no differences in terms of cell viability, compared to CSs obtained from control (pristine MC hydrogel).

Figure 1
(A) Young’s modulus of CA-crosslinked MC hydrogels. * = p < 0.05. (B) In vitro indirect cytotoxicity tests: viability of L929 cells kept in contact with 24, 48, and 120 h MC hydrogels eluates.

Figure 2
(A) Fluorescence images and (B) cell count (cells/mm²) of CSs harvested from MC, MC-L, and MC-M hydrogels 48 and 120 h after L929 cells seeding. Scale bar = 50 μm. * = p < 0.05. (C) CSs cultured for 72 h on a new substrate. Scale bar = 500 μm.

References
Light-sensitive supramolecular hyaluronan hydrogels for the intraocular delivery of therapeutic biomolecules

Yaqi Lyu, Helena S. Azevedo

Queen Mary University of London, School of Engineering and Materials Science, London, GB

Introduction
By delivering drugs into the vitreous cavity directly, intravitreal injection remains as the most acceptable and efficient way to deliver therapeutics to posterior eye since it goes beyond the physiological ocular barriers. However, frequent injections are required to maintain sufficient drug concentrations which are uncomfortable and can lead to complications, such as retinal detachment [1]. Hyaluronic acid (HA) is the main component of the vitreous, being an attractive option for the formulation of an injectable ocular delivery platform. In addition, HA is biodegradable, biocompatible and easily amenable to chemical modifications [2]. Herein, a light-sensitive supramolecular hydrogel, based on host-guest moieties conjugated HA, was prepared for the sustained and on-demand delivery of therapeutic biomolecules (proteins, peptides) for the treatment of posterior eye segment diseases.

Experimental Methods
Cyclodextrin (CD) and azobenzene (AB) were first conjugated to HA separately. HA-CD and HA-AB were mixed (1:1 molar ratio) to form a hydrogel by self-assembly. The mechanical properties and light-sensitivity of the self-assembled HA-CD/HA-AB hydrogels were characterized using a rheometer equipped with UV-visible light sources. BSA was used as a model protein to examine the release profile in vitro with or without irradiation.

Results and Discussion
A hydrogel is formed immediately upon mixing HA-CD and HA-AB via host-guest interaction between CD and AB groups (Figure 1-A). Rheology tests show a significantly higher storage modulus G’ compared to the loss modulus G”. HA-CD/HA-AB hydrogels respond to irradiation in seconds and are reversible upon UV-visible light cycles (Figure 1-B). The HA-CD/HA-AB gels are relatively soft (G’ =1-2 kPa) allowing their easy injection (Figure 1-C) while being stable in PBS for 30 days. The gels have high protein loading capacity and show sustained and on-demand release of BSA with irradiation.

Conclusion
The HA supramolecular hydrogels with high drug-loading capacity and light sensitivity represent a less invasive delivery platform for light-triggered release of drugs into the back of the eye.

Acknowledgement
This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 841783
Figure 1
Figure 1. A Schematic illustrating the assembly and disassembly of HA-CD/HA-AB supramolecular hydrogels via host-guest interaction and light irradiation, respectively; B Rheology showing reversible changes in G' and Tan(δ) of HA-CD/HA-AB hydrogels in response to UV-visible light cycles; C Injectability of HA-CD/HA-AB hydrogels into PBS (pH 7.4).

References
Hydrogel films based on bovine serum albumin and partially oxidized gellan with β-cyclodextrin/curcumin inclusion complex immobilized with applications in dermatological diseases

Camelia E. Iurciuc (Tincu)², Christine Jerome³, Marcel Popa¹,⁴, Lacramioara Ochiuz²

¹ "Gh. Asachi" Technical University of Iasi, Iasi, RO; ² "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, RO; ³ University of Liege, Liege, BE; ⁴ Romanian Academy of Scientist, Bucharest, RO

Introduction
Previous studies have reported that curcumin has antibacterial and anti-inflammatory activity and, in topical applications, was successfully used to treat rashes and skin infections. The main disadvantage is its water insolubility and low bioavailability. The inclusion complex of β-cyclodextrin with curcumin has been prepared to improve water solubility. BSA was used to improve the hydrogel film’s biocompatibility and immune response capacity.

Experimental Methods
The films were obtained by cross-linking as a result of the reaction between the bovine serum albumin amine groups with the aldehyde groups from partially oxidized gellan, working at different molar ratios -NH₂ / -CH =O. The aldehyde groups’ content in the oxidized gellan was determined indirectly by dosing the residual periodate in a reaction mixture by iodometric titration with Na₂S₂O₃. The gellan oxidation was performed with NaIO₄ to form aldehyde groups by cleaving the C2-C3 bond from the glycosidic structure. FT-IR and ¹H-NMR spectroscopy was used to demonstrate the aldehyde groups’ formation in partially oxidized gellan. Molecular weight (Mw) of oxidized gellan determined by the viscosimetric method was calculated using the Kuhn–Mark–Houwink equation (K = 7.49 x 10⁻³ and α = 0.91).

Results and Discussion
The gellan oxidation degree was 57.16% after an oxidation time of 117 h, being influenced by the amount of NaIO₄ used, time, and temperature.

FTIR spectroscopy results showed that almost all spectra of oxidized gellan a new characteristic absorption peak occur between 1730-1740 cm⁻¹ assigned to the C = O group from aldehydes that demonstrates that the oxidation reaction between gellan and NaIO₄ took place. The ¹H-NMR spectra of standard gellan and oxidized gellan (for 72 hours) were tested and compared. A peak of 10.2 ppm in the spectrum of oxidized gellan occurs in the region greater than 8.05 ppm belonging to aldehyde groups. Molecular weight (Mw) of oxidized gellan determined by the viscosimetric method in 0.1 M acetate buffer solution at 23°C decreased when the oxidation degree increased.

Different molar ratios between albumin amine groups and aldehyde groups from oxidized gellan were used to obtain the films. The covalent cross-linking reaction between the albumin amine groups with the aldehyde groups within the oxidized gellan results in forming an imine bond, also called Schiff base. Figure 1 shows schematically the structure of the obtained albumin films cross-linked with oxidized gellan.

The number of amino groups in albumin that did not participate in the cross-linking reaction decreased, and the conversion index increases when the oxidized gellan amount was higher within the films. The inclusion complex of Page 1261 of 2028
β-cyclodextrin with curcumin was included in the albumin/oxidized gellan films from the preparation stage. The inclusion complex encapsulation efficiency within the films increased when the molar ratio was higher.

**Conclusion**

The results showed that the swelling degree values depend on hydrogel film hydrophilicity and pH; its values increase at pH=7.4 and when the number of carboxylic groups increases. The antioxidant activity for curcumin-loaded hydrogel film was improved compared with free curcumin, and the polymeric matrix has a protective role for curcumin against UV radiations. The curcumin release kinetics from films was studied in two mediums of different pH (5.5 and 7.4), and the release efficiency and permeability were higher at pH=7.4, the results being consistent with the swelling degree.

![Fig 1. Schematic structure of the cross-linking reaction between albumin and oxidized gellan](image)
Therapeutical innovation for the treatment of radio-induced gastro-intestinal syndrome

Rodolphe Migneret¹, Frédéric Chaubet², Didier Letourneur¹, Teresa Simon-Yarza¹

¹ Inserm, LVTS U1148, Hôpital Bichat, Paris, FR; ² Université Sorbonne Paris Nord, LVTS U1148, Villetaneuse, FR

Introduction
Nuclear risk is a worldwide concern due to radiological and nuclear accident. Irradiation may cause severe gastrointestinal lesions named radio-induced gastro-intestinal syndromes (ri-GIS). Treatments rely on palliative and symptomatic therapies, such as anti-emetics, antibiotics, anti-ulcerants, anti-diarrheal, anti-pyretics, a supply in water, nutriments, electrolytes and finally hyperbaric oxygen therapy. This work is part of the INTRUST project whose hypothesis is that a multimodal and global approach will be much more efficient to treat ri-GIS. In particular, we want to develop an innovative treatment to
1) physically protect the mucosal barrier quickly after the irradiation,
2) locally deliver pro-regenerative therapeutics,
3) control the intestinal inflammation, and
4) fight bacteria.

Of note, chronic intestinal inflammatory diseases, such as Crohn disease and other GIS, could also benefit from this new treatment¹.

Thus, we propose to develop a novel biomaterial able to fit these specifications. In particular, polysaccharide (PS) hydrogels (HG) are particularly interesting, since they have a similar composition and properties that the intestinal mucosa and can be loaded with the chosen drugs, either by adsorption, ionic interaction or through covalent bonds. In this work we explore different strategies to covalently link anti-TNFα protein².

Experimental Methods
HG are made with two PS, pullulan (200kDa) and dextran (500kDa), and crosslinked in alkaline condition with sodium trimethaphosphate as previously described³. Two methods were followed to obtain gels coupled with proteins:
Method 1: Covalently linking the protein to the PS prior to HG formation. Divinylsulfone (DVS)⁴ was grafted onto the PS (mpS=500mg, CDVS=4.10⁻²M V=25mL and COH=100mM) and then bovine serum albumin (BSA, 66kDa, Sigma), that was used as a model drug, reacted with the vinyl group⁵ (mpS=50mg, CBSA=850µM in 2.5mL PBS). The grafted PS was washed with dialysis against DI-water after both reactions for at least 5 times during 1,5h (cut-off membrane 100kDa).
Method 2: Covalently grafting the protein after HG formation. 5mg of dried hydrogel was swollen with 2.5mL of a solution of DVS (4.10⁻³M) in NaOH (0.1M) to react for 5 minutes under agitation at room temperature. The grafted-HG was washed with water to remove the excess of DVS, then freeze-dried again. The HG grafted with the DVS, was swollen with a solution of BSA (10mg/mL) in PBS for 42h at room temperature under agitation, then washed with water to eliminate the non-grafted proteins.

Several parameters have been studied to optimize both reactions, such as concentration of DVS, concentration of BSA, time of reaction, pH and temperature. All experiments have been done in triplicates.

Results and Discussion
Method 1: Concerning the grafting of DVS on PS, the degree of substitution evaluated with NMR 1H, was 8% for dextran and 14% for pullulan in similar conditions. Concerning protein grafting, elementary analysis showed a gap in N content of 3% between grafted and non-grafted PS. This is equivalent to 619µg±5 BSA/mg of PS.
Method 2: Regarding protein binding on the HG, elementary analysis showed an increase in N and S contents in the grafted HG (0.56%±0.08 for N, 1.26%±0.11 for S, equivalent to 35µg±5 BSA/mg of PS). When compared to HG where the protein was just loaded through adsorption (21µg±11 BSA/mg of PS), we observed an increase of 60% protein loading and reduced variability within the samples.

The differences observed in the amount of protein linked to the PS compared to the HG can be due to the reduction of available hydroxyl groups, due both to crosslinking through STMP and through DVS. The addition of DVS on the HG probably causes crosslinking between hydroxyl groups as suggested by the fact that a reduction of the concentration of DVS led to higher swelling of the gels and an increase of protein content.

For future studies with anti-TNFα, method 2 will be used since anti-TNFα are potent drugs and the doses needed for a local treatment can already be reached with this method. Besides, the risk to lose protein activity during HG synthesis is avoided.

**Conclusion**

We have successfully grafted a model protein on polysaccharide HGs by using DVS by two different methods. Experiments are ongoing to graft the functional anti-TNFαMP6-XT22. After performing in vitro release studies under physiological-like conditions, in vitro and in vivo experiments will be done to prove the efficacy of the treatment.

**Acknowledgement**

This project is supported by the Agence Nationale de la Recherche (19-ASTR-0002-DA)

**References**


Synthesis of bioactive hydrogels for the control of stem cell differentiation: Impact on bone tissue engineering

Cristina Lopez Serrano, Murielle Rémy, Chanseau Christel, Laroche Gaétan, Durrieu Marie-Christine

Introduction

Successful tissue engineering strategies depend on an accurate replication of key characteristics of the cell environment. Traditionally, the focus has been put on chemical signals, which basically consists in interactions between cell receptors and ligand molecules. Over the past decades, mechanobiology has emerged as a key field in bioengineering. It is now known that cells detect and respond to the mechanical properties of their surroundings. Accordingly, the in vitro differentiation of stem cells is affected by the stiffness of the substrate on which they are cultured [1]. However, it is necessary to further define the effect of matrix stiffness in 3D cell cultures as well as the interplay between these mechanical characteristics and the presence of bioactive molecules such as adhesion or differentiation peptides. In this context, our research challenge is to develop the optimal 3D microenvironment to enhance osteoblastic differentiation of human mesenchymal stem cells (hMSCs).

Experimental Methods

To address this objective, polymer-based hydrogels are synthesized in a biologically relevant range of stiffnesses. To do that, solutions of PEG-diacylate at different concentrations are photocrosslinked under UV light to form a reticulated polymer network.

Results and Discussion

Atomic Force Microscopy (AFM) is used to measure the surface mechanical properties (Young’s modulus) of the hydrogels. XTT assays have shown negative cytotoxicity of the gels, making them good candidates as substrates for cell culture (Fig. 1). Previous research has shown that the bifunctionalization of materials with adhesion and differentiation peptides boosts the osteogenic potential of hMSCs [2]. For this reason, hydrogels are covalently grafted with RGD and BMP-2 mimetic peptides. By tagging the peptides with a fluorescent dye, we were able to assess the homogeneity and density of grafted domains using fluorescent microscopy.

Conclusion

Further work will focus on the fabrication of PEG-based 3D scaffolds with similar characteristics in terms biofunctionality and mechanical properties in order to compare the cellular response between 2D and 3D cultures.
Fig 1: Cytotoxicity test results
Cell viability tests on pure material extract and dilutions at 50 and 10% (p<0.05). The non-cytotoxic control (medium that has not been in contact with the material) is used as reference and a phenol dilution is used as a positive control of cytotoxicity.

References
Fabrication and characterization of an oxygen releasing encapsulation system for beta-cell therapy

Mette S. Toftdal1,2, Nayere Taebnia2, Firoz B. Kadumudi2, Thomas L. Andresen2, Thomas Frogne3, Louise Winkel1, Lars G. Grunnet1, Alireza Dolatshahi-Pirouz2

1 Novo Nordisk, Department of Stem Cell Delivery, Måløv, DK; 2 Technical University of Denmark, Department of Health Technology, Lyngby, DK; 3 Novo Nordisk, Department of Stem Cell Discovery, Måløv, DK

Introduction

Diabetes is a chronic and possibly fatal disease, which affects more than 400 million people worldwide—and the number is continuously increasing. Patients suffering from type 1 diabetes need exogenous injections of insulin to survive. [1] [2] Although, there are many great insulin therapies available on the market there are still some challenges connected to these such as controlling the glycemic balance. Beta-cell therapy is a potential way to overcome this challenge and numerous studies have already shown promising results. However, a major obstacle in cell therapy is the hypoxic environment created at the transplantation site immediately after transplantation. [3] [4] Calcium peroxide (CPO) has previously been investigated as an oxygen releasing agent in different cell therapy approaches and found to improve cell survival in hypoxic environments. [3] [5]

Experimental Methods

In this study, hydrogels composed of thiolated hyaluronic acid (HA), 8-arm-Poly(ethylene glycol)-Acrylate (PEGA), and CPO (Figure 1), were manufactured in order to obtain an oxygen releasing system for cell encapsulation. The hydrophilic HA is a natural polymer that, like the synthetic polymer PEGA, is known for its high biocompatibility and non-immunogenic properties. The incorporation of CPO into the hydrogel system was evaluated by FTIR and Alizarin Red staining. Hydrogels with varying concentrations of CPO were characterized in terms of their chemical, rheological, and mechanical properties. The oxygen release kinetics were assessed in an oxygen release study. Lastly, cell survival and functionality of insulin-secreting cells were investigated after encapsulation.

Results and Discussion

Hydrogels containing 0, 7.5, and 30% CPO were fabricated, and the incorporation of CPO was confirmed by both Alizarin Red and FTIR. The hydrogel system containing 7.5% CPO was found to release oxygen for at least 10 hours, whereas hydrogels containing 30% CPO released oxygen for a minimum of 30 hours. In terms of rheological and mechanical properties, all three hydrogel conditions were found to be shear thinning and suitable for beta-cell encapsulation in terms of their Young's modulus. Additionally, CPO was found to intensify the hydrogel cross-linking and thus increase the toughness and compressive modulus of the system. Insulin secreting INS-1E reporter cells were successfully encapsulated in the hydrogel system to investigate their viability and functionality. The in vitro encapsulation study indicated that CPO improved cell survival; however, the insulin secretion was found to be impaired upon incorporation of CPO in the system.

Conclusion

In summary, this work suggests that the CPO-laden tHA hydrogels have promising physiochemical properties, which may be useful in beta-cell therapy.
Figure 1
Illustration of oxygen releasing hydrogel composed of hyaluronic acid (HA), 8-arm-Poly(ethylene glycol)-Acrylate (PEGA), and calcium peroxide (CPO).

References
Development of injectable hyaluronic acid-based hydrogels

Kristine Belune¹, Dagnija Loca¹,²

¹ Riga Technical university, Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre, Riga, LV; ² Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia., Riga, LV

Introduction

Injectable composite hydrogels are becoming increasingly popular in the field of biomedicine, replacing the traditional clinical practice. In this way, a minimally invasive procedure can be performed, deeply hidden anatomical sites can be accurately implanted, and irregularly shaped bone defects can be repaired. These benefits result in reduced patient’s recovery time and risk of infection, while at the same time the procedure leaves smaller scars. Injectable hydrogels must uphold a number of requirements in order to be used for biomedical purposes and some of the pivotal ones are biocompatibility, biodegradability, stability, mechanical properties, porosity, viscosity etc. Hyaluronic acid (HA) is a natural, linear, and at physiological pH, negatively charged polysaccharide which is the major component of the extracellular matrix (ECM). HA is widely used because of its physico-chemical and biological properties such as biodegradability, biocompatibility, hygroscopicity, viscoelasticity, and mucoadhesivity. ε-Poly-L-lysine (ε-PL) is a water soluble, non-toxic, biodegradable polypeptide which provides an antibacterial effect. By adding an inorganic phase such as hydroxyapatite (HAp), which is osteoconductive, biodegradable, biocompatible and similar to the inorganic phase of human bone, it is possible to improve the mechanical properties and bioactivity of hydrogels for bone tissue regeneration. The main objective of the current research was to prepare injectable hyaluronic acid-based HAp/ε-Poly-L-lysine hydrogels and characterize them towards the gel fraction, injectability and rheological properties.

Experimental Methods

HA/ε-PL and HA/ε-PL/HAp (inorganic phase content 60wt%) hydrogels were physically crosslinked and characterized. To determine the insoluble part of the hydrogel, the lyophilized samples were immersed in 200 mL of deionized water for 48 h at room temperature. The rheological measurements were observed using Anton Paar SmartPave 102 rheometer equipped with a parallel plate geometry of 25 mm and the measuring gap of 1 mm. Time-dependent storage and loss modulus (G’ and G’’) were measured subjecting the hydrogels to 1% strain with constant frequency of 1 Hz. The force applied to the syringe plunger to inject the hydrogels was measured using Tinius Olsen 25ST material testing machine with a load cell of 2.5 kN with cross head speed 1 mm/sec.

Results and Discussion

Results indicated that with an addition of inorganic phase, statistically significant increase in gel fraction results was observed (65.36% ± 1.3% in case of HA/ε-PL and 84.11% ± 0.6% in the case of HA/ε-PL/HAp). Rheology measurements showed that the storage modulus (G’) was always greater than the loss modulus (G’’) over the entire time interval for both samples. This implies that the gel was formed immediately after the liquid phase was added to the solid phase. The addition of the inorganic phase increased the G’ value of the material, resulting in stiffer composite. Also, it was found that the maximum injection force value of HA/ε-PL hydrogel was 90.37±13.9, while for HA/ε-PL/HAp hydrogel it reached 64.62±15.5, indicating no statistically significant effect of inorganic phase addition on the force values.

Conclusion

During the research it was established that through the addition of inorganic phase to HA/ε-PL hydrogels, the gel fraction and storage modulus could be increased. Moreover, the injection force values were not statistically affected by the addition of inorganic phase to HA/ε-PL hydrogels.
Acknowledgement

The authors acknowledge financial support from the Latvian Council of Science research project No. lzp-2019/1-0005 "Injectable in situ self-crosslinking composite hydrogels for bone tissue regeneration (iBone)" and from the European Union’s Horizon 2020 research and innovation programme under the grant agreement No 857287.

References


Formation of a versatile hydrogel based on chemoselectively modified Elastin-like polypeptides (ELPs) for tissue engineering applications

Nadia Mahmoudi1,2, Bruno Paiva dos Santos1, Hugo De Oliveira1, Elisabeth Garanger2, Sébastien Lecommandoux2, Joëlle Amédée1, Bertrand Garbay2

1 Tissue Bioengineering Laboratory (BioTis), Inserm U1026, University of Bordeaux, Bordeaux, FR; 2 Univ. Bordeaux, CNRS, Bordeaux INP, LCPO, UMR 5629, Pessac, FR

Introduction
Extracellular matrix-like hydrogels are increasingly used as scaffolds for tissue engineering. Elastin-like polypeptides (ELPs) are a class of recombinant polymers of interest for hydrogel manufacture, because they are monodisperse, cross-linkable, biocompatible, biodegradable and non-immunogenic. ELPs are repeated sequences of (VPGXG) pentapeptides that are derived from the hydrophobic domain of tropoelastin. The guest residue X can be any amino acid residue but proline. ELPs are thermosensitive polymers in aqueous buffers, being soluble at a temperature below a transition temperature (Tt), and aggregating above this Tt. Our objective is to develop a bioactive ELP-based hydrogel for tissue engineering applications.

Experimental Methods
The recombinant ELP used in this study, noted ELP[M\(^{1V_{3-80}}\)], has the following sequence: MW[(VPGVG)(VPGMG)(VPGVG)]\(^{20}\). It contains a total of 21 methionine residues that were chemoselectively modified by a thioalkylation reaction to introduce an alkene function [1] for subsequent cross-linking (ELP[M(alkene)\(^{1V_{3-80}}\)]. A consequence of this reaction is the apparition of positively charged sulfonium groups. To eliminate these charges, a demethylation reaction [2] was applied leading to the derivative noted (ELP[M(dem-alkene)\(^{1V_{3-80}}\]). These three ELP derivatives were characterized by mass spectrometry, nuclear magnetic resonance, and size exclusion chromatography. Their Tts were measured by spectrophotometry at different concentrations. While the starting ELP[M\(^{1V_{3-80}}\] presented a Tt of 27.3°C at 100mM, ELP[M(alkene)\(^{1V_{3-80}}\)] did not present any thermal responsiveness due to the polar sulfonium groups. Demethylation of this derivative led to the restoration of the thermosensitivity of ELP[M(dem-alkene)\(^{1V_{3-80}}\] (Tt = 26.1°C at 100 mM). Cross-linking of ELP chains was achieved using a peptide with no specific biological activity but presenting two cysteine residues flanked at the C- and N-terminal ends allowing a thiol-ene reaction under UV irradiation in the presence of Irgacure D-2959 [3] [4]. To find the optimal conditions for hydrogel fabrication, we varied the ELP and peptide concentrations, the thiol/alkene ratio, and the UV irradiation duration. The resulting hydrogels were characterized by rheology using a temperature ramp between 10°C and 50°C.

Results and Discussion
Gels based on ELP[M(alkene)\(^{1V_{3-80}}\)] presented a constant storage modulus (G') of 0.8 kPa over all the temperature range. In contrast, the G' value measured for the gels obtained from ELP[M(dem-alkene)\(^{1V_{3-80}}\)] increased from 1.2 kPa to 1.8 kPa, illustrating gel thermoresponsiveness. Then, these hydrogels were freeze dried, and analyzed by scanning electron microscopy to visualize pore formation. Finally, preliminary experiments were performed with human cell lines to evaluate the toxicity of these ELP-based hydrogels.

Conclusion
In conclusion, we produced and characterized three different ELP derivatives with different properties. The alkene-containing versions served as hydrogel backbone for biocompatible hydrogels in vitro. This is a general strategy that allows the utilization of different biomimetic peptides that can increase the biologic functionality of the hydrogels.

References

[2] Rosselin et al., 2019, 'Expanding the Toolbox of Chemoselective Modifications of Protein-Like Polymers at Methionine Residues', ACS Macro Lett., 8, 12, 1648-1653


Natural biopolymer-based antibacterial hydrogels for tissue engineering

**Artemijs Sceglovs**1,3, Aigars Reinis2, Kristine Salma-Ancane1,3

1 Riga Technical University, Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga, LV; 2 Riga Stradins University, Faculty of Medicine, Department of Biology and Microbiology, Riga, LV; 3 Headquarters at Riga Technical University, Baltic Biomaterials Centre of Excellence, Riga, LV

**Introduction**

In recent years, development of novel biomaterials that meet certain requirements and provide multifunctional properties has become actual researcher interest in different fields of biomedicine. Furthermore, health care sector has been facing with the bacterial infections during post-surgery period and antibiotic resistance, and therefore design and development of such biomaterial class as hydrogels for tissue engineering with an antibacterial function are a main focus in biomedical research. The aim of this study is to develop and investigate novel antibacterial hydrogels based on natural biopolymers: antibacterial ε-polylysine (ε-PL) and intrinsic biocompatible hyaluronic acid (HA).

**Experimental Methods**

During experimental part parallel samples of hydrogel series based on ε-PL and HA (mass ratios of ε-PL and HA are 40:60, 50:50; 60:40; 70:30 and 80:20 wt%) were *in situ* synthesized via chemical cross-linking using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) crosslinking agents (with molar ratio of EDC:NHS = 1:1) [1]. Molecular structure and morphology of all synthesized hydrogels were evaluated using Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). In addition, gel fraction and swelling properties tests were obtained to get broad overview of physico-chemical properties. *In vitro* evaluation of antibacterial properties of samples was obtained by using modified ASTM E2149-10 standard test techniques against Gram+ (*S.aureus*) and Gram- (*E.coli*) bacterial cultures. All data were statistically processed using IBM SPSS software.

**Results and Discussion**

FTIR spectra indicated interaction between ε-PL and HA and successful formation of cross-linked copolymer via amide bond linkage. SEM micrographs of the lyophilized hydrogels revealed homogeneous and microporous structure. Cross-linking degree and swelling behavior tests showed the existence of newly formed matrix and insoluble part in synthesized samples. *In vitro* evaluation against *E.coli* and *S.aureus* revealed positive antibacterial tendency of the prepared hydrogels with the increase of mass ratio of ε-PL in presented series.

**Conclusion**

In the described study, the novel hydrogels based on chemically cross-linked ε-polylysine and hyaluronic acid copolymer system were synthesized and investigated. The evaluation of antibacterial behavior indicated inhibition ability against Gram+ and Gram-bacterial cultures. It is concluded that the developed hydrogels can be considered as promising antibacterial biomaterials for tissue engineering.

**Acknowledgement**

The authors acknowledge financial support from the Latvian Council of Science research project No. lzp-2020/1-0072 "Injectable bioactive biocomposites for osteoporotic bone tissue regeneration (inBioBone)". The authors acknowledge financial support from the European Union’s Horizon 2020 research and innovation programme under the grant agreement No 952347.

**References**
Engineering of Stable Thermoresponsive Multilayers Based on Cross-linked PNIPAM-\textit{grafted}-Chitosan and Heparin for Cell Sheet Generation

Yi-Tung Lu\textsuperscript{1}, Kui Zeng\textsuperscript{2}, Kai Zhang\textsuperscript{2}, Bodo Fuhrmann\textsuperscript{3}, Thomas Groth\textsuperscript{1}

\textsuperscript{1} Martin Luther University Halle-Wittenberg, Department of Biomedical Materials, Halle(Saale), DE; \textsuperscript{2} Georg-August-University of Goettingen, Department of Wood Technology and Wood-based Composites, Göttingen, DE; \textsuperscript{3} Martin Luther University Halle-Wittenberg, Interdisciplinary Center of Material Research and Interdisciplinary Center of Applied Research, Halle(Saale), DE

Introduction
Poly(N-isopropylacrylamide) (PNIPAM) exhibiting a lower critical solution temperature (LCST) around 32 °C can enable detachment of complete cell sheets from cell culture substrata for engineering of tissues like skin, cornea, etc. by reducing the temperature below LCST. This study focusses on the development of a thermoresponsive polyelectrolyte multilayer (PNIPAM-PEM) using layer-by-layer (LbL) technique. It can help us to understand the physical and biological properties of the sophisticated PEM systems containing PNIPAM.

Experimental Methods
PNIPAM with different sizes are covalently grafted onto chitosan (Chi) to obtain different degrees of substitution (DS) of PNIPAM-grafted-chitosan (PChi). Nuclear magnetic resonance spectroscopy and dynamic light scattering are used to analyze DS and conformational change of PChi with temperature, respectively. The PNIPAM-PEMs are built at pH4 with PChi as polycation and bioactive heparin (Hep) as polyanion. Subsequently, the systems are chemically cross-linked to stabilize the PEM. The growth behavior, thickness, and wetting properties and temperature effects of the PNIPAM-PEMs with either PChi- or Hep-terminal layer are investigated by different analytical methods. In addition, the stability of the PEMs is tested by rinsing with PBS, pH 7.4 and DMEM. Furthermore, the association of adhesive protein vitronectin with PNIPAM-PEMs at 20 and 37 °C is studied for their biological function. The application of PNIPAM-PEMs as cell culture substrate is finally examined using multipotent mouse stem cells.

Results and Discussion
PChi with higher DS and sizes show significant increase in the diameter above LCST. They have the ability to form PNIPAM-PEM with heparin. After rinsing with buffer and culture medium, the thickness significantly decreases in non-cross-linked compared to negligible changes in cross-linked PEM. As the temperature changes, the PNIPAM-PEM is slightly more hydrophobic at 37 °C than at 20 °C. This might also take account of higher adsorption of vitronectin on PNIPAM-PEM at 37 °C. Finally, higher adhesion and more spreading of cells on cross-linked PEM particularly with Hep-terminal layer are observed owing to the superior bioactivity of heparin.

Conclusion
In conclusion, the amide bonds make an irreversible bond formation between PChi and Hep to enhance stability of PEM. In addition, the PNIPAM immobilized on the surface affects the wetting properties of the surfaces. Therefore, the PNIPAM-PEMs with cross-linking provides greater stability and biological function as a cell culture system for further investigation of cell sheet generation in tissue engineering.

Acknowledgement
This work is supported by International Graduate School AGRIPOLY funded by the European Regional Development Fund and the Federal State Saxony-Anhalt.
Illustration of PNIPAM-PEM before and after cross-linking

References


Anionic hydrogel contact lenses with affinity for atropine

Inés García Del Valle, Maria Vivero-Lopez, Angel Concheiro, Carmen Alvarez-Lorenzo

Universidade de Santiago de Compostela, Departamento de Farmacología, Farmacia y Tecnología Farmacéutica I+D Farma (GI-1645), Santiago de Compostela, ES

Introduction
Topical ocular delivery of atropine is attracting growing attention. Despite atropine is used since ancient times for mydriatic and cycloplegic purposes, a better knowledge of the very wide mechanisms in which atropine may participate in the ocular tissues is notably expanding its therapeutic profile [1]. Treatment of accommodative spams and chronic ulcers, counteraction of chemical weapons, and prevention of myopia can strongly benefit of ocular controlled release of atropine. Since atropine is prevalently cationically charged (pKa 9.43) at physiological pH, the aim of this work was to elucidate the feasibility of developing anionic hydrogels suitable as contact lenses (CLs) with enhanced affinity for atropine.

Experimental Methods
2-Hydroxyethyl methacrylate (HEMA) was used as main structural monomer, and methacrylic acid (MAA) and 2-acrylamido-2-methylpropane sulfonic acid (AMPSA) were added in various proportions. After polymerization, the hydrogels were evaluated regarding swelling degree, light transmittance, mechanical properties, and ocular irritancy. The loading of all formulations was carried out by soaking in atropine (1 mg/mL in water or NaCl 0.9%) solution at 25 °C and 180 rpm. Release experiments were carried out in NaCl 0.9% for all formulations at 36 °C and 180 rpm. The amount of atropine loaded and released was monitored by HPLC considering a validated calibration curve of atropine in water.

Results and Discussion
All HEMA-based hydrogels showed physical properties adequate for CLs. The light transmittance values were above the 90% required for CLs materials and the swelling degree was in good agreement with other CLs hydrogels. When soaked in atropine aqueous solution, HEMA hydrogels loaded 1.16 (s.d. 0.05) mg/g and released the drug in one hour. Copolymerization with MAA enhanced the amount of atropine loaded up to 19.5 (s.d. 1.0) mg/g and the release was completed in one hour. Copolymerization with AMPSA notably promoted the affinity of the network for atropine, and the hydrogels loaded 43.4 (s.d. 1.2) mg/g and the release was extended for two hours. The loading was clearly driven by ionic interactions since the soaking in atropine solutions prepared in NaCl 0.9% led to remarkably lower uptake. The hen's egg-chorioallantoic membrane test (HET-CAM) indicated that atropine-loaded hydrogels can be considered as non-irritant since no lysis, hemorrhage or coagulation was observed.

Conclusion
Anionic comonomers are revealed as adequate to endow HEMA hydrogels with affinity for atropine. The proportion of anionic charges can be tuned to regulate the total amount loaded, while the nature of the anionic moieties may allow controlling drug release.

Acknowledgement
This research was funded by MINECO [SAF2017-83118-R], Agencia Estatal de Investigación (AEI) Spain, Xunta de Galicia [ED431C 2020/17] and FEDER. M. Vivero-Lopez acknowledges Xunta de Galicia (Consellería de Cultura, Educación e Ordenación Universitaria) for a predoctoral research fellowship [ED481A-2019/120].

References
PS1-08-287

PVA-based hydrogels loaded with diclofenac for cartilage replacement

Andreia Oliveira, Inês Monteiro, Pedro Nolasco, Ana C. Branco, Rogério Colaço, Célio G. Figueiredo-Pina, Ana P. Serro

Introduction
The articular cartilage may suffer deterioration mainly due to trauma, osteoarthritis and rheumatoid arthritis, leading to pain and motion difficulties. When the lesion is on an end-stage, the most common treatment relies on an invasive surgical approach (total joint arthroplasty), which is defined by the total removal of the cartilage tissue and replacement by a simple mechanical device produced with materials such as titanium, cobalt chromium alloys, alumina and ultra-high molecular weight polyethylene. However, in previous stages, a less invasive approach can be attempted, involving the replacement of the cartilage damaged areas by natural (autograft) or synthetic materials with properties comparable to articular cartilage. Hydrogels present a huge potential to be used for this application, since they have a biphasic structure and can be tailored to present mechanical properties similar to articular cartilage. In particular, polyvinyl alcohol (PVA) hydrogels, have raised special interest due to their biocompatibility, low friction coefficients and good swelling ability [1]. The later allows the incorporation of drugs and other molecules with therapeutic action, that can be locally released after implantation surgery. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to reduce pain and other inflammatory signs. This work aims to develop PVA-based hydrogels with improved mechanical and tribological properties and ability to ensure a controlled release of diclofenac, an anti-inflammatory generally used in orthopedics.

Experimental Methods
Different PVA based hydrogels were produced by the freeze-thawing method: PVA (from a solution 13.5% w/w of PVA), PVA/PAA (where PVA is mixed to PAA (polyacrylic acid) in a proportion 10:3 (w/w)), PVA/PAA+PEG (where the previous hydrogel is immersed in 100% PEG (polyethylene glycol) for 1 h and then dried at 60 °C for about 5 days) and PVA/PAA+PEG+A (where the previous hydrogel is submitted to an annealing treatment at 120ºC for 1h). The hydrogels were characterized in terms of swelling behavior, mechanical properties (compression and tensile tests), wettability (captive bubble method) morphology (scanning electron microscopy) and friction coefficient (pin-on-plate tests). The best performing hydrogel was loaded by soaking with diclofenac (0.2% w/v in PBS, 3 days, at 37ºC) and its release ability was studied, as well as its irritability for tissues (HET-CAM test).

Results and Discussion
The results showed that the addition of PAA increased the swelling capacity of the material in about 43%. Contrarily, PEG-doped and annealed hydrogels presented lower and comparable values of swelling, with a reduction of about 52% in relation to the PVA values. Regarding wettability, all hydrogels are hydrophilic (~45º). Materials with PAA showed a reduction of ~50% in the tensile elastic modulus and ultimate tensile strength relatively to PVA, while the immersion in PEG and the annealing treatment led to higher elastic modulus (0.7–1.2 MPa), elongation-to-break (298–335%), tensile strength (3.0–3.6 MPa), and toughness (5.4–6.3 MJ/m²). For the compressive tests, it was found that PVA/PAA presented the smallest elastic modulus (~0.2 MPa), being the material that suffers the highest deformation under loading. PVA/PAA+PEG and PVA/PAA+PEG+A did not show significant differences relatively to PVA. All the materials demonstrated a viscoelastic behavior at compression, recovering their original dimensions when unloaded. Through SEM analysis, it was observed that both PVA and PVA/PAA have similar porosity, but the
latter material presents slightly larger pores. The addition of PEG into the PVA/PAA hydrogel and the annealing treatment led to considerably less porosity. PVA/PAA materials presented the highest friction coefficient value (0.13), while PVA/PAA+PEG+A led to the lowest value (0.06). Given the superior mechanical and tribological behavior of the last hydrogel, it was selected for the drug release studies. The results showed a significant improvement in the diclofenac release profile relatively to PVA (Figure 1). In fact, while PVA released a small amount of the anti-inflammatory in a few hours, PVA/PAA+PEG+A was able to release a much higher quantity of drug in a sustained way during at least 3 days. The irritability tests demonstrated that this hydrogel does not induce lysis, hemorrhage or coagulation.

**Conclusion**

In conclusion, the addition of PAA to PVA, followed by immersion in PEG and an annealing treatment led to a significant improvement of the mechanical properties of PVA and induced lower friction coefficients. Furthermore, such hydrogel revealed a high potential to be used as a drug delivery platform at local level of the anti-inflammatory diclofenac, leading to a controlled release for more than 3 days, without presenting any irritant behavior for the biological tissues.

**Acknowledgement**

To Fundação para a Ciência e a Tecnologia (FCT) for funding through the project PTDC/CTM-CTM/29593/2017 (CartHeal) and the unit projects UIDB/00100/2020 (CQE), UIDB/04585/2020 (CiEM), UID/CTM/04540/2020 (CeFEMA) and UIDB/50022/2020 (IDMEC/LAETA), and for the PhD grant of A.S. Oliveira (PD/BD/128140/2016) and A.C. Branco (SFRH/BD/145423/2019).

**References**

Unity is strength: using graphene-based materials for stiff and strong PEG hydrogels

Helena P. Ferreira1,2,3, Duarte Moura1,2,4, Andreia T. Pereira1,2, Patrícia Henriques1,2,4, Fernão Magalhães4,5, Inês Gonçalves1,2,4

1 i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Bioengineered Surfaces Group, Porto, PT; 2 INEB - Instituto Nacional de Engenharia Biomédica, Universidade do Porto, Bioengineered Surfaces Group, Porto, PT; 3 ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, PT; 4 FEUP - Faculty of Engineering, University of Porto, Porto, PT; 5 LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Porto, PT

Introduction

Poly(ethylene glycol) (PEG) hydrogels are one of the most anti-adhesive explored to date, and have been widely used as a “blank slate” with tunable physicochemical and biological properties. However, they are mechanically weak, which prevents their use in load-bearing biomedical applications (e.g. intervertebral disc, vascular grafts). Graphene is a promising candidate to solve this challenge. It is a single-layer of sp2-bonded carbon atoms, with outstanding stability, mechanical and electrical properties. There are several graphene-based materials (GBM), varying in lateral size (diagonal size of planar sheet), thickness (number of layers) and oxidation degree (number of oxygen-containing groups). Oxidized forms, in particular, are proven to be cytocompatible [1]. Only a handful of papers report the incorporation of GBM in PEG-based hydrogels [2,3]. However, most of these works focus on the characterization of compressive or rheological properties [4], and little is shown about the tensile properties of PEG hydrogels – which is essential for their application in tissues/devices subjected to tensile stress (e.g. vascular grafts) [5]. Hence, we propose the use of GBM as nanofillers to mechanically reinforce PEG hydrogels and obtain composites that are stiffer, stronger, but as anti-adhesive as neat PEG hydrogels.

Experimental Methods

PEG dimethacrylate (molecular weight 8000 g/mol) hydrogels (15 wt%) with different GBM types – graphene oxide (GO), few-layer graphene (FLG), FLG oxide (FLGO) – and with a range of concentrations – 0.1-4 wt% GO, 1 wt% FLG, 0.1-1 wt% FLGO – were produced by chemical crosslinking (using redox initiators). The mechanical properties were evaluated by tensile tests until rupture using a texture analyzer, obtaining the Young’s modulus (YM; indicating stiffness), ultimate tensile strength (UTS; indicating strength) and elongation at break (EB; indicating ductility). GO and FLGO composites were characterized regarding GBM dispersion in the matrix (brightfield microscopy), surface roughness (SEM imaging), wettability (water contact angle) and swelling degree (water uptake). The cytotoxicity of 24h extracts and anti-adhesiveness of composite hydrogels was evaluated using human umbilical vein endothelial cells (HUVEC) according to ISO 10993-5 and 10993-12, assessing cell metabolic activity (resazurin assay) and morphology (phalloidin/DAPI staining and fluorescence microscopy).

Results and Discussion

We started by evaluating the effect of various GBM parameters, particularly oxidation degree, thickness and concentration, in the tensile reinforcement of PEG hydrogels. Considering oxidation degree, the incorporation of oxidized forms (1% FLGO) reinforced PEG hydrogels, unlike reduced forms (1% FLG). Regarding thickness and the effect of concentration, both single-layer (GO) and few-layer (FLGO) forms were able to reinforce hydrogels. The incorporation of 4% GO resulted in stiffer and stronger composites, with 162 kPa YM and 174 kPa UTS (9x and 18x increase, respectively, from neat PEG hydrogels). 1% FLGO composites presented a 174 kPa YM and 177 kPa UTS (9.7x and 13x increase, respectively, from neat PEG hydrogels), but had a lower EB than GO composites. Both GO
and FLGO were homogeneously dispersed in the hydrogel matrix, despite some aggregates. Although SEM images showed increasing surface roughness for higher GBM concentrations (particularly GO), they caused no alterations to the hydrophilicity of PEG hydrogels (water contact angles <20°). Neat PEG hydrogels have a great swelling degree (149% vs. samples after gelation and 1898% vs. dried samples). The swelling degree of composites tended to decrease for higher GBM concentrations, but remained >50% vs. samples after gelation and >787% vs. dried samples.

The extracts obtained from GO and FLGO composites caused no cytotoxicity on HUVEC, presenting cell viability ~100%, and phalloidin staining revealed a normal morphology (vs. control medium). Also, upon direct contact with composites, HUVEC did not adhere, as confirmed by resazurin assay and fluorescence microscopy. These results show that PEG/GBM composites maintained the high anti-adhesiveness and cytocompatibility of neat PEG hydrogels.

Conclusion

Overall, these results show that oxidized forms of graphene can be used as nanofillers to reinforce PEG hydrogels. GO and FLGO composite hydrogels have great tensile properties, are hydrophilic, cytocompatible and anti-adhesive towards HUVEC. To the best of our knowledge, this is the first work to report an increase up to 1887% of the tensile properties of PEG hydrogels using GBM, while still maintaining appreciable ductility. This work paves the way for the exploitation of PEG hydrogels for load-bearing applications, such as vascular grafts or catheters.

Acknowledgement

The authors acknowledge the funding from FCT and FEDER projects PTDC/CTM-COM/32431/2017, UIDB/04293/2020 and UIDB/00511/2020. Helena Ferreira acknowledges FCT for her PhD fellowship (2020.04712.BD).

References


Development of a new magnetically responsive device for localized cancer theranostics

Paula I.P. Soares, Adriana Gonçalves, Beatriz Correia, João Magalhães, Jorge C. Silva, João P. Borges

NOVA School of Science and Technology, FCT-NOVA, CENIMAT/i3N, Materials Science Department, Caparica, PT

Introduction
Cancer is a major worldwide problem, with an estimated 19.3 million new cases and 10 million cancer deaths in 2020. Cancer diagnostic and treatment have significantly improved in recent years, which increased life expectancy. Nevertheless, current treatment limitations and the elevated incidence, mortality, and cancer disease heterogeneity lead to growing concern and expectation over the development of new and improved cancer diagnostic and/or treatment.[1] Consequently, an unmet medical need in cancer treatment is: How to effectively deliver drugs to the tumor site and improve their efficacy while reducing side effects?

In this context, cancer theranostics emerged as a promising tool to decrease treatment delays, improve patient care, and act as a personalized cancer approach. In recent years, magnetic nanoparticles (mNPs) demonstrate a considerable potential to substantially improve theranostics platforms for cancer. Their unique properties, particularly the ability to respond to an external magnetic field, enable their use as imaging probes in the diagnostic feature and as magnetic hyperthermia agents in the treatment feature. Additionally, magnetic responsiveness can be used as a trigger for controlled drug release.[1, 2] Magneto-responsive devices can provide a real-time response upon application of an external magnetic field. An external magnetic field as the trigger is optimal since it has good tissue penetration without almost any physical interaction with the body.[3, 4]

The main goal of this work is to develop a novel magnetically responsive device for cancer theranostics application. This device is composed of mNPs incorporated into polymeric nanofibers produced by additive manufacturing techniques (electrospinning and paste extrusion). Following device implantation in a solid tumor, an external alternating magnetic field remotely activates the magnetic component (mNPs), which generates heat due to their superparamagnetic properties. mNPs concentration will be tailored to obtain localized heat in the tumoral tissue. The application of an external alternating magnetic field induces heat generation by mNPs to sensitize tumor cells and allow treatment monitoring through MRI.

Experimental Methods

Magnetic nanoparticles were prepared by chemical precipitation technique and stabilized as previously described.[5] mNPs were incorporated into a polymeric matrix using electrospinning technique. Electrospinning of selected polymers (PLA, PCL, PVA) was tested in different conditions to achieve monodisperse micro/nanofibers without defects. Optimization comprised study of solution properties, processing conditions, and environmental conditions. Magnetic properties of plain mNPs and magnetic fibers were evaluated by VSM. Characterization included scanning electron microscopy (SEM), mechanical properties (tensile tests), swelling behavior, and surface properties evaluated through static and dynamic contact angle measurements.

Results and Discussion

Superparamagnetic iron oxide mNPs with 10 nm in diameter were successfully prepared. According to the amount of oleic acid added during stabilization step, these mNPs can be easily dispersed in either hydrophobic or hydrophylc solvents, enabling their incorporating directly in polymeric solutions prior to electrospinning. Polymeric solutions containing mNPs were electrospun with the optimized conditions to produce monodisperse nanofibers without defects. PLA fibers were produced with two different morphologies: porous and non-porous fibers. Porous fibers...
displayed larger diameters (around 500 nm) than non-porous fibers (around 200 nm). PVA and PCL fibers also displayed smaller diameters (around 200 nm) (Figure 1). SEM and TEM analysis demonstrated the presence of mNPs dispersed throughout the fibers. Mechanical properties and swelling behavior of the magnetic membranes were highly dependent on fiber composition (i.e., polymer); however crosslinking degree and the presence of mNPs improved membranes' rigidity while decreasing the membrane overall swelling ability. Magnetic hyperthermia measurements were performed during 10 min of an AC magnetic field application with magnetic flux density of 300G and 418.5 kHz of frequency. The results demonstrated that the heating ability of magnetic membranes is largely dependent on mNPs concentration, although it is also influenced by membrane composition (type of polymer). Cytotoxicity assessment demonstrated that despite the concentration of mNPs incorporated, the magnetic membranes are not cytotoxic and suitable for biomedical applications.

**Conclusion**

More interesting results are being achieved under the recently financed project DREaMM (Ref. PTDC/CTM-REF/30623/2017) to engineer dual-stimuli responsive magnetic nanofibrous membranes as controlled drug release systems and magnetic hyperthermia agents for cancer treatment using additive manufacturing techniques.

**Acknowledgement**

This work is funded by FEDER funds through the COMPETE 2020 Program and National Funds through FCT—Portuguese Foundation for Science and Technology under the project POCI-01-0145-FEDER-007688 (Reference UID/CTM/50025) and PTDC/CTMCTM/30623/2017 (DREaMM).

---

**References**


Visible Light-driven Polyurethane Actuators for Bionic Hands

Lei Wu, Meng Wang

University College London, Division of Surgery and Interventional Science, London, GB

Introduction
Light-driven soft robotics made from photo-responsive materials are able to mimic the biological functions, such as swimming, walking, crawling, which have attracted intense scientific attentions recently. However, many efforts should still be taken in the field of developing photo-responsive materials with good mechanical and biological performance and simple synthetic process for constructing potential light-driven soft robotics in biomedical applications.

Experimental Methods
Synthesis of the photo-responsive polymer.
Design and fabrication of the light-driven soft actuators.
Characterization of chemical, thermal and mechanical properties of the photo-responsive polymer.
Characterization of the performances of the light-driven soft actuators.

Results and Discussion
Herein, we developed a new visible light-responsive elastomer (i.e. PAzo) by introducing photo-responsive molecule (i.e. azobenzene derivatives) into the main chain of the biocompatible polyurethane (i.e. PCL-PUU). Casting PAzo onto the commercial Kapton sheet, a visible light-driven bilayer actuator was fabricated and systematically characterised. Specially, two bending modes of the actuator were observed under the illumination with different light intensities. The correlated actuation mechanisms with photothermal and photochemical coupling effects were thoroughly studied by experiments, theoretical calculations and FEA simulations. Furthermore, based on this actuator, an application of visible light controlled soft robotic fingers for playing piano was demonstrated.

Conclusion
The present work may guide the design and manufacture of light-driven wearable robots or assistive devices for medical rehabilitation and assistance.

Acknowledgement
This work is financially supported by the UK Engineering and Physical Sciences Research Council (EP/R02961X/1). L.W. appreciates a UCL Graduate Research Scholarship/Overseas Research Scholarship (UCL GRS/ORS).
Automated fabrication of cell-instructive hydrogels

Anna Siedel1, Nicholas Dennison1, Maximilian Fusenig1, Uwe Freudenberg1, Carsten Werner1,2

1 Leibniz-Institut für Polymerforschung Dresden e.V., Max Bergmann Center für Biomaterialien, Dresden, DE; 2 Technische Universität Dresden, Center for Regenerative Therapies Dresden, Dresden, DE

Introduction

Polymer hydrogels can be instrumental to generate defined sets of exogenous signals that direct the fate and functionality of embedded cells. Biohybrid polymer networks containing sulfated glycosaminoglycans have been shown to offer particularly valuable options and are therefore increasingly used in the preparation of materials to enable regenerative therapies in vivo and in advanced human tissue and disease models in vitro.[1] Scalable, standardized production of such cell-instructive biohybrid hydrogels at low compositional variation, high batch-to-batch reproducibility, and material compliance can benefit from the adaptation of commercial synthesis and liquid handling robots. Using an exemplary, well-established peptide-functionalized multi-armed poly(ethylene glycol) (starPEG)-heparin hydrogel system[2], we herein report on an approach to set up a dedicated workflow for the fabrication of reactive hydrogel precursors and cell-laden hydrogels.

Experimental Methods

Hydrogel precursor synthesis was carried out using a customized BioSyntheSizer3.1 platform (GeSiM, Germany) for pipetting, pH adjustment, temperature control and vacuum/inert gas applying, to implement a synthesis procedure from our previously reported manual method.[1] (N-hydroxysulfosuccinimide) (sulfo-NHS) and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were added to a heparin solution cooled to 4 °C one after the other (Fig. 1). Carboxylic acid groups were activated for 20 min, followed by addition of N-(2-aminoethyl) maleimide trifluoroacetate and 1 h reaction time at 20 °C. The prepared solutions were pipetted automatically and temperature-controlled by the synthesis robot through a coolable vial rack. The sequence protocol accurately maintains activation and reaction time. Two equal precursor solutions prepared in parallel were then combined by injection into the dynamic dialysis device. The dialysis medium was sodium chloride solution and Milli-Q water. The purified precursor solution was aliquoted automatized and freeze-dried afterwards. The final product was analyzed by nuclear magnetic resonance of protons (1H-NMR).

The obtained reactive heparin-maleimide precursor and matrix metalloproteinase (MMP)-cleavable peptide-end-functionalized starPEG were crosslinked in aqueous solution using a commercial liquid handling robot (TECAN Fluent, Switzerland) to form a hydrogel network, optionally containing cells to be cultured within these matrices. The hydrogels were imaged using the microscope Opera Phenix (Perkin Elmer, USA) and the analysis software Imaris (Andor Technology, United Kingdom).

Results and Discussion

An automated multistage workflow was developed to synthesize hydrogel precursors, form hydrogels formation, and perform cell culture experiments with cells embedded in the gel matrices. The protocol combines two robotic fluid handling systems and a microscope for automated sample imaging and cell analysis. The customized heparin maleimidation, including purification, was implemented by the BioSyntheSizer3.1. Controlled cooling was achieved by a thermostat (instead of an ice-bath as used in manual synthesis). The sequence protocol enables compliance of activation and reaction time with constant mixing conditions. Optimized purification of the precursor by dynamic dialysis provided a purity and yield comparable to previously established manual protocols. Subsequent liquid aliquotation and automated sterile freeze-drying facilitated further processing. As a proof of principle, the synthesized precursor was applied to a recently developed high throughput combinatorial pipetting scheme in a 384-well plate,
including formation of the cell-hydrogel-dispersion, medium addition and exchange. Cell viability and tube formation of human umbilical vein cells (HUVECs) were analyzed and quantified automatically. The results confirm comparable network performance in this cell culture experiment.

**Conclusion**

Our newly developed automated hydrogel precursor synthesis method can ensure the quality and scalable production of GMP-compliant biohybrid hydrogel materials. Automated hydrogel preparation, cell culture and analysis can further empower combinatorial approaches to biomedical applications of cell-instructive materials.

**Acknowledgement**

This project was made possible with support of the BMBF VIP+ Project funding and the collaborative research center Transregio 67 (German Research Foundation).

---

**References**


Low-Intensity pulsed ultrasound stimulation enhances chondrogenic differentiation of ASCs in a 3D hydrogel

Cristina Manferdini1, Elena Gabusi1, Diego Trucco1,2,3, Paolo Dolzani1, Yasmin Saleh1, Andrea Cafarelli2,3, Leonardo Ricotti2,3, Gina Lisignoli1

1 Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, IRCCS Istituto Ortopedico Rizzoli, Bologna, IT; 2 The BioRobotics Institute, Scuola Superiore Sant’Anna, Pisa, IT; 3 Department of Excellence in Robotics & AI, Scuola Superiore Sant’Anna, Pisa, IT

Introduction
Articular cartilage injuries have a limited potential to heal and, over time, may lead to osteoarthritis, an inflammatory and degenerative joint disease associated with activity-related pain, swelling, and impaired mobility [1]. Regeneration and restoration of the joint tissue functionality remain unmet challenges. Stem cell-based tissue engineering is a promising paradigm to treat cartilage degeneration [1]. In this context, hydrogels have emerged as promising biomaterials, due to their biocompatibility, ability to mimic the tissue extracellular matrix and excellent permeability [2]. Different stimulation strategies have been investigated to guarantee proper conditions for mesenchymal stem cell differentiation into chondrocytes, including growth factors, cell-cell interactions, and biomaterials [3]. An interesting tool to facilitate chondrogenesis is external ultrasound stimulation. In particular, low-intensity pulsed ultrasound (LIPUS) has been demonstrated to have a role in regulating the differentiation of adipose mesenchymal stromal cells (ASCs) [4]. However, chondrogenic differentiation of ASCs has been never associated to a precisely measured ultrasound dose. In this study, we aimed to investigate whether dose-controlled LIPUS is able to influence chondrogenic differentiation of ASCs embedded in a 3D hydrogel.

Experimental Methods
Human adipose mesenchymal stromal cells at 2*10⁶ cells/mL were embedded in a hydrogel ratio 1:2 (VitroGel RGD®) and exposed to LIPUS stimulation (frequency: 1 MHz, intensity: 250 mW/cm², duty cycle: 20%, pulse repetition frequency: 1 kHz, stimulation time: 5 min) in order to assess its influence on cell differentiation. Hydrogel-loaded ASCs were cultured and differentiated for 2, 7, 10 and 28 days. At each time point cell viability (Live & Dead), metabolic activity (Alamar Blue), cytotoxicity (LDH), gene expression (COL2, aggrecan, SOX9, and COL1), histology and immunohistochemistry (COL2, aggrecan, SOX9, and COL1) were evaluated respect to a non-stimulated control.

Results and Discussion
Histological analysis evidenced a uniform distribution of ASCs both at the periphery and at the center of the hydrogel. Live & Dead test evidenced that the encapsulated ASCs were viable, with no signs of cytotoxicity. We found that LIPUS induced chondrogenesis of ASCs embedded in the hydrogel, as demonstrated by increased expression of COL2, aggrecan and SOX9 genes and proteins, and decreased expression of COL1 respect to the non-stimulated control.

Conclusion
These results suggest that the LIPUS treatment could be a valuable tool in cartilage tissue engineering, to push the differentiation of ASCs encapsulated in a 3D hydrogel.

Acknowledgement
This work received funding from the European Union's Horizon 2020 research and innovation program, grant agreement No 814413, project ADMAIORA (AdvanceD nanocomposite MAterIals fOr in situ treatment and ultRAsound-mediated management of osteoarthritis).

References


Towards fiber orientation in collagen hydrogels for annulus fibrosus tissue engineering

Ana L. Castro\textsuperscript{1,2,3}, Sitara Vedaraman\textsuperscript{4,5}, Daniela F. Duarte Campos\textsuperscript{5,6}, Mário A. Barbosa\textsuperscript{1,2,3}, Raquel M. Gonçalves\textsuperscript{1,2,3}, Laura de Laporte\textsuperscript{4,5,7}

\textsuperscript{1} i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, PT; \textsuperscript{2} INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Porto, PT; \textsuperscript{3} ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, PT; \textsuperscript{4} DWI—Leibniz Institute for Interactive Materials, Aachen, DE; \textsuperscript{5} Department of Advanced Materials for Biomedicine, Institute of Applied Medical Engineering, RWTH Aachen University, Aachen, DE; \textsuperscript{6} Center for Molecular Biology Heidelberg University, Heidelberg, DE; \textsuperscript{7} Department of Technical and Macromolecular Chemistry, RWTH Aachen University, Aachen, DE

Introduction
Intervertebral disc (IVD) herniation involves annulus fibrosus (AF) failure, and is a process still under investigation. Most of the ongoing research relies on rodent in vivo models or on bovine in vitro organ cultures that do not mimic the human IVD herniation. A bioengineered in vitro model that could recapitulate human AF at distinct stages of herniation progression would be of paramount importance to better understand AF failure. Replicating tissue-oriented organization in vitro has been widely studied in the recent years, with both natural and synthetic materials in combination with magnetic fields to establish anisotropic conditions \cite{1-3}. More specifically, magneto-responsive polyethylene glycol (PEG) microgels have demonstrated to form a unidirectional hydrogel, called Anisogel, which aligned cell growth \cite{2}. In this study, we aim to develop an AF 3D model based on collagen type I hydrogel with oriented PEG microgels, with the final goal to align collagen fibers and direct cell growth to improve our knowledge on IVD herniation.

Experimental Methods
Collagen hydrogels precursor solutions (3.2 mg/ml) are prepared and gelation is induced after pH adjustment with NaOH at 37°C for 45 min. PEG microgels are produced in rod-shape, following a previously established protocol using a mold-based soft lithography \cite{2}. Two microgel size conditions are evaluated (2,5x2,5x25 and 2,5x2,5x50 μm\textsuperscript{3}) at a volume concentration of 0.45% and 0.6%, respectively, inside the entire collagen gel. An external magnetic field of 70 mT is applied to allow for microgels orientation within the collagen hydrogel precursor solution. Microgel orientation and collagen fiber formation are evaluated by two-photon microscopy and histologic analysis using Sirius Red staining under polarized light. The hybrid hydrogel mechanical properties are evaluated by rheological studies.

Results and Discussion
To establish the best conditions for microgel alignment inside a collagen precursor solution, the protocol has been optimized: an initial incubation at 4°C for 10 min, prior to the incubation at 37°C for collagen polymerization, all in the presence of a magnetic field. The two-photon microscopy confirmed microgel orientation in these conditions for both microgel sizes. Polarized light evaluation of Sirius Red stained samples showed that the two tested microgels conditions did not impair fiber formation in collagen hydrogels. On going analysis of these samples is being performed to evaluate collagen fiber orientation. Rheological analysis showed similar storage modulus for collagen control hydrogels and oriented collagen hydrogels with 2,5 x 2,5 x 50 μm\textsuperscript{3}. Further studies are currently being conducted for 2,5 x 2,5 x 25 μm\textsuperscript{3} microgels.

Conclusion
The experimental results so far indicate promising conditions to establish a 3D unidirectional collagen hydrogel that could be further used to modelling AF structure. Further studies are being conducted to understand if the anisotropy
given by the PEG microgels is accompanied by collagen fiber alignment and how AF cells response to this physical guidance.

Acknowledgement
AL Castro acknowledges Fundação para a Ciência e a Tecnologia (FCT) for the PhD Grant (SFRH/BD/147300/2019). S Vedaraman, DF Duarte Campos, and L De Laporte thank the support from the Universities of Excellence funding line (Grant no. EXS-PFLS010).

References
Design of Polypeptide-Polysaccharide based Hydrogels for Bone Tissue Regeneration

Inta Kreicberga¹,², Jevgenija Luginina³, Kristine Salma-Ancone¹,²

¹ Riga Technical University, Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga, LV; ² Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, LV; ³ Riga Technical University, Institute of Technology of Organic Chemistry, Faculty of Materials Science and Applied Chemistry, Riga, LV

Introduction
In recent years development of bioinspired natural biopolymer-based hydrogels is a research hotspot in the field of bone tissue regeneration because their structure can mimic the native features of the extracellular matrix (ECM) of bone tissue. Hyaluronic acid 1 (HA), as one of the main components of ECM, is an optimal material to design biomimetic cell-interactive hydrogels, as well ε-poly-L-lysine (ε-PL) is an excellent candidate for preparing antibacterial hydrogels.

The aim of this study was to evaluate the impact of various aqueous solvents on the zero-length chemical cross-linking reaction and formation of HA/ε-PL hydrogels.

Experimental Methods
The bioinspired hydrogels were synthesized using zero-length carboxyl-to-amine crosslinkers 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) at room temperature in various aqueous solvents - deionized water, sodium dihydrogen phosphate buffer, sodium chloride, sodium hydroxide and hydrochloric acid solution. The intermediate products, final products and by-products were characterized by FTIR, NMR, UV/VIS spectroscopy. The swelling ratio of the synthesized hydrogels was evaluated.

Results and Discussion
The crosslinking reaction was analyzed step by step and the structure of the formed intermediates 3, by-products and final product 4 was investigated. FTIR and NMR spectra revealed that the chemical crosslinking reaction occurred in all aqueous solvents by the formation of stable hyaluronic acid N-hydroxysuccinimide ester intermediate 3 which further reacted with the primary amino groups of ε-PL 2 and leaded the formation of the crosslinked hydrogels 4. The crosslinking reaction was influenced by the amount and pH of aqueous solvent, as well as the reaction time. During the chemical cross-linking reaction, a parallel reaction of EDC hydrolysis occurred in all solvents, forming a reaction by-product - EDC urea derivative. The synthesized hydrogels showed high swelling ability up to 700% and reached an equilibrium swelling state within 2 h.

Conclusion
The impact of the various aqueous solvents on the zero-length chemical crosslinking reaction and the formation of hydrogels were evaluated. In the development of biomaterials, it is important that this reaction can be performed in a biocompatible media, such as a 0.9% NaCl saline fluid at room temperature. Our research shown that the efficiency of this chemical cross-linking reaction can be modeled by nature of solvent, solvent amount and reaction time, adjusting the degree of cross-linking.

Acknowledgement
The authors acknowledge financial support from the Latvian Council of Science research project No. Izp-2020/1-0072 “Injectable bioactive biocomposites for osteoporotic bone tissue regeneration (inBioBone)".
Hyaluronic acid and ε-poly-L-lysine cross-linking reaction
Preparation of poly(3-hydroxyoctanoate) porous patches containing diclofenac modified oligomers as potential materials for biomedicine

Katarzyna Haraźna¹, Anna Walczyk¹, Ewelina Cichoń², Małgorzata Witko¹, Bartosz Leszczyński³, Andrzej Wróbel³, Maciej Guzik¹

¹ Polish Academy of Sciences, Jerzy Haber Institute of Catalysis and Surface Chemistry, Cracow, PL; ² AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Cracow, PL; ³ Jagiellonian University, Faculty of Physics, Astronomy and Applied Computer Science, Cracow, PL

Introduction
Poly(3-hydroxyoctanoate) (P(3HO)) is an elastomeric material, which belongs to medium chain length polyhydroxyalkanoates (PHAs) polymer family. P(3HO) is accumulated in bacterial fermentation processes, under nutrient deficiency conditions. Thanks to several interesting properties such as biocompatibility, biodegradability as well as mechanical properties, our research focused on P(3HO) use in design and construction of patches, which can be used in skin regeneration processes, especially in wounds caused in sport injuries. Successfully decorating of molecules derived from decomposition of P(3HO) with biomolecules such as non-steroidal anti-inflammatory drugs, i.e. diclofenac, can be effective method, which may help deliver drugs directly to place of injury [1-5].

Experimental Methods
Strain of Pseudomonas Putida KT2440 was used in order to generate P3HO polymer in a 5L fermentor. As a carbon source in fermentation process the mixture of octanoic and butyric acid was used. After successfully finished bioprocess, the polymer was isolated from dried cells and purified by chemical methods [1-2].

The oligomers containing diclofenac (oli-P(3HO)-Dic) were prepared using modified protocol described by Haraźna et al. In this order, the reaction was carried out in melt and was catalysed using 4-toluenesulfonic acid monohydrate [3]. The structures of obtaining oligomers were confirmed by electrospray ionization tandem mass spectrometry (ESI-MS), infrared spectroscopy (IR) and nuclear magnetic resonance (1H NMR) techniques. Moreover, the amount of covalently attached diclofenac in prepared conjugates was established by 1H NMR.

In the next experiment, the prepared diclofenac modified oligomers (oli-P(3HO)-Dic) were incubated in phosphate buffered saline (PBS) (pH = 7.4, 100 mL) at 37 °C for 54 days [4]. The collected at appropriate time intervals supernatants were subjected to ultra high pressure liquid chromatography tandem with mass spectrometry (UHPLC-MS) analyses.

Finally, the porous polymeric patches were prepared by solvent casting combined with porogen leaching techniques [5]. In this purpose, different ratio of polymer to porogen were taken. As porogen, sodium chloride with 100 - 300 µm grain size was used. At the same time, analogous patches containing 100 µg of oli-P(3HO)-Dic / 1 g of P(3HO) were prepared. Description of the prepared samples is shown in Picture 1.

The porosity of obtaining patches were determined using X-Ray microtomography (µCT). The structures of materials were observed using scanning electron microscopy (SEM). Moreover, the mechanical properties of prepared materials i.e. tensile strength, Young modulus and elongation at break were determined.

Results and Discussion
1H NMR analysis showed a small amount of physically bound diclofenac (0.37 mg / 100 mg oligomers). Moreover, based on mentioned analysis, the content of the covalently attached drug were determined (22.6 mg / 100 mg oligomers). In order to define the amount of releasing diclofenac, oligomers were incubated in PBS (pH = 7.4, 100 mL) at 37 °C for 54 days. After mentioned time, 27.49 ± 2.69 % of diclofenac were released.
For the prepared soft foams, mechanical tests were carried out. These analyses showed that a small addition of diclofenac-modified oligomers causes a twofold increase in Yong’s modulus, tensile strength and elongation at break. The high value of elongation at break of patches no. A-Dic containing diclofenac modified oligomers (102.96 ± 22.30 %) in compared to patches no. A (56.83 ± 6.52 %) proves the excellent application properties of prepared functionalised polymeric foams. It is desirable that the potential patches should characterise with adequate strength, preventing it from tearing at the slightest tension. Moreover, the µCT analysis showed the porosity of foams no. A and no. B at 97 % and 96%, respectively. Additionally, it was shown that in the case of foams no. A, a homogenous pore size was observed, compared to foams no. B.

**Conclusion**

Therefore, the best structural and mechanical parameters for foams of 5% mas. P(3HO) were obtained, we decided to choose those patches as a future dressing material that will undergo in-depth characterisation in vivo. For this purpose, two series of foams – first composed of P(3HO) (no. A) and second one – composed of P(3HO) and oli-P(3HO)-Dic (no. A-Dic) were prepared. Due to the possibility of long-term release of active substances directly into the place of injury, the materials submitted for in vivo tests are an excellent alternative to commercially available wound dressings.

**Acknowledgement**

Research funded by the NCRD Poland, grant Lider no. LIDER/27/0090/L-7/15/NCBR/2016. KH also acknowledges the support of InterDokMed project no. POWR.03.02.00-00-1013/16.

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Amount of P(3HO) (%mas.)</th>
<th>Amount of poregen (%mas.)</th>
<th>Amount of oli-P(3HO)-Dic (µg/ Lg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5</td>
<td>95</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>15</td>
<td>85</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>25</td>
<td>75</td>
<td>-</td>
</tr>
<tr>
<td>A-Dic</td>
<td>5</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>B-Dic</td>
<td>15</td>
<td>85</td>
<td>100</td>
</tr>
<tr>
<td>C-Dic</td>
<td>25</td>
<td>75</td>
<td>100</td>
</tr>
</tbody>
</table>

**References**

2:30 p.m. – 4:00 p.m.

Poster floor

**PS1-09 | Biomaterials for Cardiovascular Applications**
PS1-09-305

BioV²alve - Development of a biohybrid textile-reinforced minimal-invasive venous valve implant

Caroline Emonts¹, Alexander Kopp², Heinz-Werner Henke³, Philipp Bruners⁴, Stefan Jockenhoevel⁵, Thomas Gries¹

¹ RWTH Aachen University, Department of Medical Textiles, Institut für Textiltechnik, Aachen, DE; ² Meotec GmbH, Aachen, DE; ³ Innovative Tomography Products GmbH, Bochum, DE; ⁴ Universitätsklinikum Aachen, Klinik für Diagnostische und Interventionelle Radiologie, Aachen, DE; ⁵ RWTH Aachen University, Department of Biohybrid & Medical Textiles, Institute of Applied Medical Engineering, Aachen, DE

Introduction
With a prevalence of 3-5% the chronic venous insufficiency is one of the most common adult diseases in the western population and shows a considerable socio-economic significance. By insufficient venous valve closure, the returning blood flow to the heart is disrupted thus leading to a local overpressure in the venous system of the legs. The most common symptoms are dilated veins, varicose veins, heavy and painful legs with swelling, skin and tissue changes and in the final stage chronic lower leg ulcers. Nowadays, the current therapies are mainly symptomatic. Surgical and interventional approaches to reconstruct the venous valve function have not been successful due to the special coagulation situation in the venous flow. In this setting, the aim of BioV²alve project is to develop a biohybrid implant to restore the venous valve function.

Experimental Methods
Two textile structures form the main components of the innovative venous valve implant, namely a braided magnesium structure and a warp-knitted mesh. The braided magnesium stent frame offers an initial fixation of the synthetic biohybrid valve in the vessel. After ingrowth of the valve structure in the vessel wall, the magnesium stent degrades. The warp-knitted mesh which forms the two leaflets of the valve. The warp-knitted mesh serves as reinforcement and it will be covered by a hydrogel.

Three different designs of single-wire manually braided stents are investigated regarding their mechanical properties measured by a radial force tester. Furthermore, the crimp ability and dilatation of the stents is tested as well as the possibility to incorporate the textile valve structure.

For the warp-knitted meshes PET yarns of different fineness are used. The drapability and the morphological characteristics as the wall thickness, the pore size and the porosity are evaluated. The mechanical characteristics are tested by means of tensile testing in lengthwise and circumferential direction. Furthermore, the suture retention is tested to ensure a safe processing of the textiles.

Results and Discussion
All stent designs show a radial force in the range of commercially available venous stents so that the suitability for the application in lower limb veins is ensured. Regarding the function as valve delivery structure, two stent structures demonstrate a better performance due to less elongation during the crimping process and therefore less risk of damaging the synthetic valve.

The mesh structures with finer PET yarns show a better drapability and handling during the further processing. The porosity and pore size are decreased so that the textile offers better support for the hydrogel coating of the valve.

Conclusion
The data illustrates that the biodegradable magnesium wire is suitable to manufacture new stent designs for venous valve implants. Furthermore, it is shown that the incorporation of textile reinforced valve leaflets comparable to native
venous valves is possible. Both textile processes, braiding and warp-knitting offer a high flexibility in adjusting the textile characteristics to the requirements of the implant.

Acknowledgement
The Project “BioV²alve” is supported by the European Regional Development Fund North Rhine-Westphalia (EFRE.NRW).

Prototype of the BioV²alve venous valve implant
The venous valve implant is consisting of the braided magnesium stent (a), the warp-knitted mesh (b). Functional testing of the valve function (c). Radial force testing of the magnesium stent (d)
Ultrasound responsive polymer microbubbles for an efficient targeted treatment of thrombotic diseases

Louise J. Fournier, Rachida Aid-Launais, Abderrahmane W. Aissani, Veronique Ollivier, Murielle Maire, Olivier Couture, Didier Letourneur, Cédric Chauvierre

1 INSERM U1148, LVTS, Université de Paris, X Bichat Hospital, PARIS, FR; 2 Université de Paris, UMS34 FRIM, PARIS, FR; 3 Laboratoire d'Imagerie Biomédicale Sorbonne Université, UMR 7371 - U1146, PARIS, FR; 4 INSERM U1148, LVTS, Université Sorbonne Paris Nord, VILLETANEUSE, FR

Introduction
Cardiovascular diseases are the first worldwide mortality rate, it is fair to admit research in this field is a priority. Our team focus on thrombosis innovative targeted treatments and molecular diagnostic tools. Indeed, current treatments to prevent blood clots from obstructing vessels are intravenous injection of rt-PA (plasminogen activator) and in the last recourse, invasive mechanical removal called thrombectomy [1]. Nevertheless, systemic injection of the plasminogen activator has to be in high dose, potentially toxic, due to its short half time in the bloodstream. These limitations engage teams to find innovative and targeted treatments to overcome collateral bleeding or surgical complications. Our strategy focuses on developing innovative carriers for the targeting treatment, and visualization of the blood clot. We synthesize targeting polymer microbubbles (MB) in a one-pot, scalable, and not described in the literature protocol. Our targeting agent is a sulfated polysaccharide extracted from brown algae, the fucoidan, already used in clinical trial phase IIa. It was chosen for its affinity for P-selectin, expressed protein on activated platelets and activated endothelial cells. Commercial microbubbles are used in clinics for their echogenic properties and used as contrast agents. In addition, the ultrasound insonation can also induce sonothrombolysis [2], a mechanical perturbation of the MB that can accentuate the clot disruption. In short term, the objective is to incorporate the plasminogen activator into the MB, for a complete theragnostic tool.

Experimental Methods
Microbubble synthesis is obtained via acoustic cavitation of an aqueous phase containing our targeted agent with a surfactant. The organic phase containing the monomer of isobutyl cyanoacrylate is injected into the aqueous solution at a controlled speed. The insonation is performed with the Branson Sonifier SFX 500, and during the insonation process, perfluorobutane is injected into the aqueous solution to be imprisoned inside the MBs. The obtained foam is centrifuged 3 times and filtered to 5 µm. MB characterizations were performed by size measurements with granulometry analysis on Mastersizer 3000, and concentration measurements with image analysis of calibrated counting cell with Fiji Software. Those parameters allowed us to control the shelf stability over the weeks of the MBs. Also, the morphology of the MBs was observed by Scanning Electron Microscopy (SEM). Fucoidan content was determined by a quantitative analysis with methylene blue colorimetric dosage. Targeting tests were performed ex vivo in static and fluidic conditions with human whole blood. In brief, for the static test, platelet-rich clots are obtained ex vivo by blood centrifugation and incubation at 37°C of the platelet-rich plasma. Clots are then put in contact with MBs suspension and observed by fluorescence microscopy after multiple washing. The fluidic model consists of coating microfluidic channels with collagen in order to promote platelet aggregation. Whole human blood is then injected at arterial flow speed. When platelet aggregates are formed, the channel is rinsed and the MB suspension is injected. After another rinsing, microbubble colocalization to the aggregate is observed by fluorescence microscopy. Echogenicity evaluation is performed with the comparison of commercial MBs (SonoVue) to our MBs. MBs are guided through a channel in an agar phantom setup. Signals of echogenicity and response to burst for sonothrombolysis are compared.

Page 1298 of 2028
Results and Discussion
We were able to develop an undescribed synthesis protocol for polymer MBs with acoustic cavitation reducing the time of hydrodynamic cavitation. The MBs mean size is below 6 µm and the range of production is around $10^{10}$ MBs per synthesis. Those MBs showed great shelf stability in terms of size distribution and concentration over two months. SEM images showed a good size distribution and rounded shape morphology. Fucoidan quantification reported similar incorporation to previous work in the lab from another method of MBs synthesis with around $10^{15}$ g of fucoidan per MB [3]. We are currently optimizing the targeting experiment in static and fluidic conditions but preliminary results are encouraging. Echogenic characterization preliminary experiment provided similarities with commercial contrast agent in terms of echogenicity. In order to validate these results, more synthesis conditions will be evaluated and the objective is to extend the phantom model to an ex vivo model to observe the influence of the targeted MBs with therapeutic ultrasound onto a blood clot.

Conclusion
We are thrilled to present an innovative synthesis protocol of MBS allowing echogenic and targeting properties for the treatment of thrombotic diseases. Perspectives result in the incorporation of the therapeutic agent [4] to allow its release on the targeted site and enhance thrombolysis with US insonation associated with the biochemical action of the plasminogen activator.

Acknowledgement
This work is supported by the ANR-20-CE18-0005-01 FightClot and ED MTCI doctoral school grant 2019.
Ultrasound signal of MBs under SVD filter in agar gel in vitro
MBs were guided through an agar gel and recorded with an ultrasound transducer. Matlab image processing allowed the reduction of agar gel signal (SVD: singular value decomposition). Parameters 35 dB, MB concentration of $10^8$ ml$^{-1}$.

References
Enhanced Endothelization on Nanostructured 316L Stainless Steel for Cardiovascular Stent Application

**Yasar K. Erdogan**\(^1,2\), Batur Ercan\(^1,3\)

\(^1\) Middle East Technical University, Department of Biomedical Engineering, Ankara, TR; \(^2\) Isparta University of Applied Science, Department of Biomedical Engineering, Isparta, TR; \(^3\) Middle East Technical University, Department of Metallurgical and Materials Engineering, Ankara, TR; \(^4\) Middle East Technical University, BIOMATEN, METU Center of Excellence in Biomaterials and Tissue Engineering, Ankara, TR

**Introduction**

Coronary artery disease is one of the most leading causes of death in the world. 316L stainless steel was widely used to fabricate cardiovascular stents to open up clogged arteries due to its mechanical properties, corrosion resistance and acceptable biocompatibility [1]. However, its major drawback is the nickel ion release from its surfaces which may lead to toxic and allergenic effects. Additionally, the use of bare metal can lead to stent restenosis due to limited endothelization and thrombus formation. To minimize the aforementioned pitfalls of using 316L stainless steel stents surface modification techniques have been adapted in recent years [2,3,4]. In this study, we were fabricated oxide based nanostructures on 316L stainless steel via electrochemical anodization technique.

**Experimental Methods**

Prior to anodization, samples were cleaned in acetone, ethanol and deionized water for 10 min, respectively. The anodization was performed in ethylene glycol monobutyl ether solution containing 7.5 vol % perchloric acid applied potential at 15–90V voltage. Surface chemistries were examined using X-ray photoelectron spectroscopy. Roughness of the surfaces investigated using atomic force microscopy. Contact angle measurement were performed using sessile drop method of the surfaces by goniometer. The nickel ion release from surfaces were detected by ICP-MS. For cell culture experiments, HUVECs were seeded onto sterile 316L stainless steel samples at a density of 10 000 cells/cm\(^2\) and cells were cultured up to 5 days in vitro under standard cell culture conditions (37 °C and 5% CO\(_2\) atmosphere). The 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was conducted at the 1st, 3rd and 5th days of culture to determine cellular density on the sample. For cellular imaging, HUVECs were seeded onto sterile 316L stainless steel samples at a density of 3 500 cells/cm\(^2\) and incubated at 37 °C/ 5% CO\(_2\) for 3 days. After 3 days in vitro, culture media were aspirated, and cells were fixed using a 4% paraformaldehyde solution for 20 min at room temperature. The fixed cells were permeabilized with 0.2% Triton X-100 for 30 min and blocked with 5% BSA for 30 min. When immunostaining for vinculin was complete, actin fibers were stained with red fluorescence phalloidin. Then, a 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) solution was added onto the samples. Cellular images were captured with a Zeiss confocal microscope (LSM800, Germany). Migration of HUVECs in vitro was measured using the wound healing assay. Briefly, cells were seeded in 24-well plates at a density of 1 x 10\(^5\) cells/cm\(^2\) and cultured in the 5% CO\(_2\) incubator at 37 °C overnight. Cell monolayers were then scratched using a 1000 µL sterile pipette tip. Images of the wound area were taken at 0, 4, 8 and 12 hours after the scratch with Zeiss confocal microscope. Image J software was used to measure scratch areas.

**Results and Discussion**

We successfully obtained nanostructures on the stent surfaces in two different morphologies namely nanodimples and nanopits. The feature size of these nanostructures were controlled between 20 to 300 nm in diameter. Water contact angle analysis showed that nanopit surfaces had more hydrophilic properties than nanodimple surfaces. While NP25 and NP200 nanopit surfaces had contact angles of 45.0±7.8° and 34.1±9.8°, ND25 and ND200 nanodimple surfaces had contact angles of 75.0±6.6° and 77.1±6.5°, and non-anodized surfaces had 57.7±7.1°. The surface roughness
of anodized samples were higher compared to non-anodized 316L stainless steel surfaces. The 200nm nanopit surface nanophase roughness values measured 18.6±2 nm while non-anodized surface roughness had 1.2 nm. XPS analysis results showed that nanopit samples were composed of Fe₂O₃, Cr₂O₃ and NiO while nanopit surfaces additionally had CrₓPᵧ and FeP. Furthermore, surface chemistry significantly affected nickel ion release from the surfaces and exhibited the lowest release in nanopit surfaces having 200nm diameter. In vitro tests revealed that nanostructured surfaces enhanced adhesion and proliferation of endothelial cells compared to non-anodized surface. In addition, fluorescence and scanning electron microscopy images revealed that endothelial cells were spreading more on anodized surfaces compared to non-anodized surfaces. Cellular migration experiments indicated faster endothelial cell migration on nanodimple and nanopit surfaces compared to non-anodized surface at 0, 4, 8 and 12 hr in vitro.

**Conclusion**

In this study, we were fabricated nanostructure on 316L SS by anodization technique. The nanotopography on surface influence cell-material interaction and improved hemocompatibility. Especially, nanopit surfaces having 200nm feature size is a promising candidate for rapid re-endothelialization, and minimized nickel ion release from 316L stainless steel surfaces for stent applications.

**Acknowledgement**

The authors would like to thank The Scientific and Technological Research Council of Turkey (Grant no: 118M652) for providing financial support. Also, the authors would like to thank METU Central Laboratory for XPS measurements and BIOMATEN for water contact angle analysis and confocal images.
[1] Huang, Q., Yang, Y., 2015, ‘Reduced platelet adhesion and improved corrosion resistance of superhydrophobic TiO2-nanotube-coated 316L stainless steel’, Colloids and Surfaces B: Biointerfaces, 125, 134-141,


Experimental and Computational Investigation of the Performance of Braided PLLA Stents

Agnese Lucchetti¹, Ted J. Vaughan², Thomas Gries¹

¹ RWTH Aachen University, Institut für Textiltechnik, Aachen, DE; ² National University of Ireland Galway, Biomechanics Research Centre (BMEC), School of Engineering, Galway, IE

Introduction

Despite advances in stent development over the past decades, issues such as thrombosis, in-stent restenosis and vessel remodeling impairment are still associated with permanent metallic stents [1]. Bioresorbable devices have the potential to overcome these drawbacks, being in the patient just for the time needed. Braiding is a well-established technology in the stent manufacturing and brings advantages especially in terms of a superior device flexibility and a major conformability to the anatomic shape of the lesion [2]. When it comes to device development, a full understanding of the influence of each manufacturing parameter and geometrical feature is of utmost importance. In this setting, an experimental study is carried out both to understand the influence of the most significant braiding parameters on the performance of PLLA braided stents and to validate the developed computational model that will be then used not only for further device optimization but also for investigating the device performance in more complex scenarios (e.g. patient specific vessels).

Experimental Methods

Experimental study: The PLLA stents were manufactured in-house using a Steeger Braiding Machine (Körting Nachfolger Wilhelm Steeger GmbH & Co. Wuppertal, Germany). The geometrical features under analysis were the stent diameter, number and diameter of the filaments and the braiding angle (α), as summarized in Figure 1a-b. Once braided, the device structure was fixed through a heat setting process, whose optimal conditions were found to be 140 °C and 10 min. The stents were characterized by means of a radial force test, performed according to DIN EN ISO standard 25539-2-2012. More specifically, all the stents were tested at a constant temperature of 37 °C and crimped to around 50% of their initial diameter. For the 5-mm diameter stent a comparison with a nitinol (NiTi) stent as published by McKenna et al. [3] was also considered.

Computational model: The stent geometry was reconstructed with PyFormex to resemble the 4/24/70/100 stent, more specifically featuring a 4-mm diameter, 24 filaments, a 70-degree braiding angle and a 100-µm filament diameter. The Abaqus/Explicit software (Dassault Systemes Simulia, Providence, RI, USA), was employed to mimic the radial compression bench test. The device was discretized with 4896 B31 elements. The PLLA was modelled as an elastoplastic material and its mechanical properties were determined from tensile testing of filaments. Ten crimping plates were modeled as rigid bodies to resemble the radial force testing machine. These were displaced to crimp the stent by 50% and subsequently released, as to mimic the experimental test. A friction coefficient of 0.1 and 0.05 was considered for the stent self-contact and the stent-plates contact, respectively.

Results and Discussion

Experimental study: The experimental curves (Figure 2a-c) show that the radial behavior of the stents with lower braiding angle is far from the one of the NiTi stent. However, at a diameter of interest of 4 mm (dashed line in Figure 2a-c), two stents, namely 5/24/60/150 and 5/48/70/100 showed respectively a radial resistive force of 0.17 N/mm and 0.12 N/mm and a chronic outward force of 0.047 N/mm and 0.031 N/mm; therefore, higher or comparable with the NiTi device. For smaller diameters, the stents show as expected higher forces (Figure 2d), but also a higher residual elongation, up to 20%. This may be linked to the bigger influence of friction among the filaments for such small calibers.
Computational model: The model developed up to now properly catches the initial stent stiffness, while it slightly overestimates the stent radial resistive force (max. 10%) in a range of interest of 2.5-3 mm (Figure 2e). The lower hysteresis of the computational curve compared to the experimental one can be linked to the friction between the device and the plates.

Conclusion
This study showed that the mechanical performance of PLLA braided devices subjected to a radial load is in a similar range to the one of the NiTi stent for specific braided configurations. Further studies will be carried out with the aim of finding the right combination of braiding parameters and pattern to further bridge the gap between permanent and bioresorbable devices also with the help of the validated computational model.

Acknowledgement
This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 813869. This publication reflects only the author’s view and the REA is not responsible for any use that may be made of the information it contains.

The authors would like to thank Boston Scientific Ltd., Co. Galway, Ireland for their support and collaboration on this work.

<table>
<thead>
<tr>
<th>Sample parameter</th>
<th>Diameter [mm]</th>
<th>Element count [x]</th>
<th>Bending angle [°]</th>
<th>Pitched distance [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5-41 100</td>
<td>5</td>
<td>24</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td>5.5-45 100</td>
<td>5</td>
<td>40</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>5.5-46 100</td>
<td>5</td>
<td>40</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>5.5-48 100</td>
<td>5</td>
<td>40</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>5.5-49 100</td>
<td>5</td>
<td>40</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>5.5-51 100</td>
<td>5</td>
<td>40</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>5.5-53 100</td>
<td>5</td>
<td>40</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>5.5-55 100</td>
<td>5</td>
<td>40</td>
<td>75</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 1
(a) Summary table of the different braiding parameters investigated; (b) example of manufactured stents and detail of the braiding patterns.
Figure 2
(a-c) Radial test results for the 5-mm stents featuring different manufacturing parameters; (d) radial test results for the 4 and 2.5-mm stents and (e) comparison of the computational and experimental force-displacement curves with a detail of the uncrimped (i) and crimped (ii) stent configurations.

References
Preliminary Trials of Idealised Crimping Test Rig for Bioresorbable Vascular Scaffolds

Jordan J. Barr, Gary Menary, Alex Lennon

Queen's University Belfast, Belfast, GB

Introduction
Coronary heart disease (CHD) is the leading cause of death worldwide\(^1\). Current treatment with metallic stents, leaves the treated vessels permanently caged and leads to long term adverse effects. Polymeric bioresorbable vascular scaffolds (BVS) are a promising alternative which negate the problem of permanent caging by being fully resorbed into the body after performing their function. Currently the polymers used in BVS are limited by their mechanical properties being lower than that of their metallic counterparts. This leads to increased BVS strut thickness and similar long term adverse events\(^2\).

Understanding and tailoring the processing steps of BVS may hold the key to solving this problem. While some of the processing steps have been heavily researched, the crimping process has been considered in much less detail. Crimping involves the radial compression of an as-cut scaffold onto a balloon catheter\(^3\). During this process the BVS undergoes significant deformation, particularly at the U-bend tips. While there is agreement that changes in morphology and molecular orientation occur due to the deformation, the conclusions drawn are conflicting with some authors attributing the lasting strength of BVS to these changes\(^3\) and others attributing it to increased localised degradation and premature failure\(^4\).

Crimping takes place within an enclosed environment and therefore analysis of the deformation occurs post-crimping. An idealised crimping test rig and method has been proposed which will replicate the crimping process and allow opportunity for analysis during crimping such as digital imaging correlation or x-ray scattering techniques. This test rig, its setup, and initial trials are the focus of the work presented here.

Experimental Methods
The crimping process has been idealised by focusing on an individual U-bend. Individual U-bend specimens were produced from Poly(l-lactide) (PLLA), PURASORB PL 38 (Corbion, Amsterdam, Netherlands). Initially, 1mm thick sheets were extruded. These were then biaxially stretched, using a custom built biaxial stretcher\(^5\) to replicate the stretch blow moulding process in BVS production, to a thickness of ~150 µm. The U-bend specimens were then laser cut from these thin sheets which are representative of current BVS strut thickness and dimensions.

The test rig, Figure 1, idealises the crimping process by imposing a displacement on the U-bend in one plane of motion. Computational modelling has shown the stresses and deformation experienced during this process to be representative of those experienced during modelling of a full crimping process. The U-bend specimens are clamped in the grips, with one stationary grip connected to a Honeywell FSG 0-5N load cell (Honeywell, Nort Carolina, USA) and one moving grip which imposes the deformation through the linear motion of a Zaber X-LSM025A motorised linear stage (Zaber, Vancouver, Canada).

Results and Discussion
Initial trials seen in Figure 2, show promising results with accurate displacements being imposed, while improvements in the laser cutting process, replication of crimping deformation and recording of load data are all currently a work-in-progress. Further modification of the rig to replicate the crimping process more fully will involve, the addition of an oven-like chamber around the specimen to maintain the temperature. With the rig fully functional, a Design of Experiments approach will be taken to compare the impact of both biaxial and crimping processing factors such as temperature, strain rate, stretch ratio and crimping rate. The incorporation Page 1307 of 2028
of DIC techniques present a challenge due to the small size of the specimens and will require work to find an appropriate speckling technique.

**Conclusion**

The idealised crimping method provides an opportunity for greater analysis of BVS during the crimping process and through this, a greater understanding which can be used to help optimise and produce more useful BVS in the future.

![Figure 1](image1.png)

**Figure 1**
Idealised crimping test rig model indicating the key components. Call out showing U-bend in its (A) uncrimped state and (B) post-crimping deformed state.

![Figure 2](image2.png)

**Figure 2**
U-bend specimen in (A) uncrimped state and (B) crimped state.

**References**


Small diameter artery scaffolds via additive manufacturing of thermoplastics and pro-angiogenic hydrogels

Julia Fernández-Pérez¹, Kenny van Kampen¹, Rebeca Rivero¹, Patricia Y.W. Dankers², Carlos Mota¹, Matthew B. Baker¹, Lorenzo Moroni¹

¹ Maastricht University, MERLN Institute, Maastricht, NL; ² Eindhoven University of Technology, Department of Biomedical Engineering, Eindhoven, NL

Introduction

Millions of people worldwide suffer from coronary diseases. Patients are commonly treated with a bypass using autologous tissue, usually from the saphenous vein or the internal thoracic artery. These procedures are not always possible due to insufficient tissue availability and also may induce secondary site morbidity. Currently developed tissue engineered grafts rely on lengthy culture periods, which are costly and not always off the shelf. It is for these reasons that in situ tissue engineering approaches are gaining interest. These scaffolds are intended to be implanted acellularly and encourage the body to repopulate and remodel them over time.

Experimental Methods

In this study, we have fabricated scaffolds by Additive Manufacturing of polycaprolactone (PCL), PCL-6UPy₂-urethane or polycarbonate(cyclohexyldiamide) onto a rotating mandrel as described by van Kampen et al. [1]. The tubular structures were produced with variety of fiber alignments by modifying the extruder speed. These scaffolds supported the adhesion and proliferation of HUVECs and human vascular smooth muscle cells in vitro. Cells repopulating the media layer aligned along the fibers and laid their own extracellular matrix and the intima layer consisted of a fully endothelialized lumen. To improve the mechanical properties of these constructs, an external layer was added with sine auxetic patterns, which intend to replicate the crimped collagen in the vessel wall.

Results and Discussion

Furthermore, a pro-angiogenic hydrogel was developed to emulate the adventitia layer. Two hydrogels systems have been designed: one based on alginate-norbormene using a di-thiol MMP-cleavable sequence as cross-linker and one using alginate-hydrazone and oxidized alginate. The first system is degradable by cell proteolytic activity and is composed of static covalent cross-links, while the second system exhibits viscoelastic properties due to its dynamic covalent cross-linking. Both systems were functionalized with the VEGF-mimicking QK peptide and with cell-adhesion molecules (fibronectin-derived RGD and/or laminin-derived YIGSR). The angiogenic capacities of these hydrogels was tested in 3D culture of HUVEC cells and in a rat aortic ring ex vivo model.

Conclusion

Future work will focus on combining the polymeric scaffolds with the hydrogels to obtain a multilayered construct.

Acknowledgement

This work is supported by the partners of RegMedXB and powered by Health-Holland, Top Sector Life Sciences & Health.

References

3D Bioprinting of Cardiac Pacemaker Cells - Paving the Way Towards Functional Pacemaker Constructs

Sophie Kussauer\textsuperscript{1,2}, Christian Polley\textsuperscript{3}, Phillip M. Barkow\textsuperscript{3}, Thomas Distler\textsuperscript{4}, Rainer Detsch\textsuperscript{4}, Praveen Vasudevan\textsuperscript{1,2}, Dominik Schneidereit\textsuperscript{5}, Oliver Friedrich\textsuperscript{5}, Aldo R. Boccaccini\textsuperscript{4}, Robert David\textsuperscript{1,2}, Hermann Seitz\textsuperscript{2,3}

\textsuperscript{1} University Medical Center Rostock, Department of Cardiac Surgery, Rostock, DE; \textsuperscript{2} University of Rostock, Department of Life, Light and Matter, Rostock, DE; \textsuperscript{3} University of Rostock, Chair of Microfluidics, Rostock, DE; \textsuperscript{4} University of Erlangen-Nuremberg, Institute Biomaterials, Department of Material Science and Engineering, Erlangen, DE; \textsuperscript{5} University of Erlangen-Nuremberg, Institute of Medical Biotechnology, Department of Chemical and Biological Engineering, Erlangen, DE

Introduction

Cardiovascular diseases, which are the most common cause of death worldwide, also affect the cardiac conduction system. Dysfunctions, such as the sick sinus syndrome (SSS), require adequate treatment strategies of high relevance. Engineered cardiac tissues could offer a smart alternative to existing therapies (established electronic pacemaker) through a superior adaptation to the body, especially in infants, or when functioning as \textit{in vitro} models for drug development. 3D bioprinting of cardiomyocytes has been widely used to generate contracting cardiac patches and organoids using either isolated primary cardiomyocytes (CM) or iPSC-derived CMs \cite{1-2}. In contrast, pacemaker cells have not yet been applied for bioprinting of organoid constructs with tailored biological and mechanical properties.

Experimental Methods

Against this background, we have bioprinted 3D constructs containing fully characterized ESC-derived pacemaker cells \cite{3-4}, which were enriched with fibroblasts as a basis for future repair of SSS and drug testing applications. The cell mixture was suspended in an oxidized alginate-gelatin (ADA-GEL) hydrogel \cite{5} and 3D printed with an Allevi 1 Bioprinter (Allevi, Philadelphia, USA) into 3D scaffolds. The scaffolds were ionically and enzymatically crosslinked using CaCl\textsubscript{2} (0.1 M) solution combined with microbiological transt glutaminase (0.025 g/ml). 3D bioprinted constructs were cultivated under standard tissue culture conditions (37°C and 5 % CO\textsubscript{2}). Structural and functional measures were performed after 0, 7, 14, 21, and 28 days. We examined the integrity of our printed pacemaker constructs by analyzing the morphology and cell-material interaction (light microscopy), the expression of specific marker proteins (immunohistochemistry), the presence of ordered myofibrillar sarcomeres via second harmonic generation (SHG) imaging, as well as spontaneous contractility (beating frequency) and underlying electrophysiological mechanism via calcium imaging.

Results and Discussion

3D scaffolds containing living pacemaker cells and fibroblasts were successfully printed. Until 28 days after printing, cultivation and characterization revealed both functionality of pacemaker cells and proliferation of fibroblasts. Light microscopic video recordings with a corresponding evaluation of the beating frequency showed spontaneous contractions of pacemaker cells and clusters in a physiological range. Additionally, the flux of Ca-ions during spontaneous activity could be observed, supporting the functionality of the pacemaker cells. On the microscopic level, sarcomeric, contractile structures could be identified using SHG multiphoton imaging. A high cellular attachment within the cell biomaterial system could be achieved. Vimentin-expressing fibroblasts exhibit a typical elongated morphology, whereas troponin-positive pacemaker cell clusters showed cell protrusions and partly spindle-shaped single cells.
Conclusion
Overall, the study shows that highly specialized cardiac cells such as pacemaker cells of the sinus node can be successfully 3D printed using ADA-GEL hydrogel. This approach opens up new strategies in cardiovascular regenerative medicine with a high potential for tissue engineering of biological pacemakers and the development of advanced drug testing models.

Acknowledgement
S.K. and C.P. contributed equally to this work. This research was funded by Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – SFB 1270/1 – 299150580 and the European Structural Fund (ESF/14-BM-A55-0024/18).

References
PS1-09-319

In-Silico Assessment of the impact of material Thrombogenicity in a Left-Ventricular Assist Device

Hristo Valtchanov\textsuperscript{1}, Rosaire Mongrain\textsuperscript{1}, Renzo Cecere\textsuperscript{2}

\textsuperscript{1} Mcgill University, Mechanical Engineering, Montreal, CA; \textsuperscript{2} Mcgill University Health Centre, Cardiology, Montreal, CA

Introduction
Non-physiological flow conditions in biomedical devices with blood contacting surfaces result in blood damage in the form of hemolysis – damage to blood cells- and thrombosis – the formation of blood clots that can occlude blood flow or contribute to the risk of stroke and thromboembolism \textsuperscript{1}. A recent prospective study\textsuperscript{2} has counter-intuitively found that incorporation of hemocompatible materials in blood exposed devices has no impact on rates of thrombosis, stroke or infection, despite in-vitro evidence to the contrary\textsuperscript{3}. This study assesses the impact of hemocompatibility – measured as the rate of adhesion of activated platelets to a given biomaterial – on thrombogenicity relative to other design parameters such as flow rate, rotor speed and geometry.

Recent studies have demonstrated the ability of computational fluid dynamics to simulate the intrinsic blood coagulation process at the macroscale with high accuracy.\textsuperscript{4} The ability to assess hemocompatibility in-silico can be used to address this question by simulating conditions difficult to reproduce in a lab environment. Hence simulation is useful in assessing the practical efficacy of material choice of blood exposed devices in reducing thrombosis relative to geometry and operating conditions.

Experimental Methods
CFD simulations are executed of a left-ventricular assistive device (LVAD), in which material hemocompatibilities, flow rate and geometric features are varied in a quasi-random manner. A reduced model of the intrinsic coagulation process is incorporated following the methodology of Wu et al., \textsuperscript{5}, in which 10 Advection-Diffusion-Reaction equations are used to simulate platelets in the active/inactive, non-deposited/deposited, and stabilized states, as well as the production, usage or release of Thrombin, Prothrombin, Antithrombin, Adenosine Diphosphate, and Thromboxane. Hemocompatibility is accounted for by setting a rate of platelet deposition at the wall, which is a function of the material of the blood-exposed surface.

For the purposes of this study, a constant rate of platelet deposition is used, which is varied to represent material hemocompatibility of various materials. In addition, inflow rate, LVAD rotor speed, and geometry are varied. LVAD geometry is based on an open-hub LVAD being developed at McGill university, and geometric variations are limited to the blade angle and number of blades in the rotor section sections (which can cause large variations in wall shear-stress). Simulations are executed in an automated way using a parameterized CAD geometry. Parameter values are chosen using the SOBOL algorithm which randomly samples points in a hypercube in an equidistant manner.

Results and Discussion
Simulations are performed for mass flow rates between 1-5 L/min, rotor speeds varying between 1000-8000 RPM, and thrombogenicity ranging between nitinol (high thrombogenicity) and PVC (low thrombogenicity) [goodman]. Figure 1 shows the formation of a thrombus in a RPM case on a baseline geometry using the PVC material.

Conclusion
CFD simulations were executed in order to simulate thrombogenesis in a left-ventricular assist device. Material hemocompatibility was varied, as well as operating conditions and rotor geometry to establish sensitivity of material selection relative to other key design parameters. It was found that operating conditions played the most significant role in determining whether thrombogenesis occurred, followed by material hemocompatibility.
Figure 1
Thrombus formation in an open hub LVAD at 8000 RPM using nitinol.

References
2:30 p.m. – 4:00 p.m.

Poster floor

**PS1-10 | Biomaterials for Drug Delivery**
Nanostructured dense collagen-polyester composite hydrogels as amphiphilic platforms for drug delivery

Xiaolin Wang¹,², Olivier Ronsin³, Nicolette Farman⁴, Tristan Baumberger³, Frédéric Jaisser⁴, Thibaud Coradin¹, Christophe Helary¹

¹ Sorbonne Université, Laboratoire de la Chimie de la Matière Condensée, Paris, FR; ² Macau University of Science and Technology, School of Pharmacy and State Key - Laboratory of Quality Research in Chinese Medicine, Macao, CN; ³ Sorbonne Université, Institut des NanoSciences de Paris, Paris, FR; ⁴ Sorbonne Université, INSERM - Centre de Recherche des Cordeliers, Paris, FR

Introduction
Collagen based hydrogels are broadly used in tissue engineering as they are biocompatible, biodegradable and are the natural support of cells. In many reported cases, collagen hydrogels were fabricated from low concentrated solutions and suffered from several limitations such as poor mechanical properties and fast degradability. To circumvent these limitations, dense collagen hydrogels have been developed to increase the hydrogel stability and enhance the mechanical properties. Unfortunately, these biomaterials are poor drug delivery systems due to the poor affinity between the therapeutic molecules and the collagen network, thereby limiting their utilization to prevent infection or modulate inflammation¹. Associating collagen with biodegradable hydrophobic polyesters constitutes a promising method for the design of medicated biomaterials. Current collagen-polyester composite hydrogels consisting of pre-formed polymeric particles encapsulated within a low concentrated collagen hydrogel suffer from poor physical properties and low drug loading.

Herein, an amphiphilic composite platform associating dense collagen hydrogels (40 mg/mL) with FDA approved polyesters was developed. Polymers with different hydrophobicity (PLGA, PLA, PCL) and chain length (from 7 to 60 KDa) were tested. An original method of fabrication was disclosed based on in situ nanoprecipitation of polyesters impregnated in a pre-formed 3D dense collagen network². Physical and mechanical properties of composite hydrogels were analysed with regard to the polyester nature and chain length. In addition, their ability to deliver a lipophilic model drug (Spironolactone) in a sustained and controlled manner was also evaluated.

Experimental Methods
Dense collagen hydrogels were fabricated by placing concentrated collagen solutions at 40 mg.mL⁻¹ under ammonia vapours. After several rinses, hydrogels were dehydrated using Tetrahydrofuran (THF) baths and incubated overnight in polyester and spironolactone solutions. PLGA, PLA and PCL with different chain lengths (from 7 to 60 kDa) were used. Then, the nanoprecipitation within the collagen network was triggered by incubation in PBS (Figure 1). First, the quantity of polyesters immobilized within hydrogels and the swelling properties were assessed. Then, the ultrastructure of composites was analysed by scanning and transmission electron microscopy. Their mechanical properties were evaluated by rheology. Last, the drug release kinetic from composites was studied over one month and their cytotoxicity evaluated on fibroblasts using a live/dead assay.

Results and Discussion
The nanoprecipitation enabled the immobilization of a large amount of polyesters, reaching 50 % of the hydrogel dried weight for PLGA 60 kDa. The polymer hydrophobicity negatively impacted the nanoprecipitation whereas the long chains improved the polymer immobilization. The presence of polyesters negatively impacted the swelling properties. However, all composite hydrogels exhibited a high degree of hydration (80%). When PLGA was used, the hydrogel stiffness was increased but its deformability dropped. On the opposite, PLA or PCL did not modify the hydrogel mechanical properties compared to pure collagen. The ultrastructure analysis
revealed the presence of polydisperse nano/microparticles at the surface of collagen fibrils. These particles were smaller for PLGA and more intimately linked to collagen. These results suggest a strong interaction between PLGA and the collagen network.

Compared to pure collagen hydrogels, the drug loading in composites was 5 times higher and similar in all composites. The release kinetic of spironolactone was quasi constant for PLGA 7kDa and PCL 14 kDa over the first two weeks. Unlike pure collagen hydrogels, no burst release was detected (Figure 2B). For PLGA and PLA, the chain length negatively impacted the drug delivery. The drug release relied on diffusion for PCL and PLA whereas a combination and erosion was involved in drug release for PLGA. The released drug from composites retained its activity, thereby evidencing the absence of degradation during the composite synthesis.

Hence, the choice of polyester allows to tune the hydrogel stiffness, its deformability and the drug release kinetic of lipophilic drugs. To complete this study, cell viability experiments showed the absence of cytotoxic effect of composites hydrogels on fibroblasts and keratinocytes regardless of the polyester used.

**Conclusion**

Taken together, composite hydrogels fabricated by nanoprecipitation are promising biomaterials. The ease of fabrication, wide range of accessible compositions and positive preliminary safety evaluations of these collagen-polyesters will favour their translation into clinics in wide areas such as drug delivery and tissue engineering.

**Acknowledgement**

This work received financial support from the National Research Agency of France (ANR, No.14-CE16-0010) and the Science and Technology Development Fund of Macao (FDCT 0009/2019/A, 0083/2019/A2).

We would like to thank Agathe Ribas, Claudia Almeida, Charles Marcelin and Julien Bachelet for their precious help in this project.
Properties of collagen/polyester composite hydrogels

Molecular weights of polyesters (A). Ultrastructure (B), mechanical properties (C) and drug release profile (D) of collagen/polyester composite hydrogels.

References


PS1-10-323

P28-functionalized Gefitinib-loaded PLGA nanoparticles inhibit lung cancer progression \textit{in vivo}

Ana R. Garizo$^{1,2}$, Flávia Castro$^{2,3}$, Andreia Almeida$^{2,3}$, Nuno Bernardes$^{1,4}$, Cláudia Martins$^{2,3}$, Tiago Dias$^1$, Cristina Barrias$^{2,3}$, Arsénio M. Fialho$^{1,4}$, Bruno Sarmento$^{2,5}$

$^1$ iBB-Institute for Bioengineering and Biosciences, Biological Sciences Research Group, Lisboa, PT; $^2$ i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, PT; $^3$ INEB-Instituto de Engenharia Biomédica, Universidade do Porto, Porto, PT; $^4$ Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, PT; $^5$ CESPU-Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Gandra, PT

Introduction

Lung cancer is still the main cause of cancer-related deaths worldwide, making up almost 20% of all cancer deaths (GLOBOCAN, 2020). The most common type is non-small-cell lung cancer (NSCLC) and its treatment includes surgical resection, chemoradiotherapy and targeted therapy. The discovery of key oncogenic events in NSCLC patients, like the mutations on epidermal growth factor receptor (EGFR), have improved the therapeutic strategy, with the introduction of tyrosine kinase inhibitors, such as Gefitinib (GEF) (1). Nevertheless, its poor solubility in gastric fluids weakens bioavailability and therapeutic activity (2). Therefore, the development of novel GEF delivery systems to increase its bioavailability and distribution in tumor site is highly demanded.

Here, we designed an innovative strategy for GEF delivery, by functionalizing PLGA nanoparticles (NPs) with p28, a cell-penetrating peptide derived from Azurin, a bacterial protein which have both demonstrated largely their multi-targeted anticancer activity \textit{in vitro} and \textit{in vivo} (3). Additionally, their simultaneous administration with anticancer drugs increased the drug effectiveness, leading to the reduction of dose-related toxicity (4). These findings support to the design of a p28-functionalized delivery system to effectively penetrate the membranes of cancer cells while deliver GEF.

Experimental Methods

The conjugation of p28 peptide to poly(lactic-co-glycolic acid) (PLGA) polymer was performed by maleimide-thiol click chemistry and was evaluated by HPLC, before the formulation of polymeric NPs by nanoprecipitation. The nanosystems physical-chemical characteristics were evaluated by dynamic light scattering and laser doppler anemometry. The cytocompatibility of empty NPs, anticancer potential of NPs-p28-GEF and respective controls (NPs-GEF and NPs-p28) were evaluated using A549 NSCLC cells through resazurin assay. Further the cancer cells targeting ability of NPs-p28-GEF was evaluated by flow cytometry and confocal imaging. Lastly, their therapeutic potential was evaluated \textit{in vivo} using a A549 xenograft model through intravenous and subcutaneous administration.

Results and Discussion

The conjugation efficiency was around 80% and upon production, the physical-chemical characteristics of the PLGA-PEG-p28 nanoparticles were 78nm±2 for size; 0.14±0.01 for polydispersity index (PDI) and -17mV±1 for ζ-potential. Our data indicated that p28 potentiates the selective interaction of these nanosystems with lung cancer cells (active targeting). Thus, the GEF anticancer drug was encapsulated in these NPs (size: 60nm±3; PDI: 0.23±0.03; ζ-potential: -17mV±1; GEF association efficiency: 42%±2) showing a decrease in A549 cell metabolic activity around 25% compared to non-functionalized GEF encapsulated nanoparticles (size: 58nm±3; polydispersity index: 0.22±0.01; ζ-potential: -15mV±1; GEF association efficiency: 47%±1; GEF loading: 3±1). Regarding \textit{in vivo} studies, while NPs-p28 and NPs-GEF decrease A549 tumor growth in 42% and 36%, respectively, NPs-p28-GEF presented a higher
anticancer effect, by reducing the tumor burden in 58% comparing to non-treated animals, which suggest a synergistic effect of p28 and GEF.

**Conclusion**

This work supports that p28 peptide on the surface of NPs can efficiently direct target cancer cells, minimizing the cytotoxic effects on healthy tissues caused by non-specificity of drug administration alone. Importantly, this delivery system could provide a promising strategy for chemotherapy, able to inhibit lung tumor progression in vivo.

**Acknowledgement**

This work presented was financed by Portuguese funds through FCT - Fundação para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior in the framework of the project "Institute for Research and Innovation in Health Sciences", UID/BIM/04293/2019, and also through funding received by Institute for Bioengineering and Biosciences (IBB) from FCT (UID/BIO/04565/2020). ARG thanks FCT for the PhD fellowship (SFRH/BD/122636/2016). FC acknowledges Fundação para a Ciência e a Tecnologia (FCT), Portugal for the financial support in the framework of the project “p28Nano - Cell penetrating p28 peptide-mediated delivery of nanomedicines for cancer treatment” (PTDC/BTM-SAL/30034/2017).

P28-functionalized GEF-loaded PLGA nanoparticles development and in vivo validation.

(A) Scheme of composition of PLGA-p28-Gef NPs, prepared by nanoprecipitation. (B) NPs were analyzed by transmission electron microscopy. (C) Experimental timeline of in vivo experiments. CBA nude mice, injected with A549 cells, were intravenously treated with saline solution (CTR) NPs-p28, NPs-GEF, NPs-p28-GEF and free GEF, 3 times/week, for 2 weeks. (D) The percentage of tumor growth was normalized to each tumor values at the first treatment. (E) Area under de curve for the period between day 16 and day 40.

**References**


Exploiting the Chemotherapeutic and Immunomodulatory Effect of a Glioblastoma-targeted Nanodrug Using a Novel Tumor Niche-recapitulating 3D Spheroid Construct

Claudia Martins1,2,3, Catarina Barbosa1, Marco Araújo1, Maria Oliveira1, Jonathan W. Aylott3, Bruno Sarmento1,4

1 University of Porto, Institute for Research and Innovation in Health, Porto, PT; 2 University of Porto, ICBAS - Abel Salazar Institute of Biomedical Sciences, Porto, PT; 3 University of Nottingham, School of Pharmacy, Nottingham, GB; 4 CESPU, Institute of Research and Advanced Training in Health Sciences and Technologies, Gandra, PT

Introduction
Glioblastoma (GBM) is the most common and lethal type of primary brain tumor. The 5-year survival of GBM patients is still limited to a dismal 5%, highlighting the need to advance more effective GBM therapies. GBM tissue presents an abnormal expression of the L-type amino acid transporter 1 (LAT1), for which histidine (His) is an inexpensive and powerful targeting ligand [1]. Although His is expected to provide higher accumulation of drug nanoparticles (NPs) into GBM cells via LAT1 binding, consequently enhancing the anti-tumor response, it has been poorly explored in GBM-targeted therapies. Thus, this project proposes GBM-targeted, His-functionalized polymeric NPs loaded with docetaxel as a therapeutic with capacity to induce immunogenic cancer cell death [2]. On the other hand, a recent paradigm shift in the field of GBM immune environment revealed that the majority of tumor-associated macrophages in GBM are infiltrating bone marrow-derived macrophages, and not microglia [3]. Therefore, the herein project also aims at providing a first-time developed donor-isolated macrophage/GBM crosstalk 3D spheroid construct to simultaneously study drug chemotherapeutic and immunomodulatory effects.

Experimental Methods
Carbodiimide and carbamate hydrolysis chemical strategies were employed to synthesize a polymeric conjugate based on poly(lactic-co-glycolic) acid (PLGA) and His-functionalized polyethylene glycol (PEG), to serve as the NP core and shielding, respectively. The PLGA-PEG-His polymeric conjugate was characterized by various techniques such as NMR, optical contact angle measurements, FTIR and MALDI-TOF MS. The PLGA-PEG-His polymeric conjugate was further used to manufacture docetaxel-loaded NPs, through a previously established microfluidic technique of high reproducibility and easy scaling up [4]. Docetaxel-loaded PLGA-PEG-His NPs were fully characterized for physicochemical properties. Regarding the 3D spheroid construct, agarose micro-molds were used for high-throughput spheroid assembly. GBM cell binding of unloaded PLGA-PEG-His NPs was evaluated by flow cytometry in different cell lines (U251, U373, U87) to select the best cell model for the spheroid construct core. Human monocytes were isolated from healthy blood donor buffy coats provided by São João Hospital (Portugal). The 3D spheroid construct was optimized for the optimal total cell density (2500, 5000 and 1000 cells/spheroid) and tumor cell:monocyte percentage (50:50, 35:65, 20:80, 5:95), and visualized by microscopic techniques using H&E staining and immunohistochemistry. A preliminary assay was run to investigate the chemotherapeutic effect of docetaxel-loaded PLGA-PEG-His NPs compared to the free drug control.

Results and Discussion
The chemical synthesis of the PLGA-PEG-His polymeric conjugate achieved 90% conjugation efficiency, as demonstrated by NMR; optical contact angle measurements indicated an intermediate PLGA-PEG/His hydrophilicity for the conjugate; FTIR confirmed an amide formation; MALDI-TOF MS revealed an unique ionization profile for the conjugate compared to the PLGA-PEG and His controls (Fig. 1A/B, C, D and E, respectively). Docetaxel-loaded PLGA-PEG-His NPs demonstrated scale-independent 250 nm size, 0.2 polydispersity index, 70% drug entrapment.
efficiency and a controlled drug release over 48 h. The GBM cell binding of unloaded PLGA-PEG-His NPs was 2.5-times higher than non-His-functionalized NPs in all tested cell lines. Regarding the 3D spheroid construct, U251 was selected as the tumor cell model, no H&E necrosis was observed in all tested total cell densities, and only the 50:50 and 35:65 tumor cell:monocyte percentage conditions assembled into a spheroid. Immunohistochemistry revealed the spatial distribution of tumor-associated vimentin, extracellular matrix fibronectin and CD68 macrophage marker within the 3D spheroid construct (Fig. 2A). Docetaxel-loaded PLGA-PEG-His NPs drastically disturbed the morphology of the spheroid tumor core, suggesting a significantly higher level of cytotoxicity compared to the same dose of the free drug control (Fig. 2B).

Conclusion
This work has allowed the exploitation of His functionalization to synthesize cost-effective GBM-targeted NPs with capacity to undergo a significantly higher accumulation within tumor cells and disrupt the tumor core of a first-time proposed donor-isolated macrophage/GBM crosstalk 3D spheroid construct. Ongoing work is expected to open avenues regarding the immunogenic properties of the docetaxel-loaded PLGA-PEG-His by studying spheroid macrophage M1/M2 polarization.

Acknowledgement
Grant SFRH/BD/137946/2018 from the Portuguese Foundation for Science and Technology (FCT).
First-time developed donor-isolated macrophage/GBM crosstalk 3D spheroid construct. (A) Spatial distribution of tumor-associated vimentin, extracellular matrix fibronectin and CD68 macrophage marker. (B) Chemotherapeutic effect of docetaxel-loaded PLGA-PEG-His NPs compared to the free drug control.

References
Determination of Gentamicin Sulfate Release from the Calcium Phosphate Bone Cements

Eliza Tracuma\textsuperscript{1,2}, Janis Locs\textsuperscript{1,2}, Marite Skrinda\textsuperscript{1,2}, Dagnija Loca\textsuperscript{1,2}

\textsuperscript{1} Riga Technical University (RTU), Institute of General Chemical Engineering, Rudoffs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Faculty of Materials Science and Applied Chemistry, Riga, LV; \textsuperscript{2} Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, LV

Introduction

Gentamicin sulphate (GENTA) is common antibiotic of choice due to its wide range of antibacterial spectrum against gram-negative and gram-positive bacteria (MIC 0.5-8 µg/mL), low level of resistance and rapid concentration-dependent bactericidal effect. Local GENTA concentration for prophylaxis against Staphylococcal species (spp.) must be in the range of 128 µg/mL to 200 µg/mL (MIC\textsubscript{90}%) [1], [2], [3]. Antibiotic loaded calcium phosphate bone cements (CPC) are widely used in orthopedic, while local antibiotic delivery helps to reduce the possible surgical site infection risk and the side effects from systemic antibiotic therapy [2]. Furthermore, the combination of CPC and antibiotics can provide dual-effect - induce bone regeneration and simultaneously prevent bacterial infections in post-surgery period. In vitro antibiotic release studies in deionized water (diH\textsubscript{2}O) from six clinically available CPC demonstrated that GENTA cumulative release varied from 36% to 85% and not all of the drug could be extracted within period of 21 days [4]. Numerous analytical methods have been developed for the determination of GENTA content in water solutions, including ultra-performance liquid chromatography equipped with evaporative light scattering detector (UPLC-ELSD), ultraviolet–visible spectroscopy (UV-VIS), mass spectrophotometry etc. [2], [5].

The aim of the current study was to prepare controlled release GENTA delivery systems based on the CPC and evaluate the GENTA release profiles within the period of 14 days. During the study two analytical methods (UV-VIS and UPLC) were developed and their applicability for the determination of GENTA release from CPC was evaluated. Moreover, influence of CPC matrix on the determination of GENTA release profiles was assessed.

Experimental Methods

Drug delivery system was prepared through mixing the α-TCP powder with GENTA containing sodium salt solution. GENTA containing cements were set for 24 h and placed into 1 mL of PBS (pH=7.4) at 37 and 100 rpm. All dissolution media (1 mL) were taken at appropriate intervals (24, 48, 120, 216 and 336 h) and used for the determination of GENTA release kinetics. GENTA release profiles from the prepared CPC were studied via UPLC-ELSD at λ=650 nm, using Aquity UPLC BEH C18 column and UV-VIS at λ=332 nm, using GENTA derivatization with o-phthalaldehyde.

Results and Discussion

Obtained results indicated that although UPLC-ELSD method is linear, accurate, precise, and robust towards the GENTA content determination in water solutions, when it comes to the analysis of GENTA content in CPC, interfering background signals twice bigger as the signal for GENTA (C\textsubscript{1}) peek were observed, thus making the analytical method not applicable for the determination of GENTA content in CPC matrix. For that reason, UV-VIS method was applied and no interfering signals coming from CPC matrix were found. The cumulative GENTA release from CPC was characterized by a burst release within the first 24 h (4.4 ± 0.2%) followed by a gradual stabilization of released GENTA (8.2 ± 0.3% (120h) and 11.7 ± 0.5% (336h)). During the research it was found that GENTA has substantial affinity to the CPC matrix, strongly influencing the active substance release into the PBS.

Conclusion

During the research it was established that UPLC/ELSD method could not be used for the characterization of GENTA release profile, if the drug is incorporated in the CPC matrix. According to the obtained results, the amount of GENTA
released from CPC, clearly exceeded the MIC for microorganisms (0.5-8 µg/mL) during the period of 14 days and released GENTA concentration showed no risk of cytotoxicity (<30 µg/mL) [1], [2], [3]. It was observed that CPC have the tendency to absorb the drug molecules, leading to presumption that only a part of the drug can be extracted from the CPC matrix, most likely due to the sulphate ion bound to the CPC via the Van der Waals interactions.

Acknowledgement
This project has received funding under the grant agreement No.1.1.1.5/ERANET/18/01 (DD-SCAFF). The authors also acknowledge financial support from the European Union’s Horizon 2020 research and innovation programme under the grant agreement No 857287.

References
Chitosan functionalized with different ligands for the preparation of nanocarriers for active drug targeting

Marcel Popa1,2, Delia M. Rata1, Anca N. Cadinoiu1, Leonard I. Atanase1

1 Apollonia University of Iasi, Biomaterials, Iasi, RO; 2 Romanian Academy of Scientist, Bucharest, RO

Introduction
Conventional drug administration has well-known disadvantages: rapid variation of their concentration in the body or at the site of the disease, so that the duration of storage at the therapeutic level is short and requires repeated administrations, reaching high levels of concentration, can cause toxic side effects (cytotoxicity, neurotoxicity, nephrotoxicity) and fast development of cellular resistance, etc. The association of drugs especially with polymeric carriers, in nanoparticles (NPs) eliminates some of the disadvantages, ensuring a constant and longer release over time of the biologically active principle. However, even in these conditions, the systemic administration of drug carriers does not ensure the highest effectiveness due to reaching the affected areas only by passive target. Ensuring an active target is desirable and this effect can be obtained by achieving an interaction between the drug carrier and the site of the disease. Such an interaction can be performed if the receptor on the membrane of diseased cells is known, capable of recognizing a certain ligand on the surface of the drug carrier. Active drug targeting can be achieved by attaching specific ligands to the surface of nanocarriers that bind to specific molecules or cells. Generally, as specific ligands can be used: aptamers, antibodies, folic acid, and peptides.

Experimental Methods
To target respiratory disorders, CMCS was functionalized at the -COOH group by amidation with the amine group of two specific peptides, CGSPGWVRC and indolicidin (IN). The selection of the two peptides was made based on the fact that long endothelial cells have unique cell surface molecular characteristics that allow the binding of specific peptides, especially CGSPGWVRC peptides. The reaction took place first by esterification of CMCS with N-hydroxy succinimide (NHS) in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) followed by amidation with the peptides. The amidation reaction was confirmed by FTIR and H-NMR spectroscopies. The peptide grafting yield, in both cases, is in the range of 1 to 10% as a higher functionalization degree is not desired because it will sterically hinder the amine groups of CS which are essential for the synthesis of the N/MPSs. For the active targeting of tumor cells, the aptamer AS1411-NH2 (Apt-NH2), recognizable by the nucleolin - receptor -, intensely expressed in the tumor cell membrane, was used as ligand. CMCS was functionalized in the presence of the EDAC / NHS system, by amidation of the amino group from the aptamer to the -COOH group from the CMCS. Schematically, the reaction is shown in fig. 1.

Results and Discussion
The structure of the aptamer-functionalized carboxymethyl chitosan (CCA) was characterized by FTIR, 1H, and 13C NMR spectroscopy. For the determination of the elemental composition of the product a scanning electron microscope equipped with an EDAX elemental analysis system has been used. It was found that in the aptamer-functionalized carboxymethyl chitosan (CCA) there are approximately 11% AS1411-NH2 aptamer, as illustrated in fig 2.

Conclusion
The obtained results prove the successful functionalization of the CMCS with different ligands which can be further use as precursors for the preparation of nanocarriers for the active drug targeting.

Acknowledgement
This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS-UEFISCDI, project number PN-III-P4-ID-PCE-2020-2009, within PNCDI III.

**Fig 1**: Schematic representation of the chitosan functionalization

**Fig. 2**: EDAX spectrum for aptamer-functionalized carboxymethyl chitosan (CCA)
Tween 60 cationic niosomes for drug and gene delivery

Axel Kattar\(^1\), Natalia Carballo-Pedrares\(^2\), Carmen Alvarez-Lorenzo\(^1\), Angel Concheiro\(^1\), Ana Rey-Rico\(^2\)

\(^1\)Universidade de Santiago de Compostela, Santiago de Compostela, ES; \(^2\)Universidade da Coruña, A Coruña, ES

Introduction

Niosomes have gathered increasing interest in recent years as a self-assembled nanocarrier for transport and delivery of material to different human tissues\(^1\). The versatility of the particles associated to their stability make them an interesting choice for this purpose. Niosomes are able to encapsulate hydrophobic drugs such as epalrestat or form a nioplexe with DNA plasmids. The aim of this work was to explore the suitability of cationic niosomes for two different applications: topical ocular administration of epalrestat to counteract sorbitol accumulation in diabetic eyes\(^2\), and gene delivery towards immortalized human mesenchymal stem cells (iMSCs) for gene therapy\(^3\).

Experimental Methods

Niosomes were prepared combining Tween 60, cholesterol and DOTMA (7 and 15%) and applying a modified reverse phase evaporation method. The surfactant, helper lipid and charge modifier were dissolved in ethanol. Ethanol was removed leaving a thin film, which was subsequently hydrated with MilliQ water. Subsequently, the dispersion was sonified for 90 seconds to form niosomes. Empty niosomes can be loaded with epalrestat if it is added to the hydrating water or combined with plasmids to form nioplexes. Niosomes and their nioplexes with a reporter DNA plasmid (placZ) were characterized within a wide range of cationic lipid/DNA ratios. Transfection efficiency and cytotoxicity profiles from resulting nioplexes were evaluated in iMSCs cultures.

Results and Discussion

Niosomes loaded with epalrestat had a size of 104.5 nm, a PDI of 0.351 and a zeta potential of +15mV. The niosome size, PDI and zeta potential were stable over a month. Niosomes provided sustained release of epalrestat for several days. Empty niosomes complexed with a reporter DNA plasmid (placZ) had a size around 370 nm. Their transfection efficiency in iMSCs was comparable to the commercial reagent Lipofectamine while retaining a higher cell viability.

Conclusion

In both cases of drug delivery and gene delivery niosomes have particle size and surface charge to be considered as suitable carriers. Further experimentation will focus on permeation through ocular tissues for epalrestat loaded niosomes and the applicability of nioplexes in primary cell cultures of MSCs.

Acknowledgement

This project is funded by the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie Actions (grant agreement – No 813440). The work was also partially supported by MINECO [SAF2017-83118-R; RTI2018-099389-A-100], Agencia Estatal de Investigación (AEI) Spain, Xunta de Galicia [ED431C 2020/17], and FEDER.

References


Page 1328 of 2028
Multivalent fully biodegradable PEG-dendrimers as versatile nanotherapeutics: application in gene therapy

Victoria Leiro¹, Ana P. Spencer¹,², Natália Magalhães¹, Ana P. Pêgo¹,³

¹ i3S/INEB - Instituto de Inovação e Investigação em Saúde/Instituto de Engenharia Biomédica, Universidade do Porto, Porto, PT; ² FEUP – Faculdade de Engenharia, Universidade do Porto, Porto, PT; ³ ICBAS/FEUP, Universidade do Porto, Porto, PT

Introduction

Dendrimers are promising tools for the development of a plethora of nanotherapeutics, due to their intrinsic and unique structural characteristics: globular, well-defined, ultra-branched and controllable nanostructure, low polydispersity, and multivalency [1]. However, the repeated administration of non-degradable dendrimers can lead to toxicity due to their bioaccumulation [2]. In addition, in drug delivery applications, such as gene therapy, carrier stability can result in low biological performance due to an insufficient intracellular cargo (nucleic acid) release. Thus, biodegradable dendrimers have been eagerly awaited [3,4]. Here, we present a novel family of versatile, biosafe, water-soluble, and fully biodegradable PEG-dendritic nanosystems [5], which overcomes the limitations of the most used dendrimers, advancing the current state of the art of dendritic delivery systems and therapeutics. These new dendrimers display the possibility of multifunctionalization at both their dendritic arms and PEG end. Moreover, in this work, their function to serve as nucleic acid (siRNA) vectors for gene therapy is also thoroughly explored in several cell lines, including in hard-to-transfect neuronal cells even when in full tissue explants (dorsal root ganglia) [5b].

Experimental Methods

Fully biodegradable (fb) PEG-dendrimers were synthesized and later decorated at their arms, by click chemistry, with different amine groups. Moreover, these were further functionalized with a fluorescent tag (Cy5.5) at their PEG end, through a reactive functional group at their PEG end. All dendrimers and conjugates were characterized by NMR and FTIR.

siRNA dendriplexes were prepared at different N/P ratios (from 5 to 80). siRNA complexation ability of dendrimers was assessed by SybrGold® exclusion assay. The dendriplexes were characterized regarding size, polydispersion index (PdI) and zeta potential by dynamic light scattering, and their morphology was studied by transmission electron microscopy. Moreover, their ability to protect siRNA from endonuclease degradation was evaluated by polyacrylamide gel retention assay. Relative metabolic activity (resazurin), as well as haemolysis and coagulation assays in the presence of dendrimers and dendriplexes were evaluated in U2OS and ND7/23, and red blood cells, respectively. Cellular association/uptake and silencing assays were carried out in U2OS and ND7/23 cells expressing the fusion protein eGFP-Luciferase and evaluated by flow cytometry. Furthermore, their capacity to transfect embryonic dorsal root ganglion (DRG) explants was studied using confocal microscopy.

Results and Discussion

Generation 3 of fb PEG-dendrimers were successfully synthesized, characterized, and functionalized with: i) different amine moieties at their dendritic arms to allow the nucleic acid complexation; and ii) a fluorescent tag at the PEG end in order to be able to track their biological pathway by fluorescence techniques. This strategy ensures the suitable exposure of the corresponding PEG end-linked molecule to the surface of the dendritic nanosystems, and it is extrapolatable to many other molecules, such as targeting moieties. The developed amine-terminated dendrimers efficiently complexed and protected siRNA in dendriplexes showing sizes, PdI’s, surface charge and morphologies very suitable for cellular uptake, no toxicity in several cells and primary
neuronal cultures, and non-thrombogenic and haemocompatible character. Moreover, these fb nanosystems were successfully cell-internalized, including in hard-to-transfect neuronal cells even when in full tissue explants (dorsal root ganglia) (Figure 1). Importantly, full biodegradability was found crucial for an efficient nucleic acid intracellular release and attainment of excellent transfection efficiencies.

Conclusion

New versatile, biocompatible and fully biodegradable PEG-dendrimers, as well as their bio-function as nucleic acid vectors are presented. They allowed an efficient complexation and protection of siRNA, as well as a successful siRNA transfection in different mammalian cells, including hard-to-transfect primary neuronal cultures. Our fb dendritic nanosystems can be efficiently functionalized with different ligands at both their PEG end and dendritic arms, thus the present study puts forward then as versatile and targeted vectors for further developments in nanotheranostics.

Acknowledgement

FCT (PTDC/NAN-MAT/30898/2017); siRNAC (NORTE-01-0247-FEDER-033399).

References

Polyethylene glycol based-hydrogel synthesis and characterization for anti-inflammatory drug delivery

Sidzigui Ouedraogo¹, Arnaud Ponche¹, Laurent Pieuchot¹, Mathilde Grosjean², Benjamin Nottelet², Noëlle Mathieu³, Karine Anselme¹

¹ Université de Haute Alsace, Institut de Science des Matériaux de Mulhouse (IS2M), UMR 7361, Mulhouse, FR; ² Université de Montpellier, Institut des Biomolécules Max Mousseron (IBMM), UMR 5247, Montpellier, FR; ³ Institut de Radioprotection et de Sûreté Nucléaire (IRSN), Laboratoire de radiobiologie des expositions médicales (LRMed), Paris, FR

Introduction
Radiotherapy of abdomino-pelvic tumors induces in 20% of patient’s late side effects involving a chronic inflammation. This is accompanied by the depletion of the mucus layer and bacterial permeability [1]. No curative treatments are available. In the aim to provide a therapeutic solution to this inflammation, we develop a biodegradable self-rolled patch that could be applied locally by colonoscopy without surgery. This innovative biomaterial is based on the combination of hydrogel and elastomeric layers. The association of two different layers confers tunable mechanical and bio-resorption properties to the patch and allows the unidirectional release of anti-inflammatory drugs (AI): Budesonide and Prednisolone toward the ulcerated zone after its unrolling. Targeting anti-inflammatory drugs selectively to the inflamed zone may improve therapeutic outcomes and minimize systemic toxicity. Polyethylene glycol-based (PEG) hydrogels are choice biomaterials as scaffolds for drug delivery carries [2]. However, long-term drug release from PEG-based hydrogels remains challenging. Understanding the relationship between mesh size (ξ) and precursor solution composition is critical for the design of drug delivery hydrogel. Here we report a PEG-based hydrogel synthesis and nanostructure characterization to achieve long-term and efficient release of Prednisolone for the development of self-rolled patches.

Experimental Methods
An efficient solvent free microwave-assisted method is used to synthesize Poly(ethylene glycol) diméthacrylate (PEGDM) [3]. Proton nuclear magnetic resonance spectroscopy (H-NMR) is conducted to assess the efficiency of functionalization. Hydrogels are then formulated by photo-reticulation of the synthesized product (cure process parameters: UV (365 nm, 90µW/cm², 20min) in water with photo initiator Irgacure 2959). Particular attention is paid to modifying the molecular weight of PEG (6000 Da and 20,000 Da) and the concentration of PEGDM hydrogels to study the effects of these parameters on the nanostructure and the behavior of the hydrogels. For each molecular weight of PEG, three modalities of concentrations (10%, 30% and 50%) are selected. Equilibrium swelling ratios (Qs) of PEG-based hydrogels composition is measured and used in estimating the equilibrium polymer volume fraction (v²s) and mesh size (ξ) with equilibrium swelling theory at 37°C [4] [5]. To assess drug release, the formulated hydrogels are first loaded in concentrated drug solutions for AI impregnation. Then, loaded hydrogels are soaked in fresh PBS at 37°C for in vitro drugs release. The amount of Prednisolone release was quantified by Reverse Phase – Hight Performance Liquid Chromatography (RP-HPLC) coupled to UV light detection.

Results and Discussion
H-NMR analyses validate a simple, efficient, and fast method for the functionalization of PEG with methacrylate moieties with a chemical yield of more than 91% for the two sizes of linear PEG (6 and 20 kDa). Analysis of experimental data of the six formulated PEGDM hydrogels show a correlation between the mesh size of a swollen cross-linked network and its equilibrium polymer volume fraction (Figure 1) as established by Peppas and al. [5] on poly(vinyl alcohol) and poly(2-hydroxy ethyl methacrylate) networks.

Page 1331 of 2028
Formed hydrogels ensure an efficient AI encapsulation, with no chemical modification, and a release over seven days. Increasing hydrogel molecular weight improves the total amount of Prednisolone released (Figure 2).

**Conclusion**
Six different formulations of PEG-based hydrogels were synthesized and explored. The photo-crosslinked hydrogel synthesis protocol is validated.

This preliminary study provides a basis for the design of star-shaped block copolymer degradable hydrogels. The setting of the optimal mesh size is under study. Likewise, the work presented in this document will be also compared with the polymer-budesonide formulation.

**PEG-based hydrogels mesh size comparison**

Figure 1: A) Linear PEGDM functionalized monomer B) PEGDM20 kDa based hydrogel dried and swollen in water C) Influence of molecular weight and PEGDM hydrogels % on mesh size. Bars represent average ± standard deviation for n = 8 samples, statistical significance is tested by one way ANOVA with Tukey’s HSD post-hoc test.

**Cumulative release of Prednisolone from PEG-based hydrogels**

Figure 2: Release of encapsulated Prednisolone from hydrogels formed using 6 and 20 kDa 30% Irgacure 2959 0.1% molecular weight. Bars represent mean± standard deviation for n = 3 samples.
References


Development of Fibrin Matrices for Sustained Drug Delivery

Karina Egle\textsuperscript{1,3}, Ingus Skadins\textsuperscript{2}, Arita Dubnika\textsuperscript{1,3}

\textsuperscript{1} Riga Technical University, Institute of General Chemical Engineering, Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre, Riga, LV; \textsuperscript{2} Riga Stradins University, Department of Biology and Microbiology, Riga, LV; \textsuperscript{3} Headquarters at Riga Technical University, Baltic Biomaterials Centre of Excellence, Riga, LV

Introduction
Fibrin acts as a local reservoir for growth factors and as a provisional matrix to attract cells that support the regenerative process [1]. Commercially available fibrin matrices have an advantage of a simplified method for fibrinogen activation, bypassing the clotting cascade. Usually, a dual syringe applicator to combine fibrinogen solution with a thrombin-calcium chloride (CaCl\textsubscript{2}) solution is used [2]. Previous studies have not shown antibacterial efficacy for commercially available fibrin matrices. Infections are known to be a common phenomenon, so antibiotics are needed to reduce them. Because the resulting fibrin matrix is a good environment for microbial growth, the addition of antibiotics to one of the components of fibrin matrice has been shown to reduce postoperative infections [3]. The aim of this study was to find suitable parameters for obtaining a fibrin matrix that would retain its shape and cross-links over time. Also to study the effect of preparation technology on the properties of fibrin matrices and their ability to provide drug delivery. Two types of antibiotics were used in this study.

Experimental Methods
Samples were prepared from thrombin (from Bovine plasma), fibrinogen (from Bovine plasma) and 40 mM CaCl\textsubscript{2}. The 2-syringe method was used for uniform mixing. In order to find the most suitable preparation technology, the given samples were prepared using 3 methods:

\textit{Method 1} - Fibrinogen was mixed separately in a syringe with 1 mL CaCl\textsubscript{2} solution and in a second syringe thrombin with 1 mL CaCl\textsubscript{2} solution.

\textit{Method 2} - 1 mL of CaCl\textsubscript{2} was added to the fibrinogen to form a suspension 1. In another syringe, 1 mL of H\textsubscript{2}O was added to the thrombin to form a suspension 2.

\textit{Method 3} - Fibrinogen was mixed with 2 mL of CaCl\textsubscript{2} to form suspension 1 in a syringe. The thrombin weighed in the second syringe was then added to suspension 1, and the contents of the two syringes were mixed together. In all methods, the two substances were mixed together to obtain a homogeneous mass.

As for the drug, during preparation, it is added to fibrinogen and then mixed with thrombin-CaCl\textsubscript{2}. As drugs Vancomycin hydrochloride (V-HCl) (as modular material) and Clindamycin phosphate (CLP) were used. Samples were compared by gel fraction, drug release, swelling, and microstructure.

Results and Discussion
Less CaCl\textsubscript{2} solution in the sample provides more cross-linked sample and prevents it from losing shape over time. Examining the phase composition of the fibrin matrices, it was observed that the amount of added CaCl\textsubscript{2} is completely eliminated and does not form a separate intense peak in the sample. The gel fraction of V-HCl / fibrin matrices is 84.01 ± 0.54 %, which is higher than for CLP / fibrin matrices (72.84 ± 1.23 %) and for fibrin matrices without drugs (76.72 ± 0.91 %).

The swelling results showed that for drug-free fibrin matrices and CLP / fibrin samples, equilibrium was reached after 1.5 hours, while for V-HCl / fibrin matrices it was observed after 2.5 h. This confirms that the V-HCl / fibrin matrix samples are best cross-linked and does not allow water to disrupt the cross-links between fibrin matrix components. Also the release kinetics from samples with V-HCl and CLP were tested and results were compared. The release of
V-HCl from fibrin matrices was monitored for 312 hours (13 days). The results show that 77% of the total V-HCl was released during this time.

Conclusion
To obtain a fibrin matrice, CaCl₂ must be added first to the thrombin and then mixed with fibrinogen to form a homogeneous mass. V-HCl / fibrin matrices have the highest gel fraction, as in this case V-HCl acts as a crosslinking agent that improves crosslinking of the samples, but weakens CLP.

Acknowledgement
This project has received funding from the Latvian Council of Science research project No. lzp-2020/1-0054 “Development of antibacterial autologous fibrin matrices in maxillofacial surgery (MATRI-X)”.

References
Poly(sebacic acid) as a carrier of azithromycin delivered via inhalation for the treatment of bacterial lung infections

Konrad Kwiecień¹, Katarzyna Reczyńska¹, Karolina Knap¹, Daria Niewolik², Katarzyna Jaszcz², Elżbieta Pamuła¹

¹ AGH University of Science and Technology, Department of Biomaterials and Composites, Kraków, PL; ² Silesian University of Technology, Department of Physical Chemistry and Technology of Polymers, Gliwice, PL

Introduction

Conventional therapy methods of pulmonary bacterial infections – oral or intravenous administration of antibiotics – are not always effective due to limited drug delivery to the lower respiratory tract. Moreover, such treatment requires high doses of drugs that may cause side effects and the development of antibiotic resistance. One of the possible ways to solve these problems is to use biodegradable microparticles with a short degradation time as inhaled antibiotic delivery systems [1]. Poly(sebacic acid) (PSA) is considered material for such purposes thanks to its controllable degradation kinetics. This work aimed to obtain PSA microparticles (MP) with a diameter in the range of 1-5 µm, i.e. the optimal size for pulmonary administration via inhalation [2] loaded with azithromycin (AZ), i.e. the antibiotic used in the treatment of pulmonary infections. The second goal was to evaluate the encapsulation efficacy of AZ, characterize surface and microstructural properties of MP as well as to assess their compatibility with human lung epithelial cells.

Experimental Methods

PSA was obtained from sebacic acid via polycondensation. MP were manufactured using solid-in-oil-in-water (S/O/W) emulsification, where S: AZ in a different weight ratio to PSA; O: PSA dissolved in dichloromethane (DCM); W: water solution of poly(vinyl alcohol) (PVA). AZ was dispersed in PSA solution using ultrasounds. MP were obtained by adding the oil phase to the water phase and evaporation of the organic solvent under constant stirring. Then, MP were washed in UHQ-water to get rid of PVA residues, and freeze-dried. MP were observed using both optical and scanning electron microscopes (SEM) to assess their microstructure. Diameter size (Z-ave), polydispersity index (PdI), and zeta potential (ζ) were characterized via dynamic light scattering (DLS), while AZ encapsulation efficacy by using high-performance liquid chromatography (HPLC). Biocompatibility was evaluated using AlamarBlue assay and live/dead fluorescent staining on human lung epithelial cells (A549 and BEAS/2B) after 24 h contact with MP dispersed in Dulbecco modified Eagle’s medium (DMEM) at different concentrations.

Results and Discussion

Obtained MP were round and of regular shape. Their surface was smooth even at high magnification (fig. 1). The Z-ave were in the range of 1.46-2.45 µm, PdI was on average 0.3, and ζ value varied between -15.5 and -6.3 mV. Obtained values showed no correlation with AZ concentration.

The average sizes of MP may vary due to the limited repeatability of the emulsification method. However, Z-ave of all of the obtained MP were within the range of 1-5 µm, meeting the condition of an appropriate size for inhalation, as shown in fig. 1. Satisfactory drug loading was also obtained.

In vitro tests of PSA microparticles without the addition of AZ showed (fig. 2) that, depending on the type of cells, the MP are not cytotoxic at low concentrations: ≤ 100 µg/ml (A549) and ≤ 50 µg/ml (BEAS-2B). The LD50 (the dose causing 50% mortality) of MP for A549 and BEAS/2B cells was 359 µg/ml and 149 µg/ml, respectively.

Conclusion

The results of this study show the potential of PSA as a material for inhalable drug carriers in the treatment of pulmonary bacterial infections. Chosen manufacturing parameters allow obtaining uniform MP of sizes within the...
range required for inhalation. Regular shape and low roughness may suggest no progressive degradation in the process of MP formation. Manufactured MP were also cytocompatible with model epithelial lung cells, suggesting their potential usefulness as pulmonary drug delivery systems. Further studies will focus on evaluating the degradation of PSA matrix, drug release kinetics, and assessment of antimicrobial properties of the system.

Acknowledgement

This study was supported by National Science Centre, Poland (project No 2019/35/B/ST5/01103) and by the Program "Excellence Initiative – Research University" for the AGH University of Science and Technology.

References


Electrospun brinzolamide carrier – potential antiglaucoma drug delivery platform

Olga Cegielska¹, Paweł Sajkiewicz¹, Maciej Sierakowski²

¹ Institute of Fundamental Technological Research Polish Academy of Sciences, Laboratory of Polymers and Biomaterials, Warsaw, PL; ² Cardinal Stefan Wyszyński University in Warsaw, Institute of Biological Sciences, Warsaw, PL

Introduction
Pharmacological treatment of glaucoma is based on aqueous solutions and suspensions. Their precorneal residence time is very short, which results in low drug bioavailability and makes frequent dosing obligatory. The drug spreading over the eye is partially absorbed to the bloodstream, which further reduces the drug rate absorbed at the destination site [1].

In the last few years attention has been drawn to the potential nanofibers in ophthalmology [2,3]. Nanofibers are well known for providing sustained and controlled delivery of active ingredients [4]. Non-woven of any shape can be put near the pupil. When made of proper materials, they can be less prone to clearance and provide more localized delivery than traditional formulations [3].

An electrospun delivery system of poorly soluble antiglaucoma drug brinzolamide (BRZ) based on mucoadhesive hydroxypropyl cellulose (HPC), polycaprolactone (PCL) and beta-cyclodextrin (β-CD) has been formed. Cyclodextrins improve water solubility of hydrophobic ingredients, increasing its permeability to target tissues [5]. In combination with hydrophilic and hydrophobic polymer, a sustained drug delivery is expected to be achieved. To evaluate the drug delivery system potential basic studies of the nanofibers, complexation ability between cyclodextrin and brinzolamide, and permeability of brinzolamide through lamb corneas were performed.

Experimental Methods
HPC/PCL/β-CD/BRZ nanofibers have been formed via electrospinning from hexafluoroisopropanol solutions. Their morphology was studied using scanning electron microscope (SEM). Phase solubility study was performed on a series of aqueous solutions. Additional samples containing HPC beside BRZ and β-CD were prepared to evaluate HPC influence on complexation. After incubation in elevated temperature the samples were filtered and brinzolamide amount was measured using high performance liquid chromatography (HPLC).

Supramolecular structure study of the complex was performed using Fourier transform infrared spectroscopy with attenuated total reflection sampling technique (ATR FTIR) in search of changes in spectra compared to pure components indicating interaction between them.

Brinzolamide permeation from the nanofibers through lamb corneas was studied using Franz diffusion cells. Corneas with nanofibrous samples on top were mounted on acceptor chambers filled with phosphate buffered saline (PBS). Donor chambers were put on top and the cells were put in water baths on heated magnetic stirrers. At predetermined time points samples were collected and replaced with pure PBS. Commercial brinzolamide solution was tested for a reference; 15 repetitions were made for each delivery platform to obtain reliable data. Brinzolamide content in the samples was measured using HPLC.

Results and Discussion
Proper morphology fibers with a diameter from several dozen nm up to about 1 µm were obtained using selected processing/materials parameters.
Cyclodextrins increased brinzolamide solubility in PBS, although above a certain β-CD/BRZ ratio BRZ solubility started to decrease, probably due to complexes aggregation. HPC significantly increased brinzolamide solubility. The complex spectrum was similar to the pure β-CD spectrum; among others the peak corresponding to OH stretching vibration was narrowed and shifted, which is interpreted as evidence of a successful complexation. Permeation of BRZ from the nanofibers was slow and sustained. There was no burst release. Permeation profiles of BRZ from the nanofibers and commercial solution were similar. Considering large drug loss from the later and significantly reduced loss expected with the nanofibers, the system potential for a more effective prolonged delivery is very high.

**Conclusion**

Complexation takes place between β-CD and BRZ, favored by HPC. Effective gradual BRZ permeation from the nanofibers through animal corneas is achieved. In further study mucoadhesion of the nanofibers will be evaluated.

**Acknowledgement**

This work was supported by the Polish National Science Center (grant number 2019/35/N/ST5/03882). Part of investigations was carried out with the use of CePT infrastructure financed by the European Union (agreement POIG.02.02.00-14-024/08-00).

**References**


PS1-10-343

Quantitative assessment of the comparative nanoparticle-uptake efficiency in a range of biological fluids.

Tiago Santos1,3, Pedro L. Granja1,3, Zsombor Lacza2, Peter Hamar2

1 Universidade do Porto, Instituto de Investigação e Inovação em Saúde (i3S), Porto, PT; 2 Semmelweis, Department of Orthopaedics, Budapest, HU; 3 Universidade do Porto, Instituto de Engenharia Biomédica (INEB), Porto, PT

Introduction
Nanoparticles (NPs) potentially provide a powerful tool for specific treatments of diseases, acting as a drug delivery transport. However, a deep understanding and control of how NPs interact with biological systems is a key driver to assure the safe implementation of nanomedicine [1, 2]. The overall idea of this work was to provide new leads in the development of such a field, finding tools for various biomedical applications. What the biological cell actually "sees" when interacting with a nanoparticle will influence the mechanism of internalization. Thus, whether the cell is presented with the bare particle or the particle dispersed in a biological medium, thus covered by a protein corona, results in different uptake behaviour [3, 4].

Experimental Methods
For this project, multiple cell lines were used and the ultimate goal was to control and quantify uptake of a series of negatively charged carboxylate modified polystyrene of different sizes (20, 40, 100, 200, 500 and 1000 nm), understanding the endocytic pathways required for NPs internalization and their final sub-cellular destination, they were dispersed in different biological fluids (no serum, fetal bovine serum, human albumin, SPRF).

Results and Discussion
Although the depletion of serum during the exposure to NPs is quite accepted to lead to enhanced NPs deposition on the cell layer resulting in increased cell uptake, we have in our possession outstanding preliminary results that shows that serum fraction of platelet-rich fibrin (SPRF), presents an outstanding potential as source of proteins (for larger NPs-corbora complex). Indeed, our results show an increase on the NP uptake when compared to same-sized NPs in the serum free condition. Truly, from our knowledge this has never been previously described, therefore understanding the reasons for this abnormal uptake behaviour will lead to a better understanding on the mechanistic of endocytosis and consequently, to a better control and precise drug delivery. Also, in terms of the proteome profiler analysis, data from the serum of interest (SPRF) showed interesting differences in terms of pro and anti-inflammatory regulatory responses in which we intend to go deeper, in order to initially investigate what are the differences between the sera and therefore be able to explain the abnormal uptake behaviour mentioned in Fig 1.

Conclusion
We found that internalization of NPs is highly size-dependent for all cell lines studied, with the different cell types showing very different uptake efficiencies for the same materials. Moreover, our studies showed that, in a physiologically relevant environment, highly complex protein coronas are established and modulate biological effects at the nanoparticle-cell interface in an innovative way (Fig 1), which has important implications for nanomedicine.

Acknowledgement
NA
Figure 1
Number of microspheres internalized when dispersed in No serum, FBS, SPRF and Human Albumin, for 500nm NPs across all cell lines. Mean values and standard deviations of triplicate experiments are given.

References
Multifunctional ready-to-use starPEG-heparin cryogels for the sustained delivery of signaling proteins

Jana Sievers¹, Ralf Zimmermann¹, Jens Friedrichs¹, Dagmar Pette¹, Yanuar D. Limasale¹, Carsten Werner¹,², Petra B. Welzel¹

¹ Leibniz-Institut für Polymerforschung Dresden e.V, Dresden, DE; ² Technische Universität Dresden, Center for Regenerative Therapies Dresden, Dresden, DE

Introduction
Efficient, safe and spatiotemporally controlled delivery of signaling proteins, while avoiding adverse effects is a key factor for various biomedical applications, such as in vitro and in vivo tissue engineering or drug delivery. Besides conventional hydrogel-based systems, macroporous cryogels have gained increasing attention due to their attractive material properties, including injectability and effective supply of the cells with nutrients and oxygens. However, most of the reported cryogel systems, neither allow a sustained protein release, nor tuning of the release properties independently of other cell-instructive material properties or ‘off-the-shelf’ usability. Moreover, a detailed understanding of the implication of the cryogels’ macroporous architecture on signaling protein release is still missing.

To address these challenges, in this work a ready-to-use starPEG-heparin cryogel system, containing different amounts of the protein-affine heparin component, was developed and thoroughly investigated.

Experimental Methods
In extension to our previous work [1], a set of multifunctional cryogel scaffolds were prepared by covalently crosslinking end-functionalized multi-armed polyethylene glycols (starPEG) and the natural glycosaminoglycan heparin. For the evaluation of signaling protein release, both experimental analyses based on enzyme-linked immunosorbent assay and mathematical modeling based on a reaction-diffusion model were applied. A detailed characterization of the global and local material properties of the different cryogel scaffolds was performed using methods, such as scanning electron microscopy, atomic force microscopy-based nanoindentation and uniaxial compression measurements. Beyond that, signaling protein and cell adhesion-peptide pre-functionalized cryogels were prepared utilizing an additional lyophilization step and tested for their storability and ready-to-use potential. Therefore, the bioactivity of the loaded signaling proteins was investigated in in vitro neuronal differentiation experiments.

Results and Discussion
The potential of the developed cryogel system for systematically tunable long-term delivery of different signaling proteins is shown. We demonstrate that by varying the heparin concentration of the macroporous cryogels, a fine-tuning of the protein release kinetics was made possible, while keeping the structural and mechanical properties of the scaffolds constant. The experimental and theoretical analysis of the signaling protein delivery allowed us – for the first time – to show the impact of the unique macroporous cryogel structure on protein release, leading to local differences in protein concentration. These findings are truly relevant for the application scenario, as they enable better control over the specific signaling protein concentrations that cells within the cryogels or in their surrounding will be exposed to. As a proof-of-concept for their ready-to-use potential, the pre-functionalized cryogels, prior stored in the dehydrated state, were demonstrated to induce the neuronal differentiation of colonizing pheochromocytoma cells, thus confirming the bioactivity of the loaded proteins.

Conclusion
The here developed tunable starPEG-heparin cryogel system overcomes otherwise reported limitations of cryogels, i.e. too-rapid release, limited loading capacity, and insufficient control over the release characteristics. Together with
the ease of handling and mechanical stability, this makes our multifunctional cryogel system attractive as easily storable and applicable systems for precision delivery of signaling proteins in therapeutic in vivo or high-throughput in vitro applications.

References
2:30 p.m. – 4:00 p.m.

Poster floor

PS1-11 | Antibacterial Biomaterials
Activity of biomolecules and vancomycin on collagen scaffolds after RBX treatment

Ulrike Ritz\textsuperscript{1}, Joy Braun\textsuperscript{1}, Stefanie Eckes\textsuperscript{2}, Michelle F. Kilb\textsuperscript{2}, Katja Schmitz\textsuperscript{2}, Daniela Nickel\textsuperscript{3}

\textsuperscript{1} Universitätsmedizin Mainz, Department of Orthopedics and Traumatology, Mainz, DE; \textsuperscript{2} Technical University of Darmstadt, Organic Chemistry and Biochemistry, Darmstadt, DE; \textsuperscript{3} University of Cooperative Education, Berufsakademie Sachsen, Glauchau, DE

Introduction
Infection as well as disturbed tissue regeneration after trauma are still challenging problems in surgery. Biomaterials as carriers for antibacterial as well as tissue inducing factors are needed to fight bacterial infection and on the other hand to induce tissue regeneration. Many materials exist, that could be used as carrier, but collagen is one of the most abundant proteins in the body and therefore a suitable biomaterial for Drug Delivery. However its stability is weak. Therefore, due to its weak stiffness and huge swelling degree the treatment with Rose Bengal and green light might improve the mechanical properties. A problem that might occur is that such a photochemical crosslinking could affect the activity of adsorbed biomolecules as well as drugs. To investigate the influence of RBX treatment, BMP-2, SDF 1α and Vancomycin were coated on two collagen scaffolds (Atelocollagen and Collagen Solutions) with subsequent light treatment and activity testing.

Experimental Methods
Collagen scaffolds were coated with BMP-2, SDF 1α and Vancomycin by adsorption and treated with rose bengal and green light. Scaffolds were incubated for different time points and release of bioactive molecules measured by specific ELISAs. The functionality of released BMP-2 and SDF-1 was tested on human osteoblasts and HUVEC, respectively, by viability assays. In case of BMP-2 the functional activity was also tested by the alkaline phosphatase activity (ALP). In case of SDF-1 migration assays were performed to demonstrate the still present chemotactic effect of SDF-1 after treatment with RBX.

or Vancomycin the coated collagens were investigated regarding their bacterial growth-limiting effect on Staphylococcus aureus.

Results and Discussion
Continuous release of bioactive molecules could be shown over 24 hours. This release was hardly effected by rose bengal crosslinking. Comparable to this results no effects on the inhibition capacity of bacterial growth was detected when collagen scaffolds coated with Vancomycin with or without following RBX treatment. Proliferation on both tested collagensheets increased after SDF-1 (HUVECs) or BMP-2 (human osteoblasts) relase. Although RBX treatment reduced this effect, it was still significantly higher when compared to original sheets without growth factors. Whereas humane osteoblasts showed a significant higher ALP activity on Collagen Solutions with BMP-2 (with or without RBX treatment) no differences were measured on Atelocollagen. Additionally no significant differences in the used concentrations (100 and 500 ng) were measured after RBX treatment. HUVECs showed a higher migration when tested with SDF 1α containing collagen solution scaffolds. Although this effect was again a little bit reduced after RBX treatment, it was still significantly higher when compared to non treated sheets. The SDF-1 effect on atelo collagen sheets was much less. This is the first study using human cells with RBX treated collagen sheets and demonstrating the functionality of bound growth factors after RBX treatment. We could show that the effect of RBX not only depends on each molecule or drug used but also on the scaffold. We detected differences in the effects of growth factors when bound to and released from collagen solution or
atelocollagen. For this reason it is important to evaluate the influence of the photochemical crosslinking method for each application. In this context the bioactivity of biomolecules was influenced by this treatment.

**Conclusion**

The use of RBX for crosslinking is a useful tool to improve the mechanical properties of collagen for Drug Delivery. It depends on the scaffold used whether the bound growth factor keeps its functionality. However, when using the "right" scaffold, this method can be further developed for creating laminates with different loadings for application in the treatment of open fractures after trauma.

**Acknowledgement**

This project is funded by DFG (Project number: 400569699).

**References**


Phytotherapeutic hybrid scaffold via combined 3D printing and electrospinning for antibacterial wound dressing

Irem Unalan, Aldo R. Boccaccini

Friedrich-Alexander-University Erlangen-Nuremberg, Institute of Biomaterials, Department of Materials Science and Engineering, Erlangen, DE

Introduction
Recently, a new family of hybrid scaffolds, which are fabricated via a combination of 3D printing and electrospinning techniques, have been prepared, which exhibit enhanced mechanical properties, biological activities, and extracellular matrix (ECM)-like three-dimensional (3D) structures for tissue engineering applications [1,2]. In this approach, electrospun nanofibers are included to support cell attachment while the scaffold structure is designed to mimic the natural ECM by 3D printed struts. In the present study, hybrid scaffolds further developed to provide biological functions and antibacterial activity to avoid the increasing prevalence of bacterial infections. In this regard, phytotherapeutics [3] such as essential oils (EOs) are considered alternative natural antibacterial agents to overcome the potential drawbacks of standard antibiotics [3].

Experimental Methods
This study's objective was to develop new strategies to fabricate phytotherapeutic containing hybrid scaffolds using a combination of 3D printing and electrospinning as a novel antibacterial wound dressing. In this study, firstly, polycaprolactone (PCL) struts were fabricated successfully by 3D printing. Then, EO-loaded PCL-gelatin (GEL) electrospun nanofibers were deposited on the printed PCL struts' surface. The physical and mechanical properties, total phenol content, antibacterial, the antioxidant and biological activity of the hybrid scaffolds were evaluated.

Results and Discussion
SEM images illustrated that the hybrid scaffold's morphology was uniform, bead-free nanofibers and a unit layer structure consisting of interconnected pores of ~410 μm size formed by the printed PCL structure. Also, our investigations indicated that hybrid scaffolds did not have cytotoxic effects on normal human dermal fibroblast (NHDF) and keratinocyte (HaCat) cells. On the other hand, the hybrid scaffolds exhibited antibacterial properties against S. aureus (gram-positive), and E. coli (gram-negative).

Conclusion
In conclusion, the novel family of phytotherapeutic hybrid scaffolds may have potential in wound healing applications and can be considered as a promising biomaterial for avoiding bacterial infections without using antibiotics.

Acknowledgement
Irem Unalan is funded by the Deutscher Akademischer Austauschdienst (DAAD), program of Research Grants—Doctoral Program (Section ST21, 91652927).

References
Localized delivery of antibiotics through 3D core/shell hydrogel scaffolds to treat/prevent infections and promote healing of a chronic skin burn wound

Ashwini Rahul Akkineni¹, Janina Spangenberg¹, Michael Geissler¹,³, Hubert Buechner², Anja Lode¹, Michael Gelinsky¹

¹ Technische Universität Dresden, Medical Faculty Carl Gustav Carus of TU Dresden, Dresden, DE; ² Heraeus Medical GmbH, Wehrheim, DE; ³ Technische Universität Dresden, Institute of Natural Materials Technology, Dresden, DE

Introduction
Chronic skin wounds, as result of burns, are often prone to infections. Invasive bacterial infections caused by Staphylococcus aureus (gram positive), Pseudomonas aeruginosa and Acinetobacter (gram negative) at the wound site are found to be leading cause of death in burn patients. Additionally, development of antibiotic resistant bacteria such as methicillin-resistant Staphylococcus aureus (MRSA) in recent decades had further complicated treatment of skin wound infections caused by burns [1].

Current clinical care of infected chronic burn wounds include systemic and topical treatments for managing pain and infection at the injury site along with facilitating skin regeneration by excision and grafting (with allografts or skin substitutes). Traditional, systemic administration of antibiotics is usually associated with inadequate local availability of the drug, thus not being effective in deterring aggressive infections. Whereas inhibitory concentration of antibiotics can be achieved by topical application - leading to effective management of the infection. However multiple applications are needed to maintain a minimum inhibitory concentration to completely eradicate the infection and its recurrence. To overcome these challenges, our current work explores the many capabilities of 3D core/shell bioprinting to locally deliver desired concentrations of antibiotics through 3D bioprinted hydrogel scaffolds.

Experimental Methods
High viscous ink alginate/methylcellulose (ALG/MC [2]) and alginate/methylcellulose/laponite (ALG/MC/LAP [3]) along with low concentrated alginate (3% w/w) were used as shell and core parts, respectively. The core ink was loaded with 10 mg/ml antibiotics (Vancomycin, Clindamycin and Gentamicin). Scaffolds consisting of individual antibiotics were fabricated using core/shell 3D extrusion printing technique [4]. Release of the antibiotics was quantified by a semi-quantitative method i.e. agar diffusion assays. Briefly, antibiotic loaded scaffolds were incubated in 0.9% saline solution at 37°C. At specific time intervals (2 h, 6 h, 1, 2, 3 & 7 days) the release solution was collected and 20 µl each were added onto a sample carrier. The sample carrier was placed on an agar plate previously colonized with S. aureus, and incubated at 36°C for 18 h. The released antibiotic would diffuse into the surrounding agar creating a zone of inhibition (ZOI) as the bacteria are killed by the antibiotic around the sample carrier. The ZOI was found to be directly proportional to the amount of antibiotic in the release solution, hence a standard curve was developed by plotting area of ZOI for known antibiotic concentrations which was used to quantify the antibiotic concentration in the release solutions.

Results and Discussion
Antibiotics loaded hydrogel scaffolds were successfully fabricated by core/shell3D extrusion printing technique (Fig. 1A-C). Over a period of 7 days, all the antibiotics were released in biologically active form. It appears that the cumulative release of Vancomycin was highest compared to Clindamycin and Gentamicin. The release of Vancomycin and Clindamycin was sustained and significantly lower when ALG/MC/LAP ink was used to fabricate
the scaffolds. A complete arrest of Gentamicin release was observed for ALG/MC/LAP scaffolds. Furthermore, by changing the core and shell dimensions, it was shown that release kinetics of antibiotics can be varied.

**Conclusion**

In our current work, we could successfully print 3D core/shell hydrogel scaffolds with various antibiotics loaded in the core. Their release in inhibitory concentrations was observed till 7 days, indicating sustained release characteristics. Inclusion of the nanoclay laponite in inks further significantly reduced the burst release of loaded antibiotics. Furthermore, release kinetics could also be tuned by simply changing the dimensions of the printing needles. It can be envisioned that such a versatile delivery system could potentially open new strategies to treat skin burn or wound infections.

![3D core/shell scaffolds and antibiotic release over 7 days](image)

**Figure 1:** A scaffold with the shell composed of ALG/MC/LAP and the core with 3% alginate in blue ink after 1 layer of 3D printing (A). A magnified image of the core/shell strand (B). Core/shell scaffold (C) and full strand (i.e. no core) scaffolds of 4 layers after finishing 3D printing (D). Cumulative release of Vancomycin from core/shell scaffolds with ALG/MC and ALG/MC/LAP as shell (E) and the corresponding ZOI’s formed in Agar diffusion assays (F).

**References**


Evaluation of the co-adjuvant antimicrobial effect of lyocell/silver knitted fabrics modified with Nisin Z

Tânia D. Tavares1, André Catarino1, Carla Silva2, Joana C. Antunes1, Helena P. Felgueiras1

1 University of Minho, Centre for Textile Science and Technology, Guimarães, PT; 2 University of Minho, Centre of Biological Engineering, Braga, PT

Introduction
The resistance of bacteria to traditional antibiotics is raising a serious global public health problem with huge economic and societal consequences. Antimicrobial peptides (AMPs) have been raising great interest as alternatives to antibiotics. Nisin Z belongs to the lantibiotics class of AMPs and is produced by the non-pathogenic bacteria Lactococcus lactis. This AMP has a significant antibacterial activity against Gram-positive bacteria [1]; however, its activity against Gram-negative bacteria is limited and can be improved with the addition of the non-antibiotic chelating agent ethylenediaminetetraacetic acid (EDTA), known to assist in cell permeabilization [2]. Nisin Z has been mostly studied for food preservation applications, with very little research being done on its potential for biomedicine. Lyocell is the only regenerated cellulosic fiber known as green and renewable that resorts to commercial eco-friendly manufacturing processes. The combination of lyocell and silver, recognized for its antibacterial effectiveness, in textile dressings, has been shown effective against bacteria colonizing chronic wounds [3]. The purpose of this work is the functionalization of silver-enriched lyocell knitted fabrics with Nisin Z, in the presence and absence of EDTA, against two of the most common Gram-negative bacteria present in chronic wounds, Escherichia coli and Pseudomonas aeruginosa.

Experimental Methods
MIC of Nisin Z-EDTA against E. coli (ATCC 25922) and P. aeruginosa (ATCC 25853) was assessed by the broth microdilution method [4]. Testing started with Nisin Z at 4 mg/mL and EDTA at 200 µM, both prepared in distilled water (dH2O). Nisin Z was serial diluted (1:2) in Mueller Hinton Broth (MHB) to a final volume of 25 µL. EDTA was used at equal volume and combined with the peptide. Finally, 50 µL of the bacteria suspensions prepared at 2×10⁷ CFUs/mL in MHB were added. Free Nisin Z, free EDTA, agent-free bacteria suspensions and culture media were used as controls. Samples were incubated for 24 h at 37ºC and 120 rpm. MIC was established as the concentration at which bacteria did not show any growth.

Lyocell spun yarns with 150 dtex and silver-plated polyamide multifilament yarns with 33-44 dtex were used to produce seamless weft fabrics via knitting machine MERZ model MBS, with a E28 gauge, diameter of 33.02 cm and 1152 latch needles. Lyocell (control) and lyocell/silver-plated fabrics were engineered. Fabrics were characterized via bright field microscopy (fabric pattern) and Fourier-transform infrared spectroscopy (FTIR, chemical structure). Nisin Z with and without EDTA addition was immobilized onto the engineered fabrics at 2×MIC via dip coating. Fabrics were immersed for 72 h at 120 rpm in the Nisin Z or Nisin Z-EDTA solutions for complete saturation. Loading amount was verified by UV-visible spectroscopy. Weakly bonded molecules were eliminated with dH2O washes. Kirby-Bauer diffusion testing and time-kill kinetics studies, up to 24 h of incubation, were conducted with the two microbial cells in MHB.

Results and Discussion
MICs for Nisin Z-EDTA against E. coli and P. aeruginosa were determined at 31.25 and 62.5 µg/mL, respectively; without EDTA, Nisin Z only exhibited activity against E. coli, and at 250 µg/mL. As seen, the activity of Nisin Z against Gram-negative bacteria is limited, particularly against P. aeruginosa, requiring EDTA to destabilize the outer bacterial membrane and facilitate peptide permeabilization [2].

Page 1350 of 2028
Seamless fabrics with a defect-free and uniform pattern were successfully produced from lyocell and lyocell/silver-plated yarns. Here, silver was used as an additive with the goal of preventing cell overexposure to its ions and, consequently, cytotoxic effects (considering the purpose of the final application). Cellulose characteristic chemical groups were detected via FTIR, confirming the fabric composition and presence of free -OH groups. Binding with Nisin Z and EDTA via hydrogen bonding was confirmed as well by UV-vis spectroscopy. The antimicrobial potential of the modified fabrics was also verified. Diffusion of the functionalized active cues against the two microbial cells was observed in the lyocell-based fabrics, with a formation of a halo. Yet, their combination with silver was more effective (halo 2x larger). Similar observations were made via time-kill kinetics studies, with Nisin Z-loaded lyocell/silver fabrics being quicker in acting against the selected microorganisms. EDTA was seen to play a determinant role against *P. aeruginosa*.

**Conclusion**

Data revealed that the addition of EDTA with Nisin Z is essential to improve its activity against Gram-negative bacteria. Overall, the potential of these fabrics and peptide to work in synergy for prospective wound dressing applications was demonstrated.

**Acknowledgement**

This research received funding from the Portuguese Foundation for Science and Technology (FCT) under the scope of the projects PTDC/CTM-TEX/28074/2017 and UID/CTM/00264/2021. TDT also acknowledges FCT for PhD grant 2020.06046.BD.

**References**


New gels preparation based on peptide-peptide co-assembly

Alexandra Croitoriu, Loredana E. Niță, Alexandru Serban, Alina G. Rusu

Petru Poni Institute of Macromolecular Chemistry, Iasi, Romania, Department of Natural Polymers, Bioactive and Biocompatible Materials, Iasi, RO

Introduction
Low molecular weight gelling agents (LMWG) are small molecules that can associate following a hierarchical process of self-assembly forming 3D networks generated by non-covalent interactions. Different molecular entities, such as organic molecules, proteins, peptides and DNA, have the ability to create complex structural systems through spontaneous diffusion and specific association between molecules, dictated by non-covalent interactions. The versatility of the amino-acid structures provides flexibility in the design of the peptide chains. Peptides can perform specific biological functions due to their structure and ability to assemble. Among the multiple self-assembled peptide hydrogels reported to date, phenylalanine-derived nanostructures provide efficient aromatic core self-assembly. Spontaneous self-assemblies in aqueous solution consist of peptide nanotubes from individual entities with unique properties and the technique approached was that of the pH switch. Taking into account these aspects, this study aims to prepare different gels by co-assembly of peptides derived from phenylalanine with other peptides. The final purpose is to obtain structures with double function: capacity for hydrogelation and antimicrobial character.

Experimental Methods
The technique approached was that of pH switch. Thus, peptide (Fmoc-Ph-Ph) was dissolved in a NaOH solution. In the next step, HCl or citric acid was added until the pH is neutralized. In parallel another peptide (Fmoc-Lys) was dissolved following the same procedure. The two peptides were mixed by using two approaches: in the first approach the solution of peptide was mixed immediately after the preparation. In the second approaches the peptides solution was mixing after 30 minutes (to allow time for the formation of the self-assembly process to begin and then to start the co-assembly process) (Figure 1).

Results and Discussion
The ability to self-assemble of phenyl peptide, as well as to co-assemble with other peptides was evaluated by combining different characterization techniques such as FT-IR, SEM microscopy. The gels obtained by the use of peptide as LMWGs, show stability, withstanding the vial inversion test (Figure 2).

Conclusion
The study results evidenced the dependence of the hydrogel formation on more than only one single factor. The self-assembly process of peptides derived from phenylalanine was generated by the hydrophobic π-π interactions of the fluorenyl moieties, and hydrogen bonds from the carbonyl group. The co-assembly process was evidenced by FT-IR spectroscopy and SEM microscopy. All resulted data support the formation of inter- and intra-molecular physical bonds that ensure the formation of fibrils and their organization in the 3D network. The swelling studies confirmed the superabsorbent nature of the prepared hydrogels, and recommend the systems for incorporation into the network matrix of drugs by diffusion process.

Acknowledgement
This work was financially supported by the grant of the Romanian National Authority for Scientific Research, CNCS-UEFISCDI, project number 339PED/2020, PN-III-P2-2.1-PED-2019-2743 “New hybrid polymer/peptide hydrogels as innovative platforms designed for cell cultures applications”, within PNCDI III.
Figure 1
Schematic representation showing the co-assembly principle of the two peptides

Figure 2
Visual images of different variant of synthesized gels
Infection-resistant medical device technologies from sustainable sources

Zili Ji, Nicola Irwin, Colin McCoy, Eneko Larraneta, Louise Carson

Queen's University Belfast, School of Pharmacy, Belfast, GB

Introduction
Infection caused by bacterial colonization is the most common and serious challenge associated with biomedical devices, which harms patients' health and poses a significant financial burden to healthcare systems. Lignin is a low cost, readily available naturally occurring material which has shown great prospects in its antimicrobial activity 1. In addition, hydrogel polymer systems have been investigated as coating materials due to their reported biocompatibility 2, and their low friction surfaces can efficiently decrease the trauma caused by the devices during insertion, and removal from the body. It is therefore of great interest to develop lignin-loaded hydrogel systems that would combine the benefits offered by hydrogels and lignin to use as efficient anti-infective coating materials for the surface of biomedical devices. The aim of this study is to investigate the anti-infective properties of lignin-loaded hydrogels as candidate antibacterial coating materials for medical devices. The antibacterial properties of lignin-loaded hydrogels were compared with those which loaded with tetracycline hydrochloride, a widely used broad spectrum antibiotic.

Experimental Methods
A series of lignin-loaded (0, 1, 5 and 10 wt%) hydroxyethylmethacrylate-co-methacrylic acid copolymer systems were prepared by free radical polymerization. Some of the copolymers were additionally loaded with tetracycline hydrochloride (TET) to evaluate their drug loading and drug release ability, and also indicates antibacterial property of lignin as comparison. Lignin-loaded polymers were compared using Fourier Transform Infrared Spectroscopy (FTIR), scanning electron microscopy (SEM), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and static contact angle measurement. Equilibrium swelling analysis and in vitro pH-mediated drug release were also performed. Antibacterial properties of the polymers, TET-loaded polymers and silicone discs, as controls, were assessed in zone of inhibition (ZOI) test and bacterial adherence test incubated with Proteus mirabilis ATCC 51286 and Staphylococcus aureus ATCC 29213. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests of TET against P. mirabilis and S. aureus were also performed.

Results and Discussion
ATR-FTIR analysis confirmed polymerization of the polymers. From SEM results, lignin-loaded copolymers demonstrated relatively even surfaces in comparison with silicone. DSC and TGA results show that lignin-loaded polymers degraded at higher temperature, which indicates their better thermal stability. The water contact angle of the copolymers was measured as within the range of 50°-75°. The copolymers exhibited pH-dependent swelling behaviors, and their water content plateaued by 48 h. In pH 9 buffers, the swelling rates of all the polymers and amount of released lignin of lignin-loaded polymers were much higher than those in pH 6 buffers. Zone of Inhibition test results indicated that the presence of TET significantly increased the zone size against both bacteria. The average zone diameter against S. aureus was 2 cm, while against P. mirabilis the zone diameter was approximately 0.4 cm. No clear zones were shown in the non-TET-loaded group. The MIC value of TET against S. aureus was tested to be 0.63 mg·L⁻¹ and for P. mirabilis was 78 mg·L⁻¹. The TET-loaded samples successfully reduced the bacterial adherence of S. aureus to below the limit of detection (LOD) after 4h and 24h incubation. Similarly, lignin-loaded polymers significantly decreased the relative bacterial adherence to below the LOD after 24 h incubation. Lignin-loaded polymers reduced planktonic cells and bacterial adhesion as TET-loaded samples after 24 h incubation.
Conclusion

The surface properties of the copolymers confirm their potential to be used as candidate hydrophilic coating materials for urinary catheters. The highly thermal stable copolymers reveal their potential for sustained release of loaded drug to urinary tract and indicate their ability to tolerate typical physical sterilization methods. The copolymers will also be stable during storage and under less risk during fabrication. Their pH-mediated swelling behaviors can be used as a factor to predict the drug release patterns of drug-loaded copolymers in urinary tract under infected and normal conditions. The finding of microbiological assessments indicated that released lignin possesses the ability to kill planktonic \textit{S. aureus} cells and inhibit the bacterial adhesion on the polymer surfaces. This work suggests that lignin-loaded polymers can be used as alternative biomedical device coating materials to reduce the device related infections in a low cost, sustainable and greener way.

References


AMP-grafted PLGA-PEG Nanoparticles to Fight Bacterial Wound Infections

António Ramôa¹²³, Luís Moreira¹²⁴, Cátia Teixeira⁵, Victoria Leiro¹², Paula Gomes⁵, José das Neves¹², M. Cristina L. Martins¹²³, Cláudia Monteiro¹²

¹ i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, PT; ² INEB, Instituto de Engenharia Biomédica, Universidade do Porto, Porto, PT; ³ Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, PT; ⁴ Escola Superior de Biotecnologia, Universidade Católica do Porto, Porto, PT; ⁵ LAQV-REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Porto, PT

Introduction
Chronic wounds such as diabetic ulcers represent a major healthcare problem. Infection by antibiotic-resistant bacteria contributes greatly to wound chronicity and is responsible for 33,000 estimated deaths per year in the European Union. Current treatments based on iodine and silver antiseptics or topical and oral antibiotics are inefficient, toxic, or may induce resistance. Therefore, alternatives are urgently needed. Antimicrobial peptides (AMP) are a promising alternative, as they act through non-specific mechanisms and cause virtually no resistance. In this work, we developed AMP-grafted poly(D,L-lactide-co-glycolide-polyethylene glycol (PLGA-PEG) nanoparticles. AMP grafting will increase stability, local concentration and infection targeting due to AMP cationic character. MSI-78(4-20) was used as active peptide. This short AMP is a cost-effective and highly selective compound, which maintains activity when grafted to polymer surfaces¹².

Experimental Methods
MSI-78(4-20)-PLGA-PEG NPs composed of PLGA-PEG and PLGA-PEG-maleimide (PLGA-PEG-Mal) were produced by nanoprecipitation and functionalized with cysteine-modified MSI-78(4-20) through a thiol-maleimide Michael Addition reaction. The obtained NPs were characterized in respect to their size distribution, zeta potential and AMP grafting through AMP quantification using the fluorescamine assay, Fourier-transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR). Antimicrobial activity was assessed against Pseudomonas aeruginosa and Staphylococcus aureus, two of the most common pathogens in chronic wounds using minimal inhibitory and bactericidal concentration (MIC and MBC) assays.

Results and Discussion
NPs with approximately 100nm in size did not change significantly upon AMP covalent binding, while an increase in surface charge from -2.8mV ± 0.8 to 8.6mV ± 1.8 was observed. FTIR showed the characteristic peaks of the AMP and NMR displayed a significant decrease of the Mal peak, confirming covalent tethering. AMP maintained antimicrobial activity when bound to the NPs with a MIC and MBC of 8-16 mg/mL and 16-32 mg/mL, respectively against P. aeruginosa, and 32-64 mg/mL and 64->64 mg/mL against S. aureus.

Conclusion
The AMP MSI-78(4-20) was successfully grafted onto the surface of PLGA-PEG NPs maintaining antimicrobial activity against P. aeruginosa and S. aureus. These data support that the formulation may be useful for future treatment of infected chronic wounds.

Acknowledgement
The authors acknowledge financial support from AntiINFECT: Bioengineered Advanced Therapies for Problematic Infected Wounds (POCI-01-0145-FEDER-031781) and Bio2Skin Advanced (2021-24): NORTE-01-0247-FEDER-047225). P. Gomes acknowledges FCT for financial support to the LAQV-REQUIMTE research unit.
The authors also acknowledge the support of BN - Biointerfaces and Nanotechnology i3S Scientific Platform were FTIR and DLS were performed.

Zeta-potential of AMP-PLGA-PEG NPs

AMP-PLGA-PEG NPs were produced using different percentages of PLGA-PEG-Mal (10%, 20% and 40%). Surface charge increases with increased amounts of PLGA-PEG-Mal suggesting an increase in functionalization. The adsorption control sample (40%AMPads) also presents an increase in charge suggesting that a small portion of the peptide may not be covalently linked, however, results for this sample are highly inconsistent, demonstrating that covalent binding is a better strategy than adsorption.

1H NMR spectra (400 MHz, CDCl3) of AMP-PLGA-PEG-NPs

AMP signals from the peptide could not be detected in the 1H-NMR spectrum (neither in CDCl3 (C) nor in DMSO-d6), due to the very small amounts of peptide linked. However, it was possible to observe the disappearance of the signal corresponding to the maleimide protons of the NP (6.75 ppm, identified with a red rectangle), which indirectly indicates the conjugation of AMP to the NPs.

References

Design of multi-functional PVC coating addressing the polymicrobial nature of Ventilator-Associated Pneumonia

Tânia Grainha, Diana Alves, Eugénia Nogueira, Maria O. Pereira

University of Minho, Centre of Biological Engineering, Braga, PT

Introduction
Ventilator-associated pneumonia (VAP) is a common nosocomial infection and presents a serious concern due to the high mortality and morbidity rates associated [1]. The presence of the endotracheal tube (ETT) is a risk factor for developing VAP because its surface is prone to microbial adhesion. Microbial adhesion to surfaces and consequent biofilm formation remains one of the greatest current challenges in healthcare, when microorganisms are able to reach the surface of medical devices, such as ETT, becoming the focus of persistent infections [2]. Studies have demonstrated that most biofilms associated with VAP are composed by polymicrobial communities. In general, mixed bacterial and bacterial-fungal biofilms are more resistant to antibiotic treatment than the corresponding individual biofilms. Thus, aggressive and extended antibiotic therapies may be needed to control VAP. The major concern related with this approach is the development of resistance towards antibiotics.

An alternative to deal with VAP relies, therefore, on the use of preventive measures. Given the recognized role of ETT on VAP development, one promising approach is to prevent ETT colonization through the modification of the surface materials used to design these medical devices [3].

This work aims to develop an effective strategy to fight VAP, targeting its polymicrobial nature. Accordingly, the goal is to functionalize poly(vinyl) chloride (PVC) surfaces using a polydopamine (pDA)-based strategy, in order to prevent the adhesion and biofilm formation of three important VAP-related pathogens: Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans. For that, different compounds were individually tested against single-species biofilms encompassing these pathogens.

Different compounds were firstly screened in order to characterize its antimicrobial action towards the three pathogens. The most promising were further used to functionalize PVC following two approaches: direct incorporation on the surface and indirectly through the attachment of compound-loaded liposomes. This latter strategy is being explored as an attempt to achieve a more controlled drug release, reduce the dosages and minimize eventual side effects caused by the antimicrobials. The final goal is to engage, individually or in dual or even triple combinations, the most promising compounds in the design of a broad-spectrum antimicrobial surface. This safe-by-design multi-functional surface will be tested against single-, dual- and triple-species biofilms.

Experimental Methods
PVC surfaces were functionalized with Farnesol, Ciprofloxacin and Amphotericin B using the pDA approach. Briefly, each antimicrobial compound was firstly dissolved with dopamine, being the PVC coupons immersed in the final solution. Dopamine polarization allowed antimicrobials incorporation throughout the thickness of the pDA film formed. The antimicrobial capability of the polydopamine-mediated antimicrobial PVC surfaces was tested against P. aeruginosa, S. aureus and C. albicans, the most prominent VAP-related pathogens, inspecting different concentrations of each one of the antimicrobial compounds. After a contact of 24 h, the number of viable cells adhered to the surfaces was enumerated.

Ciprofloxacin and Amphotericin were also encapsulated in liposomes and the antibiofilm potential of these liposomal formulations were inspected so far for single-species biofilms.

Results and Discussion
Farnesol, Ciprofloxacin and Amphotericin B were successfully immobilized onto PVC surfaces using pDA-based strategy and these modified surfaces displayed anti-biofilm potential. Farnesol was able to reduce S. aureus biofilm formation leading to more than 5-log₁₀ CFU reduction while Amphotericin B was effective against C. albicans ceasing its growth. Moreover, Ciprofloxacin displayed promising results by inhibiting both P. aeruginosa and S. aureus single-species biofilms, reaching more than 6-log₁₀ CFU reduction and total eradication, respectively. Ciprofloxacin and Amphotericin B were effectively encapsulated in liposomes and these antimicrobials-loaded nanocarriers showed promising results by reducing biofilm formation. Liposomal Ciprofloxacin reduced biofilm forming ability of S. aureus and P. aeruginosa while liposomal Amphotericin B reduced C. albicans biofilm formation. Although preliminary, both liposomal formulations demonstrated potential to be further used in the functionalization of PVC surfaces.

Conclusion
In conclusion, dopamine chemistry was successfully applied in the functionalization of PVC surfaces. Immobilization of Farnesol, Ciprofloxacin and Amphotericin B proved to be a promising approach to be further applied in the fight of VAP once they displayed high antimicrobial activity against single-species biofilms. The following steps will include the binding of the compounds in dual and triple combinations for broadening the antimicrobial spectrum of the polydopamine modified PVC and the study of its performance towards multi-species biofilms.

Acknowledgement
This work was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2020 unit and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020–Programa Operacional Regional do Norte. The authors also acknowledge COMPETE2020 and FCT for the project POCI-01-0145-FEDER-029841 and FCT for the PhD Grant of Tânia Grainha [grant number SFRH/BD/136544/2018].

References
Investigation of Antimicrobial Coatings for Endotracheal Tubes to Prevent Ventilator-Associated Pneumonia

Jia Li, Matthew Wylie, Nicola Irwin, Louise Carson, David Jones, Colin McCoy

Queen's University Belfast, School of Pharmacy, Belfast, GB

Introduction
Ventilator-associated pneumonia is a major type of hospital-acquired infection and is a frequent cause of mortality and morbidity in Intensive Care Units (1). Endotracheal tubes help patients breathe but can also lead to infection due to the bacterial biofilm formed on the surface (2). This study describes development of a strategy for coating of endotracheal tubes to delay or prevent biofilm formation.

Experimental Methods
Copolymer hydrogels were polymerized from 2-hydroxyethyl methacrylate (HEMA) and methacrylic acid (MAA) in different proportions (80:20 and 70:30%w/w), as reservoir coatings. Two different antibiotics, gentamicin and levofloxacin, were loaded into the coatings by immersed in gentamicin solution (50mg/ml) and levofloxacin solution (10mg/ml) for 48 hours. Copolymers loaded with gentamicin and levofloxacin were characterised in terms of drug release and resistance to microbial adherence.

Results and Discussion
The release of gentamicin and levofloxacin from the hydrogels were rapid, with 50% release occurring within the first 5 hours. Both coatings loaded with gentamicin and levofloxacin displayed anti-adherence properties against Staphylococcus aureus (ATCC29213) and Pseudomonas aeruginosa (LMG679) that both hydrogels were able to produce a 100% reduction in adherence over 24 hours (Figure 1).

Conclusion
Coatings for endotracheal tubes loaded with gentamicin or levofloxacin displayed good short-term microbial anti-adherent properties which can provide a platform to reduce the incidence of ventilator-associated pneumonia.

Acknowledgement
The authors would like to acknowledge China Scholarship Council for funding support.
References

New natural solvent improves beta-lactam antibiotic efficacy

Belén Olivares¹,², Bernardo J. Morales³, Fabián A. Martínez⁴, Paola R. Campodónico¹, Marcelo Ezquer¹

¹ Universidad del Desarrollo, Instituto de Ciencias e Innovación en Medicina, Santiago, CL; ² Universidad Nacional de Cuyo, Programa de Doctorado en Biología, Mendoza, AR; ³ Universidad Pedro de Valdivia, Facultad de Ciencias de la Salud, Santiago, CL; ⁴ Universidad de Santiago de Chile, Laboratorio de Resonancia Magnética Nuclear, Santiago, CL; ⁵ Universidad del Desarrollo, Instituto de Ciencias e Innovación en Medicina, Santiago, CL; ⁶ Universidad Nacional de Cuyo., Programa de Doctorado en Biología, Mendoza, AR

Introduction

World Health Organization declared bacterial infections by resistant pathogens a global crisis and place it among its priority lines of research. The aim of the present work is to improve the antibacterial efficacy of available molecules. We focus on beta-lactam antibiotics, a very useful therapeutic class of antibiotics, but unstable in the aqueous solvents of conventional formulations. That condition represents a clinical problem leading to therapeutic failure and antimicrobial resistance. We designed a new vehicle based on a natural deep eutectic solvent consisting on betaine and urea (BU). BU is capable of improving the stability of some beta-lactams including imipenem (IMP) the most unstable of the beta-lactam family. The main goal of the present work is to evaluate the therapeutic efficacy, the kinetics of release of IMP dissolved in BU, and evaluate the in vitro toxicological and immunogenic profile of the new solvent. Furthermore we studied the protective mechanism of the effect observed.

Experimental Methods

Killing curves of Pseudomonas aeruginosa incubated at 37°C, with IMP dissolved in BU in comparison with its aqueous solutions. Viability measures were made counting bacteria by flow cytometry using SytoBC®/propidium iodide. The kinetic of IMP release was evaluated using a diffusional test based on a bicameral devise model and data were analyzed applying Peppas Krosmayer equation. Cytotoxicity was evaluated measuring viability of primary fibroblasts cultures exposed to BU. The immunogenicity was evaluated through a human monocytic cell line THP-1 model measuring the expression of TNF and IL-6 using lipopolysaccharides as a proinflammatory positive control. The mechanism of intermolecular interaction was studied by selective NOESY H¹ - H¹ Nuclear Magnetic Resonance (NMR) applied to IMP-BU solution.

Results and Discussion

IMP-BU reduced the P. aeruginosa counting units forty times compared to IMP aqueous solution (mean and SEM 1.87x10⁷ ±3.91x 10⁶ and 7.5x10⁸ ± 3x10⁷ respectively) in 24 hours. The value of the diffusional coefficient obtained (n=1,01) indicates a case II release, consisting in a linear and progressive kinetic release. On the other hand, the cytotoxicity assay of BU showed an IC50 of 59.4 mg/ml,wich is higher than the reported for others solvents of this class. Furthermore the proinflammatory citokines expression levels indicates no inmunogenic response of BU. Finally the NMR analysis indicated especific interactions between de drug and the molecules of the solvent.

Conclusion

BU could represent a new biosafe funcional excipient to maintain IMP stability and therefore improve its efficacy.

Acknowledgement

Dirección de Investigación y Doctorados. Universidad del Desarrollo.
New eutectic solvent preserves beta-lactam antibiotic activity

Graphical representation of the observed effect related with the preserved efficacy when IMP is dissolved in betaine:urea (BU).

References
PS1-12 | Nanobiomaterials
PolyVax: Polymeric Nanoparticles as mRNA vaccines for prophylaxis and cancer therapeutics

Cristina Fornaguera, Coral Garcia-Fernandez, Laura Olmo, Marta Díaz-Caballero, Marta Guerra-Rebollo, Salvador Borrós

Institut Quimic Sarria (IQS) - Ramon Llull University (URL), Grup d'Enginyeria de Materials (Gemat), Barcelona, ES

Introduction
Vaccination has been one of the main success of modern society, remarkably improving the human beings’ half-life thanks to the eradication of many infectious diseases. Traditional vaccines were composed of entire or fractions of the infectious agent. However, they account with unsolvable disadvantages, such as safety issues and low immunogenic potential that requires the addition of adjuvants. In this context, the use of mRNA for immunizing purposes has shown an enhanced performance, as demonstrated by the very fast approval of two mRNA vaccines preventing SARS-CoV-2 infection. Beyond success in preventing viral infection, mRNA has also spread the use of vaccination for therapeutic cancer applications, and currently, the bibliography demonstrating research studies using mRNA vaccines is huge. Nevertheless, the tech transfer is still limited. In this context, PolyVax aims to solve current issues of mRNA vaccines by the development of a mRNA vaccine based on proprietary patented polymeric nanoparticles, with demonstrated safety, efficacy and selectivity to immune target cells.

Experimental Methods
Nanoparticles with a selective transfection to dendritic cells, encapsulating spike antigen epitopes or tumor associated antigens mRNA were synthesized and lyophilized by a simple, scalable and GMPzable process. Their safety and efficacy were tested in vitro and in vivo. Biodistribution of different routes of administration and specific immune response generation was tested in healthy mice.

Results and Discussion
Based on our experience of biomaterials engineering, we developed a library of oligopeptide-end modified poly (beta aminoesters) (OM-pBAE) by two-step Michael addition of primary amines to acrylates, followed by oligopeptides end capping. These proprietary OM-pBAE demonstrated high efficiency on the encapsulation of various mRNA encoding for different proteins (antigenic and reporter), by electrostatic interaction between the cationic polymers and the anionic nucleic acid, for the formation of small nanometric (<180nm) nanoparticles, able to be freeze-died without losing their integrity and functionality for, at least, 9 months. By the selection of the appropriate oligopeptide composition, we found a formulation that selectively targets dendritic cells after parenteral administration. In addition, this formulation is able to promote dendritic cells maturation and to generate a specific immune response against SARS-CoV-2 encoded spike antigens and against tumor-associated antigens in mice. In addition, tech transfer studies demonstrated the capability to produce the vaccine in an easy to scale up and under GMP procedure.

Conclusion
In conclusion, we have been able to design a platform for the production of mRNA vaccine based on polymeric nanoparticles able to overcome the main limitations that traditional vaccines present.

Acknowledgement
Author acknowledge funding received from Generalitat ed Catalunya, ISCIII and MINECO. CGF acknowledges IQS for their predoctoral grant.
Graphical abstract
Schematic representation of the functioning of PolyVax, as demonstrated by an example in non-small cell lung cancer (NSCLC) treatment.

References
Functional composite biomaterials based on iron nanoparticle-loaded hydrogels for 3D biofabrication

Christoph Rehbock, Yaya Li, Stephan Barcikowski

University of Duisburg-Essen, Essen, DE

Introduction
Nanocomposites constitute an interesting class of materials for tissue engineering, where the embedding of nanoparticles into a biocompatible polymer adds novel functionality and hence, improves viability and proliferation of relevant adhered cells. Our group has developed an elegant method for the fabrication of nanocomposites based on laser ablation in liquids in polymer and monomer solutions, yielding nanocomposites with outstanding purity and biocompatibility [1]. In previous works we could already demonstrate improved cytocompatibility of these materials through two separate pathways I) local changes in surface charge and stiffness [2] and II) Release of metal ions [3].

Experimental Methods
Nanoparticles were implemented into the hydrogel by an in situ embedding procedure using laser ablation in liquids. The obtained hydrogels were characterized using surface zeta-potential measurements, TEM and confocal microscopy. Contact angles were measured with the captive bubble method to probe the hydrophilicity of the composites. Ion release from the composites was measured by ICP-MS analysis. Protein adsorption was determined by quantification of residual proteins in supernatants via UV-Vis spectroscopy. Suitability for 3D additive manufacturing was demonstrated using a microextrusion-based compact 3D-printer.

Results and Discussion
In our most recent work we focused on alginate and PEG hydrogel-based biomaterials loaded with iron nanoparticles and examined the mechanism of metal ion release from these biomaterials. We show that the nanoparticles were evenly distributed in the gel matrix and hydrophilicity and surface charge were increased. We identified a unique release behavior of iron ions from alginate-based composites, primarily driven by oxide solubility and interactions between iron ions and the alginate hydrogel matrix. Furthermore, we could find a significantly enhanced adhesion of model serum proteins to the biomaterial surface, which, interestingly already occurs at very low nanoparticle loadings (< 0.1 wt%). This seems to indicate that a mechanism based on locally elevated iron ion concentrations is responsible for the enhanced biocompatibility of these nanoparticle-loaded biomaterials. [4] We further verified the suitability of these materials for 3D bioprinting applications where the nanoparticles had no adverse effects on printability. [3] [4] These findings were complemented by recent examinations with hematopoietic stem and progenitor cells in 2D and 3D culture. Here we found a significant influence of iron nanoparticles in the biomaterial on the early stage of in vitro erythropoiesis relevant for applications in in vitro blood farming.[5]

Conclusion
Our findings demonstrate that the loading of hydrogel-based bioinks with a few ppm of soluble metal nanoparticles can have a pronounced impact on biocompatibility. Interestingly, we could further demonstrate that the commonly used combination of iron nanoparticles with an alginate matrix shows a unique iron ion release behavior, which could deepen our understanding of future biomaterial-cell interactions. Further initial 3D-printing experiments with these newly developed, nanoparticle-spiked bioinks demonstrate their further usefulness for example in tissue engineering applications.

References


Preparation and characterization of Chitosan/PVA-based nanoparticles

Leonard I. Atanase\textsuperscript{1}, Delia M. Rata\textsuperscript{1}, Anca N. Cadinoiu\textsuperscript{1}, Marcel Popa\textsuperscript{1,2}

\textsuperscript{1} Apollonia University, Biomaterials, Iasi, RO; \textsuperscript{2} Academy of Romanian Scientists, Bucharest, RO

Introduction

World Health Organization (WHO) has classified pulmonary infections as one of the main diseases affecting the human race and therefore important efforts must be consecrated to their prevention, diagnosis and treatment. Pneumonia is an inflammatory condition of the lung (alveoli) and it is caused by infection with viruses, bacteria, parasites or fungi. Although oral or injectable drugs against bacterial pulmonary infections are effective, the treatment has to be administered as combination therapy in high drug doses for long durations in order to maintain an optimum therapeutic level. As pulmonary infections are often persistent and recurrent, a rise in drug-resistant strains of infectious organisms, such as \textit{Streptococcus pneumoniae}, poses great challenges in the treatment of pneumonia in the clinical practice. In order to overcome this inconvenient, a potential therapeutic approach is to target the delivery of drugs directly to the site of infection.

Experimental Methods

An UltraTurrax homogenizer was used for the preparation of a water-in-oil emulsion. The esterification of the carboxylic groups of CMCS with the hydroxyl groups of PVA and amidation of the remaining amino groups CMCS with its carboxylic groups was carried out in the presence of DMT-MM, as an activating agent. Nano/microspheres were separated by centrifugation, purified by successive washings and freeze dried. The following parameters were taken into account: CMCS/PVA/DMT-MM weight ratio (w/w), concentration of polymer solutions (%) (w/v), emulsification and condensation reaction time, hydrodynamic regime.

FTIR spectroscopy was used to confirm the reaction between PVA/CMCS in the presence of DMT-MM. N/MPs diameter/morphology was evaluated by DLS, TEM and SEM. Particles stability was studied measuring the Zeta potential (ZP) in buffer solution (PBS) at pH 7.4. SEM-EDAX elemental analysis was used in order to determine the elemental composition of N/MPs. The swelling degree in solutions that mimic biological fluids was evaluated by a gravimetric method.

Results and Discussion

A widely accepted theory in the literature states that for efficient lung deposition, the carrier’s size should be in the range of 1–5 μm. Smaller particles (<1 μm) are likely to be absorbed quickly from the airways and this poses a risk of systemic toxicity. Moreover, 80% of these smaller particles are exhaled without being deposited due to their low inertia. In contrast, larger particles (>5 μm) are cleared by the mucociliary clearance mechanism. Therefore, a particle size in the range of 1–5 μm is required to achieve efficient pulmonary drug delivery via inhalation route.

In view of the above, the main objective of this study is related to the preparation of nano/microparticles (N/MPs), based on carboxymethyl chitosan (CMCS) and poly(vinyl alcohol) (PVA). The mechanical properties brought by the PVA and the mucoadhesive properties of the CMCS represent a viable combination for the preparation of porous N/MPs. These N/MPs were prepared by a condensation reaction of the polymers in the aqueous phase of a water-in-oil emulsion (polymer’s aqueous solution dispersed in ethyl acetate), as illustrated in the following Fig 1: The size of the obtained particle was in the submicronic range, as a function of the molar mass of PVA. The FTIR and EDAX analysis has confirmed the presence of both PVA and chitosan in the composition of the particles. The ZP values confirmed the high stability of these particles in an aqueous medium.

Conclusion
Based on the obtained results, these biocompatible particles are suitable for the loading of specific drugs in view of the potential treatment of pulmonary diseases by a pulmonary inhalation method.

**Acknowledgement**

„This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P4-ID-PCE-2020-2009, within PNCDI III“
Understanding the influence of nanogels’ structure on protein corona formation

Federico Traldi, Anjumanara B. Arefa, Arianna Fornili, Marina Resmini

Queen Mary University of London, School of Biological and Chemical Sciences, London, GB

Introduction
The use of nanomaterials as drug delivery systems and diagnostic tools has attracted considerable interest from scientists in the last decades. However, the limited knowledge of the behaviour of nano-systems in biological media and their interactions with biomolecules is hindering their application in clinical settings. The formation of “protein corona” has been linked to a significant, and still vastly unpredictable, change in the behaviour of many nanoparticle systems both in vitro and in vivo, posing significant obstacles to the rational design of nanomaterials. There is a clear need to understand how the physical-chemical properties of nanoparticles affect their interactions with proteins and how the chemical structure can be used to tailor this behaviour.

Experimental Methods
In the present work, we investigated how morphological and chemical properties of polymeric nanogels affect their interactions with proteins. Nanogels are attractive drug delivery platforms thanks to their high colloidal stability, bioavailability, and the stimuli responsive properties that can be introduced by using specific monomers. However, few studies have attempted to elucidate the relationship between the structure of nanogels and protein corona formation. A series of acrylamide-based nanogels were synthesised using high dilution radical polymerisation. Nanogels surface charge was modified by the addition of charged co-monomers such as acrylic acid and the crosslinker methylenebisacrylamide was added to the formulation in concentrations of 5, 10 and 20 molar%. The impact of these parameters on the affinity of nanogels for model proteins such as lysozyme was investigated using a combination of techniques such as dynamic light scattering and spectrophotometry. Experimental investigation was complemented with the computational analysis of protein affinities for functional groups responsible for the protein-nanogel interactions.

Results and Discussion
Our findings suggest that both structural and morphological properties of nanogels significantly affect their ability to interact with the tested proteins. Although protein corona of nanogels was found to be more sensibly affected by the nanogel surface charge, more subtle changes in the matrix were also able to significantly alter the affinity of nanogels for the proteins.

Conclusion
These data improve our understanding of the correlation between nanogels chemical composition and protein corona formation and may enable a more rational design of drug delivery systems.

References
Biocompatibility assessment of magnetoelectric cobalt ferrite CFO-BCZT nanoparticles for non-invasive deep brain stimulation

Maurizio Gulino,1,2 Donghoon Kim3, Xiangzhong Chen3, Qiao Tang3, Sofia D. Santos2, Salvador Pané3, Ana P. Pêgo1,4

1 i3S/INEB - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal, Porto, PT; 2 FEUP - Faculdade de Engenharia, Universidade do Porto, Porto, Portugal, Porto, PT; 3 Multi-Scale Robotics Lab (MSRL), Institute of Robotics and Intelligent Systems (IRIS), ETH Zurich, Zurich, Switzerland, Zurich, CH; 4 ICBAS/FEUP - Universidade do Porto, Porto, Portugal, Porto, PT

Introduction
Magnetoelectric nanoparticles are arising an increasing interest as innovative nanomaterials capable of effectively modulating neural circuits in vivo with high precision and minimal invasiveness compared to traditional brain stimulation devices (Guduru et al., 2015; Nguyen et al., 2020; Singer et al., 2020). Due to the recent burst of this new class of materials, few studies have been conducted to assess their biocompatibility and the impact on neuronal and glial cells.

Here, we explored rodent primary astrocytes and microglia to assess the biocompatibility of a piezoelectric ceramic composite with evidenced potential as implant material.

Experimental Methods

Functional multiferroic core-shell cobalt ferrite (CFO) nanoparticles coated with calcium/zirconium-doped barium titanate (CFO-BCZT) were synthesized with hydrothermal and sol-gel processes. Single crystalline cubic CFO core was prepared with hydrothermal method. Then BCZT shell was coated on CFO core particles using sol-gel method. Magnetoelectric properties of CFO-BCZT core-shell nanoparticles have been studied using magnetic field-assisted piezoresponse force microscopy.

The cellular responses of CFO-BCZT nanoparticles were evaluated through cytotoxicity assay, microscopy analysis and cellular uptake. Uncoated CFO nanoparticles were used as reference for the corresponding coated sample.

Results and Discussion

Crystallinity of the CFO and CFO-BCZT nanoparticles have been assessed with X-ray diffraction. Both particles showed no impurities nor secondary phase. Piezoresponse Force Microscopy scan of CFO-BCZT core-shell nanoparticles indicates that CFO-BCZT has magnetoelectric coupling, which is caused by the magnetic field induced interfacial strain between CFO and BCZT.

We show that while astrocytes viability was not affected after NPs exposure at different concentrations for 24 hours, primary microglia viability was compromised by CFO nanoparticles. As hypothesized by us, the coating decreased such effect in cells exposed to CFO-BCZT nanoparticles. Prussian blue staining revealed that CFO-BCZT nanoparticles are less internalized by microglia compared to CFO, suggesting that the BCZT shell makes the NPs less prone to microglia phagocytic activity.

Conclusion

In conclusion, these data indicate that CFO-BCZT nanoparticles not only exhibit magnetoelectric coupling effect, but they are also more biocompatible compared to uncoated ones. This sets the exploration of CFO-BCZT nanoparticles as a promising solution to overcome the current limitations of traditional deep brain stimulation systems.

Acknowledgement

The authors acknowledge the funding from the European Union’s Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreement No. 764977, in the framework of the mCBEEs project (Advanced
integrative solutions to corrosion problems beyond micro-scale: towards long-term durability of miniaturized biomedical, electronic and energy systems).

Live/ Dead assay by Propidium iodide/ Calcein staining of primary rodent microglia
Viability of primary rodent microglia after exposure to CFO and CFO-BCZT nanoparticles at 100 µg/ml for 24 hours (Data shown as mean ± SEM of quadruplicate wells and are representative of 3 independent experiments).

Prussian blue staining and nuclear fast red counterstain of primary rodent microglia
Prussian blue staining and nuclear fast red counterstain of primary rodent microglia untreated (A), exposed to CFO nanoparticles at 100 µg/ml (B) and CFO-BCZT nanoparticles at 100 µg/ml (C) for 24 hours (scale bar 10 µm).

References
Novel application of hot stage polarized light microscopy for the production of nanostructured lipid carriers via warm microemulsion templates

Felipe M. Gonzalez-Fernandez1,2, Sara Nicoli1, Paolo Gasco2, Silvia Pescina1

1 Parma University, Food and Drug Department, Parma, IT; 2 Nanovector Srl, Turin, IT

Introduction
Nanostructured lipid carriers (NLC) are lipid-based nanocarriers (< 200 nm) composed of both liquid and solid lipids, stabilized with surfactant(s) and dispersed in an aqueous media. Their range of applications is wide since they are mainly composed of highly biocompatible lipids, enhancing drug apparent solubility in water, protecting it from degradation phenomena and overall improving targeted drug delivery. Amongst the diverse production methods, a simple and easily up-scalable approach is the redispersion in cold aqueous media (~ 4 ºC) of warm oil-in-water microemulsions, i.e., self-assembled, thermodynamically stable, isotropic transparent liquids [1]. Quick identification of surfactants-lipids-water combinations that lead to microemulsions at temperatures generally above the solid lipid melting point (65-70 ºC) is a crucial step in this method. Depending on the relative proportions between components, different systems can be observed: some structures can be macroscopically assessed, while other require microscopic assessment. Previous research on the use of polarized light microscopy for the evaluation of microemulsions is available in the literature but only at room temperature [2]. Hot stage microscopy, already used in different fields, allows to heat and cool the samples in a staggered manner or maintain at a fixed temperature to evaluate their temperature behaviour [3].

In this work we report a novel application of hot stage polarized light microscopy for the characterization and identification of warm microemulsion templates for the production of NLC.

Experimental Methods
Pseudoternary phase diagrams were constructed for lipid/surfactant/water mixtures consisting of triacetin (oil), monostearin (solid lipid) and tyloxapol (surfactant) mixtures using a water titration technique. Briefly, initial mixtures containing only lipids and surfactant having relative proportions ranging from 9:1 to 1:9 were accurately weighted in transparent glass vials. Oil to solid lipid proportion was held at a constant 2:3 ratio. The mixtures were heated at 65 ºC under magnetic agitation. Aliquots of 10% ultra-pure deionized water were progressively added and the macroscopically appearance after stabilization was noted and classified. Lipid-water and surfactant-water mixtures were also analysed as control.

An optical polarizing light microscope (Nikon Optiphot 2 POL, Nikon, USA) coupled to a stage temperature microscope controller (TP 93 Linkam Scientific Inst., Miami, USA) was used to analyse the 120 different excipient combinations. A drop of warm sample was deposited on microscope slides placing a cover glass. Samples were positioned on the hot stage preheated at 65 ºC and observed under cross-polarized immediately, minimizing any possible evaporation. Photomicrographs at 400x magnification were taken.

Results and Discussion
The pseudoternary phase behaviour of the triacetin/monostearin-tyloxapol-water system was macroscopically and microscopically assessed at 65 ºC. Macroscopically, a large region containing mixtures that appear transparent to the naked eye was quickly identified, especially with increasing tyloxapol concentrations. Water addition resulted in loss of transparency, specially at high triacetin/monostearin proportions leading to coarse macroemulsions or, in some cases, phase separation.
Microscopically, the method allowed to further characterise the large transparent region previously observed, detecting both anisotropic and isotropic structures, being only the latter of interest for our application. Low water concentrations of 10 and 20% of water showed intense birefringence mainly explained by high monostearin concentration. Isotropy was observed for all samples containing lipid-surfactant ratios of 2:8 and 1:9 with independence of the percentage of water. Further anisotropic structures compatible with liquid crystal mesophases were also observed but only further characterization by other techniques (i.e. small-angle X-ray scattering) could adequately corroborate their structure.

Lipid-water and surfactant-water controls were also in close agreement with previously reported data [4]. In the case of monostearin, anisotropy compatible with a liquid-crystalline lamellar mesophase could be observed until water percentages of 50%. In the case of the tyloxapol-water mixtures, at all water concentrations a dark field was observed.

**Conclusion**

The novel method based on hot stage polarising light microscopy quickly and efficiently allowed to detect isotropic microemulsions, simplifying the development of nanostructured lipid carriers based on triacetin/monostearin-tyloxapol-water mixtures. Further research, by redispersion of the isotropic structures in cold aqueous media could help confirm the usefulness of this approach for a more efficient NLC production.

**Acknowledgement**

This project is funded by the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie Actions (grant agreement - No 813440)

**References**


SNAP loading on carbon nanotubes

Fatemeh Kabirian¹, Petra Mela², Ruth Heying³

1 KU Leuven, Department of Cardiovascular Sciences/Cardiovascular Developmental Biology, Leuven, BE; ² Technical University of Munich, Munich, Medical Materials and Implants, Department of Mechanical Engineering and Munich School of BioEngineering, Munich, DE; ³ KU Leuven, Department of Cardiovascular Sciences/Cardiovascular Developmental Biology, Leuven, BE

Introduction
Nanocarriers such as carbon nanotubes are appropriate candidates for drug delivery applications. Due to the nanoscale, they have a high surface area and they can store higher concentration of drug molecules such as a nitric oxide donor which could be used to overcome the limitations of current approaches such as burst release.

Experimental Methods
S-nitroso-N-acetyl-D-penicillamine (SNAP, C7H12N2O4S) as a nitric oxide donor was synthesized. Before formation of SNAP crystals, 25 mg of Multiwalled carbon nanotubes were immersed in 1 ml of SNAP solution for 24h at 4°C. After washing with ice cooled MQ water, the loaded MWCNTs were dried in a lyophilyzer. The amount of nitrogen was measured by performing CHNS analysis.

Results and Discussion
Immersion of MWCNTs inside the SNAP solution resulted in 2.3±0.26% nitrogen content in the loaded MWCNTs.

Conclusion
SNAP can be loaded on MWCNTs which can be promising for the development of medical devices with antibacterial properties.

Acknowledgement
The authors greatly acknowledge the FWO Vlaanderen for the postdoctoral grant (1291021N) given to Fatemeh Kabirian to support this study.
Loading of SNAP on carbon nanotubes
Comparison of two types of mesoporous silica nanoparticles loaded with gentamicin to overcome antibiotic resistance and inhibit bacterial biofilm formation

Nassim Chekireb\textsuperscript{1,2}, Jean-Sebastien Thomann\textsuperscript{2}, Anna Belcarz\textsuperscript{3}, Agata Przekora-Kuśmierz\textsuperscript{3}, Grazyna Ginalsak\textsuperscript{3}, David Duday\textsuperscript{2}

\textsuperscript{1} Université Sorbonne Paris Nord, Bobigny, FR; \textsuperscript{2} Luxembourg Institute of Science and Technology, Esch-sur-Alzette, LU; \textsuperscript{3} Medical University of Lublin, Lublin, PL

Introduction
Gentamicin is an antibiotic broadly used because it has the ability to treat different types of infections. However, after oral administration, the bioavailability of gentamicin is low and cell penetration is poor. In addition, the internalized gentamicin molecules expulsed from cells with efflux pumps or degraded by specific enzymes, resulting in a decrease of the activity sought. In case of parenteral administration, gentamicin is rapidly eliminated by glomerular filtration, and then passes to the renal cortex. Therefore, administration of repeated doses leads to renal accumulation and nephrotoxicity \cite{1}. Bacterial resistance to antibiotics, including gentamicin, is a serious threat for Health \cite{2}. It is therefore essential to find new ways of delivering antibiotics to decrease the appearance of bacterial resistance. A better control of antibiotic release through nanocarriers like amorphous mesoporous silica nanoparticles (AMSNPs) is one promising approach to limit this bacterial resistance phenomenon. The literature shows the advantages of using locally delivered antibiotics including the delivery of a high concentration of antibiotics to a localized area without systemic involvement and minimal risk of developing bacterial resistance \cite{2-3}. The aim of the present work is the use of 2 advanced synthetic strategies to obtain AMSNPs able to efficiently carry a drug such as gentamicin and their effects on the eukaryotic cell viability and growth inhibition of 4 reference bacterial strains

Experimental Methods
The AMSNPs was synthesized using sol-gel approach, the gentamicin loading was carried out by precipitation in ethanol. The morphology of AMSNPs was performed by scanning transmission electron microscope (STEM), the size and zeta potential were determined by Malvern zeta sizer. the demonstration of the presence of gentamicin on silica was established by FTIR. The AMSNPs cytotoxicity against mouse preosteoblasts was evaluated by colorimetric MTT assay. Antimicrobial efficiency was evaluated on 4 reference bacterial strains

Results and Discussion
The two types of particles have different morphology in terms of size varying from 70 nm to 110 nm, specific surface area from 494 m\textsuperscript{2}/g to 1031 m\textsuperscript{2}/g and pore size from 2.3 nm to 18 nm. Stellate silica nanoparticles are less cytotoxic than wormhole silica nanoparticles. The MIC and MBC values for both type of silica nanoparticles have shown bactericidal activity for all strains and bacteriostatic activity for S. aureus

Conclusion
Overall, this study demonstrates that both types of nanoparticles loaded with gentamicin are not cytotoxic on bon cells and efficient for bringing gentamicin into bacterial cells.

Acknowledgement
Financial assistance was provided within M-Era.Net 2 transnational research program by National Science Centre in Poland (NCN, project no. UMO-2016/22/Z/ST8/00694) and Fonds National de la Recherche Luxembourg (FNR, project no. INTER/mera/16/11454672).

Page 1378 of 2028
References


Drug delivery for neural stem cell proliferation

Eirini Epitropaki, Yujie Xie, Amanda K. Pearce, Rachel K. O'Reilly

University of Birmingham, Chemistry, BIRMINGHAM, GB

Introduction
Neurodegenerative diseases such as ischemic stroke, Alzheimer’s, and Parkinson’s can cause cellular loss, which can be tackled with the use of neural stem cells (NSCs). To promote stem cell differentiation into functional neurons, nanomedicine as a non-invasive method presents an opportunity to enable delivery of differentiating agents, while preserving the blood and brain barrier (BBB). In order for nanoparticles to achieve efficient delivery to the central nervous system (CNS), numerous physicochemical parameters must be examined. Size, morphology, and surface charge of the nanoparticles are the key parameters that determine delivery efficacy. Based on the above, the current work aimed to produce amphiphilic nanoparticles with various corona chemistries, different sizes, and biodegradable nature, in order to explore the above parameters. Different morphologies such as diamond platelets and molecular caterpillars were formed by exploiting the core crystallinity using the crystallization driven self-assembly (CDSA) technique. As the particles are developed as an intravenous carrier, the interaction between the self-assemblies and protein models must be investigated. The effect of the surface charge was explored by introducing the nanoparticles in various biological environments such as salts, cell culture medium, fish water, and model protein solutions and monitor the interactions occurring.

Experimental Methods
1. For the Synthesis of the particles, a combination of ROP (for the hydrophobic core) and RAFT (for the hydrophilic corona) polymerization was used.
2. The incorporation of the fluorophore was conducted through a Mitsunobu reaction.
3. The self-assembly took place using the flash nanoprecipitation technique (Dissolution of polymer into good solvent and direct introduction to an aquatic or biologically relevant medium while stirring intensely)
4. For the development of different morphologies, crystallization-driven self-assembly (CDSA) technique was utilized. (Dissolution of polymer into θ solvent, heating up the system and then let it cool down in order to crystallize)
5. The self-assemblies and their interactions with biological media were examined thoroughly by DLS, AFM, and confocal measurements.

Results and Discussion
• Diblock copolymers with versatile corona chemistries were easily developed through simple techniques.
• By utilizing the ratio between the hydrophobic core and hydrophilic corona, as well as the corona chemistry and the length of the semi-crystalline PLLA, different morphologies were obtained.
• The positively charged self-assemblies indicated a stable solution for 24 hours at 36.5 °C. The particles were examined in various biologically relevant mediums, where they indicated a smaller particle size of 68 nm in a PBS solution. The observed result can be explained due to the stabilization of the charges in PBS solution hence the smaller population.
• The protein corona was examined with a 4 mg/ml BSA solution, which was used as the model protein. DLS and zeta potential measurements verify the electrostatic interactions between the negatively charged BSA protein and the positively charged particles. Various layers of BSA appeared to have deposited on top of the particles creating a cluster of 1μm, as it was observed through AFM. Lastly, the correlation of the fluorescent channels
through confocal proves the interaction between the protein and the nanoparticles and verifies the protein corona formation.

Conclusion

- A library of diblock copolymers was synthesized with positive and neutral corona chemistries, with amorphous and crystalline core, and with an incorporated fluorescent ACM probe.
- Various morphologies acquired through CDSA like diamond platelets, diamonds with tails, and molecular caterpillars depending on their corona chemistry and ratio.
- Stability in body temperature of the catanionic corona particles over time was demonstrated.
- Stability of the aforementioned particles in various biologically relevant mediums, such as salts, cell culture medium, and fish water was examined.
- Electrostatic interactions of the negatively charged BSA protein with the positively charged particles were verified by DLS, AFM, and confocal measurements.

Acknowledgement

I would like to thank all the O'Reilly group members for their support, Dr. Y.Xie for the confocal images, Dr. Amanda K. Pearce for her help and guidance, and Prof. Rachel O'Reilly for her supervision.

I would also like to thank the Marie Curie NANOSTEM project for the funding and the training.

References

PS1-12-385

**In-vitro cell responses to strontium/magnesium-doped calcium phosphate nanoparticles**

**Shabnam Hosseini, Kathrin Kostka, Matthias Eppe**

*Duisburg-Essen University, Chemistry, Essen, DE*

**Introduction**

Bone is an inorganic–bioorganic composite material consisting mainly collagen and calcium phosphate. Its structure, surface roughness, chemistry, and mechanical properties of biomaterials biological matter are fascinating for many researchers.[1, 2] Calcium phosphate nanoparticles (CaP) are well known as highly biocompatible and biodegradable inorganic composite for bone regeneration.[3] On the other hand, strontium (Sr) and magnesium (Mg) are assumed to enhance the formation of bone. The substitution of strontium and magnesium into hydroxyapatite instead of calcium is an effective way to enhance the biological properties of calcium phosphate-based biomaterials.[4, 5]

**Experimental Methods**

Here, strontium-doped calcium phosphate (SrCaP) and magnesium-doped calcium phosphate (MgCaP) nanoparticles with different molar contents of 5, 10, 15, and 20% were prepared separately by precipitation from aqueous solutions as dispersed nanoparticles. The nanoparticles were characterized by dynamic light scattering (DLS), zeta potential measurements, scanning electron microscopy (SEM), atomic absorption spectrometry (AAS), X-ray powder diffraction (XRD), thermogravimetric analysis (TGA), and energy-dispersive X-ray spectroscopy (EDX). Furthermore, *in-vitro* cell culture studies as a viability test (MTT) and uptake investigation in the three different cell lines HeLa, MG63, and MC3T3 were carried out.

**Results and Discussion**

The resulting nanoparticles were monodisperse with well-controlled size (about 100 nm), morphology, and composition. The nanoparticles were well taken up by HeLa, MG63 and MC3T3 cell lines together with a low cytotoxicity.

**Conclusion**

The nanoparticles can either be used in dispersion or added to a calcium phosphate paste for injection into bone defects.

**References**


Development of glucose-responsive nanoparticles towards the design of a bioartificial pancreas for diabetes therapy

Joana Moreira Marques\(^1\,2\), Rute Nunes\(^3\,4\), Helena Florindo\(^3\), Domingos Ferreira\(^2\), Bruno Sarmento\(^1\,4\)

\(^1\) University of Porto, i3S, Porto, PT; \(^2\) University of Porto, Faculty of Pharmacy, Porto, PT; \(^3\) Universidade de Lisboa, Faculty of Pharmacy, Lisboa, PT; \(^4\) Instituto Universitário de Ciências da Saúde (CESPU), Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Gandra, PT

Introduction

Diabetes mellitus (DM) is one of the biggest health problems nowadays, as 463 million individuals suffer from DM worldwide and this number is expected to rise [1]. Type 1 DM (T1DM) is a chronic auto-immune disease characterized by insulin insufficient secretion due to β-cells destruction [1]. Since T1DM is not preventable and most of the cases are diagnosed after massive β-cells destruction [2], definitive cure consists in replacing the destroyed pancreas [3]. However, the number of available pancreas or islets is limited, in addition to the need of a lifelong immunosuppression therapy. In this work it is proposed an innovative biomimetic pancreas, comprising differentiated induced pluripotent stem cells (iPSCs) into functional insulin-producing cells, immobilized in a biofunctional matrix and glucose-responsive nanoparticles (NPs) encapsulating a GLP-1 analogue. The sensitiveness to glucose will be accomplished by the incorporation of the enzyme glucose oxidase (GOx) in the system. In the presence of high levels of glucose, GOx degrades it into gluconic acid, decreasing the surrounding pH (\(\sim 5\)). pH-sensitive engineered NPs will respond to the decrease of the environmental pH by releasing their cargo.

Experimental Methods

pH-sensitive NPs based on hybrid PLGA/polymethacrylates matrices were produced by a modified solvent emulsification-evaporation method based on double emulsion technique [4] to encapsulate GLP-1 analogue exenatide or semaglutide. Association efficiency (AE) and drug loading (DL) were determined by HPLC. Exenatide and semaglutide in vitro release profiles were assessed at different pH (5 and 7.4) and secondary structure stability after release was confirmed by circular dichroism. Human WLS-4D1 induced PSCs (hiPSCs) were differentiated into insulin-producing cells [5]. iPSCs differentiation was assessed at different stages by flow cytometry through the expression of different cell markers. The capacity of differentiated cells to produce insulin was assessed by glucose-stimulated insulin secretion (GSIS) assay.

Results and Discussion

Different NPs formulations showed a monodisperse population (PdI <0.160) with an average size ranging from 120 to 150 nm and zeta potential (ZP) around 30-40 mV for higher polymethacrylates ratios, decreasing up to -7 mV for higher PLGA proportions. Two GLP-1 analogues were encapsulated (exenatide and semaglutide) using different DL (between 5\% and 15\%). For exenatide-loaded NPs, AE values ranged from 34 \% to 49 \%, while for semaglutide-loaded NPs, AE was close to 50 \%, depending on PLGA/polymethacrylates ratios. NPs showed a pH-dependent in vitro release profile, despite showing a burst release of around 40 \% at pH 7.4 and 60\%-70\% at pH 5 after 5 minutes, depending on the peptide. Besides, both exenatide and semaglutide maintained its secondary structure after 48h of in vitro release. hiPSCs were successfully differentiated into insulin-producing cells and GSIS assay showed that cells were capable of responding to high and low levels of glucose in the media, releasing different amounts of insulin.

Conclusion

Highly-loaded glucose-responsive NPs were successfully produced. Additionally, insulin-producing cells differentiated from hiPSCs were effectively differentiated and responsive to glucose, showing its potential to be used in the artificial pancreas system as a diabetes therapy. Ongoing work is focused on encapsulation of the nanosystem
in alginate microparticles and co-encapsulation of this nano-in-micro system with differentiated pancreatic cells in an alginate-based 3D scaffold.

Acknowledgement

The work was funded by scientific project (UID/BIM/04293/2019 and PTDC/MED-OUT/30466/2017, POCI-01-0145-FEDER-030466) financed by the Portuguese Science and Technology Foundation (FCT). Joana Marques also acknowledges FCT for financial support through grant PD/BD/145149/2019 (through the PhD Programme in Medicines and Pharmaceutical Innovation, i3DU) funded by the Portuguese state budget, through the Ministry for Science, Technology and Higher Education and, by the European Social Fund within the Framework of PORTUGAL2020, namely through Programa Operacional Regional do Norte (Norte 2020).

References

  [https://www.diabetesatlas.org] (last accessed on: May 14th, 2021)
Superparamagnetic and bioactive nanoparticles for bone cancer treatment

Florestan Vergnaud¹, Xavier Kesse¹, Francis Perton³, Sylvie Begin-Colin³, Damien Mertz³, Stéphane Descamps², Charlotte Vichery¹, Jean-Marie Nedelec¹

¹ Université Clermont Auvergne, Clermont Auvergne INP, CNRS, ICCF, Clermont-Ferrand, FR; ² Université Clermont Auvergne, CHU Clermont-Ferrand, Clermont Auvergne INP, CNRS, ICCF, Clermont-Ferrand, FR; ³ Université de Strasbourg, Institut de Physique et Chimie des Matériaux de Strasbourg (IPCMS), UMR-7504 CNRS, Strasbourg, FR

Introduction
Most patients who develop primary bone tumors require a surgical intervention. In this respect, the design of a multifunctional material, used as a bone substitute, is of high clinical interest to simultaneously treat cancer and promote bone regeneration. Bioactive glass (BG) nanoparticles are a promising material for bone tissue regeneration thanks to their ability to dissolve when immersed in body fluids and precipitate into hydroxyapatite, a crystalline phase with a composition close to the one of bones mineral part.¹² By coupling the high bioactivity of large specific surface area bioactive glass with the heating ability of iron oxide nanoparticles under an alternating magnetic field (AMF),³ such multifunctional material could be used to selectively destroy remaining or resurging cancer cells (magnetic hyperthermia) before promoting bone regeneration.

Experimental Methods
In this scope, core-shell heterostructures consisting of superparamagnetic iron oxide nanoparticles surrounded by BG shells have been synthesized using a sol-gel synthesis protocol previously optimized in the group,⁴ and characterized. Their physicochemical properties such as morphology, specific surface area and composition, have been evaluated using transmission electron microscopy (TEM), X-Ray Diffraction, infrared spectrometry, N2 sorptometry, and inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Their heating ability was studied by calorimetry under AMF with multiple field parameters, and the hydroxyapatite growth kinetics was assessed after immersion in Simulated Body Fluid (SBF) at 37°C. Furthermore, in vitro cytotoxicity tests were performed on human mesenchymal stem cells (MTT tests).

Results and Discussion
As shown in Figure 1, non-agglomerated heterostructured nanoparticles have been obtained, with a large proportion of particles with only one magnetic NP as a core. These γ-Fe₂O₃@SiO₂-CaO core-shell nanoparticles show good heating ability, with a temperature increase of 4 °C within 3 min under clinically relevant magnetic field parameters (157 kHz, 23.9 kA/m, figure 2), corresponding to a heating power (Specific Absorption Rate, SAR) of about 200 W/gFe. Hydroxyapatite was observed (XRD, FTIR) after 7 days of immersion in SBF, showing an effective mineralization process, and cytocompatibility has been demonstrated up to 7 days.

Conclusion
These results suggest that γ-Fe₂O₃@SiO₂-CaO heterostructures are a promising biomaterial to fill bone defects resulting from bone tumor resection, as they have the ability to both help bone tissue repair and act as thermoseeds for cancer therapy.

Acknowledgement
Nano-BioMag project is supported by the French Agence Nationale de la Recherche (ANR) under reference ANR-19-CE09-0001.
The authors wish to thank Aurélie Jacobs (ICCF) for the cytotoxicity tests.

Page 1385 of 2028
TEM images of γ-Fe₂O₃@SiO₂-CaO core-shell nanoparticles

Figure 1. TEM images show non-aggregated nanoparticles with a diameter of about 120 ± 10 nm, with magnetic cores successfully encapsulated in bioactive glass shell.

SAR of γ-Fe₂O₃@SiO₂-CaO nanoparticles under different AMF parameters

Figure 2. Calorimetry measurements show effective heating of the heterostructures under AMF. Results are consistent with the Linear Response Theory, in so far as the SAR increases with field parameters.

References
Bioactive and biocompatible cerium-doped mesoporous nanoparticles

Ioannis Tsamesidis¹, Dimitrios Gkiliopoulos², Georgia K. Pouroutzidou¹, Evgenia Lymeraki³, Eleni Likotrafiti⁴, Jonathan Rhoades⁴, Eleana Kontonasaki⁵, Konstantinos M. Paraskevoloulos¹, Anna Theocharidou⁵

¹ Aristotle University of Thessaloniki, Department of Physics, Thessaloniki, GR; ² Aristotle University of Thessaloniki, Department of Chemistry, Thessaloniki, GR; ³ International Hellenic University, Department of Biomedical Sciences, Thessaloniki, GR; ⁴ International Hellenic University, Department of Food Technology, Thessaloniki, GR; ⁵ Aristotle University of Thessaloniki, Department of Dentistry, Thessaloniki, GR

Introduction

Metal ions, depending on their properties, present the potential to affect the porosity and biocompatibility of MSNs (MSNs). The positive effect of doping MSNs with calcium (Ca) ions has already been thoroughly evaluated. Cerium (Ce) ions are reported to have antibacterial, antioxidant, angiogenic, anti-inflammatory and antimicrobial properties [1]. These properties are some of the prerequisites to achieve tissue regeneration. It has already been argued that the addition of Ca to MSNs could improve the bioactivity of these materials. In the present study the effect of Ce addition in calcium doped MSNs on their bioactivity and biological behavior (cell proliferation, antibacterial properties and reactive oxygen species (ROS) production) was evaluated.

Experimental Methods

Mesoporous, MCM-41 type, silicate nanoparticles were synthesized with either neat siliceous walls (undoped group) or doped with Ca and Ce ions (doped group) at Ce concentrations of 1%, 2.5%, and 5%. The synthesis was performed by the method of synergistic self-organization via sol-gel reaction. The structural and porous characteristics of MSNs were determined by X-ray Diffraction Analysis and Brunauer-Emmett-Teller (BET) porosimetry, respectively. The in vitro apatite forming ability of the synthesized MSNs was evaluated by immersion in conventional simulated body fluid (c-SBF) under renewal conditions. The cytotoxicity test was performed by the biochemical method of water-soluble salt of MTT tetrazole [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. Indirect "MTT assay" with eluates of the tested MSNs in different concentrations (12.5, 60, 125, 250 μg/ml) was performed 1, 3, 5, and 7 days after incubation with human gingival fibroblasts. For the detection of intracellular reactive oxygen species (ROS) levels, we employed the cell-permeable ROS-sensitive probe H₂DCFDA on culture medium received from both cell and bacterial cultures. Measurement of fluorescence was achieved using excitation wavelength 480-520 nm and emission wavelength 570-600 nm. Oxidation of H₂DCFDA 20 μM (incubated for 1 h) in cells treated with different concentrations of MSNs was monitored by measurement of the fluorescence in 96-well black-walled microplates. For investigation of antibacterial properties, bacterial cultures of Escherichia coli O157:H7 NCTC 12079, Staphylococcus aureus ATCC 6538, and Pseudomonas aeruginosa (clinical isolate) were used. Bacterial cultures were prepared by inoculating 10 mL NB with an isolated colony from an agar plate. After 24 h incubation at 37°C, 100 μL of broth culture was inoculated into 10 mL fresh NB and incubated under the same conditions.

Results and Discussion

All MSNs presented non cytotoxic behavior at all tested concentrations. At day 1 an upregulation in mitochondrial activity and ROS production was observed for all tested materials comparative to undoped MSNs. At day 3, statistically significant positive effect on cell viability was observed for cerium-doped MSNs (p < 0.001) but with a basal production of ROS. Enhanced cell proliferation with basal production of ROS was also observed at day 5 for all the tested MSNs (p< 0.001). Finally, at day 7 cell proliferation was promoted significantly in the cases of SiCaCe1 and SiCaCe2.5 MSNs (p<0.05). Cerium can change its oxidation states (Ce⁴⁺ and Ce³⁺) in physiological fluids, condition that can modulate ROS production [1]. Excessive ROS production has been reported as a dominating factor
in cell toxicity and it constitutes an effective mechanism for antibacterial activity properties [2]. In this study, the antibacterial assay revealed that the MSNs had only a slight inhibitory effect on Staphylococcus aureus and Pseudomonas aeruginosa, which agrees with the high fibroblast proliferation rates. Apparently, either the dissolution of cerium ions from the synthesized MSNs is limited, not reaching the necessary level for a strict antibacterial effect or other equilibrium mechanisms exist between ROS generation and degradation that need further clarification. Finally, the FTIR spectra revealed the deposition of apatite after only 1 day immersion in SBF, as confirmed by the shifting of the wide peak at 900–1200 cm⁻¹ to lower wave numbers, as well as the appearance of the two bands at 610 - 600 and 560 - 550 cm⁻¹ attributed to the bending vibration of the (PO₄)³⁻ bond.

**Conclusion**

Bioactive and biocompatible Ce-doped mesoporous MCM-41 nanoparticles were synthesized through the sol gel method. Their limited antibacterial activity under the tested concentrations was correlated with a progressive reduction of ROS, which however, was associated with a significant upregulation of cell proliferation, verifying the potential use of these materials in various dental and biomedical applications. Future work will be focused on direct cytotoxicity and antibacterial testing and the study of their drug loading and release profiles.

**Acknowledgement**

“This research is funded in the context of the project “Mesoporous nanocarriers with cerium ions (Ce-doped MSNs) for controlled release of active molecules aiming at osteogenic differentiation of human periodontal fibroblasts” (MIS: 5049566) under the call for proposals “Support for researchers with an emphasis on young researchers-second cycle” of the OP “Human Resources Development, Education and Lifelong Learning” (EDB103). The project is co-financed by Greece and the European Union (European Social Fund- ESF) by the Operational Programme Human Resources Development, Education and Lifelong Learning 2014-2020

---

**References**


2:30 p.m. – 4:00 p.m.

Poster floor

**PS1-13 | Cell and Protein-Biomaterial Interactions**
Establishing a Multiplex Protein-Based Assay as a Tool for Screening Osteogenic Properties of Calcium Phosphate Biomaterials

**Maria José R. Eischen-Loges**, Zeinab Tahmasebi Birgani, Yousra Alaoui Selsouli, Hoon S. Rho, Lars Eijssen, Vanessa LaPointe, Pamela Habibović

1 Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, NL; 2 Maastricht University, Department of Bioinformatics - BiGaT, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht, NL; 3 Maastricht University, Department of Psychiatry and Neuropsychology, MHeNs School for Mental Health and Neuroscience, Maastricht, NL

**Introduction**

The intrinsic self-healing capacity of bone tissue often falls short in case of large, critical-sized defects. To aid regeneration of such large defects, autologous bone grafts are still considered the gold standard treatment. Nevertheless, the use of autografts has noteworthy drawbacks, such as limited availability, the need for a second surgical intervention and donor site morbidity. This has led to a large demand for inexpensive, off-the-shelf-available and preferably synthetic bone graft substitutes. Calcium phosphate–based biomaterials are widely used in bone regeneration applications. Nevertheless, their properties and clinical performance are in general inferior to those of autologous bone grafts. Hence, there is a need for further improvement. An important reason that has hindered the optimization of synthetic bone graft substitutes, including calcium phosphates, is a lack of reliable in vitro screening methods for biomaterials. Here, we present a multiplex protein assay as a tool for screening the osteogenic properties of biomaterials, calcium phosphates in particular, and for predicting their success in promoting osteogenic differentiation of human mesenchymal stem cells (hMSCs).

**Experimental Methods**

In order to establish the osteogenic differentiation program of hMSCs, the cells were cultured in basic medium and osteogenic medium with or without the addition of BMP-2, in basic medium supplemented with Ca²⁺ or PO₄³⁻, or on a calcium phosphate coating. A multiplex protein assay to detect analytes related to bone formation and resorption, angiogenesis, and immunomodulation was performed at multiple time points. Osteogenic differentiation was confirmed by conventional methods such as an alkaline phosphatase assay and qPCR, and extracellular matrix mineralization by Alizarin red staining. The protein profiles will be correlated to the results of osteogenic differentiation using a statistical model to determine a descriptive panel of markers. As the next step in the process of establishing a screening tool, a medium-throughput calcium phosphate library was generated by coating cell culture plates with a thin layer of calcium phosphates, doped with a range of inorganic additives at varying concentrations. HMSCs were cultured on the coatings to perform a multiplex protein assay, as well as conventional assays for data validation.

**Results and Discussion**

The multiplex analysis of differentiating hMSCs over time identified differentially expressed proteins as markers for osteogenic differentiation (Figure 1). Mineral deposition as an indicator for osteogenic differentiation and extracellular matrix mineralization was observed in hMSCs cultured in osteogenic medium, osteogenic medium supplemented with BMP-2, basic medium supplemented with Ca²⁺ or PO₄³⁻, and on a calcium phosphate coating at day 28 (Figure 2A). This observation was supported by an alkaline phosphatase activity assay. This enzyme is involved in mineral deposition and its activity was increased early on in basic medium supplemented with Ca²⁺ or PO₄³⁻, and on a calcium phosphate coating. In addition, an increase in ALP activity levels over time was also observed in osteogenic medium and osteogenic medium supplemented with BMP-2 (Figure 2B).

**Conclusion**

Page 1391 of 2028
Here, we presented our efforts towards developing a high-throughput screening tool based on protein multiplex for evaluating the osteogenic properties of calcium phosphate–based biomaterials.

**Acknowledgement**

This research has been made possible with the support of the Netherlands Organisation for Scientific Research Vidi grant (#15604), the Dutch Province of Limburg (LINK project), and the Interreg Vlaanderen / Nederland project ‘BIOMAT-on-microfluidic-chip’. PH gratefully acknowledges the Gravitation Program ‘Materials-Driven Regeneration’ (#024.003.013), funded by the Netherlands Organization for Scientific Research.

![Figure 1. Protein multiplex analysis of differentiating hMSCs over time. A protein profile for each condition was established at multiple time points and differentially expressed proteins were identified. N=2.](image-url)
Figure 2. Mineral deposition and alkaline phosphatase activity in differentiating hMSCs. A) Alizarin red staining at day 28 of culture. Scale bar 500µm. B) Alkaline phosphatase activity assay. N=2.

References
High-throughput Screening to Elucidate Biomaterial-induced Fibrosis

Torben A.B. van der Boon

University Medical Center Groningen, Department of Biomedical Engineering, Research Institute W.J. Kolff, Groningen, NL

Introduction
Nowadays, it is becoming common knowledge that the human body, its tissues and cells react to biophysical and biochemical cues located on biomaterial surfaces.[1] Identifying how these parameters influence cellular behavior is of crucial importance and will aid us in the further development of medical implant technology. Unfortunately, in many studies attempting to identify these physicochemical properties’ influence on cell behavior, investigation of individual properties is the conventional method, leaving out a significant number of other variables which are encountered in vivo, which is where cells always interact with multiple cues simultaneously.[2] We are developing an orthogonal double gradient platform which allows us to investigate just such complex situations in a high-throughput screening (HTS) fashion. The platform grants us the power to screen the cell response towards thousands of these combined parameters in single cell experiments, which will result in the optimization of material properties to enhance biomaterial and implant function. Currently, we are in the final platform optimization stage, after which we will screen silicone rubber’s susceptibility to fibrosis and scar tissue formation.

Experimental Methods
PDMS double orthogonal gradients (DOGs) are prepared by sequential imprinting – and shielded air plasma oxidation treatments in accordance with previously published methodology.[3-5] Primary human dermal fibroblasts will be cultured on the DOGs for 7 days and stained for early fibrosis biomarkers (Collagen-II and α-SMA).

Results and Discussion
Every imaginable position on the orthogonal double gradient surfaces has a unique combination of three surface parameters, possessing ‘real’, clinically relevant values. This allows us to investigate a virtually unlimited amount of different parameter combinations, within their respective ranges, on single substrates. Wavy topography gradients range from \( \lambda = 1.5 \, \mu m - 12 \, \mu m \) and \( A = 50nm - 1.5 \, \mu m \), the smallest wavelengths corresponding with the smallest amplitudes going from small to big, in a coupled fashion. Stiffness gradients range in Young’s Modulus from \( \sim 50 - 500 \, MPa \), and ‘wettability’ gradients from \( 5 - 90 ^\circ \) in water contact angle (WCA). As a ‘proof of concept’, we cultured hBM-MSCs on the platforms for 24 h, imaged the cells via automated fluorescence microscopy and identified the cell response with respect to cell density, cell spreading, and nucleus area. We have found that the synergistic effect of abovementioned parameter combinations all influence cell behavior in a different manner with regard to these relatively ‘simple’ assessable characteristics. Our next steps involve the translation of ‘hotspots’ or regions of interest (ROI) to homogeneous parameter substrates, as a last verification step in the optimization process, as well as the screening of biomaterial susceptibility to fibrosis and scar tissue formation.

Conclusion
The highly efficient cell screening tool we have created with our DOG platform allows us to screen cell response to combined physical parameter influence in a high-throughput fashion, investigating thousands of different parameter combinations in single cell experiments. It will serve its purpose to facilitate enhanced biomaterial development.
High-throughput screening approach. Different physicochemical biomaterial properties influence cell behavior in a complex manner. The screening platforms enable all parameter combinations to be present within a gradient-like range. The influence on, in this example ‘Cell density’, is identified via fluorescence immune-staining and semi-automated imaging and analysis.

References
High-resolution quantitative analysis of proteins distribution involved in cells focal adhesions formation to electrospun polymers fibers

Krzysztof Berniak, Daniel Ura, Piotr Szewczyk, Joanna Karbowniczek, Urszula Stachewicz

AGH University of Science and Technology, Faculty of Metals Engineering and Industrial Computer Science, Cracow, PL

Introduction
The study of interactions between human cells and polymers fibers is one of the most crucial elements in assessing new materials' biocompatibility in medicine and bioengineering. Poly(methyl methacrylate) (PMMA) is a commonly used polymer in biomaterials with a very high degree of biocompatibility with human cells [1]. The electrospun PMMA fibers have already been used for cell dynamics studies for tissue engineering [2]. The cell culture studies indicate the stretching of cells along the fibers, creating adhesion sites through which they temporarily connect to them [3]. These molecular structures consist of a series of proteins, including paxillin and vinculin. Immunofluorescence labeled of proteins provides vast amounts of information about the localization of these proteins at the points of cell contact with the scaffold. Within this study, we show the analysis of cell adhesion sides with PMMA fibers deposited on glass using two types of cells: osteoblasts and fibroblasts. To assess potential correlations between the distribution of focal adhesions in two different types of cells to electrospun PMMA fibers, a quantitative analysis of localizations of particular protein foci in 3D multicolor microscopy images is required. We show the potential application of high-resolution confocal microscopy with Airyscan2 to study cell-matrix interaction on electrospun polymer fibers.

Experimental Methods
To obtain a 12 wt. % solution, poly(methyl methacrylate) (PMMA, Mw=350 000 g·mol⁻¹, Sigma Aldrich, UK) was dissolved in N, N-dimethylformamide (DMF, Sigma Aldrich, UK). The solution was stirred at 700 rpm for two h on a hot plate set at 55°C (IKA RCT basic, Germany). PMMA fibers were produced via electrospinning using the apparatus EC-DIG with climate control (IME Technologies, the Netherlands) at T= 25°C and RH=40 %. A voltage of 12 kV was applied to the needle, kept at a distance of 15 cm from the grounded drum rotating at 2000 rpm. The flow rate was set to 4 ml·min⁻¹. The samples were directly deposited 10×10 glass slides.

Human osteoblast-like cell line MG63 and fibroblasts NIH 3T3 cell line were cultured in DMEM supplemented with 10% addition FBS, 2% antibiotics (penicillin/streptomycin), 1% amino acids and 1% L-glutamine under standard conditions, i.e. at T = 37 °C, RH = 95% and 5% of CO₂.

After three days of cell growth, samples of the PMMA scaffolds were fixed with 4% paraformaldehyde for 15 min. Fixed cells were permeabilized with 0.1% Triton X-100 in PBS for 5 min at T= 22oC and then incubated in a blocking solution (3% BSA in PBS). To visualize actin filaments, cells were incubated for one hour at T= 22oC with Alexa Fluor™ 633 Phalloidin. Nuclear DNA was stained with DAPI for 5 min. Selected proteins involving in focal adhesion (vinculin and paxillin) were labeled by immunofluorescence. Multicolor 3D microscopy images of cells connecting to PMMA fibers were acquired using high-resolution microscopy with the concept Airyscan2 (Zeiss LSM 900), and the image analysis was performed by using ImageJ.

Results and Discussion
Within this study, we have verified the cell's connection to PMMA fibers and glass, showing the distinct changes in paxillin and vinculin distribution. It was possible due to the imaging with the high-resolution Airyscan2 confocal microscopy technique. Both human osteoblast MG-63 and murine fibroblast NIH 3T3 cells show a high affinity for PMMA fibers. They tended to be spread along fibers. However, they also create numerous connections with each
other (Figure 1). High-resolution imaging of paxillin and vinculin shows a significant difference in the distribution of both proteins in the contact cell surface to PMMA fibers and the glass for both cell lines. It indicates that the cell uses a different mechanism when creating attachment sites to PMMA than to glass. The nature and density of such foci formation correlate with the properties of the surface scaffold on which the cells are grown.

**Conclusion**

The application of the high-resolution confocal microscopy with Airyscan2 allowed to register the places of cell adhesion to polymer fibers with very high resolution. It allowed observing changes in the spatial distribution of proteins involved in forming focal adhesion of cells to the PMMA fiber and the glass.

**Acknowledgement**

Funding: this study was conducted within “Nanofiber-based sponges for atopic skin treatment” project carried out within the First Team program of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund, project no POIR.04.04.00-4571/17-00.

![Figure 1](image)

**Figure 1**

Fibroblast NIH cells stretching along the PMMA fibers. Actin fibers stained with Alexa Fluor 633 Phalloidin (A), proteins involved in focal adhesion formation – paxillin (B) and vinculin (C) were labeled by immunofluorescence, nucleus stained with DAPI (D), transmitted light (E), and overlay of all channels (F).

**References**


Effect of silver ion incorporation into a bioactive glass surface on the adsorption of albumin

Jacopo Barberi¹, Andrea M. Giovannozzi², Luisa Mandrile², Marta Miola¹, Alessandra Vitale¹, Lucia Napione¹, Silvia Spriano¹

¹ Politecnico di Torino, DISAT, TO - Torino, IT; ² National Institute of Metrology, Quantum Metrology and Nanotechnologies Division, TO - Torino, IT

Introduction

Nowadays, bacterial infection is one of the major causes for orthopedic implants failure. While it is well known how to manufacture materials that are able to stimulate osseointegration and to firmly bond with the bone, the fight against pathogenic microorganisms is carried on mainly by antibiotics, with consequent problems of poorly localized actions and antibiotic-resistance. Therefore, novel antibacterial strategies have been deeply researched. Among those, incorporation of silver in biomaterials, such as bioactive glasses, is acknowledged as an effective way to reduce bacteria proliferation. Osseointegration of biomaterials is dependent on the surface properties of the implants and on the interactions with the biological environment. In particular, a protein layer is formed on the surface within minutes after the contact between the surface and the biological fluids and it will dictate how the cells will respond to the implanted foreign body. As consequence, it is important to understand how antibacterial modifications of bioactive materials affect their interactions with proteins. In this work, the adsorption of albumin was investigated onto a silica-based bioactive glass where silver ions were incorporated through ionic exchange (Ag-SBA2) in order to understand eventual differences with the untreated surface (SBA2)[1].

Experimental Methods

SBA2 bioactive glass (mol %: 48% SiO₂, 18% Na₂O, 30% CaO, 3% P₂O₅, 0.43% B₂O₃, 0.57% Al₂O₃) was prepared via precursors melting and casting, cut into disks and grinded (up to 1000 grit). Ag-SBA2 was prepared by soaking glass slices for 1h in 0.03M AgNO₃ solution. Protein adsorption was obtained by soaking the samples for 2h at 37°C in albumin solution in PBS, in near physiological conditions (20 mg/ml, pH 7.4). The glass substrates were characterized in terms of topography and roughness (SEM, AFM and confocal microscopy), chemical composition (EDS and XPS), surface charge and potential (solid surface zeta potential, Kelvin Probe Force Microscopy (KPFM)) and surface energy (contact angle, Owens-Wendt method). The adsorbed proteins were quantified by using different methods (BCA assay, fluorescent proteins and XPS) and the BSA layer was also imaged (fluorescent microscopy and KPFM). Substrate-protein interactions and albumin conformation were investigated, too (solid surface zeta potential and ATR-FTIR).

Results and Discussion

After silver incorporation, confirmed by chemical analysis, the surface properties of Ag-SBA2 were mostly similar to the undoped glass. In particular, topography and roughness were unchanged during soaking in the silver solution, as expected. Wettability and surface free energy, both the dispersive and polar components, were also similar between the two substrates. Instead, zeta potential titration curve showed that the incorporation of Ag³⁺ ions increased the surface potential, in particular around physiological pH (7.4).

Quantification of adsorbed BSA showed that both surfaces adsorb a similar amount of albumin, with a little higher amount on Ag-SBA2. This can be possibly related to a couple of different factors: the high affinity of silver for proteins and the presence of a more positive charge on the surface, which is able to attract the negatively charged albumin. On both surfaces, albumin forms a complete and homogeneous layer, as detected by imaging techniques. Adsorption of proteins was confirmed also by zeta potential measurement on the surfaces, with a shift of the IEP of both glasses.
towards the IEP of albumin. Thanks to ATR-FTIR measurement, it was found that albumin retains more its native conformation on the undoped glass with respect to the silver containing glass, where a more disordered structure was found. This fact can be ascribed to a greater interaction between the proteins and the doped surface, due to the presence of metal ions and more positive charges.

**Conclusion**

In conclusion, even though the incorporation of silver ions in a bioactive glass surface does not affect surface properties that are usually addressed as pivotal in protein adsorption, such as roughness and surface energy, the presence of a more positive charge on the surface of the glass and affinity of proteins towards metallic ions seems to be enough to increase adsorption of albumin and strength of the protein-biomaterial interaction. The increased interaction with proteins may be beneficial for the cells response to antibacterial silver containing materials.

**References**

**PS1-13-401**

**The effect of Zn ions in protein adsorption and cell responses**

Francisco Romero-Gavilán¹, Andreia Cerqueira¹, Iñaki García-Arnáez², Ibon Iloro³, Mikel Azkargorta³, Félix Elortza³, Marílo Gurruchaga², Julio Suay¹, Isabel Goñi²

¹ Universitat Jaume I, Departamento de Ingeniería de Sistemas Industriales y Diseño, Castellón de la Plana, ES; ² Universidad del País Vasco, Facultad de Química, San Sebastián, ES; ³ CicBiogune, Proteomic Platform, Derio, ES

**Introduction**

Zinc is an essential trace element that plays a pivotal role in bone metabolism and remodeling. Additionally, Zn can promote osteogenesis and mineralization in osteoblastic cells as well as inhibit osteoclastogenesis [1]. This ion is associated with antioxidant and anti-inflammatory functions as it can inhibit the NF-κB pathway activation [2]. As a result, this compound has great potential for the development of biomaterials for tissue regeneration.

In this study, we developed new Zn-doped sol-gel coatings to bioactivate titanium implants and characterized their physicochemical, proteomic, and biological features to evaluate the role of Zn ions in bone healing.

**Experimental Methods**

The sol-gel method was employed to synthesize Zn-doped coatings to be applied onto Ti discs. The sol-gel reactions were carried out through methyltrimethoxysilane and tetraethyl-orthosilicate precursors. Acidified water (0.1 M HNO₃) was used to hydrolyze these precursors. Increasing amounts of ZnCl₂ (0.5, 1, 1.5 % wt) were added to the mixtures. The coatings were applied by dip-coating and samples were cured at 80°C for 2 h. The samples were physicochemically characterized through SEM, FTIR, ²⁹Si-NMR, hydrolytic degradation, Zn²⁺ release, contact angle, and roughness measurements. Proteomic assays were carried out to evaluate the effect of Zn on protein adsorption. Samples were incubated for 3 h with human serum. Afterwards, the proteins adsorbed onto the coatings were eluted and analyzed through nLC-MS/MS. The osteogenic potential was tested *in vitro* with MC3T3-E1 by evaluation of cytotoxicity, proliferation, ALP activity, and the gene expression of ALP, RUNX2, RANKL, RANK, and iNOS by RT-PCR. The inflammatory potential was assessed by RT-PCR gene expression (TNF-α, IL-1β, TGF-β, and IL-4) and by ELISA (TNF-α) in RAW264.7 macrophages.

**Results and Discussion**

The Zn-enriched biomaterials were successfully obtained and applied as coatings. Chemical characterization revealed no effects on the silica network condensation by the addition of Zn. All coatings showed a similar morphology and roughness. However, the composition with Zn displayed higher contact angle values and thus a more hydrophobic behavior than the coating without Zn. A controlled degradation and Zn²⁺ release were obtained. As more ZnCl₂ was added to the sol-gel network, more degradation and Zn liberation were achieved.

Regarding the *in vitro* analyses, the materials were not cytotoxic. In addition, an increased expression of ALP, TGF-β, RANK, and RUNX2 osteogenic markers was measured for the Zn-doped coatings. A higher gene expression of IL-1β and TNF-α proinflammatory markers was detected by incubating the Zn-enriched samples with macrophages. At the same time, the expression of TGF-β and IL-4, anti-inflammatory markers, also increased. In Fig. 1, the ELISA assay showed a decreased secretion of TNF-α cytokine for the coatings with 0.5 and 1 % wt ZnCl₂, demonstrating a dose-dependent anti-inflammatory potential of this compound.

Proteomic results showed changes in the protein layer composition adsorbed onto the coatings with Zn respect to the protein layer formed onto the control surface. These differential proteins were associated with immune, coagulative and regenerative functions. Complement system proteins C1S, CO3, CO4A, and CO9 were found more adsorbed onto the materials with Zn. However, in parallel, VTNC, IC1, FHR1, and CLUS proteins, which can inhibit
this pathway and are related to anti-inflammatory functions were also found more adhered onto them. The increased affinity of these proteins for the Zn-enriched surfaces could explain the immunological activity detected by RT-PCR as well as the reduction in the secretion of TNF-α. Moreover, PLF4, PROC, and IPSP proteins, which are linked to blood coagulation, increased their affinity to the Zn-containing coatings. On the other hand, VTNc, TITIN, and CXCL7 proteins were also found more adsorbed onto the materials with Zn. CXCL7 can improve the recruitment of human mesenchymal stem cells. TITIN can improve osteoblast proliferation, while VTNC is involved in the promotion of osteogenic differentiation of mesenchymal stem cells. Thus, these protein adsorption patterns can explain the observed osteogenic responses.

Conclusion
These new Zn-doped coatings present an interesting alternative to improve the bone tissue regeneration process. The incorporation of ZnCl₂ in the sol-gel network improved their osteogenic potential in a Zn dose-dependent manner and showed an anti-inflammatory effect. In addition, the cellular responses can be correlated with the effect of this divalent cation in protein adsorption onto the biomaterial surfaces.

Acknowledgement
This work was supported by MINECO [MAT2017-86043-R; RTC-2017-6147-1], Generalitat Valenciana [GRISOLIAP/2018/091, APOSTD/2020/036, PROMETEO/2020/069], Universitat Jaume I under [UJI-B2017-37], the University of the Basque Country under [GIU18/189] and Basque Government under [PRE_2017_2_0044]. The authors would like to thank Raquel Oliver, Jose Ortega and Iraide Escobés for their valuable technical assistance and Antonio Coso (GMI-Ilerimplant) for producing the titanium discs.

![Graph showing TNF-α liberation by RAW264.7 after 2 and 4 days of incubation. Results are shown as mean ± SE. The asterisks (p ≤ 0.001 (**)) indicate statistical differences.](image)

References
Progenitor cells derived from healthy and osteoarthritic human cartilage have potential for cartilage tissue engineering

Margot Rikkers¹, Jasmijn Korpershoek¹, Riccardo Levato¹,², Jos Malda¹,², Lucienne Vonk¹,³

¹ University Medical Center Utrecht, Department of Orthopaedics, Utrecht, NL; ² Utrecht University, Department of Clinical Sciences, Utrecht, NL; ³ CO.DON AG, Teltow, DE

Introduction

Articular cartilage-derived progenitor cells (ACPCs) are a potential new cell source for cartilage tissue engineering. Unlike mesenchymal stromal cells (MSCs), ACPCs do not have the tendency to undergo terminal hypertrophic differentiation. This study aims to isolate and characterize ACPCs from human hyaline cartilage. ACPCs derived from healthy and osteoarthritic cartilage are compared. In addition, the potential to use ACPCs for cartilage tissue engineering and clinical application is assessed.

Experimental Methods

Cells were isolated from macroscopically healthy (n = 6, age 46 – 49) and osteoarthritic (n = 6, age 55 - 79) human knee cartilage. Subsequently, ACPCs were isolated from the total cell population by clonal growth after differential adhesion to fibronectin. MSCs were isolated from human bone marrow by plastic adherence after separating the mononuclear fraction using a Ficoll-paque density gradient. Healthy and osteoarthritic ACPCs were characterized by cell surface marker expression, growth kinetics, colony-forming efficiency, and multilineage differentiation. The populations were compared to full-depth chondrocytes derived from the same donors. Finally, ACPCs were cultured in 3D pellets under chondrogenic culture conditions to investigate neo-cartilage formation.

Results and Discussion

Healthy and osteoarthritic ACPCs were successfully isolated and differentiated into the adipogenic and chondrogenic lineage, but failed to produce calcified matrix when exposed to osteogenic induction media. Full-depth chondrocytes derived from the same donors were able to produce calcified matrix upon induction of osteogenic differentiation. ACPCs proliferated faster compared to chondrocytes. Both ACPC populations, as well as full-depth chondrocytes met the criteria for cell surface marker expression to define MSCs as determined by flow cytometry. Cartilage-like matrix production was successful in ACPC pellet cultures, and healthy ACPC pellets had reduced expression of hypertrophic marker type X collagen.

Conclusion

In conclusion, this study provides further insight into the ACPC population which is present in both healthy and osteoarthritic human articular cartilage. The populations show similarities to MSCs, yet ACPCs did not produce calcified matrix under well-established osteogenic and mineralization culture conditions. Furthermore, extensive culture-expansion potential of ACPCs makes these cells an ideal cell type for cartilage tissue engineering and possibly for clinical application.

Acknowledgement

This work is supported by the partners of Regenerative Medicine Crossing Borders (RegMed XB), a public-private partnership that uses regenerative medicine strategies to cure common chronic diseases. This collaboration project is financed by the Dutch Ministry of Economic Affairs by means of the PPP Allowance made available by the Top Sector Life Sciences & Health to stimulate public-private partnerships.
Investigating the impact of Ti$_3$C$_2$Tx (MXene) within the capsular bag on the development of posterior capsular opacification

Grace Cooksley$^1$, Marcus Dymond$^1$, Yury Gogotsi$^3$, Joseph Lacey$^2$, Susan Sandeman$^1$

$^1$ University of Brighton, Pharmacy and Biomolecular Sciences, Brighton, GB; $^2$ Rayner Intraocular Lenses, Ltd., Material Science and Toxicology, Worthing, GB; $^3$ Drexel University, Materials Science and Engineering and Nanotechnology, Philadelphia, US

Introduction

Posterior capsular opacification (PCO) is the most common complication arising from the corrective surgery used to treat cataract patients. A cataract forms due to the aggregation of protein over the crystalline lens of the eye and is the leading cause of avoidable blindness globally. Despite the success of the corrective surgery in restoring visual acuity, patients implanted with an artificial intraocular lens (IOL) no longer have accommodative function and risk developing PCO; a secondary cataract formed of residual lens epithelial cells (LEC) within the capsular bag that have proliferated, differentiated and migrated over the visual axis.

The two-dimensional transition metal carbides and/or nitrides (MXenes) have been investigated for biomedical applications, specifically ophthalmic devices, due to their unique combination of conductivity, hydrophilicity, flexibility, and transparency. Ward et al., (2020) used Ti$_3$C$_2$Tx in an adjustable focus lens model to provide changes in dioptric range for an accommodative IOL design. However, the benefit of providing accommodation using Ti$_3$C$_2$Tx could increase the risk of developing PCO, resulting in further postoperative treatment for patients. MXenes have yet to be investigated with regards to their impact on the developmental pathways of PCO. The aim of this study was to investigate the effects of MXene on LEC behaviour linked to the development of PCO. The study focussed on the initial wound healing response following cataract surgery. Interleukin-1 beta (IL-1$\beta$) instigates inflammatory cytokine interleukin (IL)-6 and IL-8 mediated wound healing response following cataract surgery. Excess stimulation promotes inflammation and subsequent developmental pathways of PCO. It was hypothesised that Ti$_3$C$_2$Tx would suppress activation of this inflammatory cytokine cascade using a human lens epithelial in vitro model of inflammatory cytokine activation.

Experimental Methods

Delaminated Ti$_3$C$_2$Tx, MXene was synthesised using the MILD method and characterised using dynamic light scattering and UV-spectroscopy. Coatings of Ti$_3$C$_2$Tx were spin-coated onto the IOL optic (Ossila Spin Coater). The wettability of IOL material and coatings were characterised using contact angle analyser (OCA 15plus, Dataphysics). The viability of human lens epithelial cells (HLE-B3) grown on hydrophobic and hydrophilic IOLs (Rayner Intraocular Lenses Ltd., Worthing, UK) and Ti$_3$C$_2$Tx-coated hydrophobic IOLs was characterised using Live/Dead™ stain, MTS and LDH assay. Expression of IL-6 and IL-8 by HLE-B3 cells incubated on the IOL types was quantified using enzyme-linked immunosorbent assay (ELISA). Cytokine expression was further investigated using an in vitro model of HLE-B3 cells incubated with Ti$_3$C$_2$Tx colloidal solution to determine the impact of Ti$_3$C$_2$Tx on the wound-healing response of HLE-B3 cells. The study also investigated the ability of Ti$_3$C$_2$Tx to suppress cytokine expression, by treating HLE-B3 with Ti$_3$C$_2$Tx colloidal solution after priming with proinflammatory cytokine IL-1$\beta$.

Results and Discussion

Dynamic light scattering and UV-spectroscopy confirmed the synthesis of Ti$_3$C$_2$Tx colloidal solution with an average particle dispersant index of 0.244 and flake size of 472 d nm. Contact angle analysis showed the presence of Ti$_3$C$_2$Tx coatings increased the wettability of the hydrophobic lenses. There was no significant difference in cell viability between HLE-B3 cells grown on the hydrophobic, hydrophilic, and Ti$_3$C$_2$Tx-coated hydrophobic IOLs. Moreover, the
expression of IL-6 and IL-8 did not significantly differ between the IOL types. On direct contact with Ti3C2Tx colloidal solution, HLE-B3 cells did not express IL-6 and IL-8. After priming of HLE-B3 with IL-1β, post-treatment of Ti3C2Tx suppressed all expression of IL-6 and IL-8 after only 1-hour exposure. This work demonstrated that intraocular lens material and the addition of Ti3C2Tx coatings did not impact cell viability between the IOL types. Moreover, this work showed the presence of Ti3C2Tx colloidal solution does not induce cytokine expression but, suppresses IL-6 and IL-8 expression by HLE-B3 cells, suggesting a potential role of Ti3C2Tx in cytokine adsorption.

**Conclusion**

Ti3C2Tx MXene does not influence cell viability and does not induce the expression of IL-6 and IL-8, suggesting the presence of Ti3C2Tx within the capsular bag post-cataract surgery will not promote inflammatory pathways leading to PCO development. Further work will include investigating the impact of Ti3C2Tx on the epithelial-mesenchymal transition (EMT) pathway, an established fundamental pathway in PCO development, by migration assays and the development of an *in vitro* transwell insert model of inflammation using a co-culture of HLE-B3 cells and THP-1 monocytes.

**Acknowledgement**

The authors would like to acknowledge the University of Brighton and Doctoral Training Alliance for the scholarship.
Interleukin-6 and -8 expression of HLE-B3 cells post-incubation with IOL types

Figure 2: Expression of interleukin-6 and -8 by human lens epithelial cells (HLE-B3) after incubation with hydrophobic, hydrophilic and TiC/Ti-coated hydrophobic IOLs (Rayner Intraocular Lenses, Ltd. Worthing, UK). Cytokine expression by the cells alone were subtracted from each condition. Mean ± SEM. An one-way ANOVA was conducted in GraphPad Prism 5. *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001. (n=3)

References
H19 regulates extracellular matrix components and MSC behaviour

Sara R. Moura¹,², Jorge Lopes³, Nuno Neves¹,³, Susana G. Santos¹,², Mário A. Barbosa¹,², Maria I. Almeida¹,²

¹ i3s, Instituto de Investigação e Inovação em Saúde, Porto, PT; ² ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Porto, PT; ³ CHSJ, Centro Hospitalar Universitário São João, Porto, PT

Introduction
Aging is a high-risk factor for the development of osteoporosis. Osteoporotic fractures (also called fragility fractures) are an important cause of disability and loss of life quality, with an economic impact on the health care systems. Mesenchymal stem/stromal cells (MSCs) are key players in bone regeneration/repair. Engineering MSC can be a strategy to promote commitment into the osteogenic lineage and accelerate bone healing. Non-coding RNAs (ncRNAs) are a large class of transcripts that do not encode for proteins, but can be used as tools to mediate bone homeostasis and regeneration/repair, and control MSC and pre-osteoblasts differentiation, proliferation, and extracellular matrix (ECM) composition, among other mechanisms. The aim of this study is to: 1) investigate the role of the long ncRNA H19 on the ECM proteins secreted by MSCs derived from osteoporotic patients; and 2) explore how H19-engineered MSC-derived ECM can modulate cell behaviour.

Experimental Methods
MSCs were isolated from the bone marrow of osteoporotic patients that underwent total hip arthroplasty following a fragility fracture. Cells were cultured under basal or osteogenic-inducing stimuli. H19 and ECM components expression was quantified by RT-qPCR. siRNA was used to knockdown H19 levels (siH19), by transfection. A scrambled sequence was used as control. The effect on osteogenic differentiation and ECM production was evaluated by ALP and calcium deposits histochemical staining. Protein was extracted at day 10 of differentiation using an ECM enrichment protocol. Mass spectrometry-based proteomic analysis was performed to investigate proteins and pathways targeted by H19, in particular those associated with the ECM. Protein identification was performed against entries from the UniProt database with Sequest HT search engine. Then, the ECM produced by siH19-engineered MSCs was decellularized an repopulated with MSCs and pre-adipocytes (differentiated for 10 days under adipogenic conditions), which were cultured on top of the decellularized matrix. The effect of the matrix on the MSCs or pre-adipocytes behaviour was tested by RT-qPCR for MSC lineage specific markers, and by resazurin assays.

Results and Discussion
H19 knockdown in hMSCs led to a decrease in the expression of several ECM components including COL1A1, COL1A2 and COL3A1. It also caused a decrease of osteogenic differentiation markers, including RUNX2 and ALP. Mineralization was impaired when H19 levels were decreased, compared with control. High-throughput analysis showed that distinct ECM proteins were modulated by H19, including COL1A1, FBN1 and VTN. Naïve MSC cultured on top of the decellularized matrix secreted by siH19-MSCs show a tendency to differentiate toward adipogenesis in detriment of osteogenesis. The expression levels of CEBPB (adipogenic marker) were increased, while RUNX2 (osteogenic marker) was decreased in MSCs cultured on top of siH19-MSCs derived matrices, compared with control-MSC derived matrices. Furthermore, metabolic activity was inhibited when MSCs were cultured in the siH19-MSCs decellularized matrices. In agreement with these results, CEBPB was overexpressed when pre-adipocytes were cultured in the matrices produced by hMSCs depleted in H19.

Conclusion
Page 1406 of 2028
Inhibition of H19 in MSC-derived osteoblasts changes ECM components and mineralization. Furthermore, the ECM secreted by siH19-MSCs guides the effect on cell proliferation and osteogenic/adipogenic differentiation. In conclusion, this study shows that modulation of the long ncRNA H19 impact levels of ECM components, which consequently influences cell behaviour.

**Acknowledgement**
This work was funded and supported by FCT - in the framework of the project POCI-01-0145-FEDER-031402 - R2Bone, under the PORTUGAL 2020 Partnership Agreement, through ERDF.
Marker-independent analysis of biomaterial-induced macrophage response using Raman microspectroscopy and Raman imaging

Nora Feuerer1,2, Julia Marzi1,2,3, Eva Brauchle1,2,3, Daniel Carvajal-Berrio1,3, Florian Billing2, Martin Weiss1,2, Meike Jakobi2, Christopher Shipp2, Katja Schenke-Layland1,2,3

1 Eberhard Karls University Tübingen, Research Institute of Women’s Health, Tübingen, DE; 2 NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, DE; 3 Eberhard Karls University Tübingen, Cluster of Excellence iFIT (EXC 2180), Tübingen, DE; 4 University of California Los Angeles (UCLA), Department of Medicine/ Cardiology, Los Angeles, US

Introduction
The lifespan and performance of an implant is largely dependent on our body's immune response. Chronic inflammation, encapsulation by fibrotic tissue, and aseptic loosening reduce the durability of many implant materials and cause severe pain in patients. Mainly responsible for these reactions are monocyte-derived macrophages (MDMs), phagocytic cells of our innate immune system. MDMs strongly adhere to material surfaces and orchestrate many downstream events of inflammation and healing. However, the macrophage response can be modulated by surface characteristics such as topography or E-modulus of biomaterials and implants. Existing methods that comprehensively analyze the molecular response of these cells towards an implant are invasive and in most cases marker-based. Aggressive treatments to detach adherent cells, such as scraping or digestion with enzymes, alter cell surface proteins, impair cell viability and ultimately limit the ability to accurately characterize adherent cells.

Experimental Methods
Raman microspectroscopy (RM) is a marker-independent, non-invasive method that allows the analysis of cells and tissues without the need for staining or processing. Using spectral imaging, biochemical components such as proteins, lipids and nucleic acids in single cells can be visualized, making it an ideal method to reveal differences in the biochemical composition of macrophages.

Results and Discussion
In the present study, we analyzed human MDMs in suspension by RM, revealing that especially lipid composition significantly differs in M0 (resting), M1 (IFNg/ LPS), M2a (IL-4/ IL-13) and M2c (IL-10) MDM phenotypes. To track similar biochemical alterations without potent activators such as LPS, interferons or cytokines, we cultured MDMs on titanium disks with varying surface topographies. While classical methods like cytokine expression or surface antigen analysis did not show significant differences between the samples, RM identified changes in biochemistry and lipid composition.

Conclusion
Our data shows that Raman microspectroscopy and Raman imaging allow an improved and more detailed classification of MDM response to environmental triggers such as implant material topographies or biochemical composition, providing unique insight into the activation spectrum of macrophages.

Acknowledgement
The work was financially supported by the State Ministry of Baden-Wuerttemberg for Economic Affairs, Labour and Housing Construction.
Development of a Virus-Like Particle platform for the control of cell fate

Thomas Dos santos, Tatiana Petithory, Isabelle Brigaud, Karine Anselme, Laurent Pieuchot

Université de Haute Alsace, IS2M, Mulhouse, FR

Introduction
During evolution, living organisms have developed different proteins capable of self-assembly into 3D objects with specific functions such as viral capsids or components of cell cytoskeleton. The coat protein forming the capsid of many viruses can be produced through heterologous expression systems and form auto-assembled noninfectious nanoparticles named Virus-like Particles. VLPs form repetitive and well-defined architectures that can be used as an antigen-presenting platform for the designs of new vaccines.

Here we present the development of the VLPs from the RNA bacteriophage AP205 as a genetically modified platform to control cell adhesion and fate. AP205 particles are formed from 180 copies of a coat protein. The assembled particles are very robust and tolerate insertion of small sequences into N- and C- termini that will be exposed at the particle surface.

Experimental Methods
Using cloning techniques, we fused a short adhesion peptide (RGD) to the C-terminus of the coat protein. The resulting fusion protein was then expressed in a bacterial system and purified by affinity and size exclusion chromatography. Negative staining transmission electron microscopy and DLS analysis assessed the presence of particles consistent in size with the VLPs. Immunostaining and confocal microscopy allowed us to determine the adhesion properties of our particles by measuring the spreading of cells.

Results and Discussion
Adsorption of RGD containing particles on PDMS surfaces allows mouse cells C2C12 to adhere. Regulation of the stoichiometry and concentration of RGD-particles provide control of cell spreading and cell density on culture surface. These results show that recombinant VLP-RGDs can be used to control cell adhesion. With the same methods, we were able to produce particles expressing differentiation peptides.

Conclusion
We are currently working on the osteoinductive and myoinductive properties of our new particles. We expect to use AP205 VLPs as a general modular platform to finely control cell fate.
Innovative phosphate-based bioresorbable and antibacterial bioglasses

Elisa Restivo\textsuperscript{1,2}, Diego Pugliese\textsuperscript{3}, Duccio Gallichi-Nottiani\textsuperscript{3}, Giovanna Bruni\textsuperscript{4}, Lucia Cucca\textsuperscript{5}, Davide Janner\textsuperscript{3}, Daniel Milanese\textsuperscript{6}, Livia Visai\textsuperscript{1,2}

\textsuperscript{1} University of Pavia, Biochemistry Unit, Department of Molecular Medicine; Center for Health Technologies; UdR INSTM, Pavia, IT; \textsuperscript{2} ICS Maugeri, IRCCS, Clinical-Specialized Medicine, UOR5 Laboratory of Nanotechnology, Pavia, IT; \textsuperscript{3} Politecnico di Torino, Department of Applied Science and Technology; UdR INSTM, Torino, IT; \textsuperscript{4} University of Pavia, Department of Chemistry, Physical Chemistry Section; Center for Colloid and Surfaces Science, Pavia, IT; \textsuperscript{5} University of Pavia, Department of Chemistry, Pavia, IT; \textsuperscript{6} University of Parma, Department of Engineering and Architecture; UdR INSTM, Parma, IT

Introduction
Infectious diseases are one of the most important causes of death in the World. The constant use of antibiotics has led to the development of new very resistant bacterial strains, which are a threat for public health. For this reason, noticeable efforts from different scientific fields have been devoted to achieve solutions that might contribute to attenuate this problem. In this context, research on new antibacterial materials has become a current and important goal in materials science. Several metals are well-known in literature for their antibacterial properties, both because of direct contact with the surface and the release of ions into the solution (indirect contact). Among these metals, the most extensively used in different applications are silver and copper. They show a broad spectrum of action against bacteria both upon exposure to surfaces and ions in solution. The advantage of copper resides in its lower toxicity to mammalian cells than silver. Indeed, Cu\textsuperscript{2+} ions in small quantity facilitate the activity of different enzymes and help cross-linking of collagen and elastin of bones, whereas in high concentration it can inhibit osteoblast proliferation. Therefore, it is crucial to control copper concentration for biomedical applications.

The aim of this work is to evaluate the antibacterial effects and the biocompatibility of undoped (CPG) and Cu\textsuperscript{2+}-doped calcium-phosphate glasses (CPG_Cu) which have shown in previous studies interesting degradative, mechanical and optical properties to be used in biophotonic and medical applications.

Experimental Methods
CPG and CPG_Cu were analyzed with inductively coupled plasma – optical emission spectroscopy (ICP-OES) to evaluate their content of Ca, P, Na, Mg, B, Si and Cu. The antimicrobial effect of CPGs against four of the most common causing-infections bacteria, namely Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa PAO1 and Klebsiella pneumoniae were investigated; the biocompatibility was performed using NIH-3T3 cells. These viability assays in both bacteria (after 24h) and eukaryotic cells (after 24 and 48h) were evaluated through the quantitative 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test, which was performed in both direct and indirect contact and measures dehydrogenase activity as an indicator of the metabolic state. Moreover, we investigated the viability of adherent cells with MTT and confirmed it with scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM).

Results and Discussion
The obtained results demonstrated the promising antibacterial properties of Cu\textsuperscript{2+}-doped CPG with respect to undoped CPG, in particular against Gram-negative bacteria. The particular efficacy against E. coli, P. aeruginosa and K. pneumoniae is due to the different composition of bacterial surface. The preliminary quantitative and qualitative (SEM and CLSM) studies performed using 3T3 cells showed that CPG and CPG_Cu were not toxic.

Conclusion
The results suggested that the tested bioresorbable bioglasses showed biocompatible and antibacterial properties useful for medical applications.

**Acknowledgement**

D.P. and D.J. acknowledge the Interdepartmental Center “PhotoNext” of Politecnico di Torino for the partial support of this research effort. The study was supported by a grant of the Italian Ministry of Education, University and Research (MIUR) to the Department of Molecular Medicine of the University of Pavia under the initiative “Dipartimenti di Eccellenza (2018–2022)”. L.V. and E.R. acknowledge the following members of University of Pavia: Prof. Dr. Roberta Migliavacca for providing bacterial strains; Dr. Patrizia Vaghi for CLSM analyses; Stefano Iervese and Dr. Federico Bertoglio for performing some experiments.

**References**


**Bacterial colonization of oxygen-functionalized graphenic surfaces**

**Wojciech Pajerski**, Joanna Duch, Monika Golda-Cepa, Dorota Ochonska, Monika Brzychczy-Wloch, Andrzej Kotarba

1 Jagiellonian University, Faculty of Chemistry, Krakow, PL; 2 University of Gdansk, Faculty of Chemistry, Gdansk, PL; 3 Jagiellonian University Medical College, Department of Molecular Medical Microbiology, Chair of Microbiology, Faculty of Medicine, Krakow, PL

**Introduction**

The graphene family nanomaterials are an exciting promise in biomedical applications such as tissue engineering, bone regeneration, stem cells differentiation, bio-imaging, and photothermal therapy [1]. The specific properties of graphene-based biomaterials, which play a pivotal role in regenerative medicine applications include: electrical conductivity, good mechanical properties, large surface area, and tunable surface functionalities [2]. Although, carbon biomaterials are extensively studied in recent years, interactions at biointerfaces between bacteria and carbon surfaces are still far from in-depth understanding. The major issue concerning the use of graphene-based biomaterials is the infection risk and subsequent complications causing additional treatment and higher mortality.

The aim of the study was to identify the key factors mediating the bacteria adhesion to oxygen-functionalized graphenic surfaces. The investigations were focused on the initial step of bacteria colonization, which is crucial for infection and biofilm formation.

**Experimental Methods**

In this work, we have investigated the effect of oxygen plasma functionalization of model graphenic sheets (introduction of oxygen functional groups) on changes in work function (WF), surface free energy (SFE), wettability, and bacterial adhesion. The oxygen plasma parameters were precisely adjusted (exposure time: <20 min, generator power: <60 W, oxygen pressure: 0.3 mbar) to limit the modifications to the surface without changes in the bulk structure of the material. The investigated graphenic surfaces were thoroughly characterized by SEM, RS, XPS, TG/DTA, contact angle, work function measurements, and bacterial adhesion tests.

**Results and Discussion**

The effect of oxygen functional groups introduced to graphenic surfaces on bacterial adhesion was evaluated for a series of microorganisms: *Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*. The modification of graphenic surfaces was accomplished by the application of low-temperature oxygen plasma treatment while adjusting the parameters for control surface modification (number of surface functional groups) without changing the bulk structure of the material. The obtained results clearly show that key factors of bacterial colonization are the surface work function (electrodonor properties) and the zeta potential (surface charge) of bacterial cells. The lowest colonization rate was observed for lower work function graphenic material (4.4 eV) and for bacteria with the lowest zeta potential (*E. coli*). The results were rationalized in terms of total interaction energy with the main contribution from electrostatic forces at the graphenic sheet-bacterial cell interface (Fig. 1).

**Conclusion**

It was found that even the short time of oxygen plasma modification (a few seconds) increases significantly hydrophilicity, surface free energy, and work function of graphenic materials. The changes in surface chemistry, and bacterial surface charge substantially influence the bacterial adhesion. The strong relationship between work function value and bacterial adhesion was observed for several pathogenic bacterial strains: the lower work function value,
the lower colonization rate. Additionally, the low zeta potential inhibits the bacterial colonization rate of graphenic surfaces. The results pointed out the work function lowering of the graphenic biomaterial surface as a rational strategy for the infection risk limitation.

**Acknowledgement**

This study was funded by Polish National Science Centre project, award number 2020/37/B/ST5/03451. W. Pajerski acknowledges the fellowship with the project no. POWR.03.02.00-00-I013/16.

---

**References**


Diabetic environment alters the adhesion behaviour of Staphylococcus aureus to the Ti6Al4V surface

Maria Fernandez-Grajera1,2,3, Miguel A. Pacha-Olivenza1,2,3, M. Luisa Gonzalez-Martin1,2,3, Amparo M. Gallardo-Moreno1,2,3

Introduction
The titanium alloy Ti6Al4V is a biomaterial with good mechanical properties and great biocompatibility, but one of the biggest drawbacks is infectious processes. Diabetes is a disease with a very high incidence in the elderly population. In this age group it is also common to find patients with bone replacements such as hip or knee implants usually made of titanium and its alloys. The presence of glucose and ketone bodies in cases of poorly controlled diabetes significantly alters the incidence of infections in these patients. The reasons are unclear, but diabetes has been linked to different physiological processes such as inflammatory reactions, poor vascularisation or neutrophilic chemotaxis. However, there has been little work on analysing the effect of a diabetic environment on the bacterial adhesion behaviour to biomaterial surfaces from the bacterial approaching to the surface to long adhesion times using a dynamic model. This work will evaluate the adhesive behaviour of Staphylococcus aureus to Ti6Al4V surfaces under flow with and without diabetic supplements, including different concentrations of glucose and ketone bodies.

Experimental Methods
25 mm Ti6Al4V disks were mirror polished. The strains selected have been S. aureus ATCC 29213, a gram-positive bacterium with opportunistic behaviour. Bacterial growth was carried out with different diabetic conditions, including the individual action of glucose and ketone bodies and their combined action. Bacterial adhesion experiments were performed in a laminar flow chamber, where bacteria were allowed to adhere to Ti6Al4V in a suspension of phosphate buffer saline (PBS) supplemented with the same diabetic components as those used in the growth media. The contact was allowed for 5h at a temperature of 37°C. During this time a series of live photographs were recorded and then analysed to quantify bacterial adhesion. Experiments were carried out by triplicate.

Results and Discussion
The results show that the different concentrations of diabetic components in the culture media modify the adhesion dynamics of S. aureus. In particular, the supplementation with glucose leads to an increase in the number of the final adhered bacteria, but not in the initial adhesion dynamics. However, the different concentrations of ketone bodies cause changes not only in the initial adhesion rates but also in the final coverage of the Ti6Al4V surface. Under a more realistic environment of poorly controlled diabetes, the mixture of supplements indicates the dominant role of hyperglycaemia in the adhesion rate, being the largest of all treatments. Likewise, a significant increase in the bacterial aggregation over time was observed, ketoacidosis being the dominant parameter for this behaviour.

Conclusion
This study reveals the role of hyperglycaemia and ketoacidosis in the initial adhesion process of bacteria to the titanium surface. While hyperglycaemia seems to be more associated with an increase in the final number of bacteria, ketoacidosis causes bacterial aggregation and affects both the initial stages of adhesion and the final coating of the surface.

Acknowledgement
Authors are grateful to the Junta de Extremadura and FEDER (grant numbers GR18153) and to the project RTI2018-096862-B-I00 supported by FEDER/ Ministerio de Ciencia e Innovación - Agencia Española de Investigacion. Also, MFG, would like to thank the Junta de Extremadura and the European Social Fund for its grant PD16055.
Glial Wars: the astrocyte awakens. A tissue-engineered 3D model to explore astrocytic phenotype and morphology in response to mechanical stimulus

Miguel R.G. Morais¹, Eva D. Carvalho¹,², Marco Araújo¹, Cristina C. Barrias¹,³, Ana P. Pêgo¹,²,³

¹ University of Porto, i3S-INEB, Porto, PT; ² University of Porto, Faculty of Engineering, Porto, PT; ³ University of Porto, ICBAS, Porto, PT

Introduction

Multiple Sclerosis (MS) is the leading cause of nontraumatic neurologic disability in young adults in many countries. In the acute phase of the disease, the primary pathological hallmark is the presence of focal inflammatory lesions, characterized by demyelination but relative preservation of axons. These lesions evolve into chronic active lesions with complete demyelination, formation of an astroglial scar and extensive inflammation. Astrocytes have been for long assigned a role as bystanders during demyelination. Although recent finds suggest that these cells are highly active participants in MS lesions’ development, their role during MS progress is still poorly understood. Astrocytes change their phenotype from quiescent to reactive with differentially expression of reactive genes (e.g., Glial fibrillary acidic protein (GFAP) and Lcn2) and extracellular matrix proteins (e.g., collagen IV, fibronectin), which strongly modify the properties of the lesion environment. Our working hypothesis is that the alterations in the matrix caused by reactive astrocytes negatively impact the formation of new myelin sheaths after demyelination. Currently there is no suitable in vitro model that can recreate all features of astrogliosis. Thus, there is a need for the development of new methodologies to study this phenomenon.

In this work we present a three-dimensional (3D) tissue-engineered model to study astrocyte reactivity. In order to reconstruct a more physiologically relevant environment, astrocytes were embedded within modified alginate matrices. Alginate was chosen due to its compatibility with mammalian cell culturing, possibility of introducing modifications in its the structure to increase biological responses and tunable mechanical properties.

Experimental Methods

1% (wt/v) alginate hydrogels were produced by mixing non-modified high molecular weight alginate with alginate modified with the cell adhesive peptide RGD (GGGGRGDSP) and oxidized alginate (theoretical oxidation of 10% (m/m)) with engrafted matrix metalloproteinase sensitive peptide PVGLIG (GGYGPV↓LIGGK). Final concentration of RGD and PVGLIG was set to 40 μM and 400 μM, respectively (Alg Mod). Non-modified alginate was used as a control (Alg HMW). Primary rat cortical astrocytes (P1-P2 pups) were embedded in hydrogels and maintained in culture for 7 days. A pro-inflammatory stimulus (LPS/IFNg) for 72h was used to induce an astrogliosis-like phenotype. To promote alginate hydrogels mechanical properties changes, an external BaCl₂ (50 mM) bath was performed (15 min, 37°C) at 24h post cell seeding. The mechanical properties of hydrogels were measured by rheology. Astrocytic phenotype was evaluated by gene expression and 3D image analysis using the IMARIS software and glial fibrillary acidic protein (GFAP) as the astrogliosis marker.

Results and Discussion

Astrocytes embedded in Alg Mod extend more and longer cellular processes and acquire an overall more complex morphology, when compared to astrocytes grown in Alg HMW. This is in line with a more physiologically relevant extracellular environment being achieved with the introduction of modifications into the hydrogel. Treatment with LPS/IFNg did not alter metabolic activity of cells and increased the expression of the Lcn2 and CollIV genes (after 72h of treatment), with cellular morphology remaining relatively unchanged. Preliminary results suggest...
that mechanical properties of the hydrogel are not altered in the presence of the stimulus. These results are indicative of an astrogliosis-like phenotype being achieved. After submitting cell-laden hydrogels to an external BaCl₂, matrices became stiffer (G* increased from 21.22 Pa to 144.03 Pa) and approximately 45% smaller in size. Preliminary results suggest that cell metabolic activity and viability was affected by this process, but cellular morphology remains unchanged.

**Conclusion**

In this work we present a 3D cell culture method to study astrocyte reactivity and response to biochemical and mechanical stimuli. Modifications introduced in alginate hydrogels allowed astrocytes to acquire a more complex morphology in line with what happens *in vivo*. Treatment with LPS/IFNγ did not alter metabolic activity and induced an increase in expression of *Lcn2* and *ColIV* while the morphology remained relatively unchanged. Finally, using BaCl₂ we were able to increase astrocyte-laden alginate hydrogels’ stiffness (G*), with preliminary results suggesting a negative impact on cells’ metabolic activity and viability but with morphology remaining normal. In the future we will use this 3D culture system to combine these two types of stimuli (biochemical and mechanical) and characterize astrocytic response, shedding light into the complex world of mechanobiology of the glial scar and its impact on demyelinating conditions.

**Acknowledgement**

The authors acknowledge the funding from projects UTAPEXPL/NTec/0057/2017 (FCT - UT Austin Portugal Program) and GRANT13074566 (Air Force Defense Research Sciences Program, USA). E. D. Carvalho acknowledges FCT from her Ph.D. fellowship (grant SFRH/BD/140363/2018).
Spatial topological confinement dictates the effective growth and differentiation of neural stem cells

Jagoda Litowczenko¹, Barbara M. Maciejewska¹, Jacek K. Wychowaniec¹,2

¹ Adam Mickiewicz University in Poznan, NanoBioMedical Centre, Poznań, PL; ² University College Dublin, School of Chemistry, Dublin, IE

Introduction
According to World Health Organization (WHO) neurological disorders are a leading cause of disability and the second leading cause of death worldwide, affecting 276 million and 11.6% of global disability-adjusted life years (DALYs) in 2016. Particularly challenging is the limited capacity for the repair of mature nervous system, which to this day remains a significant clinical challenge and hurdle for patients [1]. This is in part due to the lack of understanding of neural tissue re-growth development. Tissue engineering approaches are able to overcome those challenges by combining cell therapies with advanced biomaterials to restore tissue homeostasis and proper functions. Specifically, spatiotemporal guidance of axon outgrowth was found crucial for the re-wiring of neural tissue damage resulting either from traumatic injuries or progressive diseases [2]. Nerve cells are particularly susceptible to the surface stiffness and topography. In our recent studies we observed that both topographical cues and stiffness promotes morphological changes in the cells of neuronal origin [3]. Graphene-based materials due to their mechanical strength, conductivity and/or available surface for bio-functionalization are also largely exploited as scaffolds that regulate cell behaviour, including proliferation, differentiation in order to promote specific tissue regeneration [4]. Recently we also showed that surface roughness of rGO scaffolds plays a crucial role in cell attachment and proliferation [5]. Therefore strategies involving manipulation of topography, stiffness, as well as surface chemistry, are being explored to engineer guidance conduits with precise ability to direct behavior of neural stems cells.

Experimental Methods
Here we studied material neural stem cells (NSCs) interactions; in particular, we investigated the alignment and differentiation of NSCs on a series of groove-patterns made of silicon, as well as of composite graphene-based matrices, prepared by photolithography and soft lithography methods. Spatial dimensions of fabricated matrices were varied from across nano- to micro-length-scales to investigate the effects on the biological outcome of cultured cells up to 21 days. Immunocytochemistry, and detailed proteomic analysis were performed to elucidate the underlying cellular mechanisms.

Results and Discussion
We demonstrated that groove patterns topography have a strong impact not only on cell alignment and neurite outgrowth but also on differentiation of NSCs. Our results point out to specific combination of topography and substrates stiffness that can lead to selective differentiation of NSCs without any exogenous supply of differentiation stimulants. The analysis of the cell processes length revealed extensive neurite outgrowth on material patterns with dimensions resembling those of native tissue. Composite scaffolds supported cell growth, proliferation and differentiation. Immunocytochemistry staining and mass spectrometry coupled with liquid chromatography (LC-MS/MS) proteome analyses confirmed favorable spontaneous NSCs differentiation on groove pattern of specific dimensions compared to all tested topographies. ICC and proteomic studies also allowed for identification of differentiation-stage specific markers of astrocytes, oligodendrocytes and neurons, which will be discussed during the presentation.

Conclusion
Page 1418 of 2028
Our results greatly contribute to the understanding of differentiation process of cells of neuronal origin, which can ultimately lead to promising strategies for neuronal tissue engineering. In particular, underlying cellular processes and mechanism of mechano-transduced differentiation were elucidated and linked to relevant proteins revealed by LC-MS/MS proteome analysis.

Acknowledgement

The authors acknowledge financial support of the National Science Centre (NSC) grant: PRELUDIUM (2016/23/N/ST5/00955).

References


New chitosan-fibronectin hydrogel for application in tissue engineering

Carla Palomino-Durand¹, Phuong Anh Dang¹,², Maxence Gall¹, Sophie Norvez², Laurent Corté²,³, Adeline Gand¹, Emmanuel Pauthe¹

¹ CY Cergy-Paris University, ERRMECe Laboratory, Biomaterials for Health group, Cergy, FR; ² ESPCI Paris, PSL University, C3M laboratory, Paris, FR; ³ Mines ParisTech, PSL University, Centre des Matériaux, Evry, FR

Introduction
Hydrogels, a tridimensional (3D) polymeric network, are a promising biomaterial for regenerative medicine. They are attractive due to different properties, such as their biocompatibility, their permeability to oxygen, nutrient growth factors and metabolic waste and their mechanical properties similar to natural tissues. It has been also reported that cells are able to remodel hydrogels to produce a new extracellular matrix (ECM). This behavior is depending on the type of cells and on the appropriate biological cues and mechanical properties of the hydrogel (1).

Among the wide selection of natural polymers, chitosan (CS, a cationic polysaccharide) has been extensively studied as a component of hydrogels applied in tissue engineering. Nevertheless, CS polymer lacks binding sites for cell adhesion. Thus, the addition of a molecule that contains these biological signals is necessary. Fibronectin (Fn) is an ECM dimeric glycoprotein and the principal molecule involve in cell adhesion, migration, and differentiation due to the presence of binding domains (2). It has been shown that the covalent addition of Fn using EDC/NHS ameliorates the cell adhesion and cell proliferation in a CS membrane (2D) (3) and also increases mechanical rigidity in polyelectrolyte-based thin films (4).

In this work, CS/Fn hydrogels were developed using the 1-ethyl-3-[3(dimethylamino)propyl] (EDC) and N-hydroxysulfosuccinimide (sulfo-NHS) as cross-linking agents. This technique would allow at the same time, give a biological cue, and improve the mechanical property of the hydrogel. The aim of this study was to evaluate cell behavior in a 3D biomaterial to better understand the cell-material relation.

Experimental Methods
Different CS:Fn ratios were studied for hydrogel formation. First, a CS stock solution was prepared using an equal molar ratio with acetic acid. Then, a solution of human plasmatic Fn was mixed at corresponding ratio by stirring. Finally, an EDC/NHS solution was added to the mixture and the crosslinking was attended by stirring the solution for 4h at room temperature, then for 20h at 4°C without stirring. A hydrogel without Fn was also prepared.

For cell culture test, samples were prepared in sterile conditions and rinsed with phosphate-buffered saline (PBS, pH 7.4). Hydrogels cytocompatibility was evaluated with pre-osteoblast cells (MC3T3-E1) using AlamarBlue® assay. Cell adhesion and proliferation were evaluated in hydrogels with and without Fn using MC3T3-E1. Hydrogels were incubated in presence of cells for 24h and 72 h at 37°C and 5% CO₂, then samples were staining with DAPI (DNA) and phalloidin-FITC (actin) and imaged using a laser scanning confocal microscope.

Results and Discussion
Stable, homogeneous, and transparent hydrogels were obtained for all CS/Fn ratios. All hydrogels were cytocompatible (with or without Fn) with cell survival greater than 75%. After 24h of incubation, cells adhesion was observed only in CS/Fn hydrogels (Fig. 1a). We can observe a spread morphology with a high quantity of actin fibers in the cell cytoskeleton. However, hydrogels without Fn showed a spherical morphology and lack in actin fibers (Fig. 1c). After 72h of incubation, the proliferation of cells was remarkable in CS/Fn hydrogels (Fig. 1b). While in hydrogels without Fn (and without cell adhesion) there was no proliferation (1d). It has been confirmed that cell adhesion in a 3D biomaterial is also needed to promote cell proliferation and migration.

Conclusion

Page 1420 of 2028
The preliminary results demonstrated the feasibility of forming a hydrogel between CS and FN with a covalent binding and validated the need of biological cues to promote cell adhesion and proliferation. Furthermore, viscoelastic properties should be evaluated to better understand the role of mechanical properties in hydrogels.

![Figure 1.](image)

MC3T3-E1 cells on CS/Fn hydrogels (Fn covalent binding): (a) 24 h and (b) 72 h of incubation. MC3T3-E1 cells on hydrogels without Fn: (c) 24 h and (d) 72 h of incubation.

References
(1) Ahearne M. 2014 “Introduction to cell-hydrogel mechanosensing”. Interface Focus. Vol. 4
2:30 p.m. – 4:00 p.m.

Poster floor

**PS1-14 | Biomaterials for Tissue and Organ Models**
Microphysiological System to Evaluate Vasculogenesis in Calcium-releasing Biomaterials

Adrián López Canosa\textsuperscript{1,2}, Oscar Castaño Linares\textsuperscript{1,2}, Elisabeth Engel López\textsuperscript{1,3}

\textsuperscript{1} Institute for Bioengineering of Catalonia (IBEC), Biomaterials for Regenerative Therapies, Barcelona, ES; \textsuperscript{2} University of Barcelona (UB), Electronics and Biomedical Engineering, Barcelona, ES; \textsuperscript{3} Polytechnic University of Catalonia (UPC), Materials Science and Metallurgical Engineering, Barcelona, ES

Introduction
Over the last decades, most of the strategies to improve the vascularization of biomaterials based on calcium phosphates (CaPs) were based on the incorporation of well-known proangiogenic agents such as growth factors, despite their high cost and the complexity of delivering safe and effective doses [1]. A promising alternative is the use of inorganic elements that naturally occur within the body, namely metallic ions [2]. It has been shown that dissolution products of CaPs are able to induce vascularization [3], although the particular mechanism by which calcium stimulates this process is not very well understood, mainly due to the lack of suitable \textit{in vitro} models.

Experimental Methods
The design of this platform was performed using CAD software and it consists of two cell culture chambers flanked by a total of three media channels. The chambers are delimited by two lines of microposts, which are used to confine the cells. Master molds were obtained in 4" silicon wafers using standard photolithography techniques in a clean room environment. PDMS replicas were obtained from these masters and bonded to 0.17 mm coverslips with deposited aligned nanofibers, which were created by electrospinning a solution of polylactic acid (PLA) in 2,2,2-trifluoroethanol with or without CaP ormoglass nanoparticles at 25 % w/w (PLA vs PLA + NP25). To perform the process, the coverslips were fixed to a grounded rotary collector, which was set to rotate at a speed of 1000 rpm. The applied voltage was 15 kV and the distance between the tip and the collector 20 cm. Most of the deposited nanofibers were carefully removed with acetone to leave only an area corresponding to the section of one of the cell chambers.

Bone-marrow derived rat endothelial progenitor cells (rEPC) and rat mesenchymal stem cells (BM-rMSC) were obtained from the long bones of young Lewis rats (2-4 weeks old) following a previously published protocol [4]. The BM-rMSC were seeded on top of the electrospun fibers (stromal chamber) and rEPC on the wall of a 3D fibrin gel (2.5 mg/ml) loaded in the other cell compartment (migratory chamber). Cells were fixed after 3 days in culture with paraformaldehyde and stained for DAPI/phalloidin. Images were acquired with a confocal microscope (Leica SP-5) and processed using Image J to obtain the number of cells and their mean migrated distance with respect to the end of the microposts. Conditioned media was collected at day 3 and analyzed using ELISA for several pro-angiogenic and pro-inflammatory factors such as the insulin-like growth factor 2 (IGF-2), interleukin 6 (IL-6) and osteopontin (OPN). Student’s \textit{t}-test (unpaired, two-tailed distribution) was used to compare the two samples (a p-value < 0.05 (*) was considered statistically significant).

Results and Discussion
Our results show that the PLA + NP25 is able to significantly increase the recruitment of rEPC respect to the PLA control, both in terms of the mean migrated distance (92.32 ± 5.24 µm vs 69.33 ± 11.24 µm) and mean number of migrated cells (42 vs 12). The protein expression analysis of the conditioned media revealed that the PLA with calcium phosphate nanoparticles caused a significant increase in the release pro-angiogenic and pro-inflammatory factors.
factors (IGF-2, OPN, IL-6). The data obtained with our microphysiological system closely matches the results obtained for in vivo studies using the same material (subcutaneous implantation in rats) [5].

**Conclusion**

In this study, we presented a microphysiological system to evaluate the potential of a biomaterial to recruit endothelial progenitor cells. We show that a material based on PLA fibers with calcium phosphate nanoparticles is able to significantly increase the migrated distance and cell number with respect to the PLA control. This result is in good agreement with in vivo studies previously performed with the material, showing the great potential of microphysiological systems to accelerate the bone-graft substitute research.

**Acknowledgement**

This work was funded by the Spanish Ministry of Economy and Competitiveness (MINECO) through the project MAT2015-68906-R and the Spanish Ministry of Education, Culture and Sports with the FPU grant (FPU17/06161).

**References**


Human dental MSC spheroids: development of a pre-clinical imaging model to clarify cellular spheroids behavior in vivo

Jorge Dias¹,², Cristina Barrias³,⁴, Fernando J. Monteiro³,², Christiane Salgado¹,³

¹ INEB - Instituto Nacional de Engenharia Biomédica, Porto, PT; ² FEUP - Faculdade de Engenharia, Universidade do Porto, Porto, PT; ³ i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, PT; ⁴ ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, PT

Introduction

In the oral cavity, adult tooth tissues contain cells with mesenchymal phenotype and their main advantages are the easy access with limited morbidity, multipotent capacity, rapid attachment and high proliferation rate. Although, in 2D cell cultures, cell metabolism and the cell–cell interactions are reduced. On contrary, 3D cell cultures (spheroids) exhibit increased levels of cell-specific markers, regain tissue functions and gene expression profiles when compared to 2D. One major challenge in tissue engineering is to ensure the functionality of administered cells within the host environment, without premature loss and/or excessive death. In this context, spheroids will be monitored along time with the expression of luminescent/fluorescent signals, for in vivo imaging purposes. Cells transfected with GFP-Luciferase plasmids have an advantage dye-labeling particles, as it is integrated into the genome, retained in the daughter cells, and can follow cell proliferation and tracking in deep tissues. Using this optical imaging approach, the pre-clinical model will monitor the cells engraftment into the bioengineered material and evaluate the tissue regenerative process at a cellular level. In this work, we developed luminescent/fluorescent human dental MSC lines, optimized MSC spheroid formation process and characterized the cells metabolic activity/proliferation/differentiation.

Experimental Methods

Dental follicle was surgically removed from the completely intra-bone impacted third molar. This study was approved by an Ethical Committee of University of Porto (50/CEUP/2018, Portugal). Cells were collected, digested and isolated by adherent culture on plastic tissue culture substrates as described previously (1). Dental MSCs expressing GFP-Luc was developed by efficient lentiviral transduction. The lentiviral vectors was produced by HEK293T cells using a dual-promoter reporter gene (pVSVG – envelop and psPAX2 - Packaging) and pHAGE GFP-IRESG-luc (Transfer plasmid Reporter). hDFSCs was incubated with the GFP-IRESD-luc virus vectors, and were assessed on a ZOE™ Fluorescent Cell Imager and GFP positive cells were sorted by FACS Aria II, after 5 days. Spheroids were produced using commercially available MICROTISSUES® technology. Briefly, agarose (2%) micro-molds were prepared and hDFSC suspension were seeded at a concentration of 5.12 × 10⁵ cells/micro-mold, corresponding to 2000 cells per spheroid. Cells were grown during 10 days, with media being changed every 48 h. Cultures were monitored and the spheroids diameter was measured with Brightfield microscopy at each time-point. Metabolic activity of spheroid cultures was assessed using Alamar blue assay at days 1, 3, 5, 7 and 10, resazurin solution (0.2%) was added to micro-molds and supernatant collected and placed in 96-well black-plates. The obtained fluorescence values were normalized in relation to the number of spheroids placed in each micro-mold.

Results and Discussion

A major obstacle in the assessment of cell-based therapeutic strategies is the ability to track their interaction with local microenvironments and differentiation into various cell phenotypes as they occur in real-time. Transfected human dental follicle mesenchymal stem cells (GFP-Luc_DFSC) were developed and showed into 2D evaluation, adhesion capacity, proliferation rate, and osteogenic differentiation similar to non-transfected cells, as validated ALP enzyme activity and high expressio of osteopontin and BMP-2 by qPCR evaluation after 21 days. GFP-Luc_DFSC spheroids were successfully produced with a narrow size distribution with an average diameter of 150 µm (Fig. 1)
and a uniform spherical shape, and the majority were viable after 10 days, as confirmed by Alamar blue assay (Fig. 2). The osteogenic differentiation of the spheroids was performed and GFP-Luc-DFSC spheroids showed human bone osteopontin and calcium crystals presence (Alizarin red), as observed for the non-transfected cells.

**Conclusion**

In conclusion, human hDFSCs with GFP-Luc (virus vector) showed a luminescence/fluorescence signal that was integrated into the genome and, as a result, retained in the daughter cells, and enable to follow metabolic activity/proliferation rate, for 10 days (3D spheroids) and 3 weeks (2D) in vitro. The GFP-Luc-DFMSC spheroids showed, high ALP activity, a remarkable osteogenic potential and produce mineralized ECM. After these successful results, a spheroid-loaded scaffold will be tested in vivo by a well-established animal model and GFP-Luc-DFSC spheroids engraftment and survival will be followed by real-time imaging, to study their role into the bone regenerative processes.

**Acknowledgement**

This work is a result of the project NORTE-01-0145-FEDER000012, supported by North Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). In addition, it was supported by Portuguese funds through FCT/MCTES in the framework of the project UID/BIM/04293/2019.
Cell metabolic activity were followed by Alamar blue assay, the potential of hDFSC and GFP-Luc_DFSC spheroids showed similar cellular viability after 1 and 3 days. But, after 5 days, higher metabolic activity was observed in both cell types, showing similar proliferation rate.

References
Human platelet lysates-based hydrogels to assess osteosarcoma spheroid invasiveness in a tri-culture model for drug screening applications

Cátia F. Monteiro, Catarina A. Custódio, João F. Mano

University of Aveiro, Department of Chemistry - CICECO, Aveiro, PT

Introduction

To date, advances in biomimetic materials have prompted great progresses in biomedical field. However, the synthetic and animal origin of the biomaterials’ majority has opening up some concerns regarding the need for a close recapitulation of the tissues’ biochemical and biomechanical properties to expedite drug discovery and development, namely anticancer therapies. [1,2] Human methacryloyl platelet lysates (PLMA) hydrogels are herein proposed as humanized 3D platforms for tumor invasiveness modelling and explored for drug screening applications through the establishment of a tri-culture osteosarcoma (OS) model. [3,4]

Experimental Methods

To develop the 3D tri-culture OS model, MG-63 OS cells spheroids formed for 3 days in ultra-low adhesion plates were encapsulated into PLMA hydrogels at 15% (w/v), surrounded by human bone-marrow mesenchymal stem cells (hBM-MSCs) and human osteoblasts (hOB) at an established cell-to-cell ratio in which the formation of tumor invasive branches and a network of hBM-MSCs was verified. In order to address effect of the extracellular matrix and tumor-associated stroma in tumor behavior, the tri-culture model was compared with tumor spheroids cultured in a scaffold-free and scaffold-based mono-culture settings. For the three established models, a 3-day doxorubicin treatment was performed after 14 days and the cells were maintained in culture until 24 days. Fluorescent protein-transduced MG-63 and hBM-MSC cells were used to analyze tumor-stromal cells crosstalk and drug response. Cells viability and morphology, tumor spheroid area and invasiveness, and hydrogel protein release were assessed over time, as well as stem cell direction towards spheroid center. The deposition of bone-related matrix in the established OS models was also verified by immunohistochemistry.

Results and Discussion

The architecture and synergistic interaction of an invading OS tumor was recapitulated encapsulating OS spheroids in PLMA hydrogels, alone or co-cultured with human osteoblasts and hBM-MSC. PLMA hydrogels supported tumor growth and the formation of tumor invasive branches from the spheroid in both settings. Stem cell direction revealed their alignment toward OS spheroid, suggesting that tumor cell chemotactically attracted the surrounding stromal cells. Comparing to scaffold-based OS mono-culture model, the presence of stromal cells potentiated tumor invasiveness ability into the ECM-mimicking matrix. The deposition of bone-related proteins and minerals were also observed. The exposure of the established models to doxorubicin revealed a higher IC50-value of PLMA-based models, comparing with scaffold-free spheroids.

Conclusion

The proposed 3D OS models highlighted the potential of PLMA hydrogels to support an invasive tumor behavior and recapitulate tumor-stromal cell crosstalk. This physiologically predictive microenvironment can be used to study early tumor metastatic events, opening up new possibilities for humanized and personalized drug screening and validation of therapeutic agents.

Acknowledgement
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, the doctoral grant SFRH/BD/144640/2019 of Cátia F. Monteiro and the individual contract CEECIND/02713/2017 of Dr. Catarina Custódio financed by national funds through the Foundation for Science and Technology/MCTES. The authors would like to acknowledge the support of the European Research Council Proof-of-Concept Grant Agreement No. ERC-2017-PoC-789760 for the project MicroBone.

Schematic representation of human methacryloyl platelet lysates (PLMA) hydrogels-based models

(I) Platelet lysates (PL) derived from human platelet-rich plasma is chemically modified with functional moieties that photo-crosslink by ultraviolet (UV) irradiation. (II) This human-derived biomaterial is subsequently used to establish 3D OS mono- and co-culture models. The chemoresistance to doxorubicin treatment is evaluated after a culture period in which tumor cells are allowed to invade the PLMA hydrogel and synergistically interact with stromal cells.
Tumor and stromal cell organization in a tri-culture model and cell viability
(A) Tumor and stromal cell organization of control and DOX-treated tri-culture OS model over the 24 days in culture, (B) stem cell direction angle toward spheroid center, and (C) viability of MG-63 tumor spheroids cultured alone or in combination with hBM-MSCs and hOBs at 14 and 24 days of culture. Scale bar: 200 µm.

References
Recombinant scaffolds for the physiological-like expansion of lung mesenchymal stromal cell subpopulations

Arturo Ibáñez Fonseca1, Måns Kadefors1, Linda Elowsson Rendin1, Zackarias Söderlund1, Sara Rolandsson Enes1, José Carlos Rodríguez-Cabello2, Gunilla Westergren-Thorsson1

1 Lund University, Lung Biology Team, Lund, SE; 2 University of Valladolid, BIOFORGE Lab, Valladolid, ES

Introduction
Three-dimensional (3D) cell culture is one of the major promises of biomaterials in the last decades, although it has not become as widespread as it could be expected. One of the reasons is the need of tailored biomaterials that address the different needs of cells living within a scaffold, such as cell adhesion and possibility of matrix remodeling, among others. This can be easily overcome with recombinant biomaterials, like elastin-like recombinamers (ELRs) [1], which can be used to form hydrogels in situ or even more complex and controlled microstructures, as cryogels. These scaffolds can be used to culture therapeutically relevant lung mesenchymal stromal cells (lung-MSCs) [2], given that their expansion in 2D is very far from their physiological niche and this may lead to the potential loss of their regenerative profile. Therefore, we hypothesize that if specific lung-MSC subpopulations are grown and expanded within ELR scaffolds that mimic some of the features of the native tissue, the cells will keep their phenotype to a higher extent.

Experimental Methods
Lung-MSC subpopulations were sorted from healthy human lung biopsies through fluorescence-activated cell sorting (FACS). Specifically, lung-MSCs were identified as CD45−/CD235a−/CD105+/CD90+ [2]. Further sorting allowed to isolate CD13− and CD13+/N-aminopeptidase) subpopulations, which are currently being studied in our laboratory. ELRs were expressed in Escherichia coli and purified by inverse transition cycling, as previously described [1]. ELR cryogels with a controlled pore size of 200-500 µm (similar to human lung alveoli) were formed following a recently established protocol in our group (manuscript in preparation), placed in a 96-well plate and seeded with CD13− sorted lung-MSCs. On the other hand, ELR hydrogels were formed with embedded cells directly in 96-well plates, taking advantage of the thermosensitivity of the biomaterial. The metabolic activity of the cells was measured with PrestoBlue (resazurin; ThermoFisher) up to 3 weeks as a signature of cell growth. Cells were imaged inside the scaffolds by phase contrast and confocal microscopy upon actin (Phalloidin-iFluor 555, Abcam) and nuclei (DAPI, ThermoFisher) staining.

Results and Discussion
The metabolic activity of CD13− sorted lung-MSCs in the 3D scaffolds increased over time, indicating cell growth and expansion, with significant differences (***p < 0.001) in comparison to 2D cell culture (Figure 1). This contrast in cell growth is probably related to phenotypic changes, meaning that the cells cultured in 2D are probably very different from those cultured in 3D. Future studies should dig into these differences and compare them to the phenotype of freshly isolated lung-MSCs. Moreover, cell morphology showed great differences between the groups, with both CD13−low and CD13−high lung-MSCs growing well attached to the plastic surface and with a fibroblast-like spindle and spread shape in 2D, while they associated forming branches in ELR hydrogels (Figure 2). Interestingly, the cells seeded in the hydrogels grew from and through these branches in an anisotropic way, in contrast to the isotropic way found in 2D, with colonies growing in every direction at the same time. These results were also confirmed by confocal microscopy, where lung-MSCs were found to be attached to the cryogels pore walls.

Conclusion
Overall, these results validate the use of two different ELR scaffolds, in the form of hydrogels and cryogels, for the 3D expansion of CD13-sorted lung-MSCs, with the latter being a highly novel microstructure that mimics the lung alveolar space. The differences found in terms of metabolic activity and morphology in comparison with the 2D culture suggest different cell behaviors, probably more physiological-like. Future studies will focus in the phenotypic characterization of both lung-MSC subpopulations through transcriptomics and protein profiling, which might have important implications in lung-MSC culture for regenerative purposes.

Figure 1. Metabolic activity at the different seeding conditions over time
Metabolic activity in terms of Presto Blue fluorescence intensity as a signature of cell proliferation for the different cell seeding conditions over time, with CD13high results on the left and CD13low on the right.

Figure 2. CD13-sorted lung-MSCs morphology
Phase contrast (top row) images of CD13-sorted lung-MSCs grown in 2D (left) and within the ELR hydrogel scaffold (right). Confocal microscopy images (bottom row) of CD13-sorted lung-MSCs within cryogels (left) and hydrogels (right).
[1] Ibáñez-Fonseca, A, et al., Trends in the design and use of elastin-like recombinamers as biomaterials, Matrix Biology, 2019, 84, 111

[2] Rolandsson Enes, S, et al., MSC from fetal and adult lungs possess lung-specific properties compared to bone marrow-derived MSC, Scientific Reports, 2016, 6, 29160
Mimetic 3D in vitro bone metastatic niche model to study the mechanism of neuroblastoma metastasis

Sanja Aveic\(^1,2\), Max Seidelmann\(^1\), Ramin Nasehi\(^1\), Simon Janßen\(^1\), Horst Fischer\(^1\)

\(^1\) RWTH Aachen University Hospital, Department of Dental Materials and Biomaterials Research, Aachen, DE; \(^2\) Pediatric Research Institute Fondazione Città della Speranza, Neuroblastoma Laboratory, Padova, IT

Introduction
Bone metastases commonly cause severe morbidities in solid tumors and are considered negative prognostic factors. In neuroblastoma (NB), a malignancy of the developing sympathetic nervous system, bone marrow and bone metastases are particularly alarming (1). However, the molecular mechanism that sustains the growth of NB cells in bone is not yet completely understood.

Experimental Methods
Here, we present a novel 3D in vitro model to reveal the details of NB metastasis. We propose \(\beta\)-tricalcium-phosphate (\(\beta\)-TCP) casted scaffolds with tailored interconnected microchannels for the combined growth of stromal and tumor cells in 3D conditions. The characterization of the composition of the extracellular matrix (ECM) produced by stromal cells was performed using two photon microscopy along with the assessment of its impact on tumor cells growth and structure (2).

Results and Discussion
We confirmed that the organization of stromal cells and ECM component such as fibronectin, are strongly shaped by NB cells. With respect to planar counterparts, cells seeded on 3D \(\beta\)-TCP structures produce more cytokines (eg. IL-6) and define membrane localization of Connexin-43. Inclusion of endothelial cells led to ulterior enhancement of the proposed 3D niche model confirming the 3D growth conditions as a determinant factor for the morphological and functional characteristics of tumor cells. Together, our niche model confirmed that both, the geometry on the cellular scale and the chemistry of the niche material are relevant for translating realistic mechanobiological cues to the metastatic NB. In this model, tumor cells actively interact with all the components of the niche. In turn, stroma assists tumor cells to form spheroid-shaped arrangements facilitating their pro-migratory and pro-invasive patterns.

Conclusion
These results emphasize the use of the model for more systematic biological studies (at cellular and molecular level) and open a window for more sophisticated pharmacological examinations of novel drugs against metastatic NB.

Acknowledgement
This work was supported by the Italian Neuroblastoma Foundation (Fondazione Italiana per la Lotta al Neuroblastoma) [grant number 19_20FNBL], “Two-Photon Imaging” Core Facility of the Interdisciplinary Centre for Clinical Research (IZKF) within the Faculty of Medicine at the RWTH Aachen University, and by the Electron Microscopy Facility of the Institute of Pathology, RWTH University Hospital, Aachen, Germany.

References
PDAC on-chip for modeling in vitro the pancreatic cancer microenvironment

Viola Sgarminato, Chiara Tonda-Turo, Simone L. Marasso, Matteo Cocuzza, Beatrice Minervini, Giorgio Scordo, Alberto Ballesio, Gianluca Ciardelli

Introduction
Pancreatic ductal adenocarcinoma (PDAC) mainly develops in the head of the pancreas, from the progression of pancreatic intraepithelial neoplasia (PanIN) lesions that occur within the acino-ductal unit, composed by acinar and ductal cells surrounded by pancreatic stellate cells (PSCs) [1]. PSCs strongly influence tumor microenvironment by triggering an intense stromal reaction which consists in an excessive extracellular matrix (ECM) deposition within the tissue surrounding cancer cells. This stroma alteration plays a key role in tumor progression and limits the drugs perfusion representing a barrier against chemotherapy and radiotherapy [2,3]. Furthermore, the lack of prognosis, the genetic complexity and the tumor heterogeneity make the discovery of new therapeutic options extremely difficult. For this reason, the establishment of a scalable in vitro model able to recapitulate the tumoral microenvironment is urgently needed. To this aim, we developed a microfluidic in vitro model of the acino-ductal unit as a powerful tool for a deeper understanding of the early evolution steps of PDAC, identify novel diagnostic, prognostic and predictive biomarkers and, in the end, validate innovative nanomedicine systems.

Experimental Methods
The microfluidic device was fabricated by replica molding (Figure 1). Specifically, an SU-8 patterned master was custom-made using photolithography and then conventional soft lithography was applied on the SU-8 patterned master to produce several polydimethylsiloxane (PDMS) replicas. The microfluidic device was designed to contain an upper layer and a lower layer divided by an electrospun polycaprolactone (PCL) membrane. The bottom layer had three channels: a central compartment (6.38 mm length, 1 mm width and 250 µm height) delimited by micropillars, with 100 µm diameter and 250 µm height, to confine a collagen gel loaded with the PSCs and two lateral channels (20.51 mm length, 500 µm width and 250 µm height) to control the passage of nutrients. The top layer was designed to incorporate PDAC cells which are expected to create an in vivo like crosstalk with PSCs co-cultured inside the bottom layer [4].

Results and Discussion
The reproducibility of the fabrication process was confirmed by optical and scanning electron microscopy (SEM). Diffusivity tests were performed on the bottom layer to evaluate the medium diffusion from the lateral channels to the middle one containing the collagen gel. With this aim, a fluorescence time-lapse imaging was carried out by confocal microscopy and the fluorescence intensity was measured in a specific region of interest (ROI) of the middle channel. The results demonstrated a controlled diffusivity of the dye through the micropillars placed at 75 µm each other. To characterize the microfluidic device from the biological point of view, human foreskin fibroblast cells (HFF-1) were embedded in the collagen solution and seeded in the bottom layer. The cell viability was measured using Live/Dead assay performed after 48h and 72h after seeding. Figure 2 shows the cell viability of HFF-1 at 72h after seeding.
within the collagen gel compared to that of cells grown in the gel-free microchannel. These results confirm the beneficial effects provided by collagen in promoting the proliferation of stromal cells.

**Conclusion**
The here realized microfluidic device reproduces *in vitro* the tumor microenvironment and the interactions that occur between adjacent cell types *in vivo*. Moreover, it provides a model of human pancreatic ductal adenocarcinoma at early stages opening the way to the investigation of the influence of stroma on the tumor’s evolution and perform drug efficacy screening. Advanced technologies which integrate optical components in microfluidic chips could be employed for the real-time monitoring of the tumor’s evolution and for performing *in situ* genomic analysis [5].

**Figure 1**
Microfluidic device fabricated by replica molding using PDMS: top layer (A) and bottom layer (B).

**Figure 2**
Differential viability of HFF-1 cells seeded within the chip containing the collagen gel (CHIP_G) and the gel-free microchannel (CHIP_NG). Cells were grown for 72h and stained using calcein-AM for live cells and ethidium homodimer-1 for dead cells.
References


Design of a biomimetic system for 3D cancer cell cultures and anticancer drug testing

Jelena Petrovic¹,², Mia Radonjic¹,², Jasmina Stojkovska¹,², Tijana Stankovic³, Miodrag Dragoj³, Milica Pesic³, Bojana Obradovic¹

¹ University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, RS; ² University of Belgrade, Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, RS; ³ University of Belgrade, Institute for Biological Research “Siniša Stanković” - National Institute of the Republic of Serbia, Belgrade, RS

Introduction
Cancer is a complex disease mostly with lethal outcome while advances in the field of anticancer drug research are slow and challenging. Conventional methods of anticancer drug testing based on monolayer cell cultures and in vivo animal models often produce misleading results due to inability to adequately mimic the cancer complexity. Consequently, there is an urgent need for new physiologically relevant 3D biomimicking tumor models. One of the approaches includes immobilization of cancer cells in biomaterials as artificial tumor extracellular matrices followed by cultivation in biomimetic bioreactors. In the present study, the aim was to develop a 3D tumor microenvironment based on alginate hydrogel microfibers and perfusion bioreactors. Additionally, chemical engineering methods were applied in order to correlate the observed effects of culture conditions on the cultured cells.

Experimental Methods
Two types of cancer cells (rat glioma cells C6 (ATCC® CCL-107™) and human glioblastoma cells U87 MG (ATCC® HTB-14™)) were immobilized in alginate microfibers by simple extrusion. Briefly, the cell suspensions (1–8×10⁶ cell ml⁻¹) in 1.5–2.8 % Na-alginate solutions (AppliChem, Germany) were manually extruded through a blunt edge stainless steel needle (22–28 G) immersed in the gelling solution containing Ca²⁺ (0.18 M). In the first experimental series, perfusion bioreactors were each filled with microfibers (0.5 g) with immobilized C6 cells and continuously perfused at flowrates in the range 0.12–0.30 cm³ min⁻¹ over 5 days. Static microfiber cultures served as a control. In the second experimental series, microfibers with immobilized U87 cells were cultured under static conditions for 28 days and treated with 100 µM temozolomide (TMZ), while treated monolayer cell cultures served as a control. Cell viability and proliferation were determined by cell counting, MTT test and live/dead staining (CalceinAM (CAM)/propidium-iodide (PI)) using confocal microscopy.

Results and Discussion
Investigations of the effects of needle diameter and initial density of U87 cells on the cell survival have shown that successful cell immobilization in alginate microfibers (~500 µm in diameter, Fig. 1) was achieved by applying a 25G needle and a minimal cell density of 4×10⁶ cells ml⁻¹. Smaller needle sizes induced the immediate cell death probably due to high hydrodynamic shear stresses during extrusion while lower initial densities of these cells resulted in decreased cell viabilities probably due to the reduction in cell-cell interactions.

The effects of flow were investigated in short-term perfusion cultures of C6 cells immobilized at different concentrations in microfibers exposed to continuous superficial medium velocities in the range 40–100 µm s⁻¹. Positive effects of perfusion were found primarily in cultures with lower initial cell concentrations of about 2.5×10⁶ cells ml⁻¹ in which higher cell viability and proliferation were obtained as compared to control static cultures.

In order to elucidate the obtained results, a simple mathematical model of mass transport based on advection, diffusion and reaction was applied for prediction of concentration profiles of bioactive molecules of interest within a microfiber cultured in the perfusion fibrous bed bioreactor. The model indicated that substances with diffusion coefficients in the range of 10⁻⁶–10⁻¹¹ m² s⁻¹ (e.g., oxygen) are sufficiently transported by diffusion only, while for...
substances with significantly lower diffusion coefficients ($\sim 10^{-19} \text{ m}^2 \text{ s}^{-1}$) the convective transport is necessary for efficient supply, thereby stressing the importance of the use of bioreactors in in vitro tumor model systems. Finally, a preliminary study with U87 human glioblastoma cells immobilized in alginate microfibers cultured under static conditions showed an altered cell response to the anticancer drug TMZ as compared to monolayer cultures indicating that 3D systems could be potentially used as more relevant in vitro models for anticancer drug testing. 

**Conclusion**
The overall results have shown potentials of biomimetic 3D in vitro systems as a tool for acquiring more reliable data in cancer cell cultivation and anticancer drug testing. Furthermore, multidisciplinary approach to the analysis of the obtained results could aid in elucidating the underlying mechanisms and pave the way to a simpler and quicker optimization of 3D culture parameters.

**Acknowledgement**
This work was supported by the Ministry of Education, Science and Technological Development RS (Contract No. 451-03-68/2020-14/200135 and 451-03-9/2021-14/ 200007), European Commission (grant952033) and James S. Mc. Donnell Foundation 21st Century Science Initiative in Mathematical and Complex Systems Approaches for Brain Cancer (Collaborative award 220020560).
Development of a biomimetic bioactive microenvironment for osteosarcoma research

Jasmina Stojkovska\textsuperscript{1,2}, Ivana Banicevic\textsuperscript{1}, Mia Radonjic\textsuperscript{1,2}, Jovana Zvicer\textsuperscript{1}, Djordje Veljovic\textsuperscript{1}, Milena Milivojevic\textsuperscript{3}, Milena Stevanovic\textsuperscript{3,4,5}, Bojana Obradovic\textsuperscript{1}

\textsuperscript{1} Faculty of Technology and Metallurgy, University of Belgrade, Department of Chemical Engineering, Belgrade, RS; \textsuperscript{2} Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, RS; \textsuperscript{3} Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, RS; \textsuperscript{4} Faculty of Biology, University of Belgrade, Belgrade, RS; \textsuperscript{5} Serbian Academy of Sciences and Arts, Belgrade, RS

Introduction

Osteosarcoma (OS) is a type of bone cancer that often occurs at the sites of bone growth. One of the reasons for slow progress in OS therapies is poor translation of the results from in vitro cultures that are mainly performed in 2 dimensional (2D) environment to in vivo settings. Thus, development of 3D culture models of the tumor microenvironment has been indicated as mandatory\textsuperscript{1}. One of the approaches relies on tissue engineering strategies utilizing scaffolds as cell supports and extracellular matrices (ECM), and biomimetic bioreactors providing efficient supply of biochemical and biophysical signals. In this work we have developed highly macroporous composite alginate scaffolds with embedded particles of hydroxyapatite (HAP) and β-tricalcium-phosphate (β-TCP) doped with Mg\textsuperscript{2+} as a bioactive environment that will possibly stimulate OS cells to express the typical morphology and metabolic functions. First we have studied active transformation of the mineral precursors into HAP in the presence of simulated body fluid followed by biocompatibility studies of the powder and short-term seeding studies of two types of OS cell lines.

Experimental Methods

HAP and β-TCP particles doped with Mg\textsuperscript{2+} with the (Ca+Mg)/P molar ratio of 1.52 were synthesized by a hydrothermal method followed by calcination. The particles were mixed with a Na-alginate solution and the obtained mixture (1 wt% alginate, 1 wt% powder, 0.03 wt% CaCl\textsubscript{2}) was poured into dialysis tubings to provide controlled alginate gelation by diffusion of Ca\textsuperscript{2+} from the surrounding gelling solution (4 wt% CaCl\textsubscript{2}·2 H\textsubscript{2}O). The obtained hydrogel cylinders were submitted to freezing (-70°C, 24 h) followed by freeze-drying (-60°C, 24 h) and rehydration (0.8 wt. % NaCl and 0.3 wt.% CaCl\textsubscript{2}). Finally, the cylinders were cut into discs (15 mm in diameter, 6 mm thick). The precursor transformation into HAP within composite scaffolds was investigated under continuous simulated body fluid (SBF) perfusion of 1.9 cm\textsuperscript{3} min\textsuperscript{-1} over 28 days in perfusion bioreactors (“3D Perfuse”, Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, Serbia), while scaffolds under static conditions served as controls. Murine K7M2-wt (ATCC\textsuperscript{®} CRL-2836\textsuperscript{™}) and human Saos-2 (ATCC\textsuperscript{®} HTB-85\textsuperscript{™}) were used for cell studies. In the biocompatibility study, the powder were added in 12-well plates (15 mg, 0.6 cm\textsuperscript{2} surface area in each well) and then the cells were seeded onto the powder at the seeding density of 4x10\textsuperscript{5} cell cm\textsuperscript{-2}, while in the cell seeding studies, the cells were seeded manually onto scaffolds (~9 mm in diameter, ~4.4 mm thick, 30x10\textsuperscript{6} cells cm\textsuperscript{-3} scaffold volume). Culture medium (3 cm\textsuperscript{3}) was added to each well and incubated for 5 days at 37 °C in 5% CO\textsubscript{2}. The scaffolds were examined regarding porosity, particle composition (by X-ray diffraction, XRD), HAP deposition (by scanning electron microscopy, SEM), mechanical properties by Universal Testing Machine and cell viability and morphology (by Live/Dead staining and SEM).

Results and Discussion

The XRD analysis of the obtained powder has indicated β-TCP as a dominant mineral phase. SEM analysis of the resulting macroporous composite alginate scaffolds has revealed uniform distribution of the filler in the hydrogel.
matrix incorporating open and connected pores. Porosity of rehydrated scaffolds was ~54%. The presence of fillers in the scaffolds induced a significant increase in the Young's modulus (21 ± 8 kPa), as compared to control scaffolds without the fillers (16 ± 3 kPa). Over 28 days under continuous SBF perfusion, β-TCP underwent active transformation to HAP, as confirmed by XRD and SEM. HAP formation was significantly more prominent under perfusion as compared to static conditions indicating the role of mass transport in this process. Both OS cell types started to adhere and spread in the presence of the powder after 24 h of plating retaining their morphology and viability showing excellent biocompatibility of the powder without adverse effects. Similarly, the cells adhered to the scaffolds, both on the surfaces and in the pores exhibiting high metabolic activity. Low cell loss was found in this seeding method, regardless of the cell type. In the next steps, dynamic seeding in the perfusion bioreactor as well as seeding onto dry scaffolds will be investigated followed by perfusion bioreactor cultures.

**Conclusion**
The overall results have shown potentials of the applied approach based on bioactive porous scaffolds for bone tumor engineering.

**Acknowledgement**
This work was supported by the Ministry of Education, Science and Technological Development RS (Contract No. 451-03-03-68/2020-14/200135 and 451-03-03-9/2021-14/200042) and European Commission (grants 860462 (IB) and 952033).

**References**
Bioinspired hydrogel scaffolds with tailorable properties for cell encapsulation applications

Minye Jin\(^1\),\(^2\),\(^3\), Alisa Gläser\(^2\), Supun W. Mohotti\(^2\), Gülistan Kocer\(^2\), Julieta I. Paez\(^2\),\(^1\)

\(^1\) University of Twente, Developmental BioEngineering, Enschede, NL; \(^2\) INM- Leibniz Institute for New Materials, Dynamic Biomaterials, Saarbrücken, DE; \(^3\) Saarland University, Chemistry Department, Saarbrücken, DE

Introduction
Within the biomaterials field, a prominent application of hydrogels is 3D cell encapsulation. They are used as extracellular matrix mimics for the basic study of cell function, high-throughput drug screening and therapeutic delivery. Several covalent coupling chemistries have been described for simultaneous hydrogel crosslinking, biofunctionalization and cell encapsulation. Ideally, a coupling reaction for hydrogel crosslinking develops efficiently and with adequate rate under physiological conditions, is non-toxic, and presents chemical orthogonality to commonly used biomedically approved polymer backbones. For widespread application, it is derived from accessible and bench-stable precursors, and allows tailoring of materials properties to facilitate adaptability to different biomedical scenarios. Developing a hydrogel that presents all these characteristics remains a challenge. Herein, a hydrogel platform that features all these attributes is presented.

Fireflies produce their bioluminescent reaction from the oxidation of substrates called luciferins, which are formed in vivo through the condensation reaction between 2-cyanobenzothiazole (CB) and aminothiol (AT) groups. Inspired by the biochemistry of fireflies, in this work we apply the CB-AT reaction as a covalent strategy for the crosslinking of hydrogels with tailorable properties (Fig. 1a). We introduce these reactive groups into polyethylene glycol (PEG) precursors and demonstrate CB-AT crosslinking under physiological conditions. The resulting gels present rapid and tunable gelation kinetics; are mechanically stable, homogeneous at the microscale, and cytocompatible. These advantageous properties demonstrate that CB-AT crosslinking chemistry is optimal for gel preparation in the presence of living cells.

Experimental Methods
4-arm, 20 kDa poly(ethyleneglycol) (PEG) macromers carrying CB or AT functional groups were synthesized and used for crosslinking under physiological conditions (25 or 37°C, 20 mM HEPES buffer, pH 7-8) with an enzymatically cleavable dithiol crosslinker, following established protocols.\(^1\)\(^-\)\(^2\) Hydrogel crosslinking, bio-functionalization with cyclo(RGDfK(C)) cell-adhesive peptide, and encapsulation of fibroblast L929 cells took place one-pot. The cell-laden hydrogels were cultured for 1, 3 and 6 days, and cell viability was evaluated by live/dead assay over time. Mechanical properties and gelation kinetics of the resulting hydrogels were characterized in situ by shear rotational rheology.

Results and Discussion
PEG hydrogels crosslinked via the CB-AT condensation reaction were successfully prepared under physiological conditions. Gel formulation was optimized to tune gelation kinetics and mechanical strength within convenient ranges for cell encapsulation applications. Gel formulation was optimized to tune gelation kinetics and mechanical strength within convenient ranges for cell encapsulation applications.

Gels at 5 wt% PEG concentration in HEPES buffer pH 8 showed fast gelation kinetics, with gelation time < 30 s, as revealed by rheology (Fig. 1b). This gelation rate was easily adjusted by external cues, namely pH and redox modulation, to afford gelation times spanning seconds to a few minutes. This time range is adequate for gentle mixing of precursors and cell suspension, thus preventing cell sedimentation in the biomaterial. Importantly, this kinetic tunability was achieved without affecting the final mechanical strength of the gel. In addition, by adjusting molar mass, multivalency and topology of PEG precursors, final mechanical strength after swelling ranged elasticity values of soft natural tissues.
CB-AT hydrogels with cell-adhesive and cell-degradable biochemical features were fabricated and tested for culture of L929 fibroblasts. Successful encapsulation of cells in these gels was demonstrated by the high cell viability found at days 1, 3 and 6 post-encapsulation (Fig. 1c). Cells recognized the adhesive ligand and spread, enzymatically degraded the material and proliferated. These features demonstrate that these hydrogels are non-cytotoxic and support the encapsulation of cells.

**Conclusion**

The presented bioinspired hydrogels demonstrated several advantages for 3D cell encapsulation: fast and tunable gelation rate, adjustable mechanics within physiological range, good cytocompatibility and tunable bioactivity. 3D cell culture gels based on CB-AT coupling chemistry are expected to become valuable as both implantable and injectable culture models for applications in tissue engineering and regenerative medicine.

**Acknowledgement**

We thank financial support from the Deutsche Forschungsgemeinschaft (DFG, Project no. 422041745) awarded to J.I.P. and from Alexander von Humboldt Foundation (Postdoctoral Fellowship) awarded to G.K.

---

**Figure 1**

a) Molecular design of hydrogel scaffolds through CB-AT condensation. b) Demonstration of pH-regulation of gelation kinetics in HEPES buffer pH 7-8, without affecting final mechanical strength of the gel. c) Successful encapsulation of L292 fibroblasts in CB-AT hydrogels.

**References**


Molecular changes induced in melanoma by cell cultivation in 3D alginate hydrogels

Sonja K Schmidt1, Melanie Kappelmann-Fenzl2, Stefan Fischer2, Rafael Schmid3, Lisa Lämmerhirt1, Lena Fischer4, Stefan Schröfer5, Ingo Thievessen4, Dirk W Schubert5, Alexander Matthies1, Rainer Detsch6, Aldo R Bocaccini6, Andreas Arkudas3, Annika Kangelbach-Weigand5, Anja K Bosserhoff1

1 FAU Erlangen-Nürnberg, Institute of Biochemistry, Erlangen, DE; 2 Deggendorf Institute of Technology, Faculty of Computer Science, Deggendorf, DE; 3 University Hospital Erlangen/FAU Erlangen-Nürnberg, Department of Plastic and Hand Surgery, Erlangen, DE; 4 FAU Erlangen-Nürnberg, Institute of Physics, Erlangen, DE; 5 FAU Erlangen-Nürnberg, Institute of Polymer Materials, Erlangen, DE; 6 FAU Erlangen-Nürnberg, Institute of Biomaterials, Erlangen, DE

Introduction
Malignant melanoma is a highly aggressive tumor of melanocytes characterized by early metastasis from small primary tumors. Cell culture models have contributed decisively to our current knowledge of the disease, yet a variety of processes in tumor development and progression remains to be deciphered. In addition to the widely used two-dimensional (2D) cell culture, there is a continuous trend towards culturing cells in three dimensions (3D), as this better represents the physiological environment of the cells. Thus, the use of 3D cell culture models, or more precisely, the cultivation of cells in hydrogel matrices, can help to better understand essential signaling processes in the development and progression of diseases such as malignant melanoma, and to accelerate and simplify target and drug screenings. However, for this to happen, the 3D models need to be very well defined and characterized. Although alginate is widely used as a hydrogel for 3D cultivation and biofabrication due to its advantageous chemical properties, there is only little information on the transcriptome of cells in 3D alginate cultures.

Experimental Methods
In our study, we performed RNA sequencing of melanoma cells cultured on classical 2D culture dishes or in 3D alginate hydrogels. We could demonstrate and analyse changes in the transcriptome induced by this culture switch.

Results and Discussion
Controlled modulation of the tumor cell microenvironment showed an effect on cell cycle, RAS signaling, GTPase signaling, cell spreading, actin cytoskeleton formation, endocytosis, and phospholipid metabolism, among others. This highlights the strong influence of the extracellular matrix on tumor cells. Further, bioinformatic comparison of gene-expression, including previously published datasets, highlight the correlation of the switch from 2D to 3D alginate culture with tumor plasticity in progression and metastasis. Furthermore, by deeper analysis of changes in the transcriptome we found the transcriptional regulator EGR1 to play a role in melanoma plasticity and aggressiveness.

Conclusion
In summary, our study shows that cell cultivation in 3D hydrogels, combined with RNA-Seq analyses, leads to a deeper molecular understanding of cancer cells and contributes to the definition of new therapeutic targets.
Role of silicate ions in bone regeneration: A comparison between 2D and 3D bone formation models

Tim Schaefer, Azadeh Rezaei, Nefeli Vaporidou, Joel Turner, Gavin Jell

University College London (UCL), Division of Surgery and Interventional Science, London, GB

Introduction
The repair of delayed and non-union bone fractures remains a clinical challenge, particularly in elderly and diabetic patients who have a significantly increased risk of fracture and impaired regenerative capability [1]. Silicon (Si) ion releasing biomaterials have been extensively studied for orthopaedic and dental applications [2] and Si has been reported to stimulate angiogenesis, enhance osteoblast proliferation, and promote bone-related gene expression such as ALP [3]. The role of Si in promoting bone regeneration is, however, still unclear and the understanding of the in vitro effect of Si on bone nodule formation is limited. In this study, we use in vitro 2D and 3D bone nodule models, together with multidisciplinary characterisation approaches to further our understanding of the role of Si ions on bone formation.

Experimental Methods
In vitro bone nodule formation was performed as previously described [4]. Briefly, primary osteoblastic cells were isolated from calvaria of 3-day-old Sprague Dawley rats. Osteoblasts were either seeded in 24 well plates (2D) or compressed 3D hydrogels in 24 well plates in MEM-α supplemented with 2 mM β-glycerophosphate, 10 nM dexamethasone and 50 mg/ml ascorbate. Hydrogels were prepared through mixing collagen (type I), 10X MEM, and neutralising agent (HEPES+NaOH) and compressed using the RAFT™ protocol [5]. Cells in both 2D and 3D were treated with Si at concentrations of 0.5, 1, 2 and 3 mM for 21 days. Bone nodules were characterised using Raman, TEM, Interferometry, Nano-CT, VEGF ELISA, Alizarin Red staining, and Total DNA Assays.

Results and Discussion
Si ions dependent decrease in cell number was observed after 24 hours in culture, but not after 7 days (Figure 1a). An Si concentration dependent expression of VEGF (per unit of DNA) was observed (Figure 1b). The results also demonstrated a difference in Si response between 2D and 3D models (Figure 1c). Si (0.5, 1 and 2 mM) appeared to accelerate bone mineralisation, with early evidence of mineralisation observed after 7 days and with no mineralisation evident at 0 mM and 3 mM Si. The 3 mM Si treated osteoblasts had a non-uniform, distressed appearance. It was also observed that bone nodule formation occurred earlier in the 3D cell cultures compared to the 2D cell cultures in all conditions, but particularly in those containing 0.5 and 1 mM of Si (Figure 2).

The results revealed that high resolution characterisation and imaging are valuable in determining the effect of Si on the biochemical (Raman) and ultra-structure (TEM) of the bone nodules.

Conclusion
Furthering our understanding of the role of Si in bone formation, will enable the creation of improved Si-based biomaterials, with optimised release profiles for specific patient groups (e.g., those with delayed healing). The use of 3D in vitro models may offer greater similarity to in vivo responses and thereby increase the translation of bone therapies.
Figure 1
Figure 1a: DNA content determined using DNA Quantification Kit (Sigma-Aldrich). After an initial reduction in cell number, Si containing media can restore cell number to initial levels after 7 days. Figure 1b: VEGF expression normalised to the Total DNA content, shows a concentration dependent increase of VEGF expression in response to Si containing media. Figure 1c: A comparison between VEGF expression in 2D (tissue culture plastic) and 3D (RAFT™) cell cultures. Particularly at day 7 there is a significantly increased VEGF expression in 3D cell cultures containing 2 mM Si media.

Figure 2
Figure 2: Primary rat osteoblasts cultured for 7 days in media containing Si at concentrations of 0, 0.5, 1 and 2 mM in a 2D environment (tissue culture plastic) or a 3D environment (RAFT™ system with rat tail collagen). The images show earlier bone nodule formation in 3D cell cultures compared to 2D cell cultures. (Images taken using EVOS™ XL Core Thermo Fischer Scientific; scale bars = 200µm)

References
2:30 p.m. – 4:00 p.m.

Poster floor

**PS1-15 | Surface-Modified Biomaterials**
Laser texturing as a programming tool to elicit defined biological responses in Additive Manufacturing implants

Victor M. Villapun Puzas¹, Kenny Man¹, Luke Carter¹, Pavel Penchev², Sophie Cox¹

¹ University of Birmingham, Chemical Engineering, Birmingham, GB; ² University of Birmingham, Department of Mechanical Engineering, Birmingham, GB

Introduction
Aseptic loosening or bonding failure between implantable devices and bone in the absence of infection remains a significant complication, affecting up to 20% of implants [1]. Such failure leads to revision surgeries with partial or total removal of the implant and increased risk of failure than primary arthroplasty devices [2,3]. The increased costs and recovery times pose a heavy burden on both patient and healthcare system, critically calling for the development of novel implants with enhanced surface-bone interaction. In this regard, surface topology is acutely regarded as a main driver in cell-surface interactions, indicating that careful selection of metallic implant topographies can be used to enhance attachment and differentiation of eukaryotic cells. Consequently, laser ablation offers a versatile approach to texture implant surfaces with minimal effect on the accuracy of the final geometry.

Experimental Methods
A series of surface topologies based on geometrical and nature based textures were applied to enhance the biological response of Ti64 additive manufactured implants. Samples were prepared with textures in the microscale, nanoscale and mixed patterns alongside natural textures and a polished control. The physico chemical properties of all surfaces were analysed through non contact profilometry, SEM imaging and contact angle measurements. The early and late biological response was studied through protein adsorption, proliferation (Protein adsorption, Live/Dead and DNA quantification) and mineralization (Alizarin Red, Alkaline phosphatase and SEM) assays of MG63 cells up to 21 days.

Results and Discussion
Physicochemical analysis showcased the ability of the technique to carve the desired pattern effectively modifying roughness and contact angle. These variations resulted in a modification in the preferential orientation of the deposited MG63 cells after 7d of inoculation. More interestingly, a significant increase in calcium deposition for the grids, dual, triangles and shark skin patterns were shown with a heavy correlation with roughness and wettability.

Conclusion
Thus, this preliminary study demonstrated the ability of laser texturing to enhance the biological response of implantable devices, opening the door to its use in additive manufacturing technologies to tackle aseptic loosening.

Acknowledgement
This research was part of the Process Design to Prevent Prosthetic Infections (PREVENTION) project and supported by Renishaw PLC. The EPSRC (Grant code EP/P02341X/1) is acknowledged for financial support.

References
PS1-15-449

Dip- and spin- multilayered coatings onto different substrates based on catechol-functionalized polysaccharides envisaging orthopaedic applications

Ana C. Almeida¹,², Ana C. Vale¹,², Ricardo A. Pires¹,², Rui L. Reis¹,², Natália M. Alves¹,²

¹ 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho, Guimarães, PT; ² ICVS/3B's, PT Goverment Associate Laboratory, Braga/Guimarães, PT

Introduction
For effective bone healing, orthopaedic prosthesis and their components should present not only adequate mechanical properties but also important biological functions such as bioadhesiveness, bioactivity, and biocompatibility. Current trends for improving osseointegration for orthopedic implants comprise their surface modification with calcium phosphate-like, bioactive glasses, growth factors, or RGD peptides, however, these approaches still have significant limitations and drawbacks.

Experimental Methods
Addressing these important features, new biocompatible layer-by-layer coatings were constructed onto different substrates, specifically glass, stainless steel, and titanium envisaging their orthopaedic application. New adhesive and bioactive coatings based on chitosan, hyaluronic acid, and bioactive glass nanoparticles were produced by two LbL methods: spin- and dip-coating. In addition, chitosan and hyaluronic acid were catechol-functionalized in order to provide adhesive properties to the constructed coatings. Hence, polymeric (CTR) and multifunctional (MF) coatings were produced and fully characterized, with particular focus on their surface properties, adhesive strength, and cytotoxicity.

The build-up of LbL coatings with CTR and MF configurations was monitored in situ by QCM-D. Their morphology was analyzed by SEM and AFM, and their wettability was evaluated by WCA measurements. Their bioactivity was assessed by SEM. FTIR imaging was used to analyse the layer interdiffusion onto the constructed coatings. Adhesive tests were performed with LbL coatings constructed onto glass plates to test their adhesive strength. Finally, preliminary in vitro cellular assays were performed to evaluate their cytotoxicity.

Results and Discussion
Major differences were found in their structure and surface properties, in particular, the spin-coated constructions onto glass were smoother, with a more homogeneous structure and lower interdiffusion of polyelectrolytes layers, in comparison with the dip-coated ones. As expected, CTR coatings exhibited a significantly higher adhesion strength, and only MF coatings were able to induce the formation of apatite-like structures on their surfaces. In addition, for both configurations, cellular assays exhibited good L929 proliferation and viability after 7 days of culture.

Conclusion
The produced coatings could be used in a variety of applications as biocompatible interfaces between the implant and host tissues. Particularly, MF films could be potentially used as adhesive coatings for orthopaedic implants to promote osteogenesis and hydroxyapatite deposition around the implant. On the other hand, the highly adhesive polymeric coatings could be used to improve the junction between distinct orthopaedic implants and a variety of tissues in a simple and versatile way.

Acknowledgement
Portuguese Foundation for Science and Technology (FCT) and European program FEDER/FEEI through projects PTDC/BTM-MAT/28123/2017 and PTDC/NAN-MAT/31036/201. FCT exploratory project MIT-EXPL/BIO/0089/2017.
REMIX Project, funded by the European Union's Horizon 2020 Research and Innovation programme under the Maria Skłodowska-Curie Grant agreement n. 778078. European Commission Horizon 2020 programme under the following grant agreements: 692333-CHEM2NATURE and 668983-FORECAST.
Vitamin E as an anti-adhesive coating for cells and bacteria for temporary bone implants

Francesca Gamna¹, Andrea Cochis², Alessandro Scalia², Sara Ferraris¹, Lia Rimondini², Silvia Spriano¹

¹ Politecnico di Torino, Torino, IT; ² Università del Piemonte Orientale, Novara, IT; ³ Politecnico di Torino, Torino, IT

Introduction
An anti-adhesive coating might be important for any application involving temporary devices that requires poor cell adhesion to prevent tissue integration (e.g. catheters, and voice implants); in this case, the focus is on temporary orthopaedic implants, which could create difficulties once the implant is being removed from the bone. Vitamin E (or more specifically, alpha tocopherol) is a potent lipophilic antioxidant and has been very successful in hip and knee arthroplasty in conferring oxidation resistance to irradiated UHMWPE. In recent years, vitamin E has been widely discussed in the literature and has become known for its potent antioxidant, -inflammatory, -cancer, and -bacterial properties.

The novelty of the present work is to realize a coating on chemically treated titanium alloy, suitable for temporary bone implants and which is anti-adhesive to both cells and bacteria, biocompatible, with anti-inflammatory properties.

Experimental Methods
Titanium Ti6Al4V alloy samples (ASTM B348, Gr5, Titanium Consulting and Trading, 10 mm diameter discs) were ground (up to 400 grit), then washed in acetone and deionized water. The discs were chemically treated to increase nanoscale roughness, to expose OH groups, and to make the surface more suitable for coating adhesion. To reduce carbon contamination and activate the OH groups, the treated samples were irradiated with UV light.

The coating was obtained by soaking the samples for 3 h at 37°C in a solution of vitamin E in ethanol (50 mg/ml), with subsequent rinsing first in ethanol then in water. Therefore, a procedure for a proper characterization of this coating was investigated. Physical and chemical characterization was performed through specific measurement techniques such as FTIR-ATR, Z-potential titration curves, reflectance spectroscopy, contact angle measurements, and tape test. Biological characterization was performed through cellular and antibacterial assays: cell adhesion was evaluated using human mesenchymal stem cells (hMSCs), whereas bacterial adhesion was performed using both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) biofilm-producing bacteria.

Results and Discussion
The results from FTIR-ATR and reflectance spectroscopy, z-Potential titration curves, contact angle measurements, and tape test reveal a continuous coating, with hydrophobic behaviour and good mechanical adhesion to the substrate. Cellular and antibacterial tests show anti-adhesive and anti-inflammatory properties.

Conclusion
This work highlights a novel promising application of this biomolecule as a coating for temporary bone implants which reduces the risk of excessive pro-inflammatory response, has antiadhesive properties to prevent unintended osseointegration of temporary devices, and reduces the risk of implant-associated infections.
A universal nanogel-based coating approach for medical implants

Damla Keskin¹, Devlina Ghosh¹, Colin Rosman¹, Clio Siebenmorgen¹, Guangyue Zu¹, Reinier Bron¹, Abigail M. Forson¹, Theo van Kooten¹, Jelmer Sjollema¹, Max Witjes², Patrick van Rijn¹

¹University of Groningen, University Medical Center Groningen, Department of Biomedical engineering, Groningen, NL; ²University Medical Center Groningen, Department of Oral and Maxillofacial Surgery, Groningen, NL

Introduction
The use of implants has increased the quality of life, for patients. However, secondary complications like infection, inflammation, or immune response in the host body can lead to implant failure. Coatings have been regarded as an excellent possibility to induce desired responses or to prevent complications, as the bulk implant material does not need to be altered. However, to apply a coating, a diverse range of chemical approaches have to be implemented owing to the distinct physicochemical and structural properties of the different classes of implant materials. The aim of the study is to develop a universal nanogel (nGel) coating approach that can be applied to most implant materials, ranging from bioglass, polymers, metals, rubbers, and furthermore, investigate the stability of the coating in vitro and in vivo conditions.

Experimental Methods
N-Isopropylacrylamide–co-N-(3-Aminopropyl)methacrylamide hydrochloride p(NIPAM-co-APMA) core-shell nGel particles were synthesized by free-radical precipitation polymerization reaction.¹ The particles were characterized by Dynamic Light Scattering (DLS) and zeta potential, while the coating was visualized by atomic force microscopy (AFM). The coating was labeled with Fluorescein Isothiocyanate (FITC) for successful detection and imaging by In vivo Imaging System (IVIS). The stability of the coating was determined in vitro by exposing nGel coated teflon surfaces to phosphate-buffered saline (PBS) and fetal bovine serum (FBS), while mimicking physiological conditions in a shaker incubator for 21 days and in vivo by implanting methacryloxyethyl thiocarbonyl Rhodamine B (MRB) labeled nGel coated (polyvinylidene fluoride) PVDF hernia mesh in a mouse model for 13 days.

Results and Discussion
The DLS measurements showed the average hydrodynamic diameter was 540.63 ± 11.2 nm, polydispersity index (PDI) was 0.09 ± 0.08, which indicated a monodisperse colloidal suspension with no particle-aggregations² and positive zeta potential, 15.83 ± 0.11 mV at 24°C was attributed to the presence of V50 along with the protonated primary amine groups,³ introduced by APMA. On plasma oxidation, the surface of the material acquired negative charge. The positively charged nGel particles were deposited onto the surface by spray coating technique and the particles were bound to the activated surface via electrostatic interactions, while the unbound particles were washed away to obtain a homogenous uniform coating.⁴ AFM images showed a closely packed nGel-layer on all the distinct surfaces, irrespective of their varied structural and chemical properties (Figure 1). As a proof of concept, FITC was conjugated to the peripheral NH₂ groups of the coating⁵ and IVIS was used for easy traceability of the nGel coated surfaces. In vitro data analyzed by AFM and IVIS showed that the nGel coatings on teflon were stable after 21 days (data not shown), while the in vivo data demonstrated that the coatings on PVDF mesh were stable enough up to 13 days, after which the fluorescent signal was decreasing (Figure 2a). The short term in vivo test is considered as an initial proof for the stability of the nGel coating, however long term evaluations are still necessary.

Conclusion
The approach successfully formed a homogenous uniform coating on most implant surfaces, belonging to different classes of materials. On fluorescent labeling, the coating was easily traceable and imaged by IVIS. The coating on the implant surface showed high stability in both in vitro and in vivo conditions.

Acknowledgement
The project was funded by the Graduate School of Medical Sciences (GSMS) of University Medical Centre of Groningen, the Netherlands.

Figure 1. Schematic illustration of the universal nGel coating approach on different implant surfaces.

Figure 2. a) Quantitative radiant efficiency values of nGel coated PVDF meshes implanted in mice at different time points. The graph shows a correlation expressed as radiant efficiency (p/sec)/(μW/cm²) of IVIS imaged surfaces. b) Representative fluorescence images of coated mesh (fluorescent color-in the middle) and uncoated meshes (grey) taken by IVIS before the implantation in the mice.

References
PS1-15-455

Physical-chemical and biological characterization of a novel Cu and Zn co-doped Plasma Electrolytic Oxidation coating produced on Ti in pulsed conditions

Matteo Pavarini¹, Monica Moscatelli¹, Gabriele Candiani¹, Paolo Tarsini¹, Stefania Cometa², Andrea Cochis³⁴, Ziba Najmi³⁴, Vincenzo Rocchetti³, Elvira De Giglio⁵, Lia Rimondini³⁴, Luigi De Nardo¹, Roberto Chiesa¹

¹ Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering, Milano, IT; ² Jaber Innovation srl, Roma, IT; ³ Università del Piemonte Orientale, Department of Health Sciences, Novara, IT; ⁴ Center for Translational Research on Autoimmune and Allergic Diseases—CAAD, Novara, IT; ⁵ Università di Bari, Department of Chemistry, Bari, IT

Introduction

The Plasma Electrolytic Oxidation (PEO) process is a relatively new, easily scalable and environmentally friendly surface modification technique, based on the same principles of conventional anodizing, which can be exploited for the production of thick microporous coatings, typically showing a high wear and corrosion resistance, on light metals such as titanium, aluminum, magnesium and their alloys [1]. This electrochemical treatment has recently raised particular interest in the biomedical field both for its ability to produce porous biocompatible surfaces and for the possibility to easily embed additive elements, such as antibacterial or osteoinductive elements (like copper and zinc), either as ions or included particles [2].

This work is focused on the physical-chemical and biological characterization of a newly developed copper and zinc co-doped PEO coating produced on titanium to evaluate its potential applicability as a surface treatment for bone-contact devices.

Experimental Methods

Commercially pure grade 2 titanium discs were treated in an alkaline electrolyte composed of 0.1M Na₂B₄O₇, 0.1M NaOH, 0.02M Na₂SiO₃, 0.01M Zn(CH₃COO)₂ and 2mM Cu(CH₃COO)₂. The samples were treated in pulsed conditions, with a 300V final voltage and 50% duty cycle at 100Hz, and characterized both chemically through GDOES and XPS and in terms of wettability by static contact angle. Then, the coatings’ antibacterial activity was assessed by means of metabolic activity and CFU count against S. aureus, S. epidermidis and E. coli after 24h, while their cytocompatibility was tested against MG63 cells for up to 7 days.

Results and Discussion

The produced coating is characterized by a homogeneous and porous surface, with an average pore size of 950 nm and a 9% porosity. The GDOES spectrum of the sample (Fig. 1) shows how all the elements introduced through the electrolytic bath, and in particular silicon, copper and zinc, are mainly present in the outermost layers of the oxide, with an atomic percentage of 4.9%, 1.4% and 0.12%, respectively (as assessed via XPS), while boron is concentrated at the interface between the coating and the substrate, indicating that its embedding happens mostly at the beginning of the sparking process. This early internalization can be linked to the high concentration and steric hindrance of borate ions.

Moreover, the treated samples present an almost superhydrophilic behavior, with a contact angle dropping to around 3° from 89° of untreated Ti, which can be related to both the coatings’ surface porosity and surface charge.

As for the antibacterial properties related to the PEO coating, the results of the direct microbiological studies are summarized in Fig. 2. The coated specimens show a clear bacteriostatic effect even in absence of copper and zinc, feature related to the tuned surface morphology and its superhydrophilicity, which are known to limit bacterial growth.
adhesion and proliferation [3]. However, the metabolic activity and viable colonies reduction given by the doping elements is significant only against the Gram-negative *E. coli*, while on the Gram-positive strains the effect of these low surface concentrations is only marginal, with a slightly higher effect of surfaces only doped with copper, and will require further tuning. Conversely, all the coated surfaces showed a good cytocompatibility, without significant differences in terms of metabolic activity among the treated groups and against the untreated controls.

**Conclusion**

The tuned PEO coatings characterized in this work show promising physical-chemical and antibacterial properties for potential orthopedic applications, without showing in vitro evidence of potential harmful effects on bone cells. However, a deeper tuning of the antibacterial dopants’ concentrations could allow maximizing the antimicrobial features of the coatings while retaining their cytocompatibility.

**Acknowledgement**

Authors would like to thank FESR 2014-2020 project ARS01_01205 “CustOm-made aNTibacterical/bioActive/bioCoated prostheses” for the economic support. A.C. and V.R. were financially supported by the “Ricerca Locale” funding provided by University of Piemonte Orientale (SPACE project).
Antibacterial activity against *S. aureus*, *S. epidermidis* and *E. coli* of the treated samples (PEOCuZn) compared to the untreated control (cp-Ti), undoped (PEO) and single-doped (PEOZn and PEOCu) coatings. * = p < 0.05 vs cp-Ti; § = p < 0.05 vs PEO.

References
Differentiation of local inflammatory tissue response following implantation of platelet-derived human growth factor preparations in a rat animal model

Michael Schlosser¹, Andreas Hoene¹, Uwe Walschus¹, Christopher Pohl¹, Olga Hahn², Kirsten Peters², Karin Schuster³, Hans-Georg Neumann³, Cornelia Prinz³

¹ University Medical Center Greifswald, Department of General Surgery, Visceral, Thoracic and Vascular Surgery, Greifswald, DE; ² University Medical Center Rostock, Department of Cell Biology, Rostock, DE; ³ DOT GmbH, Rostock, DE

Introduction
Platelet concentrates from blood donations are used to prevent or treat bleeding in people with either a low platelet count or platelet dysfunction as well as in people receiving cancer chemotherapy. However, they have a limited shelf-life of only five days. Platelet-rich plasma is also potentially valuable in the field of regenerative medicine as platelets contain high amounts of various growth factors. Therefore, expired platelet concentrates could be an important resource to produce cell-free human growth factor (hGF) preparations, potentially useful to coat implants aimed at better implant in-growth or as treatment for chronic wounds. However, an aspect of prime importance for the biocompatibility of biologically derived biomaterials is their potential immunogenicity. Recently, we demonstrated that hGF preparations, differing in the content of particular growth factors, showed a specific IgG antibody response after single application in mice. Thus, this study aimed at examining the time course of local inflammatory tissue response following by simultaneous implantation of different platelet-derived human growth factor preparation containing collagen matrices in a rat animal model.

Experimental Methods
Biodegradable implants (diameter 4 mm, thickness 0.9 mm) were prepared by freeze-drying of 1% porcine collagen solution only (control implants; MBP GmbH, Neustadt-Glewe, Germany) and in combination with three different preparations containing platelet-derived hGF (#190104-1, #190104-2, #190104-3), followed by EtO-sterilization. Twenty-one male Lewis rats (age 100 days) received one implant of each hGF preparation and one control implant via simultaneous implantation into the neck musculature at distance of approximately 2 cm. After 7, 14 and 56 days, implants with the surrounding tissue were retrieved from seven randomly selected animals per respective experimental day. Morphometric analysis was performed on cryostate sections (5 µm) stained with Hematoxyline and Eosine (H&E) staining and primary antibodies against monocytes/macrophages (CD68+, ED1), tissue macrophages (CD163+, ED2), MHC-class II+ cells, T lymphocytes, nestin (rat-401) and the APAAP technique. For morphometric analysis of the sections, pictures were taken with a Olymp 20 camera (Olympus K.K., Tokyo, Japan) and analysed by the ImageJ software. Data were analysed by Mann-Whitney-Test or one-way ANOVA using the GraphPad Prism software.

Results and Discussion
In general, morphometric immunohistological analysis demonstrates a significant increase of all inflammatory cells investigated from the experimental day 7 until day 56 (p=0.012 – p<0.0001), but most pronounced for pro-inflammatory monocytes/macrophages (ED1), MHC-class II+ cells and for T lymphocytes. This was not seen between experimental days 7 and 14. However, no significant differences between the number of inflammatory cells in their peri-implant tissue of the different hGF preparations among one another, as well as in comparison to the control implants was obtained. For the hGF preparations #190104-2 and #190104-3, containing the highest content of TGF-β and/or IGF-1, a tendency of increased number of MHC-class II+ cells and T lymphocytes was observed for
experimental days 7 and 14, possibly indicating a developing B lymphocyte dependent induction of specific antibodies against hGF preparations and/or the porcine collagen matrix.

**Conclusion**

The morphometric immunohistological analysis demonstrates a significant increase of all analyzed inflammatory cell populations in the peri-implant tissue from experimental day 7 to day 56, which, however, did not differ between different hGF preparations investigated and controls on individual experimental days. Thus, the time-dependent increase of local inflammatory reaction might rather be induced by the porcine collagen matrix than by the different hGF preparations. Additional investigations and further studies could clarify possible implications of different amounts of TGF-ß and/or IGF-1 in preparations of hGF, which might influence both, the local inflammatory and the systemic immunological response and are therefore important for future clinical applications.

**Acknowledgement**

The study was supported by the German state Mecklenburg-Vorpommern (TBI-V-1-141-VBW-050) with contributions from the European Regional Development Fund (ERDF) and the European Social Fund (ESF).
Time course of MHC-II+ Cells
Percentage of MHC-class II+ cells in the peri-implant tissue of different human Growth Factor (hGF) coated collagen sponges (GF1-3) or a uncoated control implant (Con) on experimental days 7, 14, and 56. A significant increase was observed for all implants from day 7 to day 56 (Kruskal-Wallis Test (ANOVA); p<0.0001), however, no significant difference was seen between the different implants on respective experimental day. Boxes represent median and interquartile range of seven rats per experimental day.
Laser surface modification enhances the antibacterial performance of Ti-6Al-4V without compromising biocompatibility

James Quinn\(^1\), Ryan McFadden\(^2\), Chi Wai Chan\(^2\), Louise Carson\(^1\)

\(^1\) Queen's University Belfast, School of Pharmacy, Belfast, GB; \(^2\) Queen's University Belfast, School of Mechanical & Aerospace Engineering, Belfast, GB

Introduction
As human life expectancy continues to increase, most countries now have an aging population, who unfortunately are more susceptible to chronic musculoskeletal disorders such as osteoarthritis [1]. As osteoarthritis and associated-musculoskeletal disorders are the predominant cause of joint arthroplasty, there has been a consequent, growing requirement for such procedures.

Unfortunately, despite the profound improvements in healthcare throughout the past century, two major problems are still prevalent within arthroplasty, namely aseptic loosening and periprosthetic joint infection [2]. Laser surface modification of titanium alloys offers a promising frontier for combatting the aforementioned problems, with the laser surface treatment of one of the most common alloys, Ti-6Al-4V being discussed herein.

Experimental Methods
Ti-6Al-4V discs were prepared using a fibre laser machine with a near-infrared wavelength of 1064 nm in a nitrogen-containing environment, manufactured by SPI Lasers UK Ltd (Southampton, UK). Three different laser settings of low, medium and high wattage were used with a baseline power setting of 40W, which were tested in relation to the untreated Ti-6Al-4V (base metal).

For microbiological analysis, samples were inoculated with 3mL of either Staphylococcus aureus (ATCC 6538) or Escherichia coli (ATCC 25922) at 37\(^\circ\)C on a 100 rpm gyrotary incubator for 24h to allow biofilm formation. Following which samples were stained with fluorescent Live/Dead® BacLight™ solution for 30 minutes at 37\(^\circ\)C in the dark to capture images via fluorescence microscopy. Image analysis was conducted using ImageJ software to ascertain the ratio of live to dead bacterial cells on each of the surfaces, as well as the total biofilm percentage coverage. Statistical analysis was performed using a one way ANOVA test with Tukey's post hoc test using GraphPad Prism, version 8.3.1.

Biocompatibility was assessed by means of an MTT assay based on ISO 10993-5:2009, which functions to evaluate the in vitro cytotoxicity of materials used in medical devices [3]. Two different cell lines, RAW 264.7 (ATCC TIB-71) macrophages and L929 (NTCC clone 929, ATCC CCL-1) fibroblasts were used throughout this experiment. The culture medium was Dulbecco’s Modified Eagle Medium (DMEM) (Gibco® DMEM) for the RAW 264.7 macrophages and Minimum Essential Media (MEM) (Gibco® MEM) for the L929 fibroblasts, both supplemented with 10% foetal bovine serum (Gibco®) and 1% penicillin/streptomycin (Gibco®). Cells were diluted to a seeding density of 0.05 x 10\(^6\) cells per mL and 1mL was seeded onto 24-well cluster cell culture treated plates (Nunc 6 Well multidish). Following this, samples were aseptically placed upon the cell monolayer and re-incubated for either 24h or 48h.

After exposure, samples were removed and 1mL of MTT (tetrazolium salt 3-[4,5- dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide; Sigma-Aldrich) was added to each well and kept in a dark environment for 1.5-2h at 37\(^\circ\)C. The insoluble formazan crystals formed were dissolved using 1mL of dimethyl sulfoxide (VWR chemicals) per well. The intensity of the colour of the formazan solution formed will be proportional to the metabolic activity of the cell. The absorbance at 570nm was then measured using a UV–visible spectrophotometer (PowerWave™ XS Microplate Reader).

Results and Discussion
It is evident in figure 1 for both species investigated that there was a significant reduction in total biofilm coverage (%) for all three wattages investigated. *Staphylococcus aureus* coverage was reduced from ~70% to <30% for all wattages utilised. Similarly, with *Escherichia coli*, there was over a threefold reduction, with coverage falling from ~15% to <5% for all wattages investigated. These results suggest that laser surface treatment has a significant effect on reducing the adherence of both Gram-positive and Gram-negative bacterial upon TiG5.

Regarding live:dead ratio, there again was a significant decrease in the ratio of live/dead bacteria for all three wattages tested. For *Staphylococcus aureus*, the ratio fell from ~4 in the base metal to <1 for all for the laser treated surfaces, similarly for *Escherichia coli*, the ratio fell from ~6 to <1 for all the wattages.

As illustrated in figure 2, laser surface treatment does not compromise cell viability for either of the two cell lines investigated, at any wattage used to modify Ti-6Al-4V, in fact, a modest improvement in cytocompatibility was found in RAW macrophages exposed to Ti-6Al-4V that had undergone laser surface treatment.

**Conclusion**

Laser surface modification of one of the most commonly used alloys in arthroplasty, Ti-6Al-4V was found to improve it's antibacterial capabilities, whilst maintaining bicompatibility, thereby addressing the two most pressing concerns within arthroplasty. It is the authors belief that further work should be conducted to provide additional insight into how this technology can be employed for clinical use.
Figure 2
A comparison of the effect of laser surface modification of Ti65 on a) L929 fibroblast and b) RAW macrophage viability (%) measured via MTT cytotoxicity assay. Asterisks indicate significant differences in relation to the vehicle control.

References
Plasma surface modification for controlled cell behavior

Jana Markhoff, Stefan T. Oschatz, Michael Teske, Niels Grabow

Rostock University Medical Center, Institute for Biomedical Engineering, Rostock, DE

Introduction
Since most biomaterials cannot fully or sufficiently mimic the structure and function of the tissue to be supported or replaced, modification of these is necessary to control and direct the biological response in dependence of the target area of application. This is crucial in a way as different tissue surroundings may result in different cell responses. As for that, cardiovascular implants often tend to develop tissue fibrosis due to early adhesion and proliferation of fibroblasts and therefore delayed endothelialization of the biomaterial surface by endothelial cells which may result in implant failure. Another example is dermal tissue, whereas fibroblasts are critical in all phases of skin wound healing, but may lead to implant encapsulation and scar tissue formation, inhibiting keratinocyte function. In addition to the mechanical structuring of the material surface or the binding of growth factors, optimization of the implant surface is possible by means of chemical groups or coatings. The aim of our work was therefore to develop a plasma-based surface modification for implants which, depending on the application and target tissue, specifically controls the local cells in order to inhibit or promote their proliferation and function.

Experimental Methods
For this purpose, different plasma polymer coatings from the monomers pyrrole (pPy), allylamine (pAA) and hexamethyldisiloxane (pHMDSO) without and with the addition of different amounts of oxygen and ammonia (5 sccm, 10 sccm) to the plasma reaction chamber, respectively, were applied to conventional polystyrene cell culture plates to generate ultrathin polymer films via plasma-enhanced chemical vapor deposition (PECVD). The yielded plasma polymer coatings were characterized using ATR-FTIR spectroscopy and contact angle measurements were performed to investigate wettability of the polymer layers. Fibroblasts (HT-1080), as well as endothelial cells (E.A. hy 926) and keratinocytes (HaCaT), were used for initial cell tests. Cells were cultivated in defined cell numbers for 48 h on the plasma-modified surfaces. In addition to determining cell viability by Cell Quanti Blue assay and live-dead staining, cell morphology was assessed by phalloidin staining and scanning electron microscopy.

Results and Discussion
A surface- and cell type-dependent expression of cell viability was found, which could be confirmed by fluorescence microscopy images after live-dead staining.

Amounts of 5 sccm oxygen and ammonia, respectively, especially on pPy and pHMDSO, led to reduced fibroblast cell vitality compared to the pure plasma coating. This was also confirmed by live-dead staining showing a greatly reduced cell density and a distinctly altered cell morphology. In contrast, high proportions of ammonia (10 sccm) in pPy and pAA in particular had a stimulating influence on fibroblasts. These tendencies were not quite as pronounced in the endothelial cells. Nearly all plasma coatings showed similar levels of cell activity compared to the pure plasma coatings. Only pHMDSO with 5 sccm oxygen and 10 sccm ammonia lead to greatly reduced levels in comparison, respectively. The results of the Cell Quanti Blue assay were supported by live-dead staining.

HaCaT keratinocytes showed increased cell viability on oxygen and ammonia modified pAA surfaces, whereas pure and oxygen treated pPy as well as pHMDSO mixed with oxygen and ammonia resulted in strikingly decreased
viability. Live-Dead staining of these groups revealed no dense cell layers and further mainly rounded single cells instead of flat and typical cell clusters.

Except in the above-mentioned groups, staining of the actin skeleton, as well as scanning electron microscopy evaluation, showed no clear influence of the different plasma coatings on the cell morphology of fibroblasts, endothelial cells and keratinocytes. Nevertheless, especially endothelial cells did not cover the entire surface in a plasma-dependent manner.

Further investigations, e.g. evaluation of the synthesis of inflammation markers will be done for a more detailed characterization of the several plasma coatings.

Conclusion
Plasma-enhanced chemical vapor deposition is a versatile tool for the modification of implant surfaces while maintaining inherent material properties. The process allows the coating of even complex structures and ultra-thin polymer films with thicknesses in the submicron range. Furthermore, due to the plasma activation of the monomers in gaseous state, unique polymers are accessible which cannot be synthesized by classic polymer chemistry.

Acknowledgement
The authors would like to thank Ms. Gabriele Karsten and Ms. Martina Nerger for their technical support.
Lipid-Nanocoated Hydrophobic Particles As Aspiring Stealthy Bioprotective Carriers

Francisca L. Fernandes Gomes¹, Dorothee Wasserberg², Jasper van Weerd², Pascal Jonkheijm³, Jeroen Leijten¹

¹ University of Twente, Department of Developmental Bioengineering, Faculty of Science and Technology, Technical Medical Centre, Enschede, NL; ² LipoCoat BV, Enschede, NL; ³ University of Twente, Department of Molecules and Materials, Laboratory of Biointerface Chemistry, Faculty of Science and Technology, MESA+ Institute for Nanotechnology, Enschede, NL

Introduction
Biologically sensitive compounds with outstanding therapeutic potential usually require extensive formulation adjustments prior to pre-clinical stages. An estimated >40% of drugs on the market are poorly water-soluble, leading to lower absorption rates and thus reduced bioavailability.¹ Prodrug synthesis or biomolecule conjugation can improve pharmacokinetic behavior, but biological or chemical modifications are not always advantageous. Physical modifications are, therefore, preferred in certain cases, with coating and encapsulation being a popular solution. Bioprotective coatings or carriers are usually made of materials or composites with bifunctional properties that can accommodate sensitive compounds while exhibiting desired biological responses. Hydrophobic cargo, for instance, is frequently incorporated in amphiphilic formulations or composites made of hydrophobic cores and hydrophilic shells. Equally important are the physical properties of the carrier, whose size and surface area should be tuned according to the desired administration route, release kinetics, degradability rates, and other relevant aspects. Polycaprolactone (PCL) is an inexpensive hydrophobic polyester frequently used in biomaterials science due to its physical versatility and biodegradability. With a relatively low melting point (60°C), it has been used in the production of tissue scaffolds via electrospinning or 3D printing², and even micro- and nanoparticles for compound delivery, such as antibacterial product Nanochlorex®³. Despite this, the hydrophobic surface of PCL is prone to protein adsorption, which can trigger severe inflammation, platelet activation, tissue fibrosis, and thrombus formation.⁴ A promising approach to improving the biological response to PCL would be, therefore, to endow PCL with a bioinert surface coating.

In this work, we hypothesize that a cell membrane-mimetic nanocoating will improve the biological response to PCL microparticles by offering anti-fouling and anti-thrombogenic properties. Polymer particles of sizes below 1 µm (0.4-0.8 µm) were produced and nanocoated with a proprietary lipid formulation.⁵ First biological response tests focused on the hemocompatibility of coated vs. uncoated materials to identify possible differences in hemolysis and coagulation induction. Following work will shed light on anti-fouling properties and cytocompatibility by means of protein adsorption and cell viability studies, respectively. To the best of our knowledge, this work constitutes the first hemocompatibility studies of pristine PCL microparticles coated with a synthetic lipid formulation.

Experimental Methods
PCL microparticles of different molecular weights were produced via a double emulsion with solvent evaporation technique using probe sonication for water-in-oil-in-water emulsification. Particularly, particles made of PCL with molecular weight 10,000 (Mₓ10k) and stabilized in a 0.3% Mowiol® 40-88 solution were coated with one of the fluorescent proprietary lipid formulations of LipoCoat.⁵ Coated and uncoated materials were characterized in terms of size, surface charge, and morphology using dynamic light scattering, zeta potential, and optical and electron microscopy. Hemocompatibility testing included hemolysis and coagulation studies wherein particles were incubated at 37°C with isolated human red blood cells and platelet-poor plasma, respectively.

Page 1465 of 2028
Results and Discussion
PCL particles of different molecular weights (MW: $M_n$ 80k, 45k, 10k) exhibited different size distributions, with lower MWs resulting in lower hydrodynamic size distributions. Specifically, particles produced with PCL $M_n$ 10k showed the lowest hydrodynamic particle size range (0.4-0.8 µm) (Fig.1), and thus were selected for further coating experiments. Using LipoCoat’s fluorescent X2TR lipid formulation, the coating efficiency on the microparticles was confirmed using fluorescence microscopy (Fig.2). Zeta potential measurements of coated particles showed rather neutral, buffer-sensitive values, both characteristic of the zwitterionic lipid shell. Hemocompatibility tests show equally low hemolysis on both conditions (Fig.2) and a potential trend in delay in coagulation in coated vs. uncoated particles.

Conclusion
Hydrophobic PCL microparticles were produced and successfully nanocoated with a synthetic cell-membrane mimic. First hemocompatibility testing demonstrated low hemolysis and a potentially delayed coagulation by the nanocoated particles, providing the first results on the biological response to the tested composites. Future studies will elaborate on this response profile and clarify the suitability of these materials as bioprotective carriers in therapeutics.

Acknowledgement
F.L. Fernandes Gomes acknowledges the kind donation of blood from the TechMed Donor Service of the University of Twente and all its collaborators and donors. Authors acknowledge financial support from Health~Holland (Project LSHM19074).
Fig. 2

a-c) Confocal laser scanning microscopy images of X2TR-coated PCL particle clusters. a) pseudo-green fluorescence; b) transmission; c) overlay.
d) Hemolysis levels based on the optical density of released hemoglobin from red blood cells incubated with 200, 100, and 50 µg/mL of uncoated (U) and coated (X2TR) PCL particles.
e) Pseudo-green fluorescence image of the coated particles overlaid with a pseudo-, red-colored transmission image of an erythrocyte culture. Pseudo-colors were used for better visual contrast between particles and cells, as the original X2TR formulation fluoresces in red.

References

Effect of plasma treatment on the surface properties of polylactic acid

Veronica Luque-Agudo¹²³, Margarita Hierro-Oliva¹²³, Amparo M. Gallardo-Moreno¹²³, M. Luisa Gonzalez-Martin¹²³

¹ Extremadura University, Badajoz, ES; ² CIBER-BBN, Badajoz, ES; ³ INUBE, Badajoz, ES

Introduction
Polylactic acid (PLA), one of the most widely used biodegradable polymers in the manufacture of medical devices, is a slightly hydrophobic material. As some applications require a hydrophilic surface, there are several methods to achieve this. One of these is plasma treatment, which can generate new functional groups on the surface, increase roughness or simply remove impurities [1]. The aim of this work is to study the surface properties of PLA after plasma treatments.

Experimental Methods
PLA particles (PURASORB®, PDL 04, Corbion) were dissolved in chloroform using a rotator stirrer. Then, the solution was deposited on substrates provided by Ticare (Mozo-Grau S. A., Valladolid, Spain) and they were left to dry at room temperature for one hour. Later, samples were dried in an oven for 48 hours at 70 °C to completely remove any solvent remaining. The hydrophilization of the surface was done by applying low-temperature plasma from different gases. Hydrophobicity was quantified through water contact angle measurements (WCA) by the sessile drop method. The topography, roughness (RMS) and morphology were evaluated by using an AFM and a SEM. FTIR spectra were recorded on a VERTEX 70 spectrophotometer (Bruker®). Secondary ion mass spectrometry was performed using a ToF-SIMS (ION-TOF®) instrument.

Results and Discussion
The contact angle of the PLA before treatment is 83°, while the angle measured immediately after the different plasmas are applied was 59° and 51°, so both treatments hydrophilize the surface. As it has been shown that the plasma initiates a series of radical reactions in the polymer chains of PLA [2], possible changes in the chemical composition of the surface were evaluated. Thus, by means of FTIR it was possible to detect, in one of the treatments, and in a very short time after its application, the presence of CO and CO₂, thus confirming that these reactions have taken place. The bands assigned to these compounds disappeared when the spectra were recorded the next day, probably because the radicals generated recombine to regenerate the polymer chain. The changes in composition were confirmed by the depth profiles performed by ToF-SIMS. In addition to changes in the chemical composition, the plasma also modified the roughness of PLA. Thus, the RMS was determined by AFM before and after the treatment, and it was observed that the RMS increased by an order of magnitude. The AFM and SEM analysis showed that the polymer coating was perfectly homogeneous.

Conclusion
The application of both treatments on PLA hydrophilizes the surface, either by removing any impurities it may have, or because of changes in the chemical composition. This has been proven to be the case by means of FTIR and ToF-SIMS. It also increases the roughness of the samples after exposure to the plasma.

Acknowledgement
This work was supported by Junta de Extremadura and FEDER (grant number GR18153), project RTI2018-096862-B-I00, supported by FEDER (Fondo Europeo de Desarrollo Regional “Una Manera de hacer Europa”), Ministerio de Ciencia e Innovación del Gobierno de España, Agencia Española de Investigación, respectively. ToF-SIMS was performed by the ICTS “NANBIOSIS”, more specifically by the Surface Characterization and Calorimetry Unit of the
CIBER in Bioengineering, Biomaterials & Nanomedicine (CIBER-BBN) and the SACSS-SAIUEX of the University of Extremadura (UEx). The authors also thank Mozo-Grau S. A. for their sponsorship.

References
2:00 p.m. – 3:30 p.m.

Poster floor

PS2 | Poster Sessions 2
2:00 p.m. – 3:30 p.m.

Poster floor

**PS2-01 | Biomaterials for Biofabrication**
Evaluation of a medical-grade thermoplastic polyurethane in the conception of a 3D-printed custom-made aortic stentgraft

Marie-Stella M'Bengué1,2, Thomas Mesnard1,3, Mickaël Maton1, Valérie Gaucher2, Nicolas Tabary2, Maria-José García-Fernandez1, Jonathan Sobocinski1,3, Feng Hildebrand1, Bernard Martel2, Nicolas Blanchemain1

1 Univ. Lille, INSERM, CHU Lille, U1008 - Controlled Drug Delivery Systems and Biomaterials, F-59000 Lille, FR; 2 Univ. Lille, CNRS, INRAE, Centrale Lille, UMR 8207 - UMET - Unité Matériaux et Transformations, F-59000 Lille, FR; 3 Institut Coeur Poumon, Regional Hospital Center University of Lille (CHU Lille), 59000 Lille, FR

Introduction
The use of fenestrated stentgrafts in endovascular repair (FEVAR) of abdominal aortic aneurysms (AAA) shows limitations in manufacturing time (8-12 weeks), cost (15-20 k€), and patient customization. Providing a custom-made (CM) medical device made by 3D-printing (3DP) would solve these issues. Thermoplastic polyurethane (TPU) seems to appear as an excellent raw material. Synthesized from a macrodiol (soft segment, SS) and a diisocyanate (hard segment, HS), this polymer is already used as a biomaterial for blood contacting devices [1]. For it to be implantable in the long term, TPU must be biocompatible, sterilizable and maintain its integrity over time. In this context, the aim of this work is to assess the feasibility of using TPU as a raw material in the conception of a 3DP CM aortic stentgraft. The properties of this polymer will be evaluated through the whole manufacturing process from extrusion of raw pellets to sterilization of 3DP samples. Moreover, its susceptibility to degradation by reactive oxygen species (ROS) in a pro-oxidative medium will be investigated. Finally, biocompatibility will also be assessed through cell viability and hemocompatibility assays according to ISO standards.

Experimental Methods
Raw medical-grade TPU pellets were first extruded into a neat filament with a regular diameter of 1.75 mm. Said TPU filament was then used as a raw material for the manufacture of samples using FDM 3D-printing. 3DP samples underwent a sterilization process using gamma (γ) irradiation. Impact of sterilization at a dose of 40 kGy (γ-40 kGy) and stability of TPU have been investigated on 3DP samples. The control group (0 kGy) and the sterilized group (γ-40 kGy) were accelerated ageing test, exposed to a 25% H₂O₂ solution under reflux for 6 hours to provoke oxidation [2], which is known to occur as part of the inflammatory response system of cells to foreign bodies [3]. Surfaces of samples were observed under scanning electron microscope and surface properties were investigated by infrared ATR-FTIR spectroscopy and water contact angle measurements following the Sawsen Sessile Drop method. Molecular weight was determined by gel permeation chromatography coupled to a differential refractometer. Thermal properties were assessed by differential scanning calorimetry and mechanical behaviors were investigated through tensile tests. The response of HPMEC endothelial cells to the extract of the 3DP TPU samples was analyzed via the Alamarblue® test method according to ISO10993-5 standard. Blood compatibility was assayed according to ISO10993-4 standard using whole human blood from a healthy donor. Surface of samples after whole blood contact were observed under scanning electron microscope.

Results and Discussion
Tubular TPU samples (height = 25mm; diameter = 20 mm) were manufactured using FDM 3D printing. Gamma irradiation and H₂O₂ exposure induced macroscopic color changes as 3DP samples yellowed. After γ-sterilization, molecular weight increased as well as HS glass transition temperature that went from 53°C to 59°C. TPU samples were more resistant as stress and elongation at break values augmented. Those changes suggest that reticulation occurred after γ-sterilization leading to the 3DP samples being tougher. After H₂O₂ exposure, peak emergence in...
ATR-FTIR spectra show that oxidation degradation occurred in TPU evidenced by primary amines peaks observed at 1636 cm\(^{-1}\) [4]. Surface oxidation also induces decrease in water contact angle measurements: values that were greater than 90° before are close to 78° after \(\text{H}_2\text{O}_2\) exposure. Concerning cytocompatibility, cell viability of 151% for 0 kGy and of 144% for \(\gamma\)-40 kGy shows non-cytotoxicity of the samples and vitality of cells. Even after exposure to \(\text{H}_2\text{O}_2\), cell viability remains very high: it is of 101% for the 0 kGy-25%\(\text{H}_2\text{O}_2\) sample and 128% for the \(\gamma\)-40 kGy-25% \(\text{H}_2\text{O}_2\) sample. Hemocompatibility rates lower than 2% confirm that 3DP TPU samples do not induce critical red blood cells lysis before and after sterilization and \(\text{H}_2\text{O}_2\) exposure. SEM observation after whole blood contact shows inhibition of platelet activation after \(\gamma\)-sterilization contrary to the 0 kGy-25%\(\text{H}_2\text{O}_2\) and \(\gamma\)-40 kGy-25% \(\text{H}_2\text{O}_2\) samples where adhered pseudopodia and flat activated platelets are visible on the surface of the samples.

**Conclusion**

Our study shows the feasibility of using TPU as a raw material in the conception of a 3DP CM aortic stentgraft. The manufacturing process would seem to have low impact on the properties of TPU regarding our application as it is sterilizable and biocompatible. After \(\text{H}_2\text{O}_2\) exposure, it was shown that TPU is susceptible to oxidation degradation by ROS, however the extent of its impact on the TPU properties does not compromise the application. Further studies should investigate long term mechanical stability and toxicity analysis of degradation products and extractables.

**Acknowledgement**

This project was funded by the sustained program FEDERATE, University of Lille (I-SITE scholarship call for project “Expand”). The authors would like to thank Société de Chirurgie Vasculaire et Endovasculaire de Langue Française (SCVE).

**References**


Aligned 3D fibre matrices for the development of chondrocyte-laden anisotropic scaffolds

Angela Semitela¹, Ana Capitão², Susana C. Pinto¹, Alexandrina F. Mendes², Paula A.A.P. Marques¹, António Completo¹

¹ University of Aveiro, Centre for Mechanical Technology and Automation, Aveiro, PT; ² University of Coimbra, Centre for Neuroscience and Cell Biology, Coimbra, PT

Introduction

Current cartilage tissue engineering (TE) strategies have struggled to reproduce the collagen fibrous arrangement, that confers outstanding properties of deformability, resistance to mechanical loading and low-friction gliding to the native articular cartilage [1]. Indeed, tissue-engineered cartilage lacking this native layered structure usually possess inferior biochemical and biomechanical properties, that can compromise a proper regeneration process. In this regard, electrospinning has been widely employed for the development of fibrous scaffolds for cartilage TE, due not only to the topographic resemblance with the native nanoscale extracellular matrix, but also the versatility of the technique to manipulate the properties of the resulting structures [2]. Yet, given their nearly bidimensionality, it remains a challenge to fabricate three-dimensional (3D) electrospun scaffolds. Considerable efforts have been made in an attempt to achieve the tridimensionality of the electrospun structures, with substantial progress made through the use of post-processing strategies, such as rolling and stacking the electrospun meshes [3]. Despite their reported potential, the issue of design reproducibility is still a concern, since these strategies are not automated. Additionally, chondrocyte infiltration within the 3D structures continues to be ineffective. In this regard, in this work, automated 3D electrospinning was performed using a patented electromechanically 3D electrospinning platform [4] in order to fabricate reproducible aligned 3D fibre matrices laden with chondrocytes.

Experimental Methods

All experiments were performed in a recently developed electromechanically 3D electrospinning platform [4], where the fibres were collected and placed in a collector according to programmed alignments. All equipment and material used in these experiments were sterile or thoroughly disinfected. A 10 wt% polymeric blend of polycaprolactone (PCL) and Gelatin (GEL) in 2,2,2-trifluoroethanol mixed in a proportion of 6:4 was employed to produce the 3D scaffolds. Chondrocyte-free scaffolds were fabricated and characterized based on their topography and morphology (via scanning electron microscopy (SEM) and micro-computed tomography (μCT)), on their water absorption ability (swelling assays) and mechanical response (unconfined compression). Afterwards, an immortalized human chondrocyte cell line C28/I2 was incorporated within the fibres of the 3D scaffolds using a micropipette between the layers of polymer. Chondrocyte metabolic activity, via a resazurin reduction assay, and their distribution within the fibres, via SEM and haematoxylin-eosin staining, was later assessed after 1 day of culture.

Results and Discussion

3D scaffolds were successfully fabricated in the electromechanically 3D electrospinning platform, following three different programmed alignments (3D_1, 3D_2 and 3D_3; Figure). μCT 3D reconstruction and SEM images revealed that the resulting fibre alignment remained similar to the programmed input, demonstrating the potential of this platform to fabricate highly reproducible tissue-engineered constructs. Moreover, the versatility of this platform allowed for the development of quite different fibrous architectures. 3D_1 displayed high porosity and pore interconnectivity, while 3D_2 possessed high fibre packing density and low porosity. 3D_3 was a combination of the previous two scaffold architectures. The different fibre arrangements and porosities of the 3D scaffolds also generated distinctive swelling and mechanical behaviour. Indeed, the high porosity of the 3D_1 generated the highest...
swelling ratio, while the low porosity of the 3D_2 triggered the lowest water absorption. Nevertheless, all scaffold architectures exhibited a nearly total deformation recovery after compression up to 20% strain, which can be attributed to the presence of the hydrophilic GEL [2]. The higher amount of fibre deposition of the 3D_2 resulted in the highest compression moduli. 3D_2, on the other hand, displayed a substantially lower compression moduli, but still fell in the range of values reported for native articular cartilage [5]. The in vitro studies revealed that the high fibre density of 3D_2 and 3D_3 possessed an improved retaining capacity of the chondrocytes that were able to survive the layering process. Moreover, these architectures were able to maintain a homogeneous chondrocyte distribution.

Conclusion

3D chondrocyte-laden anisotropic electrospun scaffolds were successfully developed in a versatile electromechanically 3D electrospinning platform with a controlled and reproducible fibre alignment and homogeneous chondrocyte distribution, showing the potential of this technology for cartilage TE.

Acknowledgement

This work was supported by the Portuguese funding of Program COMPETE-FEDER, Programa Operacional Competitividade e Internacionalização through the projects POCI-01-0145-FEDER-028424 and CENTRO-01-0145-FEDER-022083. Also, by Fundação para a Ciência e Tecnologia I.P. (FCT, IP) through the projects PTDC/EME-SIS/28424/2017, UIDB/00481/2020 and UIDP/00481/2020. The authors thank to FCT for the PhD grant SFRH/BD/133129/2017.

References


PLA-Bioglass Composites for Hybrid Bioprinting of Bone Tissue

Eva Schätzlein¹, Shahed Al Zoghool¹, Nicolas Söhling⁴, Dirk Henrich⁴, Johannes Frank⁴, Edgar Dörsam³, Ingo Marzi⁴, Andreas Blaeser¹, ²

¹ Technical University of Darmstadt, Institute for BioMedical Printing Technology, Darmstadt, DE; ² Technical University of Darmstadt, Centre for Synthetic Biology, Darmstadt, DE; ³ Technical University of Darmstadt, Institute of Printing Science and Technology, Darmstadt, DE; ⁴ Goethe University Frankfurt am Main, Department of Trauma, Hand and Reconstructive Surgery, Frankfurt am Main, DE

Introduction

Hybrid bioprinting is a promising technology for biofabrication of tissue implants to be used in regenerative medicine. Two material classes, soft cell-laden hydrogels and rigid polymers are printed in parallel, creating mechanically stable but highly biofunctional composite structures. These composites combine ideal characteristics for surgical handling, high suture retention, and high primary load-bearing capacity with controllable physical as well as bio-chemical cues for cell conditioning. So far, hybrid bioprinting was mostly realized by combining microextrusion methods. In this work, we present a novel approach for combining fused-filament-fabrication and drop-on-demand 3D-bioprinting technology for rapid hybrid bioprinting of cancellous bone mimicking structures. For this purpose, polymer scaffolds made of a custom-made biodegradable PLA-bioglass composite are combined with human mesenchymal stem cells (MSC) which have significant osteogenic regenerative capabilities. We hypothesize, that the incorporation of bioglass can be used to stimulate bone growth due to its bioactive nature.

Experimental Methods

In this study, composite PLA filaments and ultrafine, cancellous bone mimicking 3D-structures with bioglass contents of up to 20 wt. % were processed, fabricated, and characterized. S53P4 bioglass with a grain size of 25 - 42 µm and corresponding weight proportions of transparent PLA granules with a grain size of about 2 - 5 mm were extruded into filaments using a commercial composer (3devo, NEXT 1.0 Advanced). The distribution of bioglass within the filament as well as within 3D-printed samples were investigated using SEM. The mechanical properties of filaments with varying bioglass content were evaluated by uniaxial tensile tests. To assess the composite's cytocompatibility, calcium release and cell viability as well as proliferation studies were conducted. Calcium release of PLA-bioglass composites in PBS was determined photometrically. Viability and proliferation potential of MSCs seeded on printed polymer composite structures supplemented with 5 wt. %, 10 wt. % or 20 wt. % bioglass were determined using an MMT assay.

Finally, macroporous 3D-structures with very fine directed strands were created based on the FFF 3D-printing process. Strand diameter and pore size were determined microscopically. Ultimately, the feasibility of the process for hybrid 3D-bioprinting was demonstrated.

Results and Discussion

Analysis of the produced PLA-bioglass filaments revealed a strong influence of the material composition and extruder settings on its diameter, morphology and mechanical properties. The PLA-bioglass filament obtained from extrusion varied in diameter in relation to the bioglass content, temperature and the extrusion speed. Filaments from 1.65 to 1.85 mm diameter were used. Further morphological investigations using SEM revealed homogeneous distribution of bioglass across both, extruded filaments and 3D-printed samples. Interestingly, the bioglass particles were mostly observed within the bulk material and were found to be only rarely exposed to the outside of the structures.

The elastic modulus of the composite material was shown to increase slightly with rising bioglass content from 44 MPa (0 wt. %) to 47 MPa (20 wt. %), while its tensile strength increased accordingly.
The release study revealed a non-linear relationship between the amount of dissolved calcium and the bioglass concentration. For instance, only minimal calcium release could be detected using composite filaments with 5 wt. % bioglass, while filaments with 20 wt. % bioglass content released a 10-times higher amount of calcium within 24 h.

A porous structure which has similar geometric properties to cancellous bone was printed. Pore sizes between 500 µm and 20 µm and strand sizes between 500 µm and 120 µm could be achieved with a 400 µm nozzle. During printing higher bioglass contents were shown to be more difficult to be process. Compared to native PLA those exhibited lower print bed adhesion and required larger nozzle diameters to avoid clogging. In addition, it was found that the hygroscopic characteristic of the incorporated bioglass affected shape fidelity. However, this effect could be overcome by prior drying the filament at 50 °C for 24 h.

Finally, the material’s suitability for hybrid 3D-bioprinting could be demonstrated successfully. With increasing bioglass content, the cell viability and proliferation potential were in average up to twice as high on 20 wt. % bioglass compared to native PLA samples.

**Conclusion**
In this study, a PLA-bioglass composite was characterized as well as a novel hybrid bioprinting process combining FFF-based 3D-printing and drop-on-demand bioprinting was developed. In future, the proposed composite material combined with the presented printing technology hold great promise for the biofabrication of hybrid scaffolds for the treatment of large bone defects in regenerative medicine.

**Acknowledgement**
The project was partially funded by the “RMU-Initiativfonds”. We thank the working group Macromolecular Chemistry and Paper Chemistry at the TU Darmstadt as well as the “Merck Lab @ TU Darmstadt” for providing access and support to the SEM.
Engineering an extracellular matrix substitute for the investigation of mesoangioblast extravasation in a 3D in vitro vessel-on-a-chip model

Mattis Wachendörfer¹, Philipp Schräder¹, Florence van Tienen², Bert Smeets², Horst Fischer¹

¹ RWTH Aachen University, Department of Dental Materials and Biomaterials Research, Aachen, DE; ² Maastricht University, Department of Toxicogenomics, Research School MHeNS, Faculty of Health, Medicine and Life Sciences

Introduction
Muscular dystrophies are a group of genetic diseases characterized by progressive skeletal muscle weakness and degeneration. The intra-arterial injection of autologous in vitro cultured, genetically corrected muscle stem cells, called mesoangioblasts, i.e. mesenchymal cells located on the vessel walls, holds potential for an effective stem cell therapy. The mesoangioblasts extravasate from the vessel through the vessel walls into the surrounding muscles, where they form healthy muscle fibers and fuse with existing fibers, thereby supplying the missing proteins and restoring muscle strength and mass. The Interreg-V EMR-project GYM – Generate Your Muscle aims to develop this autologous stem cell therapy as a novel treatment for muscular dystrophies. In our subproject we aim to develop a 3D in vitro vessel-on-a-chip model for the investigation and improvement of mesoangioblasts homing. Biofabricated functional vessel substitutes are used for the investigation of extravasation properties of injected mesoangioblasts under dynamic conditions with higher biomimicry than conventional migration assays under static conditions. The vessel substitutes are embedded in extracellular matrix substitutes (ECMs) composed of hydrogel mixtures. Here, we present the engineering approach for an ECM tailored for the investigation of mesoangioblasts migration.

Experimental Methods
Gelatin-Fibrin-blends with different concentrations of gelatin (2.5, 5, 7.5, 10 %) and varying gelatin synthesis parameters (2h at 90 °C, 12h at 90 °C) and collagen-fibrin-blends (0.16% Collagen; 0.625, 1.25, 1.95% Fibrin) were synthetized. The compressive strength of the hydrogel blends was tested in unconfined compression tests. In a degradation test, the hydrogel blends were immersed in phosphate buffered saline (PBS) and the weight of the samples and the pH of the PBS was measured after 1, 3, 4, 7, 14, 21 and 28 days of incubation. The pore structure and pore size of the ECMS were investigated using scanning electron microscopy. The contact angle of widely used bioinks for 3D Bioprinting (fibrinogen, thrombin, gelatin) to the ECMS was measured. Human Umbilical Vein Endothelial Cells (HUVECs) were seeded onto the ECMS and the proliferation of the HUVECs was investigated every second day up to 1 week. The suitability of the ECMS for mesoangioblasts extravasation was investigated in a modified Boyden chamber setup: The membranes of the upper compartment of the Boyden chamber were covered with a confluent monolayer of HUVECs which was activated with Tumor Necrosis Factor (TNF-α) for 6h. Meanwhile, mesoangioblasts were starved for 24h. The lower compartment of the Boyden chamber was filled with the ECMS (with or without growth factors (TNF-α, stromal cell-derived factor 1 (SDF-1)) and starved mesoangioblasts were seeded in the upper chamber. After 24h or 72h of migration, migrated mesoangioblasts on the lower side on the membrane were fixed and counted.

Results and Discussion
The tested ECMS provided similar compressive strength to native tissue (6-17 kPa) and showed stability during incubation for 28 days, proving their suitability for long-term experiments. The pore sizes of Gelatin-Fibrin-blends were between 0.1-1 µm dependent on gelatin concentration and synthesis, whereas Collagen-Fibrin-blends provided pore sizes from 1-10 µm with decreasing pore size in higher concentrated ECMS. Most ECMS showed suitability for Drop-on-Demand (DoD) Bioprinting as tested bioinks showed hydrophobic contact angles (experiments ongoing),
meaning bioinks could be printed with high accuracy onto the ECMs. The experiments of HUVEC proliferation and mesoangioblasts migration showed promising results in preliminary testing with experiments ongoing.

**Conclusion**

In the end, a tailored ECM which provides high biomimicry to native tissue in terms of compressive strength, stability during long-term incubation and suitability for DoD Bioprinting while supporting fast HUVEC proliferation and mesoangioblasts migration stimulated by the release of growth factors will be shown.
Functionalized 3D-printed silk-hydroxyapatite scaffolds for enhanced bone regeneration with innervation and vascularization

Vincent Fitzpatrick, Zaira Martin Moldes, Anna Deck, Ruben Torres Sanchez, Chunmei Li, David Kaplan

Tufts University, BME department - Kaplan Lab, Medford, US

Introduction
The goal of this study was to generate functionalized 3D-printed scaffolds for bone regeneration using silk-hydroxyapatite bioinks and osteoinductive, proangiogenic and neurotrophic growth factors or morphogens for accelerated bone formation.

3D printing was utilized to generate macroporous scaffolds with controlled geometries and architectures that promote osseointegration. We build on the knowledge that the osteoinductive factor Bone Morphogenetic Protein-2 (BMP2) can also positively impact vascularization, Vascular Endothelial Growth Factor (VEGF) can impact osteoblastic differentiation, and that Neural Growth Factor (NGF)-mediated signaling can influence bone regeneration.

Experimental Methods
We developed and characterized a silk-based bone cement with mechanical properties close to those of bone. We assessed our ability to 3D print this material to generate structures with a controlled geometry, macroporosity, and our ability to adapt the printing process to patient-tailored scaffolds (Fig 1).

We investigated the cytocompatibility and osteoconductivity of these constructs (Fig. 2). We further assessed functions on the 3D printed construct via the osteogenic differentiation of human mesenchymal stem cells. We also examined the migration and proliferation of human umbilical vein endothelial cells, and the proliferation of human induced neural stem cells.

Results and Discussion
The scaffolds provided mechanical properties suitable for bone and the materials were cytocompatible, osteoconductive and maintained the activity of the morphogens and cytokines.

Synergistic outcomes between BMP-2, VEGF and NGF in terms of osteoblastic differentiation in vitro were identified, based on the upregulation of genes associated with osteoblastic differentiation (Runt-related transcription factor-2, Osteopontin, Bone Sialoprotein).

In vivo studies were carried out to assess the biocompatibility and bone regeneration capability of these 3D printed constructs.

Conclusion
The specific cell responses observed here are key steps towards the development of the next generation of functionalized, 3D printed bone scaffolding to promote multicellular responses in the context of bone tissue system regeneration. These results are expected to have a strong impact in bone regeneration in dental, oral and maxillofacial surgery.
Fig. 1 Characterization of the silk-hydroxyapatite material
(a) MicroCT of a 3D printed cube. Scale bar: 1mm
(b) 3D-printed anatomical structures. Scale bar: 1cm
(c) SEM of a 3D-printed construct (left); of the surface of the filament (center); of the hydroxyapatite (HAP) powder used for the silk-HAP bioink (right). (d) Compressive strength (MPa) of silk/HAP bioinks after drying the constructs. The effect of concentration, immersion in PBS, and sintering. (n=5) (e) FTIR spectra of the HAP powder, silk/HAP immediately after printing (freeze-dried), and after drying.

Fig. 2: Osteoconductivity of the 3D printed structures
(a) Activity of the enzyme alkaline phosphatase (ALP) in hMSCs after 21 days of culture in growth or osteogenic medium, normalized to the protein content (top). Staining for ALP in hMSCs after 21 days of culture in growth (left) or osteogenic (right) medium (bottom, scale bar: 5 mm). (b) Immunofluorescent staining of osteoblastic differentiation marker osteopontin (OPN, in magenta), showing the increase in OPN expression in osteogenic medium. For reference, the nuclei (blue, DAPI) and actin cytoskeleton (white, Phalloidin-488) are shown in the top panels. Scale bar: 100 μm.

References
Biofabrication using shape-morphing materials

Indra Apsite, Esther Addo, Franco Raviglione, Leonid Ionov

University of Bayreuth, Department of Biofabrication, Bayreuth, DE

Introduction
Recent studies have shown the great potential of polymeric fiber-based self-folding films for various tissue regeneration like – skeletal muscle tissue, nerve, and blood vessels \(^1\)-\(^3\). Extra high porosity not only allows fast actuation rates due to high surface area but as well supports good nutrition and waste product exchange. The use of self-folding bilayers allows controlled deposition and alignment of the cells inside of tubular structures, which is a challenge for existing nerve guide conduits and autografts \(^4\)-\(^5\).

Results and Discussion
We have designed two systems based on electrospun mono and bilayers for skeletal muscle and nerve regeneration. As skeletal muscle tissue structure is highly complex and consists of multiple fibers in one muscle bundle, thus we designed polyelectrolyte bilayers with opposite charges that would lead to the self-assembly of tubular structures into a bundle. Electrospun bilayers consisted of a hydrophobic polycaprolactone (PCL) layer and hydrophilic polyelectrolyte layers like hyaluronic acid (HA, negatively charged) and chitosan (positive charged). The addition of a medium with pH ~6 allows immediate self-folding and assembly of the tubular structures. Cell culture experiments showed good C2C12 mouse skeletal muscle cell viability and proliferation on these scaffolds.

To improve nerve regeneration we designed conductive PCL monolayers that are able to contract and form tubular structure upon temperature increase. To prepare conductive electrospun fibers, we used polypyrrole and carbon nanotube blends. Higher conductivity was achieved for carbon nanotube mats, due to the high aspect ratio of carbon nanotubes in comparison to polypyrrole particles. Cell culture experiments demonstrated that PC12 neuron cells differentiate and form a more uniform monolayer on the conductive material rather than on the nonconductive

Conclusion
Both systems have shown great potential to be used as the next step for the biofabrication of skeletal muscle and nerve tissue.

Acknowledgement
This work was supported by DFG IO 68/11-1; IO 68/14-1.

References


Optimized parameters to print high concentrated silk fibroin by an innovative 3D freeform method

Enric Casanova-Batlle1, Carla Santiago-Corral1, Antonio J. Guerra2, Joaquim Ciurana1

1 University of Girona, Department of Mechanical Engineering and Civil Construction, Girona, ES; 2 EURECAT, Centre Tecnològic de Catalunya, Girona, ES

Introduction
Silk fibroin has been successfully used for medical devices due to its biodegradability with non-toxic end products, high tensile strength and mechanical robustness, but also because of its high flexibility potential [1]. Even though natural silk fibers have excellent strength and flexibility, regenerated silk materials generally become brittle in the dry state [2]. That is why researchers have studied the effects of its fabrication parameters on its mechanical properties to increase its flexibility, but also others parameters such as gelation time, mechanical properties or biodegradability [3]. Furthermore, post-treatment techniques and fabrications methods have been evaluated to optimise the molecular structure of the silk fibroin to obtain a more ductile material.

Direct Ink Writing (DIW) can be used to manufacture 3D printed silk fibroin applications [4]. However, it requires a silk concentration of 28-30%. Taking into account that most of the research has been carried out with low concentration silk bioinks (5-10%) and with alternative fabrication methods to the DIW process, the aim of this work is to address this lack of knowledge to efficiently 3D print silk fibroin. Therefore, an innovative method based on thermal crosslinking is proposed. The morphology, molecular structure and mechanical properties of the 3D printed silk fibroin, and thus the potential behavior of medical devices printed with this technology have been described. Furthermore, four different post-treatments techniques were evaluated on these features. This work provides the tools to fabricate biomedical applications, such as a cardiovascular stent, as the resulting silk must be flexible and at the same time have high tensile strength.

Experimental Methods
Silk fibroin was prepared at 5-7 wt % and concentrated to 35 -40 wt % as described in the Rockwood et al. protocol [5]. A home-made bioink printer was used to fabricate test-pieces for mechanical trial. The Ultimate tensile strength (UTS), the elastic modulus (E) and the strain at failure ($\varepsilon_{\text{failure}}$) were calculated from stress-strain data for the 4 post-treatment techniques. The effect of the printing parameters, such as the printing speed, the nozzle temperature, the needle gauge and the ink flow were assessed with a DOE model. Fourier transform infrared (FTIR) spectra was obtained using a spectrometer in the spectral region of 4000–350 cm$^{-1}$, to determine the molecular structure of the printed material. The morphological structure was visualized with the stereoscopic microscope. The morphologic characteristics were quantified with image processing tools.

Results and Discussion
The results show trends that interact between the studied parameters and the manufacturing process. The strut width is affected by nozzle speed, temperature and ink flow. As the higher the temperature and deposition speed, the finer the deposited line. Oppositely, the higher the flow, the wider the printed strut. Furthermore, an increase in the $\beta$-sheet molecular structure is observed when the material is printed with a high nozzle temperature. This directly affects the mechanical properties which are also enhanced during high temperature printing trials. In terms of post-treatments, it has been highlighted that methanol increases the elastic modulus. However, the material becomes more brittle. The water annealing treatment has been shown to achieve the most flexible lines, among the tested post-treatments. Nevertheless, the dried at room temperature post-treatment possesses the best compromise between the mechanical properties studied in this report.
Conclusion
This work provides the design parameters to customize silk fibroin applications manufactured through DIW, as it relates the manufacturing parameters to the mechanical, molecular and morphological properties of the resulting printed medical device.

Acknowledgement
The authors acknowledge the financial support from the European Union FEDER funds (BASE 3D: 001-P-001646) and the support of the Catalan Government (2017SGR00385).

References
Electrospun meshes as a drug delivery system to release a phlorotannins-enrich extract obtained from the seaweed Undaria pinnatifida for bioactive wound dressings application

Carolina A.M. Ferreira¹, Rafael Félix², Carina Félix², Adriana P. Januário², Sara C.N. Novais², Nuno Alves¹, Marco F.L. Lemos², Juliana R. Dias¹

¹ Centre for Rapid and Sustainable Product Development (CDRSP), Politécnico de Leiria, Marinha Grande, PT; ² Marine and Environmental Sciences Centre (MARE), ESTM - Politécnico de Leiria, Peniche, PT

Introduction
Nowadays there is an emerging demand for new wound healings therapeutics, especially with antimicrobial activities since the increasing bacteria’s resistance to antibiotics are responsible for wounds infection persistence and consequently, long-term hospital stays [1]. Electrospun fibers have been contributed for the progress in the wound care field due to their exceptional characteristics such as haemostatic capacity, a porous structure that allows the maintenance of a physiologically moist environment with exudate’s absorption, tissue hydration, and permeation of oxygen, that avoid wound desiccation responsible for scar formation, alongside with extracellular matrix (ECM) similarities [2]. Moreover, this technique’s versatility allows the encapsulation of bioactive molecules (natural compounds, growth factors, drugs, among others) which give electrospun fibers a great potential to be used as drug delivery systems [3]. New sources of natural compounds, like brown seaweeds, are been sought due to their outstanding characteristics (e.g good therapeutic properties for several diseases, lower toxicity and side effects, low-price acquisition, others). Phlorotannins, polyphenols compounds from brown seaweeds, are known to possess several activities of interest to wound healing, including, antimicrobial, anti-inflammatory, and antioxidant activities [4]. Although, their instability due to oxidation and bioavailability might be a concern for pharmacological proposes, concretely in wound healing. To overcome those drawbacks phlorotannins can be incorporating in electrospun meshes enduring their stability and extended-release, joined all requirements to create a potential and innovative drug delivery system [5]. Thus, in this study, an electrospun-based drug delivery system was created integrating the non-toxic and biocompatible properties of gelatin and chitosan, to the potentialities activities of a phlorotannins-enrich extract, obtained from an invasive seaweed from Portugal coast (Undaria pinnatifida), to improve wound healing.

Experimental Methods
Previously to its incorporation, the phlorotannins-enrich extract (1.5 mg·mL⁻¹), was evaluated for antimicrobial activity against principal wounds pathogens, such as Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli. The inhibitory activity stands out, with 43.29 ± 2.85 for S. aureus, 44.79 ± 8.12 for P. aeruginosa, 37.62 ± 4.44 and E.coli, 19.49 ± 5.42. The anti-inflammatory activity was also assessed in vitro against LPS-induced inflammation damage to RAW 264.7 cells, by inhibiting 47% of the Nitric Oxide production at a minimal concentration of 0.01 µg·mL⁻¹. A high antioxidant activity was verified, which is an added value to combat the reactive oxygen species (ROS) generated in the inflammatory stage of the wounds. Afterward, 1 and 2 wt% of the bioactive extract was included by emulsion in gelatin/chitosan electrospun meshes after the solution parameters were optimized for: a 14 wt% gelatin solution mixed with 3.6 wt% of chitosan in 70% (v/v) of glacial Acetic Acid with 2% (v/v) of triethylamine, and 4% (v/v) of 1,4 – Butanonediol diglycidil ether (BDDGE), as crosslinker agent. While the processing parameters obtained were a high tension of 20 kV, 0.2 mL·h⁻¹ of flow rate, and 12 cm from tip-to-collector. The electrospun meshes were physico-chemically characterized by SEM and FTIR, and porosity, density, water contact angle, water absorption, hydrolytic and enzymatic degradation, and mechanical properties were also analyzed. In addition, their
antimicrobial activity against *S. aureus* and *P. aeruginosa* was evaluated by disc diffusion. The biological activity, namely cytotoxicity and neonatal human dermal fibroblast (hDNF) proliferation.

**Results and Discussion**

Results showed that incorporating 1% of the bioactive extract shows to be more advantageous due to its overall activities, such higher porosity (89.22 ± 1.15 %), mimics effectively the ECM fibers diameter (302 ± 83 nm), possess higher water uptake (458.03 ± 41.52%), which facilities the oxygen, nutrient passage and allows hDNF cells proliferation. Correspondingly, it was verified that the decrease in degradation rates both in the hydrolytic and enzymatic environment, as well as the structure’s preservation over time, possibly result from an interaction between phlorotannins hydroxyl group, with gelatin and chitosan, allowing the extract to act as a crosslinking agent. Nonetheless, the extract delivery occurs in two-stages (burst and stationary), reaching a maximum release around 90%, guarantee a potential wound’s protection from external and internal factors, such as bacteria or ROS respectively. Electrospun meshes antimicrobial activity was found to *P. aeruginosa*, the main pathogen found in depth wounds.

**Conclusion**

Thus, combining the physio-chemical and biological characterization results, the drug delivery electrospun system presents a potential therapeutic solution for chronic wounds, which is the wound type with more costs to the health systems.

**Acknowledgement**

This work was supported by AMALIA - Algae-to-Market Lab IdeAs (EASME/EMFF/2016/1.2.1.4/03/SI2.750419) co-funded by the European Union, and projects PAMI (Portuguese Additive Manufacturing Initiative - Projet nº22158 – SAICT- AAC nº 01/SAICT/2016) and MATIS (Sustainable Industrial Materials and Technologies - CENTRO-01-0145-FEDER-000014) co-funded by Centro 2020.

**References**


Bioprinting of Biomimetic Nano-Hydroxyapatite Functionalized Bioinks for Bone Tissue Engineering

Margherita Montanari¹, Elisabetta Campodoni¹, Anna Tampieri¹, Kamal Mustafa², Monica Sandri¹, Ahmad Elsebahy²

¹ National Research Council of Italy, Institute of Science and Technology for Ceramics, Faenza, IT; ² University of Bergen, Department of Clinical Dentistry, Faculty of Medicine, Bergen, NO

Introduction
Recently, the conventional concept of bone tissue engineering has shifted toward biomimetic approaches in which materials, biology and technology are integrated to recapitulate the complexity of bone tissues, allowing fabrication of patient-specific and anatomically shaped constructs. Thus, the whole concept of the scaffold in terms of design, architecture and fabrication technology is moving toward 3D bioprinting of hydrogels functionalized with tissue-mimicking components.

Bioinks components choice must be made based on factors related to biocompatibility, printability, crosslinking mechanisms, and degradation. In this context, gelatin modified by methacryloyl functional groups (GelMA) has recently gained increasing attention. The most common photoinitiator used with GelMA is Irgacure® 2959 which dissociates into free radicals upon ultra-violet (UV) light irradiation. However, the formation of free radicals upon longer UV exposure may lead to DNA damage and impair cellular function. Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), instead, absorbs light in the visible region, producing benefits over the commonly used UV photoinitiators.

GelMA as is cannot, however, provide cells with proper cues to form bone-like matrix. Instead, incorporating inorganic calcium-phosphate phases into hydrogels can provide the right stimuli to generate a mineralized matrix. Nonetheless, adding mineral particles to the bioinks may result in increased viscosity, decreased cell viability and printing problems. Therefore, the current study aimed to bioprint human bone marrow-derived mesenchymal stem cells (hBMSC) in GelMA bioink that can be photopolymerized using a LED dental curing lamp (DL), functionalized with two different types of nano-hydroxyapatite (nHA), chemically and morphologically different, to evaluate their effects on the bioprinting process and outcomes.

Experimental Methods
Biomimetic magnesium doped hydroxyapatite (MgHA) and magnesium-carbonate doped hydroxyapatite (MgCO₃·HA) were prepared and characterized utilizing X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), thermo-gravimetric analysis (TGA), induced coupled plasma (ICP) and scanning electron microscopy (SEM-FEG). For cytotoxicity, 1% nHA (w/v) were suspended in cell culture medium at 37 °C under shaking for 24 h. After centrifugation and filtering, the effect of the nHA leachates on the hMBMSC viability and proliferation was evaluated by live/dead stain and CCK8 proliferation kit.

To prepare the bioinks, GelMA (5% w/v) and Gel (5% w/v) were dissolved in cell culture medium and nHA (1% w/v) added and stirred for one hour. The solutions were stored at 4 °C for 24 h. Before printing, the solutions were heated to 37 °C, and LAP and cells (5×10⁶ cell/ml) were added to the solution. The bioinks were then poured into plastic printing cartridges with a 0.51 mm metal needle and the printing was conducted with 3D-Bioblotter® directly onto sterile 6 well-plates on a cooled platform (4 °C). Square structures (1cm²) of four perpendicular layers and 1.2 mm between the strands were printed. Immediately after printing, the structures were crosslinked by DL using 2 cycles (30 s) of high-power mode of exposure (1200 W/cm²) at 0.5 cm distance. To evaluate the printing quality, non-
crosslinked cell-free structures of one and four layers were imaged with a stereomicroscope. The cell viability and proliferation were evaluated by live/dead stain and CCK8 proliferation kits.

Results and Discussion

The nHAs synthesis were optimized to obtain two morphologically and physicochemical different nHAs, respectively more crystalline and needle-like (MgHA) and more amorphous and round-shaped (MgCO₃HA). XRD and FTIR characterization confirmed the HA identity, while ICP and TGA the successful doping. The indirect cytotoxicity of 1% nHA leachates showed high cell viability and cell proliferation after 1 and 7 days, confirming the safety of these nanoparticles. The stereomicroscope images showed the ability of the GelMA-Gel-nHA to form 3D structures with good fidelity and integrity. The stability of the printed structure in cell culture medium after 7 days confirmed the successful photocrosslinking step and the efficacy of LAP as photointiator. The results of the live/dead stain and CCK8 confirmed the viability of the cells and the ability of the cells to proliferate in the bioinks after 7 days.

Conclusion

All in all, the addition of both nHAs to GelMA-Gel system enhanced the printability and stability of the structures. The use of LAP as photointiator with high energy visible light maintained high cell viability and structural stability and thus holds great promises for 3D bioprinting of mesenchymal stem cells for bone tissue engineering applications.

References

Dense extracellular matrix derivatives for the bioprinting of nucleus pulposus-like structures

**Gregor Miklosic** ¹,², Christophe Hélary ³, Stephen J. Ferguson ², Matteo D'Este ¹

¹ AO Research Institute Davos, Regenerative Orthopaedics, Davos, CH; ² ETH Zürich, Institute for Biomechanics, Zürich, CH; ³ Sorbonne Université, CNRS, Laboratoire de Chimie de la Matière Condensée de Paris, Paris, FR

**Introduction**

Intervertebral disc (IVD) degeneration is a major source of pain and disability for patients worldwide, and a significant burden on healthcare providers. Due to the inadequacy of existing treatments, further study of the IVD pathophysiology is required. It is becoming increasingly clear that conventional models, such as animal IVDs and basic in vitro cultures, fail to adequately represent this complex organ. Its heterogeneous composition and response under challenging mechanical conditions cannot be recapitulated in 2D and simple 3D environments, limiting their usefulness in explaining physiological processes. Animal IVDs on the other hand, while available as mature physiological organs, display wide variability, exhibit biological, compositional, and biomechanical interspecies differences, and present a challenge when translating observations towards human benefit.

Bioprinting, with its precise control over the cell microenvironment, offers a promising avenue for the fabrication of better, tissue engineered models. Mimicking the native tissue more closely in structure and function, they could serve as reproducible and representative specimens for further research. Moreover, they could offer unparalleled control over individual parameters, facilitating exploration of disease causality in a manner not possible with mature tissue. Unfortunately, present capacity for producing such an IVD model is limited due to the lack of suitable bioinks. Despite previous ventures, a bioink representative of native tissue in composition, physical properties, and capable of providing the necessary biochemical and mechanical cues has not been reported yet. Taking the first step towards addressing this issue, we focused our efforts on the gel-like nucleus pulposus (NP) in the centre of the IVD and developed bioinks suitable for the fabrication of NP-like structures.

**Experimental Methods**

We developed a dense extracellular matrix-based bioink, composed of a tyramine derivative of hyaluronic acid (THA) combined with unmodified type I collagen. The THA was synthesized via a one-step amidation of the carboxyl groups and combined with collagen at respective concentrations of 30 and 20 mg/ml. Due to the presence of tyramine functional groups, THA possesses double-crosslinking capabilities. An enzymatic crosslinking mechanism, triggered using a combination of hydrogen peroxide ($H_2O_2$, 4 ppm) and horseradish peroxidase (HRP, 0.1 U/ml), allows the preparation of a soft extrudable gel. Following extrusion, the gel can be further strengthened in the presence of the photoinitiator Eosin Y (0.2 mg/ml) with the application of green light. By varying the concentration of $H_2O_2$, the material can be tuned for optimal extrudability. Similarly, the intensity and duration of light exposure can be adjusted for desired final properties.

The bioink was evaluated rheologically. Its gelation behaviour was studied in a continuous oscillatory time sweep as it was crosslinked half an hour using each mechanism. Its strain-dependent response after enzymatic crosslinking was acquired using a sweep from 0.01% to 1000% strain. In an evaluation of the material's ability for elastic recovery, the high-strain conditions inside the printer nozzle and subsequent low-strain conditions after deposition have been simulated on the rheometer by cyclic exposure to intervals of 300% and 0.1% strain. Finally, a frequency sweep from 0.01 Hz to 100 Hz was conducted.

To demonstrate the bioink's printability, simple lattice-based structures were produced using a 27G needle.

**Results and Discussion**

Page 1493 of 2028
Due to its double-crosslinking mechanism, the THA/collagen bioink is a versatile tool for 3D printing. In addition to good initial extrudability, the application of light can increase the storage modulus of the printed gel more than six times, from ~0.7 to 4.6 kPa (Figure 1a). The bioink has demonstrated elastic recovery behaviour in oscillatory rheology, flowing (G'' > G') when subjected to high strains, and recovering its elastic properties when the strain is decreased (G' > G'', Figure 1b). This is corroborated with the decrease in complex viscosity observed when the oscillation frequency is increased (Figure 1c). In an oscillatory strain sweep, the bioink exhibited a transition to a flowing state at approximately 80% strain (Figure 1d).

Conclusion

Our work demonstrates that using modified matrix components, bioinks suitable for the printing of NP-like structures can be developed. The rheological properties achieved fall within the range of values previously reported for healthy human NP5. To our knowledge, this is also the first bioink simultaneously composed of biochemically suitable components representative of the native NP extracellular matrix, and beginning to approach the high concentrations observed in tissue. The presented work brings us a step closer to better, reproducible, and representative 3D printed IVD models, and the promise of new insights into the treatment of disc degeneration.

Acknowledgement

This work was conducted within the scope of the INDEED project, funded by the Swiss National Science Foundation (189310) and French National Research Agency (ANR-19-CE06-0028).

Figure 1

Oscillatory rheology of the bioink. b-d were tested after enzymatic crosslinking. a) Time sweep (f = 10 Hz, γ = 1%, T = 25 °C). Shaded regions correspond to the warm-up of the rheometer from 4 °C to 25 °C, enzymatic crosslinking, and light crosslinking. A lower temperature was employed to compensate for the heating from the light source. b) Cyclic intervals of 0.1% (low) and 300% (high, shaded) strains (f = 10 Hz, T = 37 °C). c) Amplitude sweep from 0.01% to 100% strain (f = 10 Hz, T = 37 °C). d) Frequency sweep from 0.01 Hz to 100 Hz (γ = 1%, T = 37 °C).
Figure 2
A 10 x 10 mm, 3 layers high lattice structure printed with the THA/collagen bioink through a 27G (I.D. = 0.20 mm) needle. Light crosslinking was performed at each individual layer to strengthen the structure.

References
Self-folding of fibrous scaffolds for blood vessel regeneration

Mairon Trujillo Miranda, Indra Apsite, Leonid Ionov

University of Bayreuth, Professorship Biofabrication, Bayreuth, DE

Introduction
Fabrication of 3D printed scaffolds has lately demonstrated promising results for tissue regeneration of muscle, bone, and nerve. However, the main drawback lies in the poor resolution and limited nutrition achieved for small tubular structures such as blood vessels (d=8-20 µm) [1]. Hence, 4D biofabrication has allowed the fabrication of multi-layer scroll-like structures, by making use of 2D shape-morphing materials, that react upon external stimuli and recreate 3D structures capable to mimic anisotropic structures [2, 3].

Experimental Methods
In our previous studies, polycaprolactone (PCL) was used as part of electrospun bilayers that underwent extra fast actuation [4, 5], allowing fabrication of highly porous tubular scaffolds, that improved cell seeding, growth, and alignment. Despite being biocompatible, bilayers slowly degraded due to high crystallinity of PCL. Therefore, we are presenting the fabrication of electrospun bilayers, comprising highly aligned hydrophobic poly hydroxybutyrate (PHB) fibers and random hydrophilic methacrylated hyaluronic acid (HA-MA) fibers.

Results and Discussion
High fiber alignment was obtained using a high-speed rotating drum collector. Bilayers underwent ultra-fast shape transformation upon contact with different aqueous media, allowing fabrication of conduits with diameter among 2-3 mm. Dynamic mechanical analysis (DMA) showed that in comparison to widely-used PCL, PHB fiber softness allowed mechanical property drop of the bilayer to 10MPa, which is significantly closer to the native blood arteries (2-6 MPa). Fibrous scaffolds depicted good proliferation and viability of endothelial cells (HUVECs) after a week in the culture. High fiber alignment (60-80%) enhanced HUVECs cell orientation in form of a monolayer, mimicking the intima tunica found within the native vessels.

Conclusion
New designed fully biodegradable bilayer system is highly promising for narrow blood vessel formation.

Acknowledgement
DFG. Grant Number: IO 68/17-1
References


Carvacrol-loaded 3D printed PLA scaffolds to prevent bone-associated infection

Xián Farto-Vaamonde¹, Andrea Muras², Ana Otero², Angel Concheiro¹, Carmen Alvarez-Lorenzo¹

¹ Universidade Santiago de Compostela (USC), Department of Pharmacology, Pharmacy and Pharmaceutical Technology, Santiago de Compostela, ES; ² Universidade Santiago de Compostela (USC), Department of Microbiology, Center of Biological Investigations (CiBUS), Santiago de Compostela, ES

Introduction
Bone is the second most-transplanted tissue worldwide each year. Autologous grafts are the gold standard, followed by donor grafts. However, their limited number and associated risks, along with an increasingly aging population, requires a search for alternatives (1). 3D printing, particularly Fused Filament Fabrication (FFF), has found many applications in tissue engineering and pharmaceutics (2). Scaffolds produced by FFF offers great advantages compared to conventional ones, such as complexes or personalized designs that match patient’s injury, abundant interconnected pores that allow cell migration and colonization, and high mechanical resistance of the constructs. Moreover, biodegradable polymers can be used as the scaffold materials, thus being reabsorbed by the patient’s own body and avoiding a second surgery to remove the implant. However, implantable materials are prone to contamination with microorganisms, which may result in biofilms that can be hardly managed. Carvacrol is a natural compound found in Oregano spp. with antimicrobial activity against bacteria, fungi and yeast. Along its anti-inflammatory properties, carvacrol is a potential candidate to minimize risk infection and aid the healing process on bone injuries (3). The aim of this work was to prepare poly(lactic acid) (PLA) scaffolds by FFF 3D printing and incorporate carvacrol in them as an antimicrobial agent.

Experimental Methods
PLA scaffolds were made with a 3D printer by FFF. Carvacrol was incorporated into scaffolds following two different approaches: (a) Pre-printing load: where the commercial PLA filament was soaked in carvacrol solution and then fed into the 3D printer. Presumably, the obtained scaffolds would contain carvacrol trapped within the polymer matrix; and (b) Post-printing load: where blank scaffolds are first printed and then soaked in a carvacrol solution. Carvacrol would be mainly located on the scaffold surface.
All scaffolds were characterized in terms of reproducibility, morphology, topography, mechanical properties, drug content, release profiles, crystallinity, anti-bacterial activity (planktonic and biofilm) and anti-inflammatory effects.

Results and Discussion
The scaffolds showed good fidelity with the 3D design. Carvacrol was successfully incorporated into scaffolds following the two mentioned strategies. All scaffolds showed good mechanical properties and compressive modulus values in the same magnitude as those of cancellous bone tissue (4). In a compression assay, carvacrol-loaded scaffolds suffered less plastic deformation compared to blank ones (6% vs 12% height reduction). Pre-printing loaded scaffolds showed a small burst of carvacrol in the first hours followed by a sustained release over more than one month, while post-printing loaded scaffolds showed a burst release of carvacrol in the first 48 h. Scaffolds containing higher doses of carvacrol showed anti-bacterial activity against S. aureus and P. aeruginosa, with greater effect on planktonic population.

Conclusion
Although further research is required, carvacrol is a natural and high available promising candidate to be used as antibacterial and anti-inflammatory agent in tissue engineering and regenerative medicine.

Acknowledgement

Page 1498 of 2028
This work was supported by MINECO [SAF2017-83118-R], Agencia Estatal de Investigación (AEI) Spain, Xunta de Galicia [ED431C 2020/17] and FEDER. Xián Farto-Vaamonde acknowledges Xunta de Galicia for a predoctoral research fellowship (ED481A-2018/073).

References
Development of double-network inks for extrusion-based printing: rheology and printability

Krutika Singh¹,3, Jacek K. Wychowaniec¹, Brian J. Rodriguez²,3, Dermot F. Brougham¹

¹ University College Dublin, School of Chemistry, Dublin, IE; ² University College Dublin, School of Physics, Dublin, IE; ³ University College Dublin, Conway Institute of Biomolecular and Biomedical Research, Dublin, IE

Introduction
Development of inks for extrusion-based 3D printing has been a major challenge in the field of tissue engineering¹. Understanding the relationship between physicochemical properties and composition of shear-thinning hydrogels is key for the design of well-defined large-scale and complex printed scaffolds. One solution to achieve better control over final printed structures is the use of double-network hydrogels. Each hydrogel building block contributes a specific function leading to overall materials with enhanced structures and functions. In this work, we explored the development of double-network hydrogel printable inks consisting of shear-thinning Pluronic F127 and selection of chemically-modified polyethylene glycol-based molecules for printing structures with enhanced shape on deposition, withstanding significant stress during the printing process. The outcomes from this study will contribute to the goal of developing high fidelity hydrogels with a multi-functional and stimuli-sensitive magnetic delivery system with precise control via external stimulus.

Experimental Methods
A range of ink formulations were prepared (as shown in figure 1) using Pluronic F127³ and poly(ethylene glycol) with various chemically modified end-groups. The inks were printed using a commercially-available dual-head 3D printer, Allevi-2. The quality of the prints was assessed by imaging the prints using an Olympus BX51 microscope and rheological measurements were performed on a Physica MCR301 rheometer (Anton Paar) in parallel plate geometry.

Results and Discussion
We successfully demonstrate a double-network ink consisting of poly(ethylene glycol)-based polymers with the shear-thinning agent Pluronic F127. A library of hydrogel-ink formulations with tunable viscosity were made by varying the concentrations of the polymers. To optimize the printing approach, we investigated the dependence of rheological properties of the fabricated inks via applied shear rate measurements. These measurements confirmed shear-thinning and recoverability of the inks (as shown in figure 2), suggesting suitability for 3D printing. Finally, by varying printing parameters such as feed rate and pressure and using a high-resolution printing needle with an inner diameter of ~150 µm, we printed complex structures and demonstrated that our double-network inks can provide defined architectures with good print fidelity.

Conclusion
In summary, we developed double-network hydrogel inks comprising shear-thinning Pluronic F127 and selection of chemically-modified polyethylene glycol-based molecules. We demonstrated that (i) the rheological properties of the double-network inks can be changed by varying the concentration of polymers and (ii) identify the relationship between printing parameters and print quality for the inks investigated.

Acknowledgement
The authors acknowledge support from Science Foundation Ireland (16/IA/4584).
Figure 1
Figure 1: (A) Scheme depicting the thermoreversible phase transition of F127; (B) Schematic representation of the self-assembly of double-network hydrogels using modified-PEG and Pluronic F127; (C) Factors responsible to obtain a defined architecture print.

Figure 2
Figure 2: (A) The photographs show inverted vials of 25 w/v% Pluronic F127 with increasing concentrations of PEGDA Mn=700 (from left to right). Symbols above photographs correspond to those presented in recovery curves in B; (B) Recovery test, where formulations are subjected to high shear rate of 895 s⁻¹ for 20 s; (C) Microscopic images show the effect of increasing PEGDA concentration in 25 w/v% Pluronic F127 formulations on print quality.

References
Coaxial micro-extrusion of a calcium phosphate ink with aqueous solvents improves printing stability, structure fidelity and mechanical properties

Romain Bagnol\textsuperscript{1,2}, Christoph Sprecher\textsuperscript{1}, Marianna Peroglio\textsuperscript{1}, Jerome Chevalier\textsuperscript{3}, Olivier Ligier\textsuperscript{4}, Redouan Mahou\textsuperscript{4}, Philippe Büchler\textsuperscript{5}, Geoff Richards\textsuperscript{1}, David Eglin\textsuperscript{1,2}

\textsuperscript{1} AO Foundation, AO Research Institute Davos, Davos Platz, CH; \textsuperscript{2} University of Twente, Department of Biomaterials Science and Technology/ Faculty of Science and Technology, Enschede, NL; \textsuperscript{3} University of Lyon, MATEIS UMR 5510/CNRS/INSA-Lyon, Villeurbanne, FR; \textsuperscript{4} RegenHU SA, Villaz-Saint-Pierre, CH; \textsuperscript{5} University of Bern, ARTORG Center for Biomedical Engineering Research, Bern, CH

Introduction
Micro-extrusion-based 3D printing of porous and geometrically complex calcium phosphate (CaP) structures can improve bone defects treatment through the production of personalized bone substitutes. However, obtaining sufficient printing and post-printing shape stabilities for the proficient fabrication and application of fast hardening protocols are still a challenge. In this study, the coaxial printing of a self-hardening CaP cement with water-ethanol solvents aiming to increase the ink yield stress following extrusion and the stability of printed structures was investigated.

Experimental Methods
Helicoidal overhang structures were printed with various solvents and video recorded until structural failing, to test how much the use of water-ethanol solvents would influence structure stability. Cylindrical scaffolds were printed and mechanically tested through a compression test 10 minutes after printing, to assess the influence of solvents on early scaffolds properties. A standard and fast steam sterilization process was used as hardening step on the coaxially printed CaP cement (CPC) ink. Compression tests were used on the cylindrical scaffolds after hardening. Micro-CT imaging was done on cylindrical scaffolds following post-processing, to assess the effect of solvents on their internal structure. X-Ray Diffraction analysis was used to evaluate the influence of solvents on crystalline phase composition after post-processing. Additionally, a human mandibular defect model with a theoretical 72 degrees overhang wall was printed with various solvents, and the deviation from this angle was measured following post-processing.

Results and Discussion
The use of aqueous solvents doubled the printing height before structural failing of the helicoidal overhang structures. When mechanically tested 10 minutes after printing, a 2 log increase of the cylindrical scaffolds stiffness was achieved when using aqueous solvents. Following post-processing, the coaxial-printing resulted in cylindrical scaffolds with 4 to 5 times higher compressive moduli in comparison to the extrusion process not involving aqueous solvents. This mechanical performance improvement is likely due to fast CPC setting, preventing the formation of cracks during the hardening process as observed by the micro-CT imaging. Crystalline phase composition measured by X-Ray diffraction after post-processing were similar for the different groups. In the mandibular model, using solvents significantly reduced the angular deviation from the theoretical wall angle after post-processing.

Conclusion
Thus, coaxial micro-extrusion-based 3D printing of a CPC ink with aqueous solvents increases printability and allows using an easily accessible steam sterilization cycle as a standalone post-processing technique to produce 3D printed personalized CaP bone substitutes.
1st step: Coaxial-printing without support structure

- Solvents (% v/v):
  - 50% Water/50% Ethanol
  - 75% Water/25% Ethanol
  - 100% Water

- Effect of solvent addition:
  - Post-printing stability
  - Mechanical properties

- Successful printing of helical/overhanging structures

2nd step: Post-printing hardening process compatible with clinical setting

- Steam sterilization 134°C autoclave cycle
- Hardening: Crystalline phase transformation
- Effect of solvent addition:
  - Mechanical properties
  - Scaffold cracking

- Successful production of a human mandibular defect model
PS2-01-030

3D-printing technology towards the development of 3D in vitro model of the pancreatic acino-ductal unit

Chiara Tonda-Turo, Viola Sgarminato, Gianluca Ciardelli

Politecnico di Torino, Department of Mechanical and Aerospace Engineering, POLITEOBIOMED LAB, Torino, IT

Introduction

The duct adenocarcinoma evolves from a pancreatic intraepithelial neoplasia whose mechanisms of evolution are well known and documented, while the alterations that give rise to the early lesions remain still unclear\(^1\). The currently most used pancreatic adenocarcinoma models, 3D organoids, represent imperfect reproductions since they do not mimic the native microenvironment which also includes stroma cells\(^2\). This aspect is particularly relevant because, as recent studies have demonstrated, the tumor-stroma interactions play a fundamental role in the progression of adenocarcinoma\(^3,4\). The overall purpose of this project is to overcome the current in vitro model limitations by reproducing the morphology and the composition of the pancreatic acino-ductal unit, composed of specialized cells surrounded by stroma. More specifically, this work involves the use of additive manufacturing technologies to fabricate a 3D exocrine glandular tissue model that mimics in vitro the physiological structure experienced by cells in vivo.

Experimental Methods

The acino-ductal structure was reproduced through a 3D-printing technology (ROKIT InVivo, Rokit, Seul) by extruding polycaprolactone (PCL) in a layer-by-layer manner. The fabrication process was optimized to achieve high pore interconnectivity, accuracy in geometry and precise control of pore size. Particularly, the control of printing parameters such as temperature, pressure, speed, fill density and infill angle, played a key role in the development of the complex exocrine glandular structure\(^5\). A surface functionalization was performed exploiting atmospheric plasma to enhance cell-PCL substrate interaction. Pancreatic stellate cells (PSCs) were cultured for 21 days inside the 3D structures to create a stomal compartment, then pancreatic duct epithelial cells (HPDE normal and KRAS mutation) were seeded on the glandular structure to recapitulate the physiological crosstalk among stoma and epithelium.

Results and Discussion

3D-printed structures morphology was characterized by optical and scanning electron microscopy (SEM). The images demonstrated the effectiveness of the optimization process which led to the formation of a continuous filament, defect-free structures, accurate acino-ductal geometry and highly interconnected pores. Figure 1 shows the pore interconnectivity significantly improves by varying the infill deposition angle from 90° to 45°. The structures obtained by 3D-printing result in a 15x15x3.5 mm cube and the dimensions of acinus and duct diameters are 5 mm and 2.4 mm, respectively (fig.1A). The adhesion, proliferation and growth of PSCs inside the 3D structure were monitored up to 21 days and confirmed by morphological and immunochemical analysis (fig 1B). The effect of the KRAS-mutated epithelial layer on PSCs was monitored and compared to normal condition to identify the hallmark of the early stage pathology. The cellularized final structure will provide a model of human pancreatic duct adenocarcinoma at early stages which will allow to perform genomic analysis and study the physiologic pathways involved in the tumor evolution.

Conclusion

The overall purpose to reproduce the morphology and composition of the pancreatic acino-ductal unit composed of specialized cells surrounded by stroma was achieved by creating an in vitro cellularized pancreatic acino-ductal unit.
This model will serve as a powerful tool to investigate pathological processes such as pancreatic tumors (e.g. Pancreatic Ductal Adenocarcinoma, PDAC).

![Figure 1](image)

**Figure 1**
A) Image from optical microscopy of the acino-ductal structure obtained through a 3D-printing technology. B) PSCs cultured inside the acino-ductal structure after 21 days (blue:nuclei staining, green:F-actin staining). Green autofluorescence of PCL fibres is visible.

**References**


3D printing of hydrogel constructs with static mixing tool

María Puertas-Bartolomé1,2,3, Małgorzata K. Włodarczyk-Biegun1, Blanca Vázquez-Lasa3,4, Julio San Román3,4, Aránzazu del Campo1,2

1 INM-Leibniz Institute for New Materials, Saarbrucken, DE; 2 Saarland University, Saarbrucken, DE; 3 Institute of Polymer Science and Technology, ICTP-CSIC, Madrid, ES; 4 CIBER's Bioengineering, Biomaterials and Nanomedicine, CIBER-BBN, Madrid, ES

Introduction

3D bioprinting is a powerful additive manufacturing technology for tissue engineering applications that allows manufacture of 3D constructs with extraordinary control over the scaffold design1. Bioinks based on natural hydrogels have attracted much attention because of their unique inherent properties similar to the native extracellular matrix (ECM)2. However, their weak mechanical properties usually lead to poor printing quality and low stability of the printed scaffolds. Some strategies based on extrusion printing have been performed to overcome this limitation, such as increasing polymer content or viscosity, but they can compromise cell viability due to the high shear forces and low nutrients transport. The development of low viscosity bioinks and novel strategies for its suitable biofabrication is still in demand. In this work, we present a novel bioprinting methodology based on a dual-syringe system with a static mixing tool that allows in situ crosslinking of a reactive hydrogel in the presence of living cells. Proposed in situ gelation offers several advantages for 3D extrusion printing: it provides the structural integrity necessary to obtain good printing quality, and it allows using low viscosity solutions to avoid applying high shear stress to the encapsulated cells3. Developed bioink is based on a reactive hydrogel system of two polysaccharides that are covalently crosslinked during the printing process. Finally, as a proof of concept, this novel printing approach is used for coprinting of the hydrogel system with catechol functionalized nanoparticles (NP) developed by the group with proven wound healing promoting activities.

Experimental Methods

The reactive hydrogel system used in this work consists of carboxymethyl chitosan (CMCh) and oxidized hyaluronic acid (HAox). Both hydrogel precursors are loaded in two separate syringes and simultaneously extruded into a static mixer by mechanical force (Figure 1A). During the residence time in the static mixer precursors solutions are homogeneously mixed in a 1:1 ratio, the crosslinking reaction begins via Schiff base formation, and a partially crosslinked hydrogel is finally extruded. Gelation times and viscosities were analyzed by rheology in order to optimize bioink formulation. Swelling and degradation of scaffolds were analyzed in vitro and post-printing treatment with FeCl3 was tested to increase stability. Additionally, bioactive catechol functionalized nanoparticles were coprinted within the hydrogel system and NP distribution and release from the scaffold were analyzed. L929 fibroblasts were used for bioprinting of bioink with and without NP, and cell viability, proliferation and immunostaining of the encapsulated cells were investigated.

Results and Discussion

For adequate mixing and proper printing, crosslinking kinetics of the hydrogel has to match the residence time in the mixing tool. Therefore, bioink composition was optimized by adjusting polymers concentrations, ratios, gelation times and viscosity. The final optimized formulation allowed us to print multi-layers grid square scaffolds with good printing accuracy, low deviance from the needle geometry and stable and uniform filaments (Figure 1B). It was observed that the stabilization step with Fe leads to higher long-term stability of the printed scaffolds and moderated swelling. NP did not impair the printing process and good NP distribution and sustained release were revealed. Bioprinted scaffolds showed homogeneous distribution of encapsulated cells and good biocompatibility with cell viability values
around 90-100% after 7 and 14 days of culture while maintaining their shape integrity during the whole period of cell culture (Figure 1C). Cell proliferation within the scaffolds was continuously increasing over the 14 days. Furthermore, metabolic activity of fibroblasts encapsulated into the bioprinted constructs showed that NP loaded constructs supported a more stable cell growth rate than materials without NP.

**Conclusion**

The presented methodology allows successful bioprinting of cell-laden reactive hydrogels with high cell viability. Applicability of this printing approach with a reactive system loaded with bioactive NP was demonstrated with a great potential for wound healing therapies. Furthermore, since printing conditions and type of precursors can be easily modified, this approach offers high versatility and can be adaptable to a wide range of reactive hydrogels for tissue engineering applications.

**Acknowledgement**

Authors thank CIBER-BBN and the Spanish Ministry of Economy and Competitivity (BES-2015-075161 scholarship and MAT2017-84277-R project) for supporting this work; RegenHu company (Sandro Figi, Dominic Ernst, Michael Kuster and Andreas Scheidegger) for the collaboration, development and providing the mixing tool; and Dr. Emmanuel Terriac for assistance in the confocal imaging.

![Figure 1](image)

A) Scheme of the 3D bioprinting approach using the static mixing tool. B) Images of multi-layer grid square hydrogel scaffolds printed using the described printing approach. C) Fluorescence images of immunostaining of L929 fibroblasts embedded into hydrogel bioprinted scaffolds (top) and nuclei staining with DAPI (bottom) over 14 days of culture.

**References**

Non-destructive characterization of bioinks by in situ Raman imaging

Julia Marzi¹²³, Ellena Hönig², Eva M. Brauchle¹²³, Verena Singer², Jessica Pfannstiel², Katja Schenke-Layland¹²⁴, Hanna Hartmann²

¹ Eberhard Karls University, Department of Women's Health, Tübingen, DE; ² NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, DE; ³ Eberhard Karls University, Cluster of Excellence iFIT (EXC 2180) "Image-Guided and Functionally Instructed Tumor Therapies", Tübingen, DE; ⁴ David Geffen School of Medicine at University of California, Department of Medicine/Cardiology, Cardiovascular Research Laboratories, Los Angeles, US

Introduction
Additive manufacturing using bioinks, comprising cells and biocompatible polymers, is an emerging biofabrication strategy to produce functional tissue models. Despite advancement in building increasingly sophisticated objects, biological and cellular analyses in printed constructs remain challenging and often imply endpoint analyses. Methods that enable non-invasive monitoring of embedded cells in bioprinted constructs would provide further insights on functionality and viability.

Experimental Methods
We implemented Raman imaging for molecular-sensitive investigations on bioprinted objects. Hyperspectral maps were acquired from bioinks followed by multivariate data analysis (MVA). Different aspects such as culture format (2D, 3D-casted, 3D-printed), cell type (endothelial cells, fibroblasts) and the selection of the biopolymer (alginate/NFC, gelatin, alginate/gelatin) were considered and evaluated.

Results and Discussion
Raman imaging allowed for a marker-independent identification and localization of subcellular components against the surrounding biomaterial background. Furthermore, single-cell analysis of spectral signatures, performed by MVA, demonstrated a discrimination of endothelial cells and fibroblasts and identified cellular features influenced by the bioprinting process.

Conclusion
In summary, Raman imaging was successfully established to evaluate bioinks and access cellular information in 3D culture in situ, representing a promising tool for the quality assessment of bioprinted objects.

Acknowledgement
The work was financially supported by the State Ministry of Baden-Wuerttemberg for Economic Affairs, Labour and Housing Construction and the BMBF (‘SOP Bioprint’ 13XP5071C).
Fundamental study on 3D printing fabrication of HA-PLLA composite implants

Tomokazu Hattori\textsuperscript{1,2}, Akihiro Hiramitsu\textsuperscript{1}, Atsushi Ikebata\textsuperscript{1}, Manano Itho\textsuperscript{1}, Masataka Deie\textsuperscript{2}

\textsuperscript{1} Meijo University, Dept. of Materials Science and Engineering, Nagoya, JP; \textsuperscript{2} Aichi Medical University, Dept. of Orthopedic Surgery, Nagakute, JP

Introduction
PLLA (Poly L-type Lactic Acid) of thermoplastic is a well-known biodegradable polymer, and already applied to the absorbable surgical sutures and fracture fixation devices. PLLA is also used as ecofriendly plastic filament for the FDM (fused deposition modeling) 3D printing in various engineering field. The other hand, HA (Hydroxy Apatite) is well-known as calcium phosphate ceramics for bone augmentation material with osteoconduction ability. Naturally, by the combination of PLLA and HA, biodegradable composite material was developed and has been applied for clinical medicine.

In order to develop the 3D printing fabrication of HA-PLLA composite implants, HA-PLLA composite filament was made, and small plate implants were fabricated by the 3D printer. Then in-vivo bone tissue reaction was investigated by animal experiment with 3D-\(\mu\)CT.

Experimental Methods
\textbf{Materials:} Spherical HA powder with the average diameter of 15~20\(\mu\)m (Taihei Chemical Industrial Co., Ltd. Japan), PLLA filament with diameter of 1.75mm (Mutoh Industries Ltd. Japan) were used as raw materials. Uniaxial filament extruder (Filabot EX2, USA) and the single head FDM 3D printer with the nozzle diameter of 0.4mm (MF-800, Mutoh Industries Ltd. Japan) were employed for the fabrication of HA-PLLA composite implants. And the slicer software of Slic3r and the control software of Pronterface were used for the 3D printing.

As experimental animal, Dutch rabbits were used to investigate bone tissue reaction to HA-PLLA composite implants. And the corn beam 3D-\(\mu\)CT (Rm-CT2, Rigaku co. Japan) was applied to periodic investigation of the bone tissue reaction.

\textbf{Methods:} PLLA filament was cut into small pellets with the length of approximately 2mm, and mixed with HA powder with splaying 70% alcohol solution. Then the mixture was dried up at 70°C for 24 hours. By means of weight measurement, the HA composition rate was evaluated. HA-PLLA filaments with two different HA composition rate of 10% and 20% were extruded at 185°C with an additional cooling fan and a filament drawer device.

In order to investigate in-vivo bone tissue reaction, based on Dynamic Compression mini plate system for human fingers, a small plate implant with 3 holes was designed as 16mm in length, 5mm in width and 1mm in thickness using the 3D CAD system (123D design, Autodesk Inc. USA). Then the small plate implants were fabricated by the 3D printer using the HA-PLLA composite filaments and pure PLLA filament. In addition, small screws were made of pure PLLA by heated compression molding at 180°C.

Under 3 mixed anesthesia, HA-PLLA composite plate implants were implanted at the medial aspects of proximal tibiae in Dutch rabbits, and were fixed by the pure PLLA screws. As controls, pure PLLA plate implants were also implanted in the same manner. Then 3D-\(\mu\)CT of the proximal tibiae were taken at just after the implantation (0 w), and at 2, 4, 8 and 12 weeks after the implantation. Surrounding bone tissue reaction was investigated by using a medical image processing system (Expert INTAGE, Cybernet Systems Co., Ltd. Japan).

Results and Discussion
By adjustments of extrusion speed, heating temperature and drawing speed, HA-PLLA composite filament with a diameter of 1.75mm could be made without clogged nozzle due to HA particles. The HA-PLLA composite plate implant...
implants were successfully fabricated with a little rough surface due to HA particles. The filament extrusion and 3D printing are very sensitive to the thermal conditions. It is necessary to take into account that HA particles reduce thermal conduction.

Regarding animal experiment, although PLLA is invisible on 3D-μCT, HA-PLLA composite plate implants were barely visible due to HA particles dispersed in PLLA matrix. And new bone formation was observed along to the side wall of the composite implants, then which enlarged according to the postoperative period (Fig.1, Fig.2). Thus the osteoconduction ability of HA particle dispersed in PLLA matrix was demonstrated in this in-vivo experiment. However, volume reduction of the HA-PLLA plate implant was not yet observed at 12 weeks.

**Conclusion**

Taking advantage of the 3D printing technology, HA-PLLA composite small implants were fabricated. And its osteoconduction ability was confirmed by using 3D-μCT in the animal experiments. In order to evaluate the biodegradation and further bone tissue reaction, long term follow-up is in progress.

**Acknowledgement**

This animal study was authorized by the Animal Study Ethics Board of Aichi Medical University in accordance with International Guiding Principles for Biomedical Research Involving Animals announced by the Council for International Organizations of Medical Sciences 1985 (Ref. No. 2020-87).
Fig. 2 3D-μCT image of HA-PLLA plate (HA20%) at 12 w

New bone formation was observed along the side wall of the composite implants, then which enlarged according to the postoperative period.
PS2-01-040

Development of a Laponite-collagen bioink

Max von Witzleben, Catherine Cortes Vesga, Tilman Ahlfeld, Anne Bernhardt, Michael Gelinsky

Technische Universität Dresden, Carl Gustav Carus Faculty of Medicine, Centre for Translational Bone, Joint and Soft Tissue Research, Dresden, DE

Introduction

Type I collagen is the most abundant structural protein in the human body and is a crucial component of bone extracellular matrix and therefore considered a suitable biomedical material for tissue regeneration. Therefore, the use of collagen in bioinks is highly desirable. Fibrillation of acidic collagen solutions provides an increase of stability, however, additional cross-linking is necessary to achieve sufficient mechanical stability of the printed strands. Conventional bioinks often contain only small amounts of collagen to circumvent cytotoxic side-effects of crosslinking agents like genipin or tannic acid.

Laponite is a nanosilicate and forms, dissolved in water, a shear-thinning liquid. These thickening and shear thinning properties make it interesting as an additive for bioinks, as lower shear forces allow higher cell survival during extrusion plotting. Furthermore, the different degradation products are non-toxic, on the opposite, they may have a positive effect on bone metabolism and its calcification.[1]

In the present study we therefore combined collagen and laponite to manufacture stable bioprinted constructs without using cytotoxic cross-linking agents.

Experimental Methods

Bovine derived collagen type I (GfN, Germany) was purified and lyophilized before being dissolved in acetic acid at various concentrations at 4°C. Laponite was diluted in ddH2O at various concentrations or directly added to the collagen solutions. The pH and the consistency of the multiple mixes were determined at 4°C to narrow down the possible suitable mixtures for bioplotting. These mixtures were then further evaluated with an initial printing test employing a syringe before plotting with a GeSiM Bioplotter 3.1 (GeSiM, Germany) at room temperature. Printing characteristics were analyzed with established filament fusion and filament collapse tests. Therefore, every mixture was prepared at 4°C and directly transferred to the printer. For cellular investigations, immortalized human-mesenchymal stem cells (hTERT-MSCs) were employed. VybrantTM DiI Cell-labeling (Invitrogen, USA) was used as staining and applied prior to the loading of the respective 4°C cold hydrogel with 5x10⁶ cells/g. Therefore, the cells were suspended in 500µl of media and then placed in a 5ml syringe. The mixing was performed with a custom made mixer. Subsequently, three scaffolds per time point were printed with an applied air-pressure of 55kPa and a speed of 15mm/s, each scaffold comprising of 4 layers with 0°/90° grid. After printing, 3ml of media was added to each scaffold for 10min before replacing it with fresh media (3ml). Fluorescent images of the scaffolds were taken with a fluorescent Microscope (BZ-X810, Keyence, Japan) on the days 1, 5, 7 and 14.

Results and Discussion

Laponite gels of different concentrations showed pH values above 10. The collagen solutions had a pH of 3-6 depending on the concentration of acetic acid and collagen concentration. With a mixing ratio of 50:50 wt% of collagen solution and Laponite hydrogel, different mixtures with varying pH could be prepared. Mixtures with pH below 4 and above 8 were highly inhomogeneous. At the same time, the viscosity of the mixtures liquefied significantly compared to the individual components, making it necessary to increase the concentrations of the components. At least 5 mg/ml collagen and 10 mg/ml Laponite were applied. Comparison of the characteristics of filament collapse and filament fusion tests of the individual components clearly showed the accurate printability of the Laponite hydrogel and the inaccurate and collapsing printability of the collagen solution. The 50:50 mixing ratio
was reflected in the printing behavior of the composites; the addition of Laponite improved the shape fidelity of the collagen solution, but the scaffolds collapsed at lower layer numbers (≤15) compared to the pure Laponite gel. The mixture containing 40mg/ml collagen and 100mg/ml Laponite showed the best shape fidelity. Interestingly, after immersing the Laponite/collagen scaffolds in medium, the gel fibrillated in such a fashion that crosslinking became unnecessary. HTERT-MSCs mixed into pastes survived the plotting process. Further, cells revealed distinct proliferation in bioprinted constructs over a cultivation period of 14 days.

Conclusion
Pasty Laponite-collagen gels with different concentrations could be prepared. Depending on the pH value, only a few mixtures were suitable for the printing process. With their good printability in combination with the observed proliferation of printed hTERT-MSCs the investigated mixtures qualify as bioinks.

Acknowledgement
This measure is financed by the Saxon State Ministry for Higher Education, Research and the Arts (SMWK).

References
**PS2-01-042**

**Dual-stage Crosslinked Hyaluronic Acid-based Bioink Tailored for Chondrogenic Differentiation of Mesenchymal Stromal Cells**

Julia Hauptstein\(^1\), Leonard Forster\(^2\), Ali Nademnezhad\(^2\), Jürgen Groll\(^2\), Torsten Blunk\(^1\), Jörg Teßmar\(^2\)

\(^1\) University of Würzburg, Department of Trauma, Hand, Plastic and Reconstructive Surgery, Würzburg, DE; \(^2\) University of Würzburg, Chair for Functional Materials in Medicine and Dentistry and Bavarian Polymer Institute, Würzburg, DE

**Introduction**

In recent years, biofabrication has evolved as a promising and fast-growing field for applications in general life sciences as well as in regenerative medicine. A special interest in the field focuses on hydrogels which combine cell-supportive properties with accurate 3D printability. To facilitate the generation of 3D printed constructs with an adequate shape fidelity, frequently highly concentrated polymeric hydrogels are used as cell supportive matrices. However, the resulting high network density and material stiffness can distinctly negatively affect cell bioactivity and the homogeneous distribution of extracellular matrix (ECM) newly produced by the incorporated cells. Therefore, the aim of this study was the development of a new versatile dual-stage crosslinking approach of a thiolated hyaluronic acid-based bioink, which should not only provide stand-alone 3D printability, but also facilitate chondrogenic differentiation of mesenchymal stromal cells (MSCs) as a model system and homogeneous distribution of newly produced ECM.

**Experimental Methods**

A range of thiolated HA (HA-SH) with different molecular weights was synthesized and all of them were crosslinked in two stages, utilizing adjustable amounts of polyethylene glycol-diacryl (PEG-diacryl) for pre-crosslinking via Michael addition and PEG-diallyl for thiol-ene mediated post-crosslinking after 3D printing (Figure 1A). Printability of ink formulations was empirically examined by extrusion tests at a 3D bioprinter and a mechanical testing machine. The most promising formulations were also rheologically characterized and diffusion studies were performed with FITC-labeled dextrans. Selected ink compositions were used as MSC cell carrier and long-term cell culture over 21 days was performed in 3D printed constructs. Chondrogenic differentiation of MSCs was examined by gene expression analysis and the quantification of newly produced ECM components like glycosaminoglycans (GAGs) and collagens. Differences in ECM distribution were analyzed via histological and immunohistochemical staining as well as histomorphometric quantification. Mechanical testing further validated the cartilaginous quality of the constructs.

**Results and Discussion**

In general, by adjusting HA-SH and PEG-diacryl concentration and molecular weight (M\(_W\)), a wide range of inks could be generated with regard to printability and diffusion properties. MSCs were demonstrated to differentiate chondrogenically in all bioprinted constructs. Bioinks with low M\(_W\) HA-SH (ca. 50 kDa) required a high polymer content for good printability, but resulted in only pericellular ECM distribution and overall weak mechanical properties of the final constructs after chondrogenic differentiation. In contrast, constructs made from high M\(_W\) HA-SH (> 200 kDa), requiring only a low polymer content for 3D printing, exhibited higher GAG and collagen contents, a homogeneous ECM distribution throughout the constructs (Figure 1B and C) and strongly improved stiffness after chondrogenic differentiation.

**Conclusion**
The here presented dual-stage bioink system using a modified biopolymer and two different crosslinkers can serve as an example to design bioink platforms utilizing two independent crosslinking reactions at one functional group, which allows to adjust printability as well as the material and biological properties of the printed bioinks.

Acknowledgement

This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project number 326998133 – TRR 225 (subprojects A02, A06 and B02). The authors thank the Orthopedic Centre for Musculoskeletal Research, University of Würzburg, for providing bone samples.
Dissecting the effect of bioink material cues on the biological function of bioprinted 3D skin constructs

Rúben F. Pereira1,2,3, Bianca N. Lourenço2,3, Paulo J. Bártolo4, Pedro L. Granja2,3

1 ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, PT; 2 i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, PT; 3 INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Porto, PT; 4 School of Mechanical, Aerospace and Civil Engineering, University of Manchester, Manchester, GB

Introduction
Bioprinting has been used to generate grafts for skin tissue repair and in vitro 3D tissue models for drug screening and disease modelling through the deposition of cell-laden biomaterial formulations termed as bioinks [1, 2]. Despite the progress in the design of hydrogel bioinks using a wide range of natural and synthetic materials, the bioprinting of 3D constructs resembling the architecture, composition, and function of human tissues and organs is not widespread. A major limitation in the field relies on the lack of data on the required material cues at biophysical and biochemical level necessary for the bioprinting of 3D tissues with biological function [3]. To address this need, herein, we designed a library of dual-crosslinked hydrogel bioinks which biochemical and biophysical properties can be independently modulated in order to unravel the effect of key material cues on the biological function of bioprinted 3D skin constructs.

Experimental Methods
Norbornene-modified pectin [4] was used to generate single-polymer backbone bioinks, which composition and properties were controlled by the incorporation of a cell-adhesion peptide and a cell-degradable peptide crosslinker. Constructs were extrusion bioprinted into 3D shapes with varying levels of complexity using pre-crosslinked bioinks, which rheology was tuned via either calcium- or barium-mediated ionic gelation. Dermal fibroblasts were embedded within pre-crosslinked bioinks with distinct biochemical and biophysical properties, bioprinted and photopolymerized. Cell response was analyzed at day 14 by Live/Dead imaging, cell morphology and proliferation, as well as deposition of extracellular matrix (ECM) components.

Results and Discussion
Pre-crosslinked bioinks formed homogenous and stable filaments (13-15 mM CaCl2; 15-17.5 mM BaCl2), allowing the bioprinting of 3D constructs that supported cell viability in a crosslinking density dependent-manner. Rheological characterization indicated that the yield stress and viscosity of bioinks were dependent on the cation concentration, while a high percentage of recovery ability (85-86%) was obtained upon subjecting the bioinks to regimes of low and high shear rates. Bioprinted fibroblast-laden constructs fabricated with varying levels of matrix stiffness (G′: 294 to 1098 Pa) via exposure to UV light for different time periods exhibited dramatic changes on cell spreading, proliferation and ECM deposition throughout the culture. At day 14 post-printing, dermal constructs prepared from both low and high stiffness bioinks showed cells with an elongated morphology, indicating that the ability of cells to proteolytically remodel the gel network towards spreading is not dictated by the initial hydrogel stiffness. However, a significant reduction on cell proliferation and deposition of collagen-I and fibronectin were observed in stiffer bioinks, showing that the initial stiffness restricts the ability of the cells to produce new ECM regardless of the degradable nature of the gel network.

Conclusion
The design of bioinks with tunable rheological, mechanical and biochemical properties is essential to perform systematic studies to evaluate the effect of material cues on the biological function of bioprinted constructs. Our
results identified the major role of matrix stiffness on the cell response within protease-degradable bioinks, underscoring the effect of material cues on the deposition of key ECM components in bioprinted dermal tissue analogues.

Acknowledgement

This work was supported by the project Norte-01-0145-FEDER-000012 – Structured program on bioengineered therapies for infectious diseases and tissue regeneration, supported by the Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF), and supported by the projects P2020-PTDC/BBB-ECT/2145/2014, PTDC/MEC-GIN/29232/2017, and Portugal-UK Bilateral Research Fund (PARSUK/FCT) funded by POCI via ERDF and by Foundation for Science and Technology (FCT) via OE. The authors acknowledge the support of the i3S Scientific Platforms (Bioimaging; Biointerfaces and Nanotechnology), member of the PPBI (PPBI-POCI-01-0145-FEDER-022122).

References

AVOIDING CELL DEATH IN 3D BIOPRINTING- COMPUTATIONAL FLUID DYNAMICS (CFD) SHEAR STRESSES MODELLING INEXTRUSION

Patricia Santos Beato\(^3\), Deepak Kalaskar\(^1\), Ryo Torii\(^2\)

\(^1\) University College London, Department of Surgery and interventional sciences, London, GB; \(^2\) University College London, Department of Mechanical Engineering, London, GB; \(^3\) University College London, Department of Biochemical Engineering, London, GB

Introduction

In vitro tissue modelling is a growing field which is currently being enhanced with new manufacturing techniques such as 3D bioprinting. This revolutionary technique enables the production of complex hierarchical structures while allowing high cell densities encapsulated in bioinks to be deposited in a controlled manner\(^1\). However, it has been shown that high shear stresses produced during the extrusion process can compromise cells' ability to survive\(^2\) and grow\(^3\). Currently, the printing process is optimised by trial & error, which requires large quantities of cells and materials, and time. A more efficient process of printing optimisation, e.g. towards maximum cell survival post printing, is much needed for the further advancement of 3D bioprinting. Computational fluid dynamics (CFD) modelling used to simulate the extrusion process of bioinks under different extrusion pressures, nozzle and needle diameters, is expected to play an important role in the optimisation of the printing process. The aim of this work is to develop a CFD model to establish a quantitative understanding of the association between the extrusion pressure, choice of bioink material, nozzle or needle size, and shear stress within the nozzle/needle.

Experimental Methods

The CFD model was developed using ANSYS Fluent software. The geometrical model of nozzle/needle and the air bodies combined, were made in a 2D axisymmetric manner and subsequently meshed using quadrilateral elements. After mesh sensitivity test, the simulations were conducted using different bioinks – mixtures of xanthan gum and gelatine, and gelMA 5% – to assess the optimum printing conditions for each material. The materials were characterised rheologically to find their viscosity and fitted into the Herschel-Bulkley model, when non-Newtonian, as inputs for the CFD model. The shear stresses generated were analysed in three different nozzle sizes and three needle sizes (22G–410 µm, 25G–250 µm and 27G–200 µm).

Results and Discussion

The results showed the correlation between smaller nozzle sizes and higher shear stresses generated for the same material and extrusion pressure, which was expected. Additionally, a comparison of the maximum shear stresses showed that the xanthan gum-gelatine mixtures generated lower shear stresses (140 Pa) than gelMA (845 Pa) when extruded with the same nozzle diameter and extrusion pressure. Additionally, the flow rate was assessed and compared to experimental gravitational measurements to validate the model. By examining the flow rate and the experimental results, a further correlation between the maximum pressure and maximum printing velocity that can be used without losing filament continuity was found. Although the simulations and experimental validation tests were conducted under acellular condition, the results provide a quantitative indication which can narrow down the number of possible printing parameter combinations towards a low potential cell damage and high printing output.

Conclusion

Ongoing work will be aimed at establishing the potential association between the predicted shear stress and the cell-specific tolerance of shear stress that causes cell damage in experimental printing, which will make this model a strong tool to design 3D bioprinting processes.

Acknowledgement
Support from the UKRI Engineering and Physical Sciences Research Council (EPSRC) Centre for Doctoral Training (CDT) in Bioprocess Engineering Leadership is gratefully acknowledged (EP/S021868/1).

References


**PCL Micro-Dumbbells – A New Class of Polymeric Particles Reveals Morphological Biofunctionality**

**David Sonnleitner**, Gregor Lang, Patrick Pasberg, Natascha Schäfer, Lena Fischer, Ingo Thievessen

1 University Bayreuth, Biopolymer Processing, Bayreuth, DE; 2 University Hospital Würzburg, Institute for Clinical Neurobiology, Würzburg, DE; 3 Friedrich-Alexander-University of Erlangen-Nuremberg, Biophysics Group, Department of Physics, Erlangen, DE

**Introduction**

The semi-crystalline linear aliphatic polyester Poly-ɛ-caprolactone (PCL) is a highly used material in the field of medical devices, tissue engineering and biofabrication due to its relatively low price, mechanical strength and FDA approval [1]. It can be processed into different morphologies, e.g. films, spheres and foams, but also fibers. Creating PCL fibers in the micro-, submicro-, and nanoscale is possible when electrospinning is used as processing technique [2]. The generated fibers can mimic morphological features of the extracellular matrix and are applied mostly as scaffolds for tissue engineering approaches. By precisely balancing out the electrospinning parameters we were able to apply and control for the first time the in-situ fragmentation of the generated fibers to create dumbbell shaped micro fragments from PCL.

**Experimental Methods**

Fiber fragments were created using electrospinning setup with flow rate 1 ml/h, distance 10 cm, voltage difference 5.5 kV. Morphological analysis was performed with scanning electron microscopy. PCL fragments were immersed consecutively in 1,6-Hexanediamine, 1% Glutaraldehyde and finally 2.5 mg/ml collagen solution to create a bioactive coating. Contact angle measurement was used to assess the surface wettability of the coated and uncoated samples. Dry samples of coated and uncoated fiber fragments were investigated with FTIR using the ATR Ge-crystal setup in direct contact mode. All spectra were measured in absorbance mode with atmospheric compensation enabled with a resolution of 4 cm⁻¹ and a sample scan time of 100 scans in the range from 4000-800 cm⁻¹. TdTomato-farnesyl expressing U87 reporter cells were cultured in Minimal Essential Medium supplemented with 10% FCS, 1% Penicillin 50 U ml⁻¹, Streptomycin 50 µg ml⁻¹, 1% 200 mM GlutaMAX, 1% 100 mM sodium pyruvate. Cells were washed with 1x PBS, fixed with 4% PFA/Sucrose for 15 min, following blocking with 5% normal goat serum and 0.1% Triton-X100 for 30 min. Cells were incubated with ActinGreen™ 488 ReadyProbes™ Reagent for 1 h. After three washing steps cells were mounted with Mowiol.

**Results and Discussion**

Increasing the concentration of the PCL spinning dope in the electrospinning process showed the transition from spheres to continuous fibers with an unstable intermediate state. Precise balancing of the parameters and adjustments to the molecular weight of PCL blends enabled us to control the in-situ formation of dumbbell shaped fiber fragments with diameters between 1.5 – 3 mm and an aspect ratio between 10 and 30. These fragments could be successfully separated and coated with collagen to create a more bioactive surface. The procedure was evaluated by SEM, contact angle and FTIR, verifying the successful coating. Cell culture experiments with U87 glioblastoma reporter cells on fragments and continuous fibers showed clustering of round cells in the fiber samples in SEM analysis [3]. Cells seeded on fragments showed a more homogenous distribution over the sample surface and more pronounced spreading [4]. Additional F-actin experiments showed directed growth of cells in close proximity to fragments with strong integrin activity when cells interacted with the rounded ends [5]. For continuous fibers the observed integrin activity was undirected and less pronounced.
Conclusion
In this work, we could for the first time demonstrate and control the formation of dumbbell-like PCL-structures produced by a simple electrospinning setup with a precisely balanced parameter set. Entropy-elasticity combined with the process-induced anisotropic molecular arrangement is hypothesized to be the driving force behind fiber snapping. Based on these findings, MW of PCL was identified to be crucial for the formation of such morphologies and could be applied to control the aspect ratio of fiber fragments. Considering the fact, that such morphologies might be appealing for cells as they display attractive micro-meter-range dimensions, in-vitro testing with U87 glioblastoma cells was performed. To render the surface biofunctional, collagen coating was applied, moreover leading to hydrophilization and thus improved suspendability of the fragments in water. Strikingly, it was shown that particularly the round-shaped ends of dumbbell-like fragments provide highly efficient structures for integrin-based cell adhesion. Considering, that FDA-approved PCL is one of the most widely used biopolymers in the biomedical field and electrospinning is a popular method to process it into fibers, it is highly astonishing, that this has never been shown before for any polymer. Considering, that dumbbell-like structures combine morphological features of spheres and fibers, this new class of polymeric particles will be further explored as e.g. anisotropic filler material in biofabrication and cosmetics as well as in the field of injectables and drug delivery systems.

Acknowledgement
This work has been funded by the German Research Foundation (DFG, Deutsche Forschungsgemeinschaft) – project number 326998133, SFB/TRR 225 “Biofabrication” (subprojects A07, PI: Gregor Lang and B06, PI: Ingo Thievessen).

Natascha Schaefer was supported by funds of the Bavarian State Ministry of Science and the Arts and the University of Würzburg to the Graduate School of Life Sciences (GSLS), University of Würzburg. Annalena Wieland is greatly acknowledged for her excellent assistance in generating the U87 reporter cell line.
SEM analysis of U87 glioblastoma cells after 96 h cultivation on different morphologies of collagen-coated electrospun PCL substrates. The investigated morphologies are random continuous fibers (A), aligned continuous fibers (B), mixed morphology with aligned, continuous fibers and fragments (C) and non-oriented dumbbell shaped fragments (D).

References
PS2-01-052

3D bioprinted model for breast cancer cell-stroma interaction study during radioinduced oxydative stress.

Theo Desigaux\textsuperscript{1,2}, Léo Comperat\textsuperscript{2}, Nathalie Dusserre\textsuperscript{2}, Marie-Laure Stachowicz\textsuperscript{2}, Hugo Oliveira\textsuperscript{1,2}, Jean-Christophe Fricain\textsuperscript{1,2,3}, François Paris\textsuperscript{4,5}

\textsuperscript{1} University of Bordeaux, Tissue Bioengineering, U1026, Bordeaux, FR; \textsuperscript{2} INSERM U1026, Tissue Bioengineering, ART Bioprint, Bordeaux, FR; \textsuperscript{3} CHU Bordeaux, Service d'Odontologie et de Santé Buccale, Bordeaux, FR; \textsuperscript{4} University of Nantes, Nantes, FR; \textsuperscript{5} INSERM 892 - CNRS 6299, CRCINA, Nantes, FR

Introduction

Breast cancer is the most common type of cancer in women. Depending on tumor subtype, cancer therapy can range from classical treatments based on surgery, chemotherapy and radiotherapy, to hormonotherapies, or monoclonal antibodies directed to ERBB2. Despite great advancements achieved for breast cancer treatment, cancer associated mortality is still a public health problem. There is, therefore, a need for further understanding regarding how breast cancer therapies interact with tumor cells, within the niche, to prevent resistance and improve treatment efficiency. Recently, the study of the tumor microenvironment demonstrated that these effects are in part modulated by the surrounding matrix and cellular elements. The most studied were Cancer Associated Fibroblasts (CAF) and Endothelial cells (EC). The first are essential for matrix remodeling and for secreting supporting factors to the tumor, increasing its proliferation and invasiveness. The second leads to vascularization, providing tumor with oxygen and nutrients. However, EC have also been demonstrated relevant during radio-induced oxydative stress, as their ASMase/ceramide mediated apoptosis can lead to survival signals for cancer cells, thus suggesting that this interaction can mitigate radiotherapy efficiency on cancer cells (1).

Studying these mechanisms in a more complex microenvironment is necessary to confirm this hypothesis. \textit{In vivo} models are limited by their poor versatility for microenvironment modulation, especially when considering human cell interactions. To overcome this breach between \textit{in vitro} models and human pathology, biofabrication has emerged in the last ten years as a powerful tool for cancer modeling. Here, we describe a simplified bioprinted breast cancer model containing both cancer and stromal compartments with CAFs and ECs, thus in part recapitulating the complexity of the cancer microenvironment needed for these investigations.

Experimental Methods

\textit{Cell culture} - MDA-MB-231 cells were maintained in DMEM with 10\% Fetal Bovine Serum (FBS). Breast CAFs were maintained as indicated by supplier in MSC-GRO medium. Human Umbilical Vein Endothelial Cells (HUVEC) were extracted and amplified in IMDM medium complemented with 10\% FBS and ECGS. EGM2-MV medium was used for maturation. Primary Human Skin Fibroblasts (HSF) were cultured in DMEM-F12 with 20\% FBS. GFP and mKate fluorescent MDA231 cells and HUVECs, respectively, were obtained using lentiviral vector transduction.

\textit{Gel formulation} - We based this gel on previous work from our group (2). Briefly, we used rat tail collagen methacrylate at 2mg/mL concentration with Hyaluronic Acid methacrylate at 10mg/mL. The gel was functionalized with IKVAV peptide at 1mg/mL concentration. The LAP photoinitiator was used at 0.1\% (w/v). MDA231 cells were diluted at 5M/mL in this gel for the cancer ink. For the stroma ink, we used 5M/mL of HUVECs associated with 5M/mL of CAF or HSF depending on experiments.

\textit{Bioprinting} - We used the RegenHU 3D discovery bioprinter with two-printing head for the different inks, and 400\mu m nozzle. The model was composed of two circular patterns with a cancer core surrounded by stroma, 3.5mm in total diameter.
Analysis - We used confocal imaging and immunohistochemistry for various markers (Ki67, CD31, VE-Cad) to assess for proliferation of cancer cells, maturation of endothelial cells. Quantification was made with image analysis software (FIJI and Imaris). Statistics were processed on Graphpad.

Results and Discussion
We first demonstrated a 24h post-printing viability of at least 80%, confirming that the printing process enables the creation of a viable 3D model. To improve our ink functionality, and cancer cell proliferation, we tested the impact of a laminin-derived peptide grafting. We observed a significant 2-fold increase in MDA231 metabolic activity with IKVAV peptide, at 1mg/mL, while YIGSR peptide did not show any difference, consistently with literature. We then assessed the effect of CAF/HSF and EC coculture on MDA231 proliferation. We observed a significant increase of proliferation by adding stromal elements. We then exposed our models to 5-15Gy to mimic radiotherapy, and observed a decreased in tumor size over time. Studies are currently underway to further characterize radiotherapy. Future studies will focus on how stromal elements affect the efficiency of radiotherapy.

Conclusion
Here, we describe our model successfully modeling breast cancer and its local microenvironment. Our model demonstrates to be consistent with current literature regarding the effect of microenvironment, and its ability to respond to radiotherapy. We intend to use this model to further characterize ceramide-mediated interactions, during treatment-induced oxidative stress, to further understand these complex mechanisms within the breast cancer microenvironment. We envisage the use of patient derived cancer cells to assess our model's ability to mimic patient's specific response to treatment.

Acknowledgement
This work is funded by the Foundation ARC and INSERM.

References
Ion-doped bioactive glass electrospun fibers for wound healing applications

Liliana Liverani, Theresa Reiter, Kai Zheng, Aldo R. Boccaccini

University of Erlangen-Nuremberg, Institute of Biomaterials, Erlangen, DE

Introduction
The successful treatment of chronic wounds has been the aim of several researches in the last years. In particular, the electrospinning technique has been exploited for the fabrication of advanced wound dressing and wound healing constructs, which support and improve the healing process. Bioactive glass particles can be incorporated in polymeric electrospun fibers to enhance the functionalities of those fibers addressing specific applications [1]. Besides the fabrication of those composite fibers, the combined use of the sol-gel and electrospinning methods to obtain bioactive glass fibers has been already reported and allows the fabrication of bioactive glass electrospun 3D cotton-like mats [2]. This approach has been used in the present work to fabricate copper-doped bioactive glass 3D fibrous constructs for wound healing application.

Experimental Methods
Tetraethylorthosilicate (TEOS), calcium nitrate tetrahydrate and copper nitrate hemi(pentahydrate) were used to prepare the sol for the electrospinning. A commercial electrospinning setup (Starter kit, Linari Engineering srl) was used for the fabrication of the 3D mats. The process parameters were optimized and kept in the following range: 10-15 kV, flow rate 0.4-0.6 mL/h, needle-collector distance 10-12 cm. The influence of the relative humidity was investigated since it is the main factor for the obtainment of 2D or 3D mats. The fabricated fibers were characterized from morphological (SEM), chemical (EDX, FT-IR) and biological point of views. Moreover, bioactivity assessment, by immersion in simulated body fluid solution, was performed.

Results and Discussion
The optimization of the parameters allows the identification of the suitable range and related thresholds for the fabrication of 3D mats with all the tested compositions. The average fiber diameter was comprised between 1-1.5 microns for all the compositions. All the compositions showed bioactivity, but the presence of copper delayed the formation of hydroxycarbonate apatite. Biological assays did not show significant differences between the different compositions and demonstrated that all the fibers were not cytotoxic.

Conclusion
The successful fabrication of ion-doped bioactive electrospun fibers was obtained and the presence of copper was not affecting the electrospinnability and the cytocompatibility of the bioactive glass fibers. An optimization of the electrospinning process was required for each composition, to identify the proper parameters which allowed the fabrication of 3D mats. Therefore, it is possible to extend this approach to several complex compositions, but the conditions for the electrospinnability must be considered and optimized.

References
3D printed Pluronic/PVA/Chitosan/Gelatin dressing for potential healing of chronic wounds

Ioanna Koumentakou¹, Zoe Terzopoulou¹, Anna Michopoulou², Dimitrios Bikiaris¹

¹ Aristotle University of Thessaloniki, Chemistry, Laboratory of Polymer Chemistry and Technology, Department of Chemistry, Thessaloniki, GR; ² Biohellenika Biotechnology Company, Thessaloniki, GR

Introduction
The skin is the largest organ of the body, accounting for about 15% of the total body weight in adults. Surgeries, traumas, abrasions, burns and other factors which cause cutaneous wounds require effective treatment to prevent morbidity or mortality. Wound healing is an intricate process and wound dressings form a significant segment of wound care management.

3D printing technique has shown great potential assisting and promoting the wound healing process. 3D printing facilitates the production of more flexible matrices, possessing well defined architecture and physical orientation. A wide range of biocompatible synthetic and natural polymeric hydrogels have been used for the fabrication of 3D printed dressings.

In this study, a 3D printed hydrogel based on chitosan (CS), gelatin (Gel) and poly (vinyl alcohol) (PVA) was developed for wound dressing application. CS is a naturally derived polymer, non-toxic and biodegradable. It promotes the proliferation of osteoblasts and mesenchymal cells, while it also presents antimicrobial and hemostatic properties. Gelatin was selected to improve the printability of chitosan and the tissue repair capacity of dressing due to its cell adhesion, migration, and proliferation ability. The hydrogel was reinforced with nontoxic, biodegradable, and biocompatible synthetic polymer PVA to improve the mechanical properties of the dressings.

Experimental Methods
Different ratios of CS, Gel and PVA powder were dissolved at 60 °C under magnetic stirring in acetic acid solution until complete homogenization. The mixture was then transferred to 3D printing syringes, which were centrifuged and cooled to room temperature (25 °C) to form the gel structure. The printability of hydrogels was studied, changing the parameters of 3D printer (needle size, pressure and temperature). The material which presented the optimal printability was used to fabricate dressings with different porosity. The 3D printed porous patches were cross-linked using ammonia gas, NaOH/EtOH and KOH solutions for 15 and 60 min to improve the stability of samples. The dressings dried for 24 h in an oven (30 °C) and their physicochemical properties were investigated.

Results and Discussion
Five hydrogels with different ratios of CS, PVA and Gel were synthesized and tested for their printability. The sample with 3 CS % (w/v), 3 PVA% (w/v) and 2 Gel % (w/v) presented the best printing capacity and was thus selected for the crosslinking process (Figure 1).

The successful synthesis of hydrogels was confirmed by FTIR spectroscopy, and their crystallinity was studied by XRD. Moreover, the water swelling, and stability capacity were studied before and after the cross-linking process, proving that the main impact of the cross-linkers was to increase the stability, and at the same time decrease the swelling ability of patches.

Conclusion
The combination of the 3D printed porous structures and the chemical composition of films aid cell attachment and migration which allow better tissue regeneration after injury

Acknowledgement
This research has been co-funded by the European Regional Development Fund (ERDF) and from national resources through the Operational Program "Competitiveness, Entrepreneurship and Innovation", NSFR 2014-2020, under the call "Aquaculture"-"Industrial materials"-"Open innovation in culture" (project code: T6YBP-00288).

Figure 1a
3D-printed structure of CS/PVA/GEL 3/3/2 with needle size G22

Figure 1b
3D-printed structure of CS/PVA/GEL 3/3/2 needle size G25
Fused Filament Fabrication 3D-Printing and Melt-Electro-Spinning for Tissue Engineering Scaffolds

Carsten Linti1, Michael Doser1, Goetz T. Gresser1, Josefin Weber2, Meltem Avci-Adali2

1 German Institutes of Textile and Fiber Research Denkendorf, Biomedical Engineering, Denkendorf, DE; 2 University Hospital Tuebingen, Thoracic and Cardiovascular Surgery, Tuebingen, DE

Introduction
Using additive manufacturing to create 3D-printed organs could reduce organ transplant shortage saving many lives on waiting lists. In this project, a tissue engineering approach is introduced combining fused filament fabrication (FFF) 3D printing with melt electrospinning [1]. Scaffolds made by this technique integrate nonwoven scaffolds with high porosity made by absorbable polymers with adjusted degradation profiles within more rigid supporting structures to build microenvironments for organoids with additive manufacturing methods. The surface of the electrospun scaffolds can be modified with ECM components to support adhesion and to potentially enable biochemical stimulation via creating niche microenvironments. In 2007, a milestone in regenerative medicine was achieved by creating induced pluripotent stem cells (iPSCs) from human adult somatic cells, fibroblasts [2]. Retroviral vectors were used to express the reprogramming factors Oct4, Sox2, c-Myc, and Klf4 in the cells for the reprogramming of the cells into iPSCs. These cells provide great opportunities to treat many disorders [3]. In contrast to embryonic stem cells (ESCs), the generation of iPSCs from somatic cells, their generation and application avoid ethical concerns. Building macrostructural containments and cell support structures within a single FFF 3D printing session can be one step towards manufacturing organ-like structures that can be populated with different iPSC-derived cells.

Experimental Methods
Filaments of poly-ε-caprolactone PCL (ITV Denkendorf Productservice GmbH) have been produced for FFF printing with 1.75 mm diameter. An FFF 3D printing machine (German RepRap X350pro) was modified with a high voltage electrostatic source (Eltex KNH34) and an electrostatic target as a printing bed. In this approach, PCL filament was extruded through a printing nozzle and the melt jet was established with an acceleration voltage enabling the manufacturing of fibers in the lower micrometer-scale that are deposited as nonwoven scaffold. The same extruder is used for building up complex support architectures. The melt-electrospun PCL scaffolds were characterized by SEM imaging (Hitachi TM1000) and statistically evaluated by directional-diameter-analysis (MAVIfiber2d, ITFM). First seeding experiments were performed with iPSC-derived hepatoblast precursor cells, which were differentiated into hepatoblasts on the vitronectin-coated scaffolds. After finishing hepatoblast differentiation on the scaffolds, the attachment of the cells, spreading, and proliferation were evaluated by fluorescence microscopy using ActinRed staining, which visualized the cytoskeleton. To further characterize the hepatoblasts seeded on the scaffolds, cells were stained with alpha-fetoprotein (AFP) specific antibody.

Results and Discussion
Cell culture plate inserts for 12-well plates have been 3D-printed with PCL filament containing electro-spun nonwoven as porous membranes for cell seeding (Figure 1 a + b). In Figure 1 c, a SEM picture is showing the structure of the nonwoven membrane. A printing nozzle with a diameter of 0.15 mm and an accelerating voltage of 15 kV was used. A mean-fiber-diameter was measured with 10.8 µm (distribution from 1 to 18 µm). The thickness of the membrane scaffold is about 300 µm.

First seeding experiments with iPSC-derived hepatoblast progenitor cells showed an efficient adhesion and proliferation on the scaffolds. The coating of the scaffolds with vitronectin further enhanced the adhesion of the cells. The coated scaffolds were seeded with hepatoblast precursors which were then differentiated into hepatoblasts.
on the scaffolds. Hepatoblasts attached and proliferated very well (Figure 2A). Furthermore, the coating increased the cell-cell contacts. Successful differentiation of the hepatoblast precursors into hepatoblasts could be demonstrated on the scaffolds by the expression of AFP (Figure 2B).

Conclusion
In this study, it was demonstrated that the fabrication of melt electrospun PCL fibers is possible using an FFF 3D printing process that combines additive manufacturing of supporting structures with the production of nonwoven cell culture scaffolds in a highly automated process. Using this technology, we produced new scaffolds within a cell culture insert by melt-electrospinning of PCL filament in a standard FFF 3D printing machine. These scaffolds were successfully seeded with hepatoblast precursor cells that could be differentiated into hepatoblasts. Furthermore, we demonstrated uniformly covered fiber surfaces in this first study. This may be a step towards additive manufacturing of the shelf support structures for organoids, which can be seeded with iPSCs for the cultivation of patient-specific hepatocytes to perform drug testing, cell modifications and analysis for the development of improved therapeutic approaches.

References
2:00 p.m. – 3:30 p.m.

Poster floor

**PS2-02 | Biomaterials Characterization**
The response of human gingival fibroblasts, keratinocytes and macrophages to Ti surfaces pre-incubated with whole blood

William A. Lackington, Lada Fleyshman, Yvonne Elbs-Glatz, Stefanie Guimond, Katharina Maniura, Markus Rottmar

Swiss Federal Laboratories for Materials Science and Technology (Empa), Biointerfaces Lab, St. Gallen, CH

Introduction
Classical in vitro testing platforms for implantable biomaterials offer limited predictability of clinical performance, which presents a major challenge in implant development. While classical platforms facilitate the study of biomaterial-tissue interfaces using relevant cell types, the importance of the first interaction a biomaterial encounters in vivo, that with whole human blood, is often neglected. In the context of titanium (Ti) implant soft tissue integration, for dental applications, the aim of this study was to assess how the blood-biomaterial interaction influences the tissue healing response.

Experimental Methods
Clinically available Ti implant surfaces (machined, SLA, and SLActive) were used to study the response of relevant soft tissue cells (human gingival fibroblasts, gingival keratinocytes and macrophages), with and without a pre-incubation step in whole human blood. Ti surfaces properties were characterized using contact angle measurements, to determine wettability, and atomic force microscopy, to determine surfaces roughness. Cell attachment was assessed by staining samples with F-actin and counterstaining using DAPI, and imaged using confocal laser scanning microscopy. Scanning electron microscopy was also used to assess cell attachment and morphology on Ti surfaces. Metabolic activity of cells on different surfaces was evaluated using a prestoBlue assay. Enzyme linked immunosorbent assays were performed to determine the levels of TNFα, IL-6 and IL-8 produced in response to the different surfaces, and cytokine levels were normalized to DNA quantity in each sample using a dsDNA picogreen assay.

Results and Discussion
Findings show that, depending on their surface properties, including roughness (Sₐ), contact angle and micro/nanostructures, distinct fibrin networks formed on the Ti surfaces after incubation with blood. Generally, across all cell types, pre-incubation of the Ti surfaces with blood led to enhanced attachment, metabolic activity, proliferation and matrix production. Fig. 1 shows distinct human gingival fibroblast morphologies developing only 24 hours after coming into contact with Ti SLActive surfaces in the presence and absence of a pre-incubation step with whole human blood. Ti surface properties were a key determinant of inflammatory marker (TNFα, IL-6 and IL-8) production by macrophages and pre-incubation of the Ti surfaces with blood led to significant changes in their relative amounts.

Conclusion
Collectively, these findings indicate that while material surface properties play a large role in determining the tissue healing response, the interaction between a biomaterial and whole human blood can significantly alter this response. In conclusion, this study highlights that blood-biomaterial interaction should be taken into account during implant development.

Acknowledgement
The authors would like to thank Innosuisse, the Swiss Agency for Innovation Promotion, (Grant # 40048.1 IP-LS) for funding.
Figure 1. HGF attachment to Ti surface in the absence or presence of blood. Top panels show representative scanning electron micrographs, while the bottom panel shows representative confocal laser assisted microscopy images, of HGFs interaction with Ti SLActive.
In vitro degradation testing of hydrogels – concept and case study of gellan gum degradation in water

Christine Gering, Jenny Parraga, Minna Kellomäki

Tampere University, Faculty of Medicine and Health Technology, Tampere, FI

Introduction
Over the past two decades, hydrogels have become an indispensable tool for cell culture and regenerative medicine, in part due to their swelling and degradation capabilities. Degradable hydrogels present the advantage of improved biocompatibility and flexibility concerning the diffusion and stability properties. The testing of hydrogel degradation in vitro before its targeted application is required but the evaluation methods are precarious. One of the main obstacles is the manipulation of very soft hydrogels. The samples are often very delicate and measured parameter such as mass may be problematic to determine.

Several strategies to determine mass loss of hydrogels exist, including weighing the wet mass using closed containers, and lyophilization to determine dry mass [1]. Common issues in the experimental setup include errors being introduced by handling and touching the samples, as well as remaining incubation media that is difficult to separate from the gel. Here, we propose a degradation testing setup using a holder for the hydrogel sample and present degradation test results using gellan gum-based hydrogels. As a simple concept study, we incubate hydrogels samples in ultra-pure water and monitor weight change over 24h. Our demands for the method are: 1. the hydrogels are fully surrounded by the incubation medium; 2. it is possible to remove excess liquid; and 3. the sample can be moved without harming.

Gellan gum (GG) is an anionic polysaccharide that easily forms self-supporting hydrogels in the presence of cations, and has been investigated as artificial cell matrix and other tissue engineering applications [2] We have further investigated the modification of GG for extrusion-based applications, and are using an oxidized gellan gum (GGox), prepared using sodium iodate reaction, where a portion of the rhamnose sugars in the structure have been opened and carry aldehyde groups [3].

Experimental Methods
For the testing setup, so-called 'mesh ring holders' were assembled using laser cut polyacrylate rings (ø 18.5 mm, ring width 1.8 mm, height 1.8 mm) with bonded nylon mesh. The cylindrical cast hydrogel [3] was demolded directly on top of the holder (Fig. 1B), so that a sample can then be moved by exclusively manipulating the holder rather than the soft sample. Weights of the holders have to be determined beforehand. The holder with sample is then placed into the incubation chamber on top of the sample stage, which is moved downwards (Fig. 1A). Finally, then media is added carefully and the chamber is closed with a rubber stopper.

In order to weigh the sample at a time point, the steps are reversed: The media is carefully removed with a pipette and the sample stage is moved upward so that the mesh ring can be taken. The excess media is removed by carefully blotting the ring and mesh with a swab (Fig. 1C).

Hydrogel samples of native GG and GGox (both 1% w/v) prepared with spermidine trihydrochloride (SPD, 2 and 10 mM respectively) in volume ratio of 5:1, were cast to a mold and incubated overnight [3] The samples (n=5) were placed on top of the holders as described above and 2.5 mL of ultra-pure water were filled to the chamber. The samples were weighed at 1, 2, 4, 6, 8 and 24h, and fresh water was re-filled after every measurement. The weight of the mesh holder was subtracted from ach measurement and the weight change was calculated according to:

%change = (wet weight – original weight)/(original weight)

Results and Discussion
Using the method outlined above, we observed GG-based hydrogels incubated in ultra-pure water over one day. The weight changes of the immersed hydrogels are shown in Fig. 2 and the respective mass gain and loss can be tracked easily from the continuously monitored samples. Native GG samples disintegrate, and mass loss happens swiftly after a very brief swelling phase. At high polymer concentration (1% w/v) the crosslinking and blending with ionic crosslinker may be hindered by the high viscosity of GG, and thus the integrity of the samples is poorer overall. These hydrogels were especially difficult to handle, as the samples degraded to small pieces very quickly within the observation period. However, due to the pieces remaining on the mesh holder the mass loss could be monitored regardless. In comparison, GGox hydrogels show prolonged swelling period, and mass loss only slowly begins after 24 hours. Likely, the aldehydes present in GGox form a Schiff-base pair with primary amines of SPD [4]. The higher stability and prolonged swelling phase of GGox-SPD gels can thus be understood to stem from these hydrogels having a combination of physical and chemical crosslinking [3].

**Conclusion**

In conclusion, we have presented a successful technical improvement for the tracking of hydrogel degradation by assessing the mass loss. We have shown preliminary results on GG-based hydrogels in water. In the future, this method can be adapted to also track degradation products in the media, as well the visual shrinkage or swelling of each sample.

**Acknowledgement**

This work was supported by the Academy of Finland through the Center of Excellence – Body on Chip project, while C.G. received funding from the Jenny&Antti Wihuri foundation.

---

**Figure 1**

Figure 2
Weight change curves of GG-based hydrogels.

References
Phenomenological ex-situ tracking of pitting corrosion and the mechanical integrity of a WE43 magnesium alloy

Kerstin van Gaalen¹, Felix Gremse³, Felix Benn⁴, Peter E. McHugh¹, Alexander Kopp², Ted J. Vaughan¹

¹ National University of Ireland Galway, Biomechanics Research Centre (BMEC), Biomedical Engineering, School of Engineering, Galway, IE; ² Meotec GmbH, Aachen, DE; ³ RWTH Aachen University, Department of Experimental Molecular Imaging, Aachen, DE; ⁴ Queens University Belfast, School of Mechanical and Aerospace Engineering, Belfast, GB

Introduction
Magnesium and its alloys show great potential as biodegradable alternatives to permanent metallic orthopaedic implants. However, they tend to undergo pitting-based corrosion when implanted into the body, which causes significant challenges in predicting the mechanical stability of an implant during fracture healing and degradation [1]. The objective of this study is the development of a three-dimensional automated detection framework that systematically evaluates the severity and phenomenology of pitting corrosion. With these data, first correlations are drawn to the mechanical integrity data

Experimental Methods
Cylindrical tensile test specimens were manufactured from a Magnesium WE43MEO alloy rod containing rare earths (Mg-Y-Nd, Meotec GmbH, Germany). Immersion testing (Figure 1(a)) in simulated body fluid (SBF) was performed for 28 days with weekly time points (Three samples per time step). Hydrogen gas measurement was tracked, which is linked to mass loss [2,3]. Following immersion, micro-computed tomography scanning (Skyscan 1272, Bruker, Belgium) and tensile tests (ZwickRoell GmbH & Co. KG, Germany) were conducted. Only the magnesium core was segmented since it is assumed to be the load-bearing part (Imalytics Preclinical Software, Gremse-IT GmbH, Germany). For the automated pit tracking, an algorithm was developed to track pit formation in every cross-section (Figure 1(c)) and another algorithm reconstructs the complete gauge length of a dog bone with the spatial pit identification (Figure 1(d)). The process chain was developed in python by using OpenCV for the automated image recognition.

This framework outputs measures of pitting corrosion on the specimen surface, including pit density, pit size, pit depth, and pitting factor ASTM G46-94 [4]. Linear and exponential correlations were established between several pit features and the ultimate tensile strength (UTS).

Results and Discussion
For the examined WE43 alloy, a three-fold higher corrosion rate via volume loss tracking through µCT scanning was detected than the determined one over the hydrogen evolution (2 mm/year vs. 6.5 mm/year). Tensile tests show a steady drop of the ultimate tensile strength with an increase in mass loss. In general, the suggested factors in ASTM G46-94 show a poorer correlation to the mechanical integrity than other (Figure 2). Further our results show that over time single pits merge into larger pits together. Our data set show the best correlation between the smallest tracked Magnesium core width and the remaining mechanical strength. The weakest was found for the pitting factor.

Conclusion
The presented tool automatically outputs standardised measures of pit features of circular-shaped specimens. In terms of mechanical strength, there are better pit features than the one described in ASTM G46-94 for a WE43 alloy. All factors related to the reduction of the cross-sectional area show a better correlation to UTS. Further, the pit tracking shows that small pits are formed early within the corrosion process, which eventually merge into one another
over time to form larger and deeper pits that are more harmful to load-bearing capacity. It should be noted that further testing is necessary to underline the determined correlations here.

Acknowledgement

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 813869. This document reflects the views of the author(s) and does not necessarily reflect the views or policy of the European Commission. The REA cannot be held responsible for any use that may be made of the information this document contains.

Figure 1: Pit detection framework
a) Immersion testing; b) raw µCT cross-section; c) 2D pit detection d) contour plot surface of gauge length after immersion

Figure 2: Correlation pit feature vs. Ultimate tensile strength
a) Pitting factor according ASTM G46-94(2018); b) Min. identified Mg core width

References
Fabrication of highly tuned Calcium phosphate/silk sericin composites: exploring new pathways on skin regeneration

Anabela Veiga1,2, Filipa Castro2, Rui Magalhães1, Fernando Rocha2, Ana L. Oliveira1

1 Centro de Biotecnologia e Química Fina, Porto, PT; 2 laboratory for Process Engineering, Environment, Biotechnology and Energy, Porto, PT

Introduction

Calcium phosphates (CaPs) are well established materials for bone tissue engineering. However, new possibilities arise for this “old” bioceramic. Recent studies are focusing on reinventing CaPs for skin tissue engineering [1]. Calcium has demonstrated to play an important role in the barrier function repair and skin homeostasis and serves as a modulator in cell proliferation and differentiation. In this context, the combination of CaPs with biopolymers to promote skin tissue regeneration becomes an attractive strategy, providing appropriate microenvironments for cell interaction. Recently, we have proposed -nano and – micro CaP/sericin (SS) composites as suitable candidates for tissue engineering approaches [2]. Silk SS, until recently considered unfit for biomedical use, it is now accepted as a valuable byproduct from the textile industry, able to stimulate collagen production and to elicit an antioxidant, moisturizing and anti-inflammatory effect [3].

The effectiveness of cell attachment on CaP composite particles highly depends on their physicochemical characteristics which in turn is directly depending on the processing strategy. Continuous flow regimes operated through Oscillatory flow reactors (OFRs), improve particle mixing and mass transfer processes in multiphase systems, generating a product with uniform and controlled characteristics [4],[5]. Moreover, they allow scale-up without compromising the chemical composition. In the present work, we propose, for the first time, fully controlled CaP and CaP/SS particles for skin regeneration, through a continuous coprecipitation process, using a specifically designed modular oscillatory flow plate reactor (MOPR) (WO/2017/175207). For this, the influence of the oscillation amplitude (x0) and frequency (f), residence time (τ) and initial reagents concentration were investigated on the final CaP physicochemical properties and in vitro behavior using human dermal fibroblasts (HDFs).

Experimental Methods

CaPs synthesis was carried out by mixing equal volumes of a solution of CaCl2.2H2O (Merck, 99.5%) and a solution of Na2HPO4 (Sigma-Aldrich, 99.0%) into the MOPR, using a peristaltic pump (Longer Pump, BT100-2J). SS was obtained using extraction in boiling water from cocoons of the Bombyx mori silkworm and added to the calcium precursor solution. Coprecipitation was carried out by a continuous process in a MOPR, at 37ºC. Several experimental conditions were studied with different x0, f, τ and initial reagents concentration were investigated on the final CaP physicochemical properties and in vitro behavior using human dermal fibroblasts (HDFs).

Results and Discussion
FTIR spectra of the synthetized products evidenced a typical apatite structure similar to commercial hydroxyapatite, with the exception of condition 10 where brushite peaks were identified. SEM show aggregated nano/micro particles (1-10^-2 µm) with low crystallinity that adopt a rod and plate-like shape. Uniform and aggregated nanoparticles were only obtained in condition 7. While for the f of 6 and 1.9Hz the increase in $x_0$ seems to be associated with the increase of plate-shaped particles, for the intermediate f this effect is not evident. In fact, at 4Hz there seem to be larger HAp particles surrounded by smaller particles. In condition 11, which corresponds to 5 times increase in the initial concentration of reagents, it is possible to observe large particles, in the form of well-defined and crystalline shapes. Regarding the addition of SS, the particles have a homogeneous appearance, appearing to be surrounded by a structure that could be the protein matrix. Particles with the most distinct characteristics (4,7,10,11) were selected for further analysis in cell culture. Preliminary results using MTT assay showed that all conditions studied promote cell viability.

**Conclusion**

For the first time, a study was carried out on synthesis of CaPs and on the influence of different parameters of OFRs continuous production on the final characteristics of CaPs and CaPs/SS composites. With the tested parameters it was possible to obtain particles with different physicochemical properties. In addition, preliminary *in vitro* tests on these materials show their potential as biomaterials for skin tissue.

**Acknowledgement**

This work was financially supported by: National Funds through FCT (Foundation for Science and Technology) under the project UIDB/50016/2020 of the Centre for Biotechnology and Fine Chemistry - CBQF. The authors also acknowledge Portuguese National Funds from FCT through project Base Funding – UIDB/00511/2020 of the Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE – funded by national funds through the FCT/MCTES (PIDDAC). A. Veiga gratefully acknowledges doctoral scholarship [2020.08683.BD] from FCT.

![Figure 1.](image)
Figure 2.
SEM images for the different experimental conditions studied amplified 10 000 times (1 μm)/20 000 times (500 nm), beam intensity (HV) 25.00 kV, distance between the sample and the lens (WD) around 10 nm.

References
How can have a bioactive glass antibacterial and cell viability character simultaneously?

Zsejke R. Tóth\textsuperscript{1,2}, János Kiss\textsuperscript{2}, Alexandra Feraru\textsuperscript{1,3}, Anna Szabó\textsuperscript{2}, Klara Hernadi\textsuperscript{2,4}, Emilia Licarete\textsuperscript{1}, Lucian Baia\textsuperscript{1,3}, Klára Magyari\textsuperscript{1,2}

\textsuperscript{1} Babeş-Bolyai University, Interdisciplinary Research Institute on Bio-Nano-Sciences, Cluj Napoca, RO; \textsuperscript{2} University of Szeged, Department of Applied and Environmental Chemistry, Szeged, HU; \textsuperscript{3} Babes-Bolyai University, Faculty of Physics, Cluj Napoca, RO; \textsuperscript{4} University of Miskolc, Institute of Physical Metallurgy, Metal Forming and Nanotechnology, Miskolc, HU

Introduction
One of the six most important world problems is health care. As a solution for healthy problems bioactive glasses (BG) are used in several applications, such as bone injuries and healing, cancer metastases, or wound injuries (ex. after burning) \cite{1}.

In the last 40 years, the Bioglass invented by Prof. Larry Hench \cite{2}, was modified for achieving different properties such as porosity, biocompatibility, and stability. Besides the mentioned properties, other specific properties were also achieved such as antibacterial or the cell viability character of BG. By introducing the Ag nanoparticles into the glass matrices, antibacterial behavior resulted \cite{3}, while gold nanoparticles help for achieving the cell viability character \cite{4}.

In our previous work, silver-iodide \cite{5} was used to eliminate the formation of AgCl from the Ag nanoparticles in simulated body fluid. Moreover, in another of our work, differently shaped gold nanoparticle \cite{4} was added into the BG matrix, for achieving the best cell viability.

Therefore, the aim of this work was to combine these two methods in order to achieve both antibacterial and cell viability character in only one bioactive glasses composite.

Experimental Methods
Spherical gold nanocages were synthesized with the galvanic replacement method until AgI was synthesized with the solvothermal method. The BG with Agl and gold content were synthesized by involving two methods: (1) the spherical gold nanocages and Agl microcrystals were introduced in sol one after another (BG-Agl+AuNPs), (2) Agl-Au composites were synthesized via impregnation method and added into the sol (BG-AglAu).

The as-obtained materials were characterized using UV-Vis spectrophotometer (JASCO-V650), X-ray photoelectron spectroscope (SPECS PHOIBOS 150 MCD system), transmission/scanning electron microscope (Philips CM 10 and Hitachi S-4700 microscope), and X-ray diffractometer (Shimadzu XRD 6000). Antibacterial behavior of the composites was analyzed by Pseudomonas aeruginosa and Staphylococcus aureus bacterial and their cell viability was analyzed on Human keratinocytes (HaCaT, Cell Line Service, Germany) cells.

Results and Discussion
The spherical gold nanocages contain a small amount of AgNPs that remain after galvanic replacement reaction \cite{4}. After heat treatment of the glass samples, the gold nanocages become gold nanospheres with a diameter between 11 and 25 nm, and the Agl transforms to calcium iodate and AgNPs. All obtained samples exhibited antibacterial effect against Pseudomonas aeruginosa and Staphylococcus aureus bacteria. The better results were obtained for the BG-Agl+AuNPs. The viability of HaCaT cells indicated a good in vitro tolerance for all investigated samples. The better proliferation rate of HaCaT cells was obtained in presence of BG-Agl+AuNPs.

Conclusion
We have successfully added simultaneously Au nanocages and AgI microcrystals in BG matrix. The obtained composites showed an increase in antibacterial character and in cell viability behavior than the previously obtained composites.

Acknowledgement
This work was supported by a grant from the Ministry of Research and Innovation, CNCS - UEFISCDI, project number PN-III-P1-1.1-TE-2019-1138, within PNCDI III.

References
Reaction-diffusion models to predict Hydrogel viscoelastic properties

Nicole Guazzelli, Ludovica Cacopardo, Arti Ahluwalia

University of Pisa, Centro di ricerca E.Piaggio, Pisa, IT

Introduction

Hydrogels’ viscoelastic behaviour is strongly related to liquid molecules’ interaction with the solid network. Despite having been used to simulate chemical and transport phenomena in gels (e.g. reaction, diffusion, and hindered convection), most mathematical models lack correlation with gel viscoelastic properties (e.g., relaxation time, \( \tau \)) or do not provide experimental validations [1, 2]. Therefore, in this study, reaction-diffusion models were implemented to investigate how the presence of different dextran concentrations in the aqueous phase affect liquid molecules diffusion in agarose hydrogels and how these variations can be correlated with experimental relaxation times [3].

Experimental Methods

Agarose-dextran hydrogels were prepared by dissolving 0.5% w/v agarose in different dextran solutions (0, 2, 5% w/v). The viscoelastic parameters (relaxation time \( \tau \), equilibrium modulus \( E_{eq} \), and instantaneous modulus \( E_{inst} \) ) were derived according to the procedure described in [3]. As shown in Fig.1, agarose gels were modelled as a homogenous porous medium and an aqueous phase. Moreover, dextran is considered as a spherical agglomerate that moves in the interstices of the gel together with the free water. A Reaction-Diffusion (RD) Equation (1) was implemented in Matlab R2015 and used to describe the complessive hydrogel behaviour:

\[
\frac{\partial c}{\partial t} - \nabla \cdot (D_i \nabla c) + k_r c = 0
\]  

(1)

The water-dextran binding rate \( k_r \) was estimated using viscosity curves measured as a function of temperature (Brookfield viscometer). The measurements were performed during the process of gelation of the different agarose-dextran solutions.

To consider the hydrodynamic interaction between matrix fibres and diffusing macromolecules, the Brinkman Effective medium equation (2) was implemented to evaluate the diffusion coefficient of the different species in the system [3]:

\[
D_i = \left[1 + (R H_i^2) k_i^4 \right]^{-1/2} + (1/3) \left[(R H_i^2) k_i^4 \right]^{-1} \times \text{D}_0
\]  

(2)

where \( D_0 \) is the diffusion coefficient described by the Stokes-Einstein equation, \( R H_i \) is the hydrodynamic radius of the species, and \( k_i \) is the hydrogel permeability estimated by the Carman-Kozeny equation [2].

A 3D finite element model (FEM) was used to estimate the \( R H_i \) of the dextran molecules, using Comsol 5.3 and including \( E_{eq}, E_{inst} \) and \( \tau \) in the material properties of the domain. The model parameters were optimised on the base of experimental diffusion curves, which were obtained using fluorescein isothiocyanate (FITC)-conjugated dextran (Fig 2c-d).

Results and Discussion

The model showed that, as the dextran concentration increases, the diffusion time of the liquid phase decreases (Fig.2a), reflecting the relaxation time trend shown in Fig.2b.

The results of the Reaction-Diffusion model highlighted the influence of the dextran in weakening the bonds between water and agarose, with a consequent decrease of the steric hindrance and a faster flow through the hydrogel pores. Therefore, although liquid phase viscosity increases with increasing dextran concentration [2], it contributes to reducing the resistance to flow preventing the formation of water-agarose bonds.

Conclusion

Page 1544 of 2028
The proposed Reaction-diffusion model can describe the interaction of the liquid molecules with the solid network; this interaction can be modulated by altering the liquid phase composition and the chemical affinity for the polymeric chains. The RD model can be used to 'predict' the bulk viscoelastic behaviour of the hydrogels, according to their composition. In the future, different materials can be investigated for altering both solid and liquid phase properties, providing a useful tool for the design of engineered viscoelastic hydrogels.

Figure 1.
Schematic representation of the agarose-dextran porous system.

Figure 2.
a) Liquid phase diffusion time; b) hydrogel relaxation time; c) dextran diffusion in the agarose hydrogel after 0 and d) 24 hours.

References
Osteoblastic cell response to an additively manufactured zirconium-based bulk metallic glass

Lisa Larsson1, Jithin James Marattukalam2, Björgvin Hjörvarsson2, Natalia Ferraz3, Cecilia Persson1

1 Uppsala University, Department of Materials Science and Engineering, Applied Materials Science, Uppsala, SE; 2 Uppsala University, Department of Physics, Materials Physics, Uppsala, SE; 3 Uppsala University, Department of Materials Science and Engineering, Nanotechnology and Functional Materials, Uppsala, SE

Introduction
Zr-based bulk metallic glasses (BMGs) with disordered atomic structure have shown high strength, a relatively low Young’s modulus, excellent corrosion resistance and promising biocompatibility [1] [2]. The combination of these unique properties makes the Zr-based BMGs potential candidates for orthopaedic applications, particularly for load-bearing implants. Furthermore, advanced manufacturing techniques of BMGs, such as additive manufacturing (AM) by laser beam powder bed fusion (LB-PBF), provides an effective way to bypass critical casting thickness constraints associated with conventional BMG manufacturing techniques, thereby allowing fabrication of components with almost any desired size and geometry, particularly interesting for the medical industry [3]. A very limited amount of studies has however explored the biocompatibility of newly developed systems of zirconium-based BMGs destined for LB-PBF, and the influence of the process parameters on the biocompatibility of the final built material is seldom reported. The aim of the present study was to investigate the in vitro cell response to a LB-PBF Zr-based BMG, namely Zr59.3Cu28.8Al10.4Nb1.5 (trade name AMLOY-ZR01), and to study the effect of surface roughness, resulting from process parameter variation, on the cell response and ion release of the built material.

Experimental Methods
The as-printed samples were manufactured using an EOS M100 machine with a step size of 20 W laser power within an optimal processing window (55 W, 75 W and 95 W). By changing the laser power during the AM process, the average surface roughness (Sa) of the samples was varied. The as-printed materials were characterized in terms of degree of crystallinity, surface roughness and morphology, followed by a systematic investigation of the response of the MC3T3-E1 pre-osteoblastic cell line to the different surface roughness of the as-printed samples. The ability of the materials to promote cell proliferation was evaluated by culturing the cells on the surface of the material’s discs up to 7 days. The number of adherent cells at the different time points was evaluated by measuring the activity of the intracellular enzyme lactate dehydrogenase (LDH) in the cell lysates. Cell attachment and cell viability at the different time points were assessed qualitatively by live/dead staining of the attached cells, followed by fluorescence microscopy imaging. The cell morphology was evaluated by SEM imaging of the samples after 1, 3 and 7 days of cell proliferation. To evaluate the effect of the materials on cell differentiation, cells were cultured on the material discs under differentiation conditions and the cell response in terms of the activity of the early osteogenic marker alkaline phosphatase (ALP) was evaluated. Ion release experiments of the materials were performed in cell culture medium under physiological (pH 7.5) and inflammatory (with reactive oxygen species and acidic pH) test conditions and the ion release was quantified using inductively coupled plasma - optical emission spectrometry (ICP-OES).

Results and Discussion
Material characterisation confirmed the as-printed BMGs to be X-ray amorphous with a decrease in Sa with increasing laser power during printing (varying from 11 µm to 4 µm). The surface quality was further confirmed through SEM observations, where fewer defects and porosities were observed at higher laser power during printing. The biocompatibility evaluation indicated that the AMLOY-ZR01 substrates supported cell proliferation and differentiation of the pre-osteoblastic cells MC3T3-E1, while no difference in cell response was observed when...
comparing the different surface roughnesses (Fig 1). Moreover, the cell behaviour on the BMG alloy was comparable to the results obtained with the reference material Ti6Al4V, a commonly used and well accepted biomaterial. The promising cell response to AMLOY-ZR01 is in accordance with previous biocompatibility studies on Zr-based BMGs [4], [5], demonstrating that the additively manufactured AMLOY-ZR01 alloy is a good contender among other BMGs. When comparing the ion release rates found for the different AMLOY-ZR01 materials, no clear trend between the degree of surface roughness and ion release rate was found. However, the ion release experiments showed a large increase in ion release under inflammatory conditions as compared to regular physiological conditions, indicating that future studies should investigate the response of the osteoblastic cells when the cell-material interactions take place in an environment where high levels of ions are expected to be released from the alloy.

Conclusion
The findings in this work reveal a promising biological response to the AMLOY-ZR01 alloy, with potential for orthopaedic applications. It also highlights the need of future studies where the cell response is evaluated under conditions of elevated ion release from the alloy surface, i.e. under inflammatory conditions.

Acknowledgement
The authors gratefully acknowledge funding from the Swedish Foundation for Strategic Research (SSF) within the Swedish national graduate school in neutron scattering (SwedNess); the Swedish Foundation for Strategic Research (SSF), through the project “Development of Process and Materials in Additive Manufacturing”, Reference number GMT14-0048; Sweden’s Innovation Agency VINNOVA through the Competence Centre in Additive Manufacturing for the Life Sciences (Ref. nr 2019-00029).

Figure 1.
SEM images of cells on the as-printed materials and the titanium reference material after 3 days of proliferation. Cells show typical MC3T3-E1 cell morphology, with no differences in the number of adherent cells between the different materials.

References
Experimental mechanical study of human abdominal aortic tissue with various pathologies

Anna Lipovka¹, Daniil Parshin¹, Andrey Karpenko¹,²

¹ Novosibirsk State University, Novosibirsk, RU; ² Meshalkin National Medical Research Center, Vascular and Hybrid Surgery Center, Novosibirsk, RU

Introduction
The aorta is the largest vessel in the human circulatory system, which plays the most important role in the process of blood supply to all organs and tissues. Any lesions of the aorta lead to serious consequences and even death. When the aorta ruptures due to damage to its wall, the probability of death reaches 90%, even with emergency hospitalization and surgery. The main cause of aortic rupture is an aneurysm, which most often occurs in the abdominal aorta. The risk of rupture in the presence of an aneurysm can be aggravated by concomitant cardiovascular diseases - stenosis, atherosclerosis, which cause pathological changes in the walls of the vessel, leading to a deterioration in the elastic properties of tissues and its gradual degradation.

Experimental Methods
The aim of this work is an experimental study of the mechanical properties of aortic tissues with aneurysms and concomitant pathologies. For this purpose, we used aortic tissue (Fig. 1) excised during cardiac surgery at the Meshalkin National Medical Research Center. Under conditions that ensure the best preservation of the mechanical properties of these vessels, the aortic samples are transported to the laboratory of the Lavrentyev Institute of Hydrodynamics and Science of the Siberian Branch of the Russian Academy of Sciences, where experiments are performed. Before the experiment the obtained tissue is cut into several specimen, paying attention, if possible, to the direction of the bloodflow. After that the parameters of the specimen are measured and it is placed in the uniaxial tensile machine INSTRON 5944 (Fig. 2).

Results and Discussion
The obtained results were processed, the strain-stress diagrams plotted for each experiment. Such data as ultimate stress and strain, as well as Young's modulus were identified for each type of pathology, and statistically analysed.

Conclusion
In the future it is planned to widen the variety of specimens to improve the quality of analysis. Also this work will be combined with the results of aortic wall insicion, revealing the pre-existing stress in the tissue. In prospect, these results can be used for preoperative modeling.

Acknowledgement
This study was supported by the RSF (№21-15-00091)
Fig. 1. Aorta with an aneurysm
Piece of aorta with aneurysm extracted during the surgery, before testing

Fig. 2. Testing the specimen
The specimen in the clamps in the tensile machine after the rupture
Effects of unconventional setting conditions on the mechanical strength of alpha tricalcium phosphate bone cement

Adelia Kashimbetova¹, Karel Slámečka¹, Mariano Casas-Luna¹, Carolina Oliver-Urrutia¹, Simona Ravaszová², Karel Dvořák², Ladislav Čelko¹, Edgar B. Montufar¹

¹ Brno University of Technology, Central European Institute of Technology, Brno, CZ; ² Brno University of Technology, Faculty of Civil Engineering, Brno, CZ; ³ Brno University of Technology, Central European Institute of Technology, Brno, CZ

Introduction
Calcium phosphate cements (CPCs) are constantly being studied to improve and find new formulations with better properties, as well as to explore new processing routes for pre-set blocks, granules and scaffolds, including additive manufacturing approaches. Traditionally, to mimic the physiological temperature the setting of CPCs is performed at 37 °C, but the processing of pre-set bodies is not restricted to such condition, and other conditions may be used to improve the mechanical strength and reduce the processing time. The aim of this work was to study the effects of the setting temperature and particle size on the mechanical strength of a monocomponent CPC and correlate the results with the microstructure and reaction percentage. Mechanical tests were performed in small and big samples to elucidate the effect of sample size. Moreover, some samples were mixed with gelatine solution to mimic the hybrid composition of natural bone, and the effect of gelatine crosslinking on the mechanical strength was studied in dry and wet state. Gelatine was selected because it has been demonstrated to be a versatile additive in CPCs.

Experimental Methods
The cement consisted of alpha tricalcium phosphate (α-TCP) powder, with 3 different particle sizes (F < 32 µm; M 32-45 µm; C > 45 µm), and aqueous solution of 2.5 % Na₂HPO₄, mixed at a liquid to powder (L/P) ratio of 0.37 ml/g.

The samples were allowed to set in water at 4, 22, 37, 60 and 90 °C for 10 days. Additional samples were mixed with 10 % gelatine solution at L/P ratio of 0.60 ml/g, crosslinked with glutaraldehyde and set in water at 37 °C for 10 days. Compression test was performed in dry state at a constant crosshead speed of 1 mm/min. The small samples were cylinders with diameter of 6 mm and a height of 12 mm, whereas the big samples had a diameter of 20 mm and a height of 20 mm. Some samples prepared with crosslinked gelatine were also tested in wet state. The crystalline phases and reaction percentage were determined by XRD and Rietveld analysis. The microstructure was analysed by SEM, nitrogen adsorption and porosity determination.

Results and Discussion
The set cements consisted mainly of hydroxyapatite and unreacted α-TCP. The reaction percentage increased with increasing the reaction temperature from 4 to 37 °C, then the conversion was nearly constant at temperature up to 60 °C. Above this temperature a second but slight increment of the reaction percentage was observed (Fig. 1a). The fine powder achieved clearly higher reaction percentage than the medium and coarse powders at the same temperature. The microstructure showed the entangled network of hydroxyapatite crystals with different crystal morphology and size depending on the setting temperature and powder size. Plate-like crystals that grew with temperature were observed at setting temperature between 22 and 37 °C, whereas needle-like crystals, the size of which decreased with temperature, were observed at 60 and 90 °C (Fig. 1b). Differences in porosity within a 6 % range were found in spite of cements were mixed at the same L/P ratio. The compressive strength increased with temperature up to a maximum and then decreased (Fig. 1c). The increment of the powder size decreased the compressive strength and shifted the maximum strength towards higher setting temperature. The fine powder and the reaction temperature of 37 °C resulted in the strongest material. Other trends observed are that the compressive
strength decreased with the increase in the size of the samples, increased 4.7 fold when the gelatine was added and crosslinked, and decreased 2.7 fold when the crosslinked sample was tested in wet state.

**Conclusion**

The setting temperature had an important effect on the compressive strength, conversion, microstructure, and porosity of CPCs. The fine powder resulted in higher conversion and the strongest cements set at lower temperature. Further increase in setting temperature led to the decrease in the compressive strength due to the change of crystals morphology.

**Acknowledgement**

This work was supported by the Czech Science Foundation (grant 19-22662S). A.K. acknowledges the Brno Ph.D. Talent scholarship founded by the Brno City Municipality and BUT project CEITEC-VUT/FAST-J-21-7305. C.O.U. acknowledges the CONACYT grant 2020-000021-01EXTV-00235. A special acknowledgment to CzechNanoLab Research Infrastructure supported by MEYS-CR (LM2018110).

Fig. 1
Effects of setting temperature on a) progress of the setting reaction, b) specific surface area (inverse trend than crystal size) and c) compressive strength.
Data extraction using text mining methods on polydioxanone literature

Carla V. Fuenteslópez¹, Austin McKitrick², Javier Corvi³, Maria-Pau Ginebrà², Osnat Hakimi³,⁴

¹ University of Oxford, Institute of Biomedical Engineering, Oxford, GB; ² Universitat Politècnica de Catalunya, Department of Material Science and Engineering, Barcelona, ES; ³ Barcelona Supercomputing Center (BSC), Barcelona, ES; ⁴ Universitat Internacional de Catalunya, Faculty of Medicine and Health Sciences, Barcelona, ES

Introduction

Information extraction is fundamental to scientific research, however, when done manually, it is extremely time-consuming and often comprises re-work. Alternatively, automated data extraction renders up-to-date, quick, and easy access to information. Nevertheless, there is little precedence of using automated text mining tools to extract information in the biomaterials domain[1].

Here, we compared the ability of contemporary text mining tools to extract useful information from biomaterials abstracts exported from PubMed. As a topic, we selected ‘polydioxanone’ which, despite being a widely-used biomedical material, has a relatively limited available literature, thus enabling manual validation of results.

Experimental Methods

First, we filtered abstracts using a machine learning classifier, training a Support Vector Machine algorithm on a previously-published biomaterials collection[2]. Following corpus definition and classification, we compared various information extraction tools, including the open access annotation tool PubTator[3]; MeSH tagging; an unsupervised topic discovery algorithm (hLDA); text analysis methods using R; and the Biomaterials Annotator, an open-access annotation system of biomaterials, implants and medical devices.

Results and Discussion

Results were analysed in conjunction with a manual review of the literature and existing systematic reviews. Our findings show that automated text mining tools can be useful for mapping the biomaterials literature. In the case of polydioxanone, biodegradation and biocompatibility are predominant terms across all tools. Moreover, our analysis revealed that different tools reveal diverse aspects of the polydioxanone literature. Text analysis makes it easier to follow the evolution and frequency of specific term usage - ‘scaffold’ and ‘repair’ are increasingly used, ‘suture’ and ‘graft’ decrease, and ‘stent’ remains relatively steady (Figure 1). Analysis of MeSH tags permits the identification of major topics and shows unified term usage, making it possible, for example, to identify substances commonly used alongside polydioxanone like polyglactin 910 and collagen. Topic mining allows cluster identification based on similarity of topics between publications and helps cluster dominant areas, this suggests that there are bundles of abstracts related to, possibly, infection following wound site closure (Topic 3) or orbital fracture repair (Topic 15) (Figure 2). PubTator annotates well both species (e.g., humans, rabbits, rats) and diseases, including adverse effects (e.g., infection, fibrosis, cancer). Finally, the Biomaterials Annotator pre-organises identified terms in categories, making it possible to identify, for instance, manufactured objects such as stents or clips.

The information offered by these tools provides a wider albeit less detailed insight of the literature compared with that reported by systematic reviews, which tend to focus on the safety and performance of polydioxanone. Information extraction tools yield more updated information on biomaterials and do so in less time, making them more efficient. This suggests that there is a need for information extraction tools that provide more detailed information.

We also observe a change in term usage trend, possibly associated with topics that become more or less popular over time. The term ‘suture’ exemplifies this change - during the first few years, it dominated the polydioxanone literature, but as polydioxanone became more relevant and therefore had a more widespread use in other
manufactured object’s production, sutures became less predominant. When comparing pre- and post-2010 term usage, we can observe an increased use in terms such as ‘drug’ and ‘safety’, which could suggest the inclusion of a therapeutics as an application for biomaterials, while ‘degradation’ and ‘biocompatibility’ decrease, possibly attributed to the migration of the research focus away from characterisation and compatibility of the material. On the other hand, we noted some specific challenges to indexing polymer-related literature. For example, naming is heterogeneous, and abbreviations are extremely versatile. Only one of the tools (MeSH indexing) displayed unified term usage. The lack of uniformity in term usage makes it more difficult for researchers to identify all the articles published on a specific topic, therefore emphasizing the need for indexing tools and controlled vocabularies, for example for polymer names.

**Conclusion**

Here, we compare different text mining tools, as well as systematic reviews, by using a topic with a relatively limited literature - polydioxanone. While the text mining tools provide a broader overview of the literature and do so in a significantly shorter time, the information they provide is less detailed and some term ambiguity occurs. Also, most of the tools used in this study required some degree of user proficiency, limiting their everyday use by bench side scientists. This highlights the need for field-specific and user-friendly tools.

**Acknowledgement**

This project received funding from the European Union’s Horizon 2020 programme under the Marie Skodowska-Curie grant agreement DEBBIE (project number 751277).
Topics suggested by the hLDA algorithm, by number of abstracts per topic.
Abstracts clustered by hierarchical topics using unsupervised machine learning. This network shows the proposed topics weighted by number of abstracts per topic, showing levels and associations between topics. The six terms in each topic in Level 3 are shown.

References
Physico-chemical, morphological and biocompatibility characterization of a ready-to-use 3D biocomposite scaffold for periodontal bone regeneration

Mariana Souto-Lopes¹,², Liliana Grenho³,⁴, Maria Helena Fernandes³,⁴, Fernando Jorge Monteiro¹,², Christiane L. Salgado²,⁵

¹ University of Porto, FEUP - Faculty of Engineering, Porto, PT; ² University of Porto, i3S - Instituto de Investigação e Inovação em Saúde, Porto, PT; ³ University of Porto, FMDUP - Faculty of Dental Medicine, Porto, PT; ⁴ University of Porto, LAQV/REQUIMTE - Laboratório Associado para a Química Verde/ Rede de Química e Tecnologia, Portugal, Porto, PT; ⁵ University of Porto, INEB - Instituto de Engenharia Biomédica, Porto, PT

Introduction
Despite bone’s innate self-renewal capability, some periodontal pathologic and traumatic defects’ size inhibits full spontaneous regeneration. The current research characterizes a 3D porous biodegradable nano-hydroxyapatite/chitosan (nHAp/CS, 70/30) scaffold for bone regeneration, which is prepared through a simple 3 step process: dispersion preparation, freeze-drying and supercritical CO₂ (scCO₂) solvent extraction and sterilization, resulting in a cost-effective and environment-friendly ready-to-use graft biomaterial [1].

Experimental Methods
Full in vitro characterization of the nHAp/CS scaffold was performed comparing with a CS control scaffold. Its microstructure was scanned by micro-CT, the bioactivity in simulated body fluid (SBF) was assessed using scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) elemental analysis. The biodegradation and compressive strength in PBS with and without physiologic levels of lysozyme was evaluated by dynamic mechanical analysis (DMA) for 28 days. The biomaterials innate antimicrobial abilities against Gram positive (S. aureus) and Gram negative (E. coli) bacteria were compared by CFU counts and sessile/planktonic bacteria metabolic activity. The cytotoxicity of the scaffolds leachables after 1 and 24 hours was evaluated using MG63 osteoblastic cell culture through MTT (ISO 10993-5:2009) and live/dead assays. The proliferation of MG63 cells seeded for 21 days within the porous structure of both materials was assessed by their metabolic activity (Alamar blue assay) and DNA quantification. Cells morphology and distribution were observed by SEM and laser scanning confocal microscopy (LSCM).

A pilot in vivo study was performed with CBA nude mice to evaluate the materials’ biodegradation and biocompatibility when subcutaneously implanted in the animals’ dorso. The animals were euthanized at 3 and 5 weeks and histological samples were stained with hematoxylin-eosin (HE) and Masson’s trichrome (MT) stains.

Results and Discussion
The nHAp/CS scaffold microstructure has interconnected pores and a total porosity of 70%, enabling cell migration, growth and diffusion of nutrients, oxygen and metabolites [2]. SEM revealed HAp crystals forming on the surface of the nHAp/CS scaffold after 21 days in SBF, showing its bioactivity in vitro (Fig. 1). The presence of nHAp promoted a significantly lower biodegradation rate when compared to the CS scaffold [3], though the addition of lysozyme to PBS did affect the results. DMA analysis showed that both scaffolds have viscoelastic properties, but the presence of nHAp significantly enhanced the storage modulus (E') under compression after the frequency scan (0.1 to 15 Hz). At 10 Hz (high frequency), the E’ of the nHAp/CS scaffold was 42.34 ± 6.09 MPa after 28 days of incubation in PBS (no significant difference was observed with or without lysozyme), showing that it may support bone ingrowth at low-load bearing bone defects [2]. Both nHAp/CS and CS scaffolds significantly inhibited the growth, attachment and colony formation abilities of S. aureus and E. coli (with a stronger effect on S. aureus), enhancing the relevance of...
chitosan in the grafts' composition for the naturally contaminated oral environment. Leachables from both types of scaffolds did not show cytotoxicity, with the cells' viability ranging from 86 to 124% depending on the leaching conditions. MG63 cells showed normal morphology in SEM and LSCM images, and could adhere and proliferate inside the biomaterials' porous structure [4], especially for the nHAp/CS scaffold, reaching higher proliferative rate at day 14.

The in vivo subcutaneous implantation of both scaffold types did not induce acute inflammatory response. The histological analysis showed an outer thin fibrous capsule with disorganized connective tissue and a few monocytes. Between the 3rd and the 5th week, intensive and progressive ingrowth of new vessels and collagen fibers within the porous structures were observed, especially for the nHAp/CS scaffold (Fig. 2) [5].

**Conclusion**

So far, the nHAp/CS ready-to-use scaffold showed microstructural, chemical and mechanical features making it a suitable bone graft alternative for defect sites in an adverse environment as in periodontal disease. It showed higher biocompatibility in vitro and in an ectopic in vivo model. In the near future we shall evaluate the nHAp/CS potential to support human mesenquimal stem cells proliferation and differentiation, osteoclast and endothelial cells behavior in vitro. Finally, the nHAp/CS bone regenerative properties will be assessed in vivo in rats in calvaria critical defects.

**Acknowledgement**

This work is a result of the project NORTE-01-0145-FEDER000012, supported by North Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). In addition, it was supported by FCT/MCTES in the framework of the project UID/BIM/04293/2019.
Fig. 1  
EDS elemental analysis and SEM images of nHAp/CS and CS samples after 28 days in SBF for in vitro bioactivity evaluation

References
Improved bone surgical procedures with novel gold nanoparticles based biomaterials – pilot study

Alexandra Dreanca1,2, Zsejke-Reka Toth1,3, Sidonia Bogdan2, Andra Popescu2, Cosmin Pestean2, Corina Tomà2, Alina I. Ardelean2, Ciprian Ober2, Bogdan Sevastre2, Marian Taulescu2, Luacian Baia1,4, Klara Magyari1,3

1 Babeș-Bolyai University, Interdisciplinary Research Institute on Bio-Nano-Sciences, Cluj Napoca, RO; 2 University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Cluj Napoca, RO; 3 University of Szeged, Department of Applied and Environmental Chemistry, Szeged, HU; 4 Babeș-Bolyai University, Faculty of Physics, Cluj Napoca, RO

Introduction

Biomaterials based on bioactive glass with gold nanoparticle composites have many applications in tissue engineering due to their tissue regeneration and angiogenesis capacities [1]. This paper outlines a new approach to extending the biomaterials biological properties, composites of biopolymers-bioactive glass with gold nanospheres (AuSP) / spherical gold nanocages (AuIND) in bone tissue engineering. To further obtain data; the composites were tested in vivo, in a bone experimental defect in Sprague Dawley rats. Within the framework of these criteria we also tried to evaluate the biomaterials performance, in vivo, in canine species, a pilot study of TTA procedure.

Experimental Methods

The glasses with gold nanoparticles (gold nanospheres/spherical gold nanocages) content were synthesized by sol-gel method [2]. The alginate-pullulan-bioactive glass with gold nanoparticles (Alg-Pll-BGAuSP, Alg-Pll-BGAuIND) composites were obtained by cross-linking method [2]. The composites were analyzed using FT-IR spectroscopy and transmission electron microscope (TEM).

Experimental bone defects (3 mm wide and 2 mm depth) were performed on 60 rats (2 defects/animal) [3]. The defects were filled with biomaterials (90) or left as control/sham defects (30). Bone tissue samples were harvested at 2, 4 and 8 weeks postsurgical for histopathological and immunohistochemical evaluation.

An 11-months-year-old Kangal was presented with CCL rupture, second degree patellar luxation and moderate stifle osteoarthritis. During TTA Rapid fixation (4), lateralization of the tibial tuberosity was performed in order to correct patellar luxation and a glass-polimer-gold-nanoparticle composite was added in the osteotomy defect in order to accelerate bone healing.

Results and Discussion

The FT-IR spectroscopy measurements were demonstrated the cross-linking between the biopolymers and glasses. The TEM measurements indicate that in the glass samples the diameter of the AuSP was around 25 nm, and the AuIND between 11 and 25 nm.

At 30 days, the histopathological analysis showed osotoblastic proliferation alongside neoangiogenesis concerning both compounds containing gold nanoparticles. The in vivo results, also demonstrated that the Alg-Pll-AuIND had a significant influence on mesenchymal cell proliferation, neoangiogenesis, osteoblast differentiation (BMP2 and osteocalcin immunopositivity) and new bone formation, compared to Alg-Pll-AuSP and hydroxiapatite composite, particularly at 8 weeks.

Clinical and radiological follow-up was performed 30-days after surgery. The dog had excellent clinical outcome (lameness scoring=0) and complete bone healing. The TTA-Rapid gap was filled with bone and no progression of the osteoarthritis was observed.

Conclusion
Overall, these results offer compelling evidence that the newly developed biomaterials have the potential to provide a one-step treatment for further bone regeneration techniques, offering surgical complementary aid, while enhancing bone healing.

Acknowledgement
This work was supported by a grant of Ministry of Research and Innovation, CNCS - UEFISCDI, project number PN-III-P1-1.1-TE-2019-1138, within PNCDI III.

References
Introduction
The purpose of this in vitro study was to determine the metal-ceramic bond strength between dental porcelain and cobalt-chromium (Co-Cr) metal substrates fabricated by different techniques.

Experimental Methods
Forty Co-Cr metal substrates were fabricated according to ISO 9693-1, by casting, milling, soft milling, and direct metal laser sintering. Forty additional substrates were fabricated for each technique to record the modulus of elasticity. A commercially available feldspathic porcelain was placed on the substrates, and then the specimens were also tested for metal-ceramic bond strength with the 3-point bend test, according to ISO 9693-1. The fractured specimens were observed with optical and scanning electron microscopy using electron dispersive spectroscopy to define the mode of failure. X-ray diffraction spectroscopy was conducted to determine changes in crystalline phases after fabrication and the 3-point bend test. Statistical analysis was with 1-way analysis of variance and the Tukey post hoc test ($\alpha=0.05$).

Results and Discussion
No statistically significant differences were found for modulus of elasticity among any of the groups. The metal-ceramic bond strength for casting had no statistically significant differences and the mode of failure in all groups was cohesive. The metallographic analysis of the as-received, the after porcelain firing, and the after 3-point bend test specimens revealed changes in microstructure. The crystallographic microstructure revealed that the patterns had minor changes among the groups.

Conclusion
The study revealed that all of the techniques showed similar results. The modulus of elasticity and metal-ceramic bond strengths presented no statistically significant differences, and the mode of failure was cohesive.

Acknowledgement
Special thanks to Mr A. Chatzipmatzakis for his contribution in the fabrication of the metal ceramic specimens. Also many thanks for the contribution in SEM analysis to Mr P. Tsakiridis and Mrs E. Gomoza.
PS2-02-090

Zebrafish model for graphene oxide nanoflakes biocompatibility assessment

Sara Pérez-Davila¹,², Laura González-Rodríguez¹,², Raquel Lama³, Julia Serra¹,², Antonio Figueras³, Beatriz Novoa³, Pío González¹,²

⁰ CINTECX, Universidade de Vigo, New Materials Group, Vigo, ES; ¹ Galicia Sur Health Research Institute (IIS Galicia Sur). SERGAS-UVIGO, Vigo, ES; ² Institute of Marine Research (IIM), National Research Council (CSIC), Vigo, ES

Introduction
The global acceptance of *Danio rerio*, commonly known as zebrafish, as a modern experimental animal model is increasing gradually. This model is becoming popular in the fields of toxicology and biomedical research during both adult and embryonic stages. The reason for this extensive recognition is due to advantages respect to conventional animal models like a small size, very high reproducibility, quick development, transparency of the embryo and close homology with the human genome, including the immunogenic responses. Furthermore, costs are much lower, reducing space, animal maintenance, tested materials and even experimentation time, making it in a quick, cheap, and facile model to assess toxicity of nanomaterials [1,3].

The robust zebrafish model allows the biocompatibility assessment of a wide array of nanomaterials, facilitating a rapid pre-clinical development of novel biomaterials. Among them, graphene oxide has attracted strong interest for biomedical applications due to its unique 2D structure and exceptional physico-chemical properties. Moreover, it has been shown that graphene oxide exhibits antimicrobial properties [2,5] and even osteogenic regenerative activity, which combined with other materials can become an excellent nanomaterial for the manufacture of different biomedical devices [4].

The purpose of this work is to investigate the biocompatibility of commercial graphene oxide (GO) nanoflakes on larvae zebrafish (*Danio rerio*) as *in vivo* testing model.

Experimental Methods
The percentage survival of 2 days post-fertilization (dpf) zebrafish larvae was evaluated by exposition to different concentrations of GO by bath. Inflammatory response of 3 dpf larvae was performed by microinjection of GO at a concentration of 0.1 mg/mL into the duct of Cuvier. It was performed using a transgenic zebrafish line that specifically marks neutrophils with green fluorescent protein, Tg(mpx:GFP), and the total count of neutrophils and those migrated to the area of the microinjection were counted at different times post-treatment (2 and 24 h) using the ImageJ Software. Also the expression of the pro-inflammatory genes TNFα and IL-1β and tp53 was also assessed by qPCR, 24 hours after the microinjection.

Results and Discussion
The results indicate that the *in vivo* zebrafish model served to confirm the biocompatibility of graphene oxide at 0.1 mg/mL. No significant changes were found in the survival rate compared to the control (Figure 1). Also, there was no amplification of the inflammatory response compared to the control as no increase in the expression of inflammatory genes was recorded. This also prevented an increase in neutrophils (Figure 2) or migration into the micro-injected area.

Conclusion
It can be concluded that commercial graphene oxide (GO) nanoflakes up to 0.1 mg/mL concentration is biocompatible, using *in vivo* testing zebrafish model.

Acknowledgement

Page 1561 of 2028
This research was financially supported by BLUEBIOLAB project (POCTEP INTERREG España-Portugal) and BLUEHUMAN project (EAPA_151/2016, Atlantic Area 2016). Pérez-Davila, S. is grateful for funding support from Xunta de Galicia pre-doctoral grant (ED481A 2019/314).

Figure 1
Percent survival of 3 dpf (days post fertilization) larvae zebrafish (Danio rerio) at different concentrations of GO in zebrafish water until 7 days post-treatment (dpt). (CTR: 0.1 mg/mL milliQ water as a control).

Figure 2
In vivo imaging of neutrophil of Tg (mpx:GFP) larvae after 24 h microinjection with control (CTR, milliQ water) and with GO (0.1 mg/mL).

References
Reactive Accelerated Aging Test, A Time Machine to Evaluate the Durability of Biomaterials for Neural Implants

Blanca Limones-Ahijón, José Miguel García-Martín, María Ujue González, Sahba Mobini

Centro Superior de Investigaciones Científicas, Instituto de Micro y Nanotecnología, Tres Cantos (Madrid), ES

Introduction
Neural implants are increasingly investigated to assist patients with defects or pathologies in nervous system. There is a growing research about developing new biomaterials to improve the effectiveness and biocompatibility of neural implants. For example, inflammatory responses to implantation of these devices, also called foreign body reactions (FBR), are still a challenge for their performance. During FBR, immune cells such as monocytes and macrophages are recruited and activated, leading to several consequences. One of those is the increased amount of reactive oxygen species (ROS) due to differentiation and phagocytosis of macrophages. ROS affects the performance of neural implants in the long-term by increasing corrosion and delamination. Reactive accelerated aging tests (RAAT) are used as a tool to test the durability of bioelectrodes and neural implants, mimicking the presence of ROS in vivo. In RAAT, an H₂O₂ solution with high temperature can mimic several years of implantation during a few weeks due to the increased reaction rate provided by temperature. A RAAT protocol for testing neural implants has been recently approved by FDA.

In this work, we have built a RAAT setup based on this protocol. We have studied different parameters that affect the stability of the system and we have employed RAAT to analyse the durability of different flat and nanostructured metallic layers.

Experimental Methods
The sketch of the RAAT setup is depicted in Fig. 1. The reaction chamber consists of a 750ml solution of H₂O₂ in phosphate-buffered saline (PBS) with a concentration in the range of 10-20 mM. The solution is placed in a double walled jacketed bottle (reaction chamber), kept at constant temperature of 85°C using a water bath. To compensate the H₂O₂ decay, small volumes of highly concentrated (750 mM) hydrogen peroxide are periodically introduced in the chamber using a peristaltic pump controlled by a timer. The excess volume is removed by a second pump programmed by a timer.

To check the concentration of H₂O₂, samples were taken from reaction chamber and tested by means of a classic colorimetric test using titanium (IV) oxyxsulfate sulfuric acid and measuring the absorbance at 410 nm.

To understand the role of the different parameters in RAAT setup, a MATLAB code was developed to calculate the H₂O₂ concentration evolution in the reaction chamber with time.

Results and Discussion
From the different parameters that influence the concentration in the reaction chamber, we found out that the most relevant parameter is the hydrogen peroxide decay time or half-life time (t₁/₂), as it can be seen in the calculation shown in Fig. 2 A and 2B. This t₁/₂ value determines the needed delivery volume of highly concentrated H₂O₂ solution (V_in) to keep the reaction chamber concentration within the desired range. Moreover, for a fixed V_in, the steady state values of concentration reached by the system are determined by t₁/₂. We therefore measured this decay time for H₂O₂ at 85 ºC and obtained a value of 123 min. With this decay time, introducing 1.5 ml of the reservoir each 15 min and collecting the excess volume (V_out) each 4 hours is enough to keep the concentration in the 10-20 mM range (Fig 2B).

Due to the high reaction temperature, there may be small evaporation losses of the solution. We plot in Fig. 2C the influence of a mismatch between delivery and removed excess volumes. According to this, V_in and V_out should be...
equal to reach a stable concentration value: therefore, the losses should be considered and compensated. Moreover, an additional observation was that the initial concentration of the solution has a very small effect on the reached steady state of concentration. This value depends only on the delivery and removed excess volumes (Fig 2D). It is important to remark that the concentration value always fluctuates in a range of at least 2 mM due to delivery and decay time cycles (Fig. 2D, inset).

Finally, we observed that the introduction of implants to be tested could influence the stability of the solution concentration. Once any discrepancies or variations in \(V_{in}\) and \(V_{out}\) were discarded, we attributed this effect to modifications of \(t_{1/2}\) for the degradation of hydrogen peroxide, associated with some catalytic effect of the implant materials (samples introduced in RAAT setup).

**Conclusion**

RAAT is a useful testing method for the durability of materials within a short time, however the different parameters in the setup need to be carefully monitored and adjusted for obtaining stable and reliable results. In general, system destabilizations can be fixed by modification of pump rates and controlling the mismatch between the input and output, as we showed in our MATLAB model. However, we observed unforeseen changes in the decay time of hydrogen peroxide that could be due to the effect of biomaterials on \(H_2O_2\). To solve this problem further in-depth studies are needed.

**Acknowledgement**

Funding from the European Commission (Ref. 793102-NeuPES-H2020-MSCA-IF-2017), Comunidad de Madrid (Atracción de Talento Programme, Modalidad-1 Ref. 2019-T1/IND-1335, project S2018/NMT-4291 TEC2SPACE and YEI contract PEJ-2019-AI/IND-14451 with support from FSE) and MINECO (project CSIC13-4E-1794) is acknowledged.
MATLAB simulations

Figure 2: Calculated evolution of the reaction chamber concentration with time for different parameters of the setup: A) Decay time ($t_{1/2}$), B) Delivery volume ($V_{in}$), C) Excess volume ($V_{out}$), and D) Initial concentration ($C_i$).

References

Design optimization of the mechanical heart valve for a pediatric heart assist pump – RH-PED

Przemysław Kurtyka¹,², Roman Kustosz¹, Artur Kapis¹

¹ Foundation of Cardiac Surgery Development, Artificial Heart Laboratory, Zabrze, PL; ² Silesian University of Technology, Faculty of Biomedical Engineering, Zabrze, PL

Introduction
Mechanical Circulatory Support (MCS) including the Ventricular Assist Devices (VAD) is currently considered to be the most reliable and effective treatment for patients with advanced heart failure (HF). Many new pump constructions have been recently presented. However the clinical experience has provided information that despite many undeniable benefits, VAD constructions still require improvement to minimize the risk of complications. Although the present trend of development of the mechanical circulatory support systems tends towards continuous flow [CF] centrifugal blood pumps, the extracorporeal pulsatile blood pumps are still successfully used. In addition to more physiological flow, the pump due to its placement outside of the body does not require a lot of space in the chest. Therefore, pulsatile blood pumps more often are designed especially for children, due to the increase in the incidence of heart failure in this group. The number of children with heart failure and qualification for transplantation is steadily increasing, the waiting time is usually long and the mortality while waiting for a transplant is high. In Europe, 130-200 operations of this type are carried out annually. In Poland, from 1998 to 2016, a total of 87 heart transplants were performed in group of children. Polish pediatric prostheses are currently at the stage of preclinical research using the sheep model. However the prostheses have undergone laboratory tests and have demonstrated their functionality and safety. Polish pediatric prostheses have treatment options proven in the use of POLVAD heart prostheses still in use for adults.

Experimental Methods
On the basis of the project implemented in 2012-2015, the construction of a family of Polish pediatric heart prostheses (RH-PED) was developed and a preliminary evaluation of the prototype design was performed in laboratory and during experimental tests on animals. Based on the risk analysis of the prototype pediatric system, the design and manufacturing technology of the final sterile clinical product were optimized. In the project four pump constructions were developed and differed in stroke volume: 45ml, 30ml, 20ml, 12ml. Each design required the development of new inflow valves and the optimization of commercially available outflow valves. The optimization of the structure was primarily aimed at suppressing the overloads generated on the drain and valve discs as a result of the flow and minimizing the impact of external forces. The simulations were performed using CFD module in Ansys software as the Fluid-structure interaction (FSI). During calculations a model of a liquid in the form of blood with non-Newtonian properties was used. All simulations were performed for three positions of the mechanical heart valve: open, middle and closed.

Results and Discussion
In the case of the first construction, the obtained results showed that the over-stiffening of the system, which could result in damage to the valve during prolonged work. Two constructions required additional elements made of titanium and polymers of different Shore hardness, allowing for sufficient suppression and load transfer. The final design was selected on the basis on optimization and partner's technological capabilities.

Conclusion
The research confirmed the effectiveness of the presented optimization method. Based on the numerical simulations, it was possible to select appropriate materials for the production of a pediatric prosthesis and estimate the existing stresses.

Acknowledgement
Project no. POIR.04.01.02-00-0073/17 supported by NCBiR
Osteogenic and osteoclastogenic evaluation of strontium substituted Mesoporous Bioactive Glasses

Georgia-Ioanna Kontogianni1,2, Mattia Pagani3, Giorgia Montalbano3, Sonia Fiorilli3, Chiara Vitale Brovarone3, Maria Chatzinikolaidou1,2

1 University of Crete, Department of Materials Science and Technology, Heraklion, GR; 2 FORTH, IESL, Heraklion, GR; 3 Politecnico di Torino, Department of Applied Science and Technology, Turin, IT

Introduction
Osteoporosis, a global health hazard characterized by bone fragility, decrease of bone mass and strength, results in increasing danger of fractures. This disease is the most common among bone pathologies occurring in all ages and races with higher frequency in women over 50 years old and more accurately postmenopausal women [1]. In the field of bone regeneration, considerable attention has been addressed towards the use of mesoporous bioactive glasses (MBGs) due their ability to form a strong chemical bond with bone tissue leading to the formation of new tissue. Moreover, according to the final application, MBG composition can be enriched with specific elements acting as cues to impart a specific biological effect. In particular, the incorporation of therapeutic ions, such as strontium (Sr) ions into MBGs is known to stimulate osteogenesis and bone regeneration potential [2]. In this study, we assessed the in vitro osteogenic and osteoclastogenic potential of Sr-containing MBG by investigating the cell viability, proliferation and differentiation of the pre-osteoblastic MC3T3-E1 cells and human peripheral blood mononuclear cells (hPBMCs), respectively.

Experimental Methods
MBGs with binary SiO2-CaO composition were synthesized using two different methods, the first a base-catalyzed sol-gel procedure (SG) for the production of nanoparticles, and the second based on aerosol-assisted spray-drying (SD) approach for micro-sized particles. The binary composition was further enriched with two different percentages of strontium (4% and 10% mol.) using both production methods, the SG and SD, resulting in four different types of MBGs. The obtained materials were suspended to a final concentration of 200 μg/ml and contacted with pre-osteoblastic and peripheral blood mononuclear cells. The Sr-containing MBGs were evaluated for their in vitro cytotoxicity, osteogenic and osteoclastogenic capacity. MC3T3-E1 pre-osteoblastic cells were used for the investigation of cell adhesion, viability, proliferation and osteogenic differentiation potential of MBGs. Cell viability and proliferation was assessed using the PrestoBlue™ assay. An enzymatic activity assay was used to measure the levels of alkaline phosphatase activity, while the alizarin red staining method was used to quantify the calcium deposition. Collagen secretion was measured to assess the formation of the extracellular matrix. Collagen type I is highly expressed by osteoblasts and involved in the formation of extracellular matrix (ECM) in which mineralization can occur. Real time quantitative polymerase chain reaction (qPCR) was performed to quantify changes in the gene expression of the osteogenesis-related markers ALP, Coll1-a1, osteonectin, osteopontin and osteocalcin.

Human Peripheral Blood Mononuclear Cells (hPBMC) have the capacity to differentiate into osteoclasts after treatment with M-CSF and RANKL, and were used for the assessment of the in vitro osteoclastogenic potential of the MBGs. For this, qPCR experiments were performed to evaluate the gene expression levels of the osteoclastogenesis-related markers DC-STAMP, NFATC1, OSCAR and TRAP.

Results and Discussion
Pre-osteoblasts indicated an excellent cell adhesion and spreading in direct contact with all types of Sr-containing MBGs at the concentration of 200 μg/ml. Increased cell viability and proliferation rates revealed the absence of
cytotoxicity with abundant extracellular matrix formation as evidenced from the levels of collagen secretion and calcium deposition. Increased levels of ALP activity were observed after 3 days in culture for all MBGs types. Osteogenesis-related gene expression was upregulated, with the SG-produced and the 4% Sr-containing MBGs indicating a significantly higher ALP gene expression after 14 days in culture. The results from the osteoclastogenesis-related gene expression did not show an induction of osteoclasts formation.

Conclusion
Micro and nanoparticles of MBGs, with and without strontium in their composition, were assessed for their bone regeneration and resorption potential. All types of MBGs indicated an excellent biocompatibility, promoted the osteoblastic cell maturation, and did not induce the osteoclastogenic potential of the hPBMCs.

Acknowledgement
This project has received funding from the European Union’s Horizon 2020 research and innovation program under grant agreement No. 814410.

References
Miniaturized *In Vitro* Electrical Stimulation Platform

**Sahba Mobini, María Ujué González, Jorge M. García Martínez, José Miguel García-Martín**

*CSIC (CEI UAM+CSIC), Instituto de Micro y Nanotecnología (IMN-CNM), Tres Cantos, ES*

**Introduction**

Bioelectricity facilitates sending and receiving information to/from the body to provide controlling and feedbacking systems to regulate, and monitor biological microenvironment. Also, numerous studies showed electrical stimulation (ES) promotes proliferation, migration and secretion of pro-regenerative factors in the cells and accelerates tissue regeneration, and wound healing. Moreover, ES has a great potential to be adapted as a digital medicine and/or smart therapeutic.

Growing research on the use of ES in biological systems, promotes development of *in vitro* platforms to study the role and applications of ES in contact with cells and tissues. Up to now several *in vitro* ES devices have been established, such as: devices for measuring electrical impedance of cells that is used to evaluate specific biological activities; devices for inducing specific cell responses by means of low voltage ES (e.g., cell migration, differentiation); devices for stimulating and/or recording electrical activity of electroactive cells in CMOS-based multi-electrode arrays (MEAs) and/or multipurpose setups such as lab-on-the-chip. Nevertheless, quality of electrical signals, reproducibility of experiments, increasing of throughput and accuracy of performance of current *in vitro* platforms are yet to be improved. We believe, advances in biomaterials technology, computational modeling, microelectronics, microfabrication, and electrochemistry have opened new doors to meet these needs.

Here we present the evolution of *in vitro* platforms for electrical stimulation and recording for different proposes and introduce our innovative device for low voltage direct electrical stimulation. We discussed design, fabrication and characterization of this platform and its benefits for conducting high throughput and controlled experiments within a standard cell culture vessel.

**Experimental Methods**

We designed and developed a miniaturized *in vitro* device for electrical stimulation (MiDES) of cells and tissues. MiDES is illustrated in Figure 1 A and B. The universal frame and the top lid are fabricated by means of 3D-printed polyether ether ketone (PEEK). The cell culture chamber is fabricated from SYLGARD™ 184 silicone elastomer to be detachable that provides convenient postprocessing for multiple tests. We fabricated platinum electrodes by means of sputter coating and connected them to the printed circuit board (PCB), designed for MiDES, by means of conductive epoxy (EPO-TEK H20E). PCB will be connected to a function generator with automatized or manual operation options. We measured impedance of the system and used this data for defining accurate signal form and intensity in MiDES during stimulation.

**Results and Discussion**

MiDES is designed to be compatible with slid-chamber cell culture systems such as Millicell® EZ SLID manufactured by Millipore. PCB provides 10 channels that are connected to a jumper connector and allows independent electrical delivery in each well. Therefore, each cell culture well can be stimulated separately or simultaneously. Each well supports 200 to 700 µl of culture medium and provides sufficient space for 2D or 3D culture. Live microscopy assessment can be done during electrical stimulation since the surface for cultured cells is made of a high-quality coverslip (0.17mm) attached to the bottom of the universal frame, Figure 1 C. MiDES also designed to be stackable Figure 1 D. Although there are several designs for *in vitro* setups, the majority of these experiments have been conducted without considering the electrochemical characteristics of electrodes and studying the role of electrode material in the quality of delivered electrical signal, and unwanted electrochemical reactions. Therefore, previously...
we run a full study and picked Pt electrodes among other metals (Au and Ti) to be incorporated to MiDES. We acquired electrochemical properties of MiDES system and used them to define amount signal filtration (The study on materials and signal filtration is reported separately).

Conclusion
A miniaturized in vitro device for electrical stimulation of cells is designed and developed. This patented technology will benefit in vitro electrical stimulation research to conduct multiple reproducible controlled experiments within a standard cell platform.

Acknowledgement
Funding from the European Commission (Ref. 793102-NeuPES-H2020-MSCA-IF-2017), and Comunidad de Madrid (Atracción de Talento Programme, Modalidad-1 Ref. 2019-T1/IND-1335) is acknowledged.

References
**If life gives you nanoparticles, screen them with zebrafish**

*Beatriz Custódio*<sup>1,3</sup>, Joana F. Marques<sup>2</sup>, José Bessa<sup>2</sup>, Sofia D. Santos<sup>1</sup>, Ana P. Pêgo<sup>1,4,3</sup>

<sup>1</sup> University of Porto, INEB/i3S, Porto, PT; <sup>2</sup> University of Porto, IBMC/i3S, Porto, PT; <sup>3</sup> University of Porto, ICBAS, Porto, PT; <sup>4</sup> University of Porto, FEUP, Porto, PT

**Introduction**

Stroke affects around 14 million people each year, being one of the major causes of death and long-term disability in the world, with a huge economic and social impact. Stroke survivors remain with major disabilities since there are no therapeutic options to protect neurons and promote regeneration. Therefore, there is an urgent need to develop new therapies to mitigate the extension of stroke damage.

Nanotechnology has shown potential to address these challenges, since nanobiomaterials are promising delivery platforms of therapeutics to the brain, increasing their blood stability and brain bioavailability. Despite this, the complexity of traditional *in vivo* models, often impairs the feasibility and success of pre-clinical studies, contributing to the low number of therapeutic molecules that reach the clinics.

Currently, zebrafish is classified as a complementary animal model to the classic mammalian ones. Its widespread acceptance for drug screening recently increased due to the high genome similarity to human’s, high-fertility, low-cost and easy maintenance. Here we describe the establishment of a global ischemic model by oxygen deprivation of zebrafish larvae, as well as an adaptation of a stroke model in the adult fish.

**Experimental Methods**

Zebrafish larvae (10 days post fertilization) were exposed to oxygen deprived medium. Larvae transparency allows to confirm the compromised blood-brain barrier after dye injection using up-to-date bioimaging techniques. Also, the cerebral damage extension after hypoxia is being assessed *in vivo* by motor patterns monitoring through medium-throughput behavior assays and using different neurodegeneration assays.

Also, a focal ischemic model attained by photo-thrombolysis in adult zebrafish, close to what happens in clinics, is being optimized. Neuronal apoptosis and neurodegeneration after ischemia is under analysis. Neurological outcome analysis is also being evaluated by monitoring behavior.

**Results and Discussion**

Bioimaging techniques of the larval zebrafish model, using light-sheet microscopy, ensued an accurate 3D reconstruction of the larvae brains injected with the dye (Figure 1). Also, scanning confocal light microscopy allowed the determination of the nanocarriers biodistribution. Due to the high fertility rate of zebrafish, low-cost larvae maintenance and fast imaging technologies, a high number of animals can be used, increasing the screened nanobiocarriers and the statistical power of the outcomes.

Pilot experiments performed in the focal ischemic model to determine the infarcted area using X-Ray micro computed tomography (micro-CT), showed the possibility to clearly visualize the brain using several contrast agents (Figure 2). The neurodegeneration in the affected area has been determined by immunofluorescence assays, with apoptotic and neuronal markers, showing neuronal loss close to the occlusion site.

**Conclusion**

Overall, these models are expected to open new avenues towards the assessment of the most promising and recent nanobiocarriers to deliver neuroprotective agents after an ischemic stroke. In the larval model, a medium-throughput screening will additionally permit to analyze safety, biocompatibility, biodistribution of the nanobiomaterials. Furthermore, the pharmacokinetics and neuroprotective effects of the studied nanoformulations will be validated in a focal ischemic model using adult zebrafish.
Acknowledgement
This study has been funded by the projects NORTE-01-0145-FEDER-000008, INFARMED (FIS-2015-01_CCV_20150630-88), i3S (POCI-01-0145-FEDER-007274), siRNAC (NORTE-01-0247-FEDER-033399) and by FCT FCT (PTDC/NAN-MAT/30898/2017; SFRH/BD/145652/2019 and Norma Transitória – DL 57/2016/CP1360/CT0013).
The authors would like to acknowledge the support of the i3S Scientific Platforms: Bioimaging and Advanced Light Microscopy, members of the PPBI (POCI-01-0145-FEDER-022122).

Figure 1
Live imaging of zebrafish larvae head using light-sheet microscope (3D reconstruction). 4 days post-fertilization zebrafish was systemically injected with a fluorescent dye (Evans blue) to visualize the brain vessels.

Figure 2
Micro-CT imaging in adult zebrafish. WT adult zebrafish without induction of focal ischemic not stained (A) and stained with osmium tetroxide (B) and PMA (C) in a coronal (COR), transversal (TRA) and sagittal (SAG) views.
PS2-02-104

Tensile model through the finite element to obtain the parameters of the mechanical properties of the porcine earlobe tissue.

Hilda Aguilar-Rodriguez, Javier Ortiz-Ortiz, Miguel Villagomez-Galindo, Marco A. Espinosa-Medina, Georgina Carbajal-de-La-Torre

UNIVERSIDAD MICHOACAN DE SAN NICOLAS DE HIDALGO, Facultad de Ingeniería Mecánica, Morelia, Michoacán, MX

Introduction
The skin is a highly complex biological tissue, due to its specific physical characteristics and mechanical properties. For this investigation, because of its similarity to the human, the porcine skin samples were taken from the earlobe since it is a soft area. And they were taken to a tensile test, to obtain the mechanical parameters of the tissue. Due to its mechanical behavior seen, it was chosen by hyperelastic models that help explain its mechanical behavior.

Experimental Methods
With Comsol® Multiphysics, the skin was characterized, which the constitutive behavior was adjusted to the curve and the parameters of the Ogden model for engineering stress. Based on the ASTM D638 standard, a mold was designed for a tensile model for finite element analysis.

Results and Discussion
With these results, it can be discerned that the Ogden model can determine the nonlinear behavior of the skin concerning a traditional mechanic model, likewise, using the parameters of said model a stress analysis is performed in Comsol® Multiphysics replacing traditional models of material behavior in finite element.

Conclusion
The Ogden model is a hyperelastic model that allows determining the non-linear behavior of the skin in a more punctual manner. These results will help the development of biomechanical models of the finite element that explain the behavior of the skin and reduce the analysis in-vivo models of biological tissue.

Acknowledgement
The authors would like to thank financial support from the Conacyt Project CB-2014-243236.
2:00 p.m. – 3:30 p.m.

Poster floor

**PS2-03 | Biomaterials for Tissue Engineering**
PLA 3D scaffold to improve bone reconstruction

Aurora F. Pérez Jiménez¹, Ana Monteiro¹,², Erwan Plougonven², Jérôme Hurlet¹, Christian Grandfils¹

¹ Interfaculty Research Center of Biomaterials (CEIB), University of Liège, Liège (Sart-Tilman), BE; ² Department of Chemical Engineering, University of Liège, Liège (Sart-Tilman), BE

Introduction

Three-dimensional (3D) printing, also known as additive manufacturing, is a digital fabrication process used to produce 3D objects, printing layer by layer with different materials. 3D printing has become very popular in tissue engineering due to the ability to mimic complex tissue architectures and seed them with different cell types. Considering the increasing need for new bone grafts in clinical orthopedics, a 3D scaffold mimicking natural structure features can help to improve bone reconstruction.

For the development of successful biomimetic scaffolds, the microstructure and porosity are very important to promote cell colonization and guarantee their viability. For this reason, three different scaffolds with different architectures and designs have been printed to evaluate how the pore size could affect bone reconstruction.

Experimental Methods

The scaffolds have been manufactured layer by layer using a Prusa I3 MK3 printer with a nozzle diameter of 0.2 mm. The scaffolds have been printed using an aliphatic biodegradable and biocompatible polymer called poly (lactic acid) (PLA). Micro-computed tomography (CT) was used to analyze the resulting 3D prints, its porosity and the pore sizes. Their mechanical features were assessed under static mechanical compression (Lloyd equipment).

Results and Discussion

Scaffolds have been designed to comprise an interconnected network, one of them with a uniform pore size and the two others with variable size and anisotropic characteristics. After 3D reconstruction of the CT scans, the three scaffolds showed similar porosity around 66% and the volume of each scaffold around 3200 mm³. The uniform pore scaffold exhibited a pore size between 1.007-0.975 mm³, the anisotropic pore scaffold presented pore size between 0.840-0.728 mm³ and the gradual pore scaffold showed pore size between 0.642-0.569 mm³.

Conclusion

This exploratory study has highlighted the potentialities of additive manufacturing using thermal deposition to adjust the pore size network within 3D scaffold tailored for bone reconstruction. Playing on the pattern of filament distribution for each layer has given rise to 3D objects with controlled pore size range and spatial distribution, while keeping constant the total porosity and maintaining their connectedness. Future efforts will start with in vitro evaluation before considering any preclinical trials.

Acknowledgement

Ana Monteiro would like to thank the Belgian National Funds for Scientific Research (F.R.S.-FNRS) for their Post-doctoral position. The authors also acknowledge Interreg Great Region and Wallonia for the financial support of ImproveStem Project. Aurora Perez Jimenez would like to thank the European Region Action Scheme for the Mobility of University Students for their ERASMUS + internship grant.

References


Bioengineering cytocompatible 3D printed capillary-like multilayered structures embedded in hydrogels for vascular tissue engineering

João Borges, Catarina A. Saraiva, Cristina F. Sousa, Tamagno Pesqueira, Tiago R. Correia, Maria I. Rial-Hermida, Sónia G. Patrício, João F. Mano

University of Aveiro, Department of Chemistry, CICECO - Aveiro Institute of Materials, Aveiro, PT

Introduction

Bioengineering highly organized prevascularized networks to support the growth of vascularized functional tissues is a critical unmet clinical challenge that has been gathering tremendous attention by the medical, materials science and engineering communities aiming to better emulate and repair damaged tissues [1]. In particular, the emergence of the bottom-up approaches has opened new and exciting routes to rationally design and develop robust, complex, highly organized and biofunctional 3D vascular tissue-like constructs for pursuing advanced regenerative therapies [2]. Among them, the Layer-by-Layer (LbL) assembly has proved to be highly versatile technology to functionalize any type of surface and precisely engineer highly hydrated and hierarchical extracellular matrix (ECM)-mimetic biomaterials, with fine-tuned structures, properties and functions at the nanoscale, by resorting to a myriad of biological components exhibiting complementary interactions [3]. However, the lack of a carrier platform that could encapsulate, protect, and endow the developed biomaterial structures with superior biomechanical stability to be administered in the human body via minimally invasive procedures extensively limits the use of LbL technology standalone. In this regard, hydrogels are the most promising and customizable 3D biomaterial platforms for cell encapsulation, controlled therapeutics delivery, and construction of 3D tissue-like constructs embedded with 3D printed perfusable tubular structures recreating vascular tissues owing to their numerous appealing features [4,5].

Herein, we report the bioengineering of advanced bioactive and hybrid 3D cell-biomaterial constructs encompassing (i) cytocompatible 3D printed capillary-like multilayered structures embedded in (ii) photopolymerizable natural-origin hydrogels to promote the formation of tissue-mimetic vascularized networks in vitro and be potentially used as injectable systems in tissue engineering strategies.

Experimental Methods

The build-up of electrostatic-driven bioactive multilayered thin films encompassing alternate layers of biocompatible oppositely charged chitosan (CHT) and arginine (R)-glycine (G)-aspartic acid (D)-grafted alginate (ALG-RGD) biopolymers was firstly monitored in situ by quartz crystal microbalance with dissipation monitoring (QCM-D). Following the optimal assembling conditions gathered in the QCM-D experiments, similar (CHT/ALG-RGD)n multilayers were assembled on top of 3D printed ALG microfibers. Then, either uncoated or LbL coated ALG fibers were embedded in a methacrylated xanthan gum (XGMA) pre-hydrogel solution to develop mechanically robust 3D constructs after crosslinking with UV light, as confirmed by rheological measurements. The constructs were further immersed in a EDTA aqueous solution to obtain hollow microtubular structures in which human umbilical vein endothelial cells (HUVECs) were cultured for 3 days aiming to control cell functions (Live/Dead assay).

Results and Discussion

ALG microfibers were successfully produced by 3D printing and further LbL coated with six CHT/ALG-RGD bilayers, as revealed by fluorescence microscopy by labelling CHT with rhodamine B isothiocyanate. The encapsulation of the bioactive LbL functionalized ALG microfibers within biocompatible photocrosslinkable XGMA hydrogels of the desired size and geometry, and formation of liquified multilayered microtubes that replicate vascular tissues was accomplished by EDTA-induced chelation of the ALG fiber template. The formation and perfusion extension of the hollow microchannels was unveiled by the naked-eye visible flow of a fluorescent dye aqueous solution injected.
through the microchannel across the hydrogel. With the aim of replicating the vasculature of native tissues, HUVECs were seeded in either (CHT/ALG-RGD)₆ LbL functionalized microchannels or LbL-free microchannels, both embedded into XGMA hydrogels, and the in vitro cell viability assessed after 3 days by fluorescence microscopy, revealing a higher number of viable cells in the LbL coated microchannels.

**Conclusion**

Robust, bioactive and hybrid 3D constructs were successfully bioengineered by embedding cytocompatible LbL functionalized 3D printed ALG microfibers into photocrosslinkable XGMA hydrogels, which provided biomechanical stability and support to the whole construct. The ALG sacrificial core was further liquefied into hollow microtubular structures having the capacity to withstand flow and retain the original size and geometry of the fibers, aiming to recreate native vascular tissues. HUVECs viability within the hollow multilayered microtubes was assessed by a Live/Dead assay after 3 days, revealing a higher number of live cells in comparison with LbL-free hollow microtubes. This work holds great promise to surpass the major limitation associated with the absence of the formation of a prevascularized functional network on the developed 3D biomaterials to repair damaged tissues.

**Acknowledgement**

This work was supported by the Programa Operacional Regional do Centro – Centro 2020, in the component FEDER, and by national funds (OE) through FCT/MCTES, in the scope of the project “SUPRASORT” (PTDC/QUI-OUT/30658/2017, CENTRO-01-0145-FEDER-030658), as well as by the Marine Biotechnology ERA-NET project “BLUETEETH” (ERA-MBT/0002/2015), funded by FCT under the European Commission’s Seventh Framework Programme (Grant Agreement no. 604814). J. Borges acknowledges FCT for the individual Assistant Researcher contract (2020.00758.CEECIND). This work was developed within the scope of the project CICECO – Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through FCT/MCTES.

**References**


Bioorthogonally Cross-Linked Laminin-Functionalized Hyaluronan Hydrogels for 3D Neuronal Cell Culture

Michael Jury1, Isabelle Matthiesen2, Robert Selegård1, Anna Herland2,3, Daniel Aili1

1 Linköping University, Laboratory of Molecular Materials, Division of Biophysics and Bioengineering, Department of Physics, Chemistry and Biology, Linköping, SE; 2 KTH Royal Institute of Technology, Division of Micro and Nanosystems, Stockholm, SE; 3 Karolinska Institute, AIMES, Center for Integrated Medical and Engineering Science, Department of Neuroscience, Stockholm, SE

Introduction
Laminins are central for neuronal function and is closely associated with neuronal development, affecting e.g. the direction of neurite growth and stabilization of neuronal synapses in vivo. Laminin coated substrates are thus widely used for in vitro culture of neurons to promote neuronal attachment, viability and network formation. The use of laminin-rich biologically derived extracellular matrix (ECM) based hydrogels can provide more native-like three dimensional (3D) cellular microenvironments but suffer from poor reproducibility and difficulties in tailoring the properties of the hydrogels. Engineered hydrogels for 3D cell culture can offer better reproducibility and possibilities to control composition and stiffness and can enable development of sophisticated tissue models. Presentation of laminins in 3D hydrogels for providing optimal conditions for neuronal cell culture can, however, be challenging. Most engineered hydrogel systems thus resort to laminin-derived peptides, which can be incorporated and conjugated to various polymers. Here, we show a new means of producing very tunable and stable ECM mimicking hydrogels for 3D neuronal cell culture by using full sized and covalently conjugated laminin-521 using bioorthogonal copper-free click chemistry.

Experimental Methods
Two different variants of azide were conjugated to human recombinant laminin 521 (Biolamina, Sweden) by carbodiimide coupling to lysine residues in the laminin. Bicyclo[6.1.0]nonyne (BCN) was grafted to hyaluronic acid (HA) via the glucuronic acid carboxyl-moieties. The final HA-BCN product had a derivatization degree of about 19%. Hydrogels were formed by strain-promoted alkyne-azide 1, 3-dipolar cycloaddition (SPAAC) by first combining the HA-BCN product in cell media with the azide-functionalized laminin and then incubated at 37°C for one hour to create laminin-functionalized HA-BCN. Then SH-SY5Y neuroblastoma cells or human induced pluripotent derived neuroepithelial cells (NES) were suspended in cell media containing 8-arm poly(ethylene glycol) (PEG) with terminating N3-moieties (p(N3)8). The components were then combined and incubated at 37°C and 5% CO2 for one hour prior to adding cell media.

Results and Discussion
HA-based hydrogels were formed by cross-linking with multi-arm-PEG using SPAAC. An excess of BCN groups were included in HA for conjugation of azide modified laminin-521 prior to addition of cells and p(N3)8. The storage modulus of the hydrogels was about 0.3 and 0.7 kPa at 1% (w/v) to 2% (w/v), respectively, and did not change significantly when including laminin (100 µg/ml). Functionalization of laminin with azide moieties (Figure 1a) promoted retention of laminin within the hydrogel. The release was negligible for the higher hydrogel concentration when using a short PEG linker between the laminin and the azide. Softer hydrogels and a shorter linker led to higher laminin release (Figure 1c). Scanning electron microscopy (SEM) showed that increasing the concentration of the hydrogel components from 1% (w/v) to 2% (w/v) produced a marked difference in the hydrogel structure with thicker pore walls in the latter (Figure 2a). The hydrogels supported proliferation of both undifferentiated and differentiated SH-SY5Y cells and NES, but no significant effect of laminin was seen in any of the conditions. Confocal microscopy of SH-
SY5Y in hydrogels with Cy5-labeled laminin indicate that the cells remodel the hydrogels and accumulate the laminin in their immediate vicinity after 24 hours of cell culture.

**Conclusion**

Here we show a flexible hydrogel system that enables integration and retention of recombinant laminin for neuronal 3D cell culture. Bioorthogonal copper-free click-chemistry was used for both cross-linking of the hydrogels and for conjugation of laminin. This approach enables fabrication of well-defined ECM mimicking materials that both ensures efficient cell encapsulation and supports neuronal proliferation.

**Figure 1**

a) The two variants of linkers used for conjugation of laminin. b) Storage ($G'$) and loss ($G''$) modulus (Pa) of the hydrogels. c) Cumulative release of laminin from the hydrogels over time.

**Figure 2**

a) SEM of functionalized hydrogels. b) Microscopy images of SH-SY5Y cells in functionalized hydrogels after 24 hour incubation, showing, left to right, merged image, f-Actin staining, cy5-labelled laminin and co-localization image created from previous two. Scale bar represents 100 µm.
How sintering temperature influences the physicochemical properties of tricalcium phosphate (TCP) scaffolds?

**Szymon Skibiński, Ewelina Cichoń, Joanna Czechowska, Anna Ślósarczyk, Aneta Zima**

**AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Kraków, PL**

**Introduction**

Biomaterials designed for bone regenerative medicine include three-dimensional porous scaffolds, which provide support for bone cells and thus enable the reconstruction of damaged tissues. Such materials should meet a number of requirements, for instance in terms of their phase composition, adjustment of mechanical properties to replaced tissues, porosity and appropriate pore architecture as well as biocompatibility. Among the materials used as bone substitutes, tricalcium phosphates (α-TCP, β-TCP) have unique properties that distinguish them from other compounds [1,2]. Therefore, the aim of this work was to prepare TCP-based scaffolds by a polyurethane sponge replica method with appropriated porosity, interconnectivity and mechanical strength for application in as scaffolds tissue engineering.

**Experimental Methods**

In this study, ceramic TCP-based scaffolds were prepared by a polyurethane sponge replica method. Initial β-TCP powder used for preparation of ceramic slurries was synthesized via a wet chemical method. Materials were sintered in conventional furnace at four temperatures: 1100°C, 1150°C, 1200°C and 1250°C. Influence of sintering conditions on physicochemical properties of the scaffolds was determined (phase composition, microstructure, porosity and mechanical strength). Additionally, chemical stability was evaluated in vitro.

**Results and Discussion**

XRD analysis revealed that the materials sintered at 1100°C and 1150°C consist of only one crystalline phase i.e. β-TCP meanwhile in scaffolds sintered at 1200°C and 1250°C the second phase i.e. α-TCP was noticed. Additionally, changes in the microstructure were observed in terms of shape and size of grains. In the samples sintered at temperatures above 1150°C the microcracks were observed (Figure 1). All materials possessed high total porosity (~60 vol%). Interestingly, sintering temperature did not affect significantly the compressive strength of the obtained scaffolds (from 3.2 ± 0.8 MPa to 3.9 ± 1.1 MPa). The investigated materials were chemically stable, pH of the simulated body fluid solutions were close to the physiological conditions.

**Conclusion**

It was found that the sintering temperature in the range from 1100 to 1250 °C influences on the phase composition, microstructure, porosity and the average grain size of the obtained materials. Scaffolds sintered above 1150 °C consisted of two crystalline phases, i.e. β-TCP and small amounts of α-TCP. All materials possessed similar total porosity. The best compressive strength (3.9 ± 1.1 MPa) was achieved by the materials sintered at 1150 °C. In the case of scaffolds sintered in 1200 and 1250 °C, besides of the β-TCP → α-TCP polymorphic transformation, there was no significant loss on mechanical properties. Moreover, all studied materials are chemically stable and do not show any significant pH fluctuations during 28 days of incubation in simulated body fluid. Due to favourable physicochemical properties and expected biocompatibility developed materials seem to be prosperous candidates for cancellous bone tissue engineering.

**Acknowledgement**

Research funded by the National Centre for Research and Development, Poland, grant Techmatstrateg no. TECHMATSTRATEG2/407507/1/NCBR/2019 and by the Faculty of Materials Science and Ceramics AGH UST -
University of Science and Technology, Krakow, Poland, Project No. 16.16.160.557 (2021). SS has been partly supported by the EU Project POWR.03.03.00-IP.08-00-P13/18 - PROM NAWA

![Figure 1: Microstructure of TCP scaffolds](image)

**References**


Composite electrospun fibers for tissue engineering and wound healing applications

Elisa Piatti¹, Marta Miola¹, Liliana Liverani², Aldo R. Boccaccini², Enrica Verné¹

¹ Politecnico di Torino, Department of Applied Science and Technology (DISAT), Torino, IT; ² University of Erlangen-Nuremberg, Department of Materials Science and Engineering, Erlangen, DE

Introduction
Tissue engineering (TE) and regenerative medicine are alternative therapeutic approach to organ and tissue transplants towards the repair and regeneration of diseased or damaged living human tissues. Various strategies are available for the development of scaffolds as temporary templates for tissue regeneration, providing a supporting structure for cell proliferation and differentiation. In this context, the synthesis of composite materials merging the properties of flexibility of polymers and bioactivity and rigidity of ceramic materials such as bioactive glass (BG) particles is of interest.

Among others, the electrospinning is widely used for the fabrication of micro- and nanofibrous mats, because of the versatility of this technique and the advantageous properties of the electrospun fibers, such as fibrous structure, tuneable size (in the range of sub-micrometers down to nanometers), high aspect ratio, high surface area and high porosity with interconnected pores, easy incorporation of functional components (like drugs, genes, enzymes, living cells, etc.), that allow to physically mimic the ECM of many human tissues.

Experimental Methods
Novel B- and Cu-doped bioactive glass nanoparticles (62 wt% SiO₂ - 15 wt% B₂O₃ - 9 wt% CaO - 9 wt% P₂O₅ - 5 wt% CuO) were synthetized using sol-gel methods. Composite electrospun fibers were obtained by the incorporation of these particles inside poly-ε-caprolactone (PCL) mats, electrospinning a solution of PCL, acetic acid and BG particles after a careful process optimization. EM-EDS, DLS, BET, FTIR, XRD and bioactivity test were performed on the synthetized bioactive glasses, whereas SEM-EDS, FTIR, contact-angle measurements, degradation test, mechanical test and bioactivity test were carried out on the composites.

Results and Discussion
Bioactive nanosized round-shaped particles were obtained and successfully incorporated in PCL electrospun mats. The synthetized composite fibers were indeed very rich of glass as desired, although the presence of some glass particles clusters, resulting in uneven distribution within the polymeric matrix, in agreement with previous results. The in vitro acellular bioactivity test showed a good bioactivity of both bioactive glasses (since the first days of immersion in SBF) and the fabricated composites, as revealed by the increased amounts of phosphorus and calcium during immersion in the simulated fluid solution (SBF) and the deposition of HA-like crystals on the composite surface.

All other tested properties (mechanical properties, wettability and degradation) were satisfying, but sometimes affected by the presence of glass powder clusters. The proliferation of bone murine stromal cells (ST-2 cell line) was not inhibited, confirming that the synthetized composites were nontoxic and promising for potential TE applications.

Conclusion
Because of the angiogenic and antibacterial properties of the doping ions (boron and copper), these glasses are considered novel promising angiogenic and antibacterial agents.
The synthetized composites electrospun fibers showed good biocompatibility and bioactivity and, considering their potential angiogenic and antibacterial properties (to be examined in future work), they are considered adequate for soft TE applications.

References
Chemically-modified Gellan-Gum as composite and bioactive bioink for the design of 3D printed scaffolds for bone tissue engineering

Ugo D’Amora¹, Alfredo Ronca¹, Stefania Scialla¹, Alessandra Soriente¹, Maria Grazia Raucci¹, Paola Manini², Alessandro Pezzella³, Luigi Ambrosio¹

¹ National Research Council, Institute of Polymers, Composites and Biomaterials, Naples, IT; ² University of Naples Federico II, Department of Chemical Sciences, Naples, IT; ³ University of Naples Federico II, Department of Physics “E. Pancini”, Naples, IT

Introduction
Over the past years, the linear anionic hetero-polysaccharide gellan-gum (GG) is emerging as a promising noncytotoxic biopolymer for bone tissue engineering (BTE)¹. Among its advantages, it is worth noting that GG is a natural, biodegradable, and hydrophilic polymer, which exists in abundance. Furthermore, the water-based process does not require the use of harsh chemicals. The primary structure of GG is composed by repeating units of glucose, rhamnose, and glucuronic acid. The secondary structure changes upon the temperature from a random coiled form, at high temperature, to a double-helix form, at room temperature. This peculiar transition allow producing hydrogels from GG by simply decreasing the temperature. However, the mechanical performances as well as the stability are still the limiting issue, especially considering its application as bioink for 3D printing. In particular, bioinks should be able to: i) be extruded through a thin needle, ii) retain a 3D structure and iii) create a cytocompatible environment, suitable for embedding living cells².

To this aim, GG was chemically modified in order to add functional moieties and making possible a further cross-linking of the hydrogel by UV light using a photo-initiator. Furthermore, the material was enriched by incorporation of hydroxyapatite nanoparticles (HAp) obtained by sol-gel method and eumelanin (EU) extracted from natural fonts. On one hand, HAp has widely demonstrated to be a bioactive signal towards osteogenic induction, owing to the synergic contribution of its inherent biomimicry and improved mechanical properties. On the other hand, a few studies have recently suggested that EU could promote osteoblast differentiation, inhibit osteoclast differentiation, strengthen the bone, and increase alkaline phosphatase activity and bone mineralization³.

Experimental Methods
GG (Gelzan™, Sigma Aldrich) was chemically modified by the reaction with methacrylic anhydride (Sigma Aldrich) to produce a photocrosslinkable derivative (GGMA) with a shear thinning behavior and tunable mechanical properties. The materials were fully investigated in terms of physico-chemical properties to validate the success of the functionalization. The bioprinting was performed using a 3D printer “In vivo Rokit” (Rokit Healthcare Inc.). Different formulations of biocomposites based on GGMA (2 and 4 %wt/v) and bioactive components, as HAp and EU, were developed. HAp were added at a concentration of 10 and 30 %wt referred to GGMA amount while, based on the cytocompatibility data, EU was added at a concentration of 0.3 %wt/v. The morphology of the 3D structures was investigated by scanning electron microscopy (SEM). The mechanical properties of the scaffolds were tested by dynamic mechanical analysis. The cytocompatibility was assessed by using murine fibroblast cell line according to ISO guidelines, meanwhile human mesenchymal stem cells were used to evaluate the differentiation in osteogenic lineage. The cell proliferation was assessed by confocal microscopy and Alamar blue assay. To evaluate the osteoinductive properties of 3D materials, the expression of some osteoblast-related markers, including alkaline phosphatase, type I collagen, osteocalcin and osteonectin were analyzed.

Results and Discussion
The methacrylation of GG was confirmed by the presence of carbon double bond peak at 1640 cm\(^{-1}\), known to be present in methacrylate groups but not in GG chains, as shown by Attenuated total reflect Fourier transform infrared spectra. The three different structures (GGMA, GGMA/HAp and GGMA/EU) were successfully 3D printed with a good shape fidelity, proving to be able to maintain their structure without collapse in the Z-direction. A homogenous porosity with lateral pores clearly visible was confirmed by SEM. Mechanical properties of GGMA/HAp scaffolds showed values of the storage modulus generally higher than GGMA scaffolds. Furthermore, the nanoparticles improved the nanoroughness and bioactivity. Biological analyses demonstrated a difference of cell attachment and proliferation among the different groups. In particular, the presence of bioactive signals (HAp and EU) seemed to stimulate bone tissue formation.

**Conclusion**

Here, 3D well-organized structures were produced by 3D bioprinting. The obtained results proved the potential use of these materials as bioinks for BTE. The scaffolds showed enhanced mechanical, morphological as well as good biological properties.

**Acknowledgement**

The authors would like to thank “SAPIENT – A system approach for identifying connective tissue degeneration in diabetic analogues”, Grant N° 2017CBHCWF for financial support.

**References**


Influence of pore size, morphology and fibroin molecular weight on 3D silk fibroin scaffolds

Melisa Kafali¹, Yiğithan Tufan¹, Batur Ercan¹,²,³

¹ Middle East Technical University, Metallurgical and Materials Engineering, Ankara, TR; ² Middle East Technical University, Biomedical Engineering, Ankara, TR; ³ METU Center of Excellence in Biomaterials and Tissue Engineering, BIOMATEN, Ankara, TR

Introduction

Controlling pore size, pore morphology and molecular weight distribution of silk fibroin (SF) are important for the production of 3-D scaffolds which is the crucial framework for the cell cultures.[1] For changing pore size and morphology of the scaffold, SF concentration, cooling temperature and cooling rate can be changed, and also a secondary phase can be added into the scaffold to obtain various pore sizes [2]. For instance, SF scaffolds having 1wt. % SF concentration are synthesized with 250-300µm pore size while using 5 wt.% SF concentration reduces pore size to 80-150µm [2]. Furthermore, the molecular weight distribution of SF can be altered by alkaline hydrolysis or lithium bromide salt – SF interaction [3]. In the study of Kim et al, the molecular weight distribution (MW) is altered between 263.1 – 82.7 kDa by regulating the hydrolysis time(10 - 180 min). Here, SF scaffolds are synthesized by changing distribution of MW and tuning the pore architecture to optimize mechanical, rheological, morphological, and biological properties. Three different processing parameters used in this study are dissolving time, cooling rate, and cooling temperature.

Experimental Methods

Bombyx mori cocoon shells were cut into small pieces to extract fibroin protein inside boiled 0.02M sodium carbonate solution. SF was primarily dissolved in 11M lithium bromide solution for 4h and 48h, then dialyzed against distilled water for 3 days. Afterward, dialyzed SF solution was frozen at -20°C and -80°C and lyophilized to obtain a porous scaffold and to examine further characterization. The metabolic activities of the osteoblast (ATCC – CRL 11372) at a cell density of 20.000 cells/cm² were analyzed using MTT assay. The cross-sectional morphologies of the SF scaffolds were investigated using FEI Nova Nano SEM 430 microscope. The secondary crystalline structures of the SF scaffolds were analyzed using FTIR and confirmed with XRD analysis. Mechanical properties of the scaffolds were measured based on ASTM 683D standard with Instron 5565A, Norwood, MA.

Results and Discussion

The altered parameters for SF scaffold production could divided into two groups: those that affect the MW and those that control pore morphology. Firstly, the parameter that affects MW distribution of SF was dissolution time. To generate SF solution, strong hydrogen bonds of SF were destroyed in high ionic strength salt solution (11M lithium bromide) for 4h and 48h. During this step, the MW distribution of SF was altered, and thus, the viscosity of SF solution was affected. For instance, dissolution in lithium bromide for 48h at 60°C resulted in higher MW distribution and viscosity than dissolution in lithium bromide for 48h at 60°C. In FTIR and XRD tests, the methanol-treated SF scaffolds had stronger intermolecular beta-sheet crystalline structure than the extracted and not methanol treated ones. Secondly, the parameters that affected the distinct pore morphology of SF scaffolds were cooling rate and cooling temperature. Freezing the dialyzed SF solution in isopropanol at -20°C resulted in a circular pore morphology (99.39 ± 6.66 µm). When the same process is repeated at -80°C, a lamellar-like pore morphology (266.33 ± 89.19 µm) is obtained as shown in Figure 1a-b. Pore characteristic was important in the design of SF scaffolds to provide enough space for cell migration and transportation of waste and nutrition with the surrounding environment. The osteoblast cell culture that have been prepared by using circular pore scaffolds (at -20°C in isopropanol) showed
better cell proliferation and cell migration compared to the one that have been prepared by using lamellar-like pore scaffolds (at -80°C in isopropanol) (Figure 1c). In the swelling test, since smaller pore size limits infiltration, swelling is slower in scaffolds that have been synthesized at -20°C with isopropanol. Thus, with one being 4 h⁻¹ and the other being 7.830 h⁻¹, the swelling rate is almost 2 times for the larger pore size specimen, complying with Schotts second order diffusion kinetic model. In the uniaxial tensile test, circular pore shape scaffold was resulted in 242± 39.2 kPa tensile strength while lamellar-like pore shape scaffold was reached to 167± 22.87 kPa tensile strength as shown in Figure 1d.

Conclusion
In the field of tissue engineering, SF is an important natural, proteinaceous material due to its biocompatibility, tunable degradation characteristic and excellent mechanical properties. These features of SF scaffolds are related to scaffold fabrication parameters. By altering SF is dissolving time in LiBr, it was possible to control molecular weight and secondary structure of SF and altering freezing temperature and cooling rate it was possible to control size and distribution of pores. Our preliminary results showed enhanced cellular viability and mechanical properties on scaffolds having globular-shaped pores. Thus, scaffolds SF having globular pore morphology should be further studies for tissue engineering applications.

Acknowledgement
This work was financially supported by The Scientific and Technological Research Council of Turkey (Grant no:118M652).

References

Fabrication of Methacrylate Hyaluronic Acid scaffolds with axially oriented pores

Paola Nitti, Marta Madaghiele, Christian Demitri

University of Salento, Department of Engineering for Innovation, Lecce, IT

Introduction
The aim of this work was the development and characterization of biomimetic scaffolds based on hyaluronic acid with axially oriented pore channels for nerve regeneration. Matrices with elongated microstructure, suitably oriented porosity and controlled degradation rate have the potential to improve the regeneration of peripheral nerves and spinal cord by physically supporting and guiding the growth of neural structures across the site of injury [1]. Highly porous and functionalized tubular scaffolds were successfully produced using methacrylate and photo-crosslinked hyaluronic acid. In addition, to create axially oriented pore channels, scaffold manufacturing technique based on unidirectional freezing and subsequent freeze-drying was optimized.

Experimental Methods
Hyaluronic acid (HA) was methacrylated by means of a glycidyl methacrylate (GDM) grafting reaction, with an excess of GDM equal to 10 with respect to the disaccharide unit [2]. Next, a few photocrosslinked hydrogels were synthesized by exposing 26 mg/ml methacrylate HA (HAm) to UV light in the presence of increasing concentrations of the photoinitiator Irgacure 2959 (1%, 2% and 3% wt), in order to obtain different photo-crosslinked HAm hydrogels. To create axially oriented pore channels, the optimized HAm solution resulting by rheological tests (HAm 26mg/ml + 3 % wt PhI) was freeze-dried using three different strategies. They distinguish for the methods of preparation of support plates, before inserting the gel into the tube, in: Full ice (the cavities between guide tubes were completely filled with water and frozen), ice bottom (cavities filled only for 1/3 of their height) and no ice (water added after filling of tubes with gel). In addition, three HAm concentrations (26, 35, 50 mg/ml) and three different sequences of photo-crosslinking and freeze phase were studied: (i) photo-crosslinked of HAm, filled tube, cavities filled with water, frozen and freeze-drying; (ii) filled tube, cavities filled with water, frozen and freeze-drying, UV exposure of scaffold; (iii) filled tube, cavities filled with water, frozen, UV exposure and freeze-drying. All scaffolds produced were observed by SEM and the evaluation of the weight loss % in PBS at 37°C up to 28 days of soaking was carried out.

Results and Discussion
The metacrylated and photo-crosslinked hyaluronic acid hydrogel was optimized and characterized. By assessing the variation of photo initiator concentration, the best rheological properties were achieved by hydrogel produced with PhI at 3% by weight. Starting from these results, the optimized solution based on HAm 26 mg/ml + PhI 3% was used for the production of tubular nerve scaffold by freeze-drying technique. From different strategies of freeze-drying tested, scaffolds with elongated and suitable porosity have been obtained using strategy called NO ICE, in which water at room temperature is placed around the material. The use of material in which photo-crosslinking was interposed between two successive stages of freezing and the control of temperature gradients during freezing, allows the columnar growth of crystals thus obtaining a structure totally oriented and aligned according to the longitudinal axis of the scaffold (Figure 1 and 2 ). The scaffolds produced using method described above and with three different HAme concentrations were tested up to 28 days of soaking in PBS. The scaffolds HAme 26 mg/ml and HAme 35 mg/ml showed loss of structure and swelling after 1 day compared to scaffold HAme 50 mg/ml, which presented good stability.

Conclusion
Highly porous and functionalized scaffolds were successfully produced using methacrylate and photo-crosslinked hyaluronic acid, using tubular guides and water at room temperature as a means of controlling the columnar growth of ice crystals within the hydrogels, which subjected to the freeze-drying process left room for an engineered scaffold. In order to mediate between the high concentration of methacrylate hyaluronic acid, necessary for stabilizing the support in a physiological environment, and the growth of columnar crystals, an alternative method of production has been developed and optimized.

![Figure 1](image1.png)

**Figure 1**
Optimized production method

![Figure 2](image2.png)

**Figure 2**
Internal longitudinal section scaffold in Hame 50 mg/ml + 3 % wt PhI, produced using optimized production method.

**References**


Heatable PLA/CaP scaffolds for bone therapy through electromagnetic induced hyperthermia of iron oxide nanoparticles

Laura González-Rodríguez\textsuperscript{1,2}, Sara Pérez-Davila\textsuperscript{1,2}, Stefano Chiussi\textsuperscript{1,2}, Julia Serra\textsuperscript{1,2}, Pío González\textsuperscript{1,2}

\textsuperscript{1} CINTECX, Universidade de Vigo, New Materials Group, Vigo, ES; \textsuperscript{2} Galicia Sur Health Research Institute (IIS Galicia Sur). SERGAS-UVIGO, Vigo, ES

**Introduction**

Calcium phosphate (CaP) based scaffolds are very demanded in bone tissue engineering for repairing, replacing or regenerating defects in human bones or teeth due to their similar composition to the mineral part of the human bone. In fact, CaP content improves the attachment, differentiation, and proliferation of significant cells, in particular fibroblast-like and osteoblast cells. Moreover, it has been shown that CaP granulates embedded in a biodegradable polymer matrix, like polylactic acid (PLA), enhance the mechanical properties of organic scaffolds, mimicking as close as possible the bone mechanical strength [1].

An innovative strategy in the scaffold development for bone therapy is the possibility of incorporating an adjuvant effect through a hyperthermia process. The induced heating can enhance the bone regeneration [2], stimulate the vascular irrigation [3] and even can play an important role in the elimination of tumour [4] and microbial cells [5]. Local hyperthermia can be generated using different methods, among them, the use of iron oxide nanoparticles (Fe\textsubscript{3}O\textsubscript{4}NPs) activated by electromagnetic fields in the radiofrequency range (Figure 1).

The purpose of this work is to develop CaP/PLA heatable scaffolds through electromagnetic induced hyperthermia by using embedded iron oxide nanoparticles.

**Experimental Methods**

Scaffolds were produced by 3D-FDM printing based on a mixture of commercial PLA pellets and marine origin CaP granulates in different concentrations, with a CaP weight percentage up to 30%. After that, Fe\textsubscript{3}O\textsubscript{4} nanoparticles (5 mg/mL) were deposited on the scaffolds in several volumes. Biocompatibility of different concentrations of Fe\textsubscript{3}O\textsubscript{4} nanoparticles, from 0.1 mg/mL to 0.05 µg/mL, was assessed by \textit{in vitro} tests using MG63 osteosarcoma cell line. Cytotoxicity was evaluated using MTS assay after 24 h. Hyperthermia was induced by an electromagnetic inductor working at 340 kHz and operating with different current intensities, ranging from 60 to 99 A. The temperature rise on the scaffolds was recorded by an optical pyrometer.

**Results and Discussion**

\textit{In vitro} biocompatibility tests demonstrate a threshold of non-toxicity for Fe\textsubscript{3}O\textsubscript{4} NPs concentrations below 0.05mg/mL. PLA/CaP scaffolds embedded with Fe\textsubscript{3}O\textsubscript{4} nanoparticles were submitted to different electromagnetic radiation intensities. Hyperthermia induced on the scaffolds can be tuned by selecting the appropriate current of the inductor. As shown in Figure 2, for PLA/CaP30% scaffolds, a temperature rise up to 23°C is reached for 99 A current intensity. The PLA/Fe\textsubscript{3}O\textsubscript{4}NPs scaffolds were used as a control.

**Conclusion**

The biocompatible concentration threshold of Fe\textsubscript{3}O\textsubscript{4} nanoparticles has been determined. The generation of local hyperthermia in PLA/CaP/Fe\textsubscript{3}O\textsubscript{4}NPs scaffolds by electromagnetic induction has been demonstrated.

**Acknowledgement**

This research was financially supported by BLUEBIOLAB project (POCTEP INTERREG España-Portugal) and BLUEHUMAN project (EAPA_151/2016, Atlantic Area 2016).

Page 1593 of 2028
Figure 1.
Scheme of the hyperthermia therapy approach.

Figure 2.
Temperature rise dependence on the applied current intensity, for PLA/CaP/Fe₃O₄NPs scaffolds.

References


Design and manufacture of biocompatible scaffolds with fused filament fabrication and electrospinning for MCF7 breast cancer cells

Xavier Gallardo\textsuperscript{1,2}, Caitlin Burns\textsuperscript{1,2}, Teresa Puig\textsuperscript{1}, Joaquim Ciurana\textsuperscript{2}

\textsuperscript{1} New Therapeutic Targets Laboratory (TargetsLab) - Universitat de Girona, Department of Medical Sciences, Girona, ES; \textsuperscript{2} Product, Process and Production Engineering Research Group (GREP) - Universitat de Girona, Department of Mechanical Engineering and Industrial Construction, Girona, ES

Introduction

Two–dimensional culture mediums have traditionally been used in the vast majority of studies involving cell culture. This methodology, however, is not representative of the true conditions in which cells proliferate in the organism. Cells rely on the extracellular matrix (ECM) not only for architectural support, but for biochemical and biomechanical signals for morphogenesis, differentiation and homeostasis.\textsuperscript{1} Three–dimensional (3D) scaffolds allow for the assimilation of physiological cellular conditions in cell culture, leading to closer replication of in vivo environment,\textsuperscript{2,3} this includes cellular morphology, differentiation, proliferation rate, protein synthesis and gene expression, as well as drug metabolism.\textsuperscript{4,5}

This study aims to compare the proliferation of MCF7 breast cancer cells in the different materials available for 3D cell cultures, polylactic acid (PLA) and polycaprolactone (PCL), as well as the different techniques for scaffold synthesis by comparing fused filament fabrication (FFF) and electrospinning methods.

Experimental Methods

Fused filament fabrication (FFF) and electrospinning were compared using two different aliphatic polyesters, PLA and PCL; resulting in four 3D scaffold models for cell culture: FFF–PLA, FFF–PCL, electrospun PLA and electrospun PCL.

FFF printing technique was used to manufacture 3D scaffolds in order to compare PLA and PCL materials. Three scaffolds were printed for both PLA and PCL with deposition angles of 90\(^\circ\). The resulting scaffolds were cultivated with MCF7 breast cancer cells; at 3 and 6 days the adhesion and proliferation were measured using colorimetric assay (MTT).

Electrospinning printing technique was also used to manufacture 3D scaffolds to compare PLA and PCL materials. Again, three scaffolds were printed for both PLA and PCL, both with a material concentration of 15\% w/v. The resulting scaffolds were also cultivated with MCF7 cells at 3 and 6 days, the adhesion and proliferation were measured using colorimetric assay (MTT).

These same MCF7 cells were also cultured on a traditional 2D culture medium as a control for both FFF and electrospinning techniques.

Results and Discussion

Cell adhesion and proliferation of MCF7 breast cancer cells were observed at 3 and 6 days after seeding on the 3D scaffolds.

At 3 days, cell adhesion and proliferation on all 3D scaffold cell cultures was observed to be significantly less than in the traditional 2D culture, which presented increased cell proliferation. When comparing the 3D scaffold models amongst themselves, scaffolds manufactured with PCL through electrospinning presented increased proliferation when compared to both FFF–PLA, FFF–PCL and PLA electrospun scaffolds (Figure 1). Six days after initial seeding, adhesion and proliferation were measured again, where no significant differences were observed when comparing traditional 2D culture and all four scaffold groups. When comparing 3D culture mediums, an increased adhesion and proliferation was observed when using electrospun PCL scaffolds (Figure 2).
Conclusion

In summary, cellular culture of MCF7 breast cancer cells (as determined by cell adhesion and proliferation) on 3D scaffolds, using both FFF and electrospinning with either PCL or PLA, does not differ from traditional flat 2D cell culture. When comparing the four groups of 3D scaffolds, the one that yielded the highest cellular proliferation was the electrospun PCL scaffold.

Figure 1

Figure 1. Cell adhesion and proliferation of MCF7 breast cancer cells at 3 days, comparing traditional flat 2D culture medium to PLA and PCL electrospun scaffolds with PLA and PCL-FFF scaffolds. No statistical significance was found, each bar represents n=3 samples.

2D: Traditional two dimensional culture, ESP PCL: electrospun polycaprolactone, ESP PLA: electrospun polylactic acid, SCF PCL: fused filament fabrication scaffold with polycaprolactone, SCF PLA: fused filament fabrication scaffold with polylactic acid

Figure 2
Figure 2. Cell adhesion and proliferation of MCF7 breast cancer cells at 6 days, comparing traditional flat 2D culture medium to PLA and PCL electrospun scaffolds with PLA and PCL-FFF scaffolds. Statistical significance is represented with an asterisk (*) where p<0.05, each bar represents n=3 samples.

2D: Traditional two dimensional culture, ESP PCL: electrospun polycaprolactone, ESP PLA: electrospun polylactic acid, SCF PCL: fused filament fabrication scaffold with polycaprolactone, SCF PLA: fused filament fabrication scaffold with polylactic acid

References
PS2-03-130

3D printed β-TCP/ S53P4 bioactive glass composite scaffolds coated with tea tree oil: optimization and evaluation of the antibacterial activity

Ana P. Nogueira Alves1, Rodrigo L. Moraes Saldanha de Oliveira1, Igor Maia Ferreira2, Elisángela Guzi de Moraes2, Antonio P. Novaes de Oliveira2, Marcela Arango Ospina3, Malte Hartmann3, Aldo R. Boccaccini3, Eliandra de Sousa Trichês1

1 Federal University of São Paulo, Laboratory of Bioceramics / Department of Science and Technology, São José dos Campos, BR; 2 Federal University of Santa Catarina, Laboratory of Glass-Ceramic Materials/ Department of Mechanical Engineering, Florianópolis, BR; 3 University of Erlangen-Nuremberg, Institute of Biomaterials, 91058 - Erlangen, DE

Introduction
The treatment of bone defects, arising from infections, is a big challenge for the orthopedic field. 3D printing is a promising technology to produce scaffolds with customized architecture able to stimulate and support bone growth [1]. Bioceramics, such as β-TCP and S53P4 bioactive glass, used for scaffold manufacturing, have shown a great biological response [1]. However, a multifunctional scaffold, able to inhibit microbial proliferation on the defect site, is of increasing interest not only to avoid infection recurrence but also to decrease the dosage of antibiotics, preventing complications due to resistant bacteria [2]. Tea tree oil (TTO) has aroused interest as antimicrobial agent to minimize the use of antibiotics [3]. Therefore, combining the regenerative potential of a bioceramic scaffold with the antimicrobial properties of TTO could result in an innovative biomaterial capable of stimulating tissue growth and treating infections. In this context, this study aims to produce and characterize 3D printed β-TCP/ S53P4 scaffolds coated with TTO.

Experimental Methods
The scaffolds were produced by the “Direct Ink Writting” (DIW) method and the morphological and chemical characterizations by means of XDR, SEM, FT-IR were carried out.

The scaffolds were coated by immersion in an ethanol/TTO solution with a TTO concentration of 5, 10 and 15% (v/v) until the ethanol evaporated. The release of TTO was evaluated with a UV-vis spectrophotometer. Samples in triplicate were incubated in PBS under constant agitation at 90 rpm and 37°C for up to 14 days. After the TTO release evaluation, antibacterial and in vitro bioactivity assays are performed. The antibacterial activity of the coated scaffolds was evaluated against S. aureus.

Results and Discussion
Figure 1 shows the XRD pattern of β-TCP/S53P4 scaffolds. It is possible to notice the formation of different crystalline phases due to the sintering temperature. SEM images of the scaffold show the fracture surface of the filaments and the pore geometry pattern (Figure 2), the obtained porosity of sintered scaffolds was 48%.

Preliminary turbidity assays showed the potential antibacterial properties of the coated scaffolds with higher TTO concentration during the first 7 hours in contact with bacteria.

Conclusion
It was possible to produce β-TCP/S53P4 scaffolds using Direct Ink Writting method and coat them with Tea Tree Oil. The XRD pattern shows the β-TCP phase and others due to the sintering temperature.

SEM images show the pattern in the filaments and pore geometry.

The other assays are in progress and we will hand in the results as soon as possible.

Acknowledgement
This project was supported by FAPESP agency (process number: 2019/08141-9, 2020/12944-7 and 2019/19594-4).
XRD pattern of β-TCP/7.5 SS3P4 scaffolds

β - β-TCP (JCPDS 09-0169); α - α-TCP (JCPDS 09-0348); δ - Na15·78Ca3(Si6O12)(high-combeite (JCPDS 01-0781650); '*' - CaSiO3 (wollastonite) (JCPDS027-888); '#' - NaCaPO4 (rhenanite) (JCPDS 029-1193)

SEM images of β-TCP/7.5 SS3P4 scaffolds

a) cross section area structure, b) details of the filament and c) filament surface.

References


2:00 p.m. – 3:30 p.m.

Poster floor

**PS2-04 | Biomaterials for Orthopedic Applications**
Continuous peptide gradients in 3DP scaffolds to control osteochondral differentiation

Ivo Beeren, Sandra Camarero-Espinosa, Piet Dijkstra, Ravi Sinha, Carlos Mota, Matt Baker, Lorenzo Moroni

Maastricht University, Biofabrication group, MERLN institute, Maastricht, NL

Introduction
Articular cartilage has little capacity for repair and damage can easily progress to osteoarthritis. The current clinical treatment is microfracture or autologous tissue transplantation; however, this often results in fibrocartilage formation. Hence, tissue engineers are designing a new supportive base at the site of defect – an osteochondral (OC) construct – capable of enhancing OC regeneration. The OC region is described by gradual transitions between cartilage and bone via multiple zones each with its specific cell type and matrix composition. Continuous gradients are required to create biomimetic tissue engineering constructs. We hypothesized that a construct with gradual control over human mesenchymal stromal cells (hMSC) differentiation is needed to improve gradual tissue formation for osteochondral applications. To this end, we designed a 3D printed (3DP) construct with gradients of differentiation-inducing peptides on the surface. We employed the gradient printing of polymers with orthogonal functional groups for post-fabrication functionalization with different peptides. Here, we will show our work from polymer synthesis, to preliminary in vivo experiments.

Experimental Methods
Polycaprolactone (PCL$_{45k}$) polymers were modified with an azide via end-group modification or with a maleimide via ring-opening polymerization (figure 1A). The countercurrent polymer gradients were created by a custom-made print head$^1$. The print head has two reservoirs with individual control over pressure and temperature, which is used to mix materials in a certain ratio (figure 1B). The material gradient in a single construct was confirmed by $^1$H nuclear magnetic resonance (NMR) and colored dyes. To confirm surface availability of the functional group, we attached complementary fluorescent dyes to single material scaffolds. An alkyn-TGF-$\beta$1 binding peptide$^2$ and a thiolated BMP-2$^3$ derived peptide were attached onto the surface to direct hMSC differentiation osteogenically and chondrogenically, respectively. hMSC differentiation on single and dual-material scaffolds was investigated by evaluating gene and protein expression such as RUNX2, Sox9 and extracellular matrix molecules. Furthermore, biochemical assays and histological analysis were performed. Finally, the scaffolds were implanted in a rat subcutaneous model to assess biocompatibility and cellular infiltration.

Results and Discussion
Synthesis and characterization of PCL-derived polymers with a terminal azide or maleimide was accomplished on a multi-gram scale (figure 1A). We were able to mass-produce constructs (figure 1C) in a high throughput fashion with our custom-made print head (18 scaffolds per run). Our material gradient was visualized by blending a dye into one of the polymer melts (figure 1D). $^1$H NMR analysis confirmed having functional groups and polymers on opposing sides of the scaffolds by the appearance of the methylenes of the maleimide (c and d) and depletion of the azide (b, figure 1E). Spectrofluorometric measurements confirmed surface attachment of fluorescent dyes in the nmol/cm$^2$ range. We assumed similar reactivity for our peptides as they based on the same click chemistry. Currently, we are looking into TOF-SIMS measurements to also confirm peptide binding, but encouraged by the spectrometry results we proceeded with cell experiments. We now aim to measure the up/downregulation of characteristic differentiation markers, such as gene expression or protein expression that are influenced by the peptide signals attached to the surface. In our rat model, we observed high cellular infiltration with or without attached peptides.
Conclusion
In this project, we have successfully printed 3D constructs with a continuous material and functional group gradient without creating discrete interfaces. Fluorescent dyes validated availability of the functional groups on the surface. Moreover, due to the nature of click chemistry, we made a chemical versatile system in which numerous modified peptides or bioactives could be attached onto the surface. The surface gradients can be exploited to improve biomimicry for OC constructs by gradually directing cell differentiation.

Acknowledgement
We thank the European Research Council (H2020-ERC grant CELL HYBRIDGE, #637308) for providing financial support to this project.

Creating a continuous material gradient in a 3DP scaffold
Figure 1: A) PCLA (yellow) and PCLM (blue) were synthesized as base materials for this study. B) Schematic of the FAST printer developed in our institute. Individual control over pressure and temperature in each reservoir determine the inflow in the mixing channel. C) An example of a continuously produced scaffold consisting out of two materials by our printer. D) A dye was blended in one of the polymer melts to visualize the material gradient. E) 1H NMR evaluation of the top and bottom slices of the scaffold in C.

References
[1] Sinha, R et al. 2021, A hybrid additive manufacturing platform to create bulk and surface composition gradients on scaffolds for tissue regeneration, Nature communications, 12, https://doi.org/10.1038/s41467-020-20865-y
Co-axial 3D printed composite biopolymer scaffolds based on AlgMA/GelMA for bone tissue engineering

Amanzhol Turlybekuly, Aruzhan Naren, Dana Akilbekova

Nazarbayev University, Department of chemical and materials engineering, Nur-Sultan, KZ

Introduction
Trabecular bone is a dynamic, multifunctional tissue that goes through continuous remodeling process by osteoclasts and osteoblasts paired action [1], [2]. Polymeric scaffolds that mimic essential extracellular complexity and cellular processes of trabecular bone cavities can be used to mimic the natural bone regeneration process and create three-dimensional (3D) in vitro models to study bone osteogenesis and pathology. For that purpose, 3D bioprinting (3DP) provides the ability to manufacture reproducible 3D structures with well-defined spatial cell location [2]. Here, we describe a coaxial 3DP capable of printing simultaneously two bioinks that can contain separately various cell types [3], [4]. High fidelity 3DP of cell-laden scaffolds depends on the bioink that possesses favorable physical characteristics such as shear thinning to be printable and at the same time the ability to retain its shape after printing. In present study we used alginate (Alg) and gelatin (Gel) based bioinks with phototunable mechanical properties for creating 3D engineered bone tissues.

Experimental Methods
Co-axial nozzle was developed according our design and produced by SLA FormLabs 3B printer. The advantage of this co-axial nozzle is the ability to use of standard conical nozzles (18G-27G) as inner (core). Moreover the design of co-axial nozzle allows to control the core filament diameter by regulation of pressure (as an issue the current velocity). Methacrylated Gel and Alg were printed using co-axial nozzle adapted for the Cellink, Inkredible bioprinter. GelMA was used for creating core filament diameter, because it has higher mechanical properties and works as scaffold support. During printing, a lattice-rod model was extruded at the pressure of 30kPa for core nozzle and 34 kPa for shell nozzle. The scaffolds were photo-crosslinked with UV for 5 min and 0,25 M CaCl2 solution for 10 min. Rheological properties, printability, mechanical properties and scaffold morphology were systematically investigated.

Results and Discussion
Rheology studies showed the shear-thinning behavior for all AlgMA and GelMA based bioinks. Higher GelMA content decreased the degradation rate after 14 days in culture medium.

Conclusion
In summary, the co-axial nozzle for Cellink Inkredible bioprinter was developed. The composite AlgMA/GelMA scaffolds were produced by use co-axial nozzle. The composite scaffolds are beneficial by tunable mechanical properties that provide control over biodegradation. The disadvantage of that method is a lower degree of core element dual crosslinking by calcium due to gradient diffusion from a shell. Future studies will investigate the mechanical properties enhancement of core element by increasing photoinitiator concentration. The construction of a co-axial nozzle allows controlling core filament diameter by increasing the pressure in the printhead and bioink consumption.

Acknowledgement
The research project was supported by Nazarbayev University "Tissue engineering of 3D in vitro human organotypic bone model" SEDS2020020 grant.
References


PS2-04-136

Optimization of novel 3D composite scaffolds for bone reconstruction

Ana Monteiro1,2, Aurora Perez Jimenez1, Erwan Plougonven2, Stéphanie Lambert2, Christian Grandfils1

1 University of Liège, CEIB-CEntre Interfacultaire des Biomatériaux, Liège, BE; 2 University of Liège, Chemical Engineering Department, Liège, BE

Introduction

Custom-made scaffold-mediated regeneration is an emergent field in bone reconstruction. However, the optimization of 3D degradable implants with suitable biological response and mechanical features still remains challenging today. The focus of this study was to tailor a 3D scaffold made from a polymeric macroporous matrix filled with hydroxyapatite (HA) and amphiphilic hydrogel. Accordingly, three different implant designs composed of PLA have been developed by 3D printing with various interconnected porous in an optimal range of 300 to 600 µm. In a second step, the scaffolds have been filled with an amphiphilic gel containing HA in order to optimize the dissolution rate of the gel and its ability to enhance cell colonisation and proliferation.

Experimental Methods

3D matrix manufacturing was performed by Prusa I3MK3 printer using degradable PLA filament. The resulting scaffolds were characterized by Micro-computed tomography (CT) in order to analyse their porosity (total area, volume and size distribution of the pores). Hydrogel dissolution studies were assessed in PBS buffer at 37 °C and cell permeability were evaluated via diffusion of different polystyrene beads (2 to 10 µm).

Results and Discussion

The scaffold design was satisfactory optimized with pore size around 600 µm. The scaffold porosity plays an important role in the vascularization and cell proliferation, hence, in the integration of these implants in the body. On the other hand, the amphiphilic gels were quickly dissolved in PBS buffer at 37 °C, indicating that future efforts should be devoted to increase the cohesiveness of the physical reticulated polymeric network.

Conclusion

In conclusion, this work represents an attractive strategy for bone reconstruction by proposing composites made of a polymeric scaffold filled with an inorganic HA dispersed in a soft biocompatible hydrogel. The opened porosity of this biocomposite should promote cell colonization. However, further efforts should be required to adjust the stability of the amphiphilic gel to promote cell colonization and tissue rebuilding.

Acknowledgement

Ana Monteiro and Stéphanie Lambert thank the Belgian National Funds for Scientific Research (F.R.S.-FNRS) for their Post-doctoral position and Senior Associate Researcher position, respectively. The authors also acknowledge Interreg Great Region and Wallonia for the financial support of ImproveStem Project.

References

https://doi.org/10.1016/j.compositesb.2020.108238


https://doi.org/10.1038/natrevmats.2016.71
Elastic Polymer-Coated Nano- and Macroporous Bioactive Glass Scaffolds for Bone Regeneration

Nina Ehlert\(^1,5\), Marvin Lietzow\(^1,5\), Karen Besecke\(^1,5\), Lisa Gülker\(^1,5\), Monika Seegers\(^1,5\), Henning Hartwig\(^2,5\), Meike Stiesch\(^2,5\), Sarah Gniesmer\(^3,5\), Andreas Kampmann\(^3,5\), Andrea Hoffmann\(^4,5\), Peter Behrens\(^1,5\)

\(^1\) Leibniz University Hannover, Institute of Inorganic Chemistry, Hannover, DE; \(^2\) Hannover Medical School, Dental Prosthetics and Biomedical Materials Science, Hannover, DE; \(^3\) Hannover Medical School, Mouth, Jaw, and Facial Surgery, Hannover, DE; \(^4\) Hannover Medical School, Orthopedic Clinic, Hannover, DE; \(^5\) Lower Saxony Centre for Biomedical Engineering, Implant Research and Development, Hannover, DE

Introduction
Bone replacement for critical-sized defects like after infection or cancer debridement is still a major issue in bone surgery. Autografts are still the materials of choice but have disadvantages like low availability and donor site morbidity. Therefore, the aim of the presented work is the development of a biodegradable implant material for non-load-bearing implantation sites which supports the formation of new bone and in the end is replaced by it.

Experimental Methods
In a first step nano- and macroporous bioactive glass scaffolds are produced via a foam templating procedure. Polyurethane foam is repeatedly soaked with a bioactive glass precursor solution. After coating and drying the initial foam is removed by calcination at 600 °C [1]. To overcome the inherent brittleness of the bioactive glass, the replicas are coated with the biodegradable polymer polyglycerol sebacate (PGS) [2]. Additionally, we developed a new phosphate derivative of the latter and tested it in comparison to PGS.

Results and Discussion
The bioactive glass replicas exhibited nanopores with a diameter of 5 nm and a high inner surface area for further functionalization. For example, the nanoporous system could be equipped with strontium ions to support bone formation. The three-dimensional interconnected macroporous structure of the initial foam template is well reproduced. The polymer coatings (PGS and the phosphate derivative) on the bioactive glass replicas led to an elastic composite material, which was proven via compression tests. This improvement enables typical press-fit implantation in the defect without collapse of the macroporous structure, which is a crucial factor for good biointegration. Release experiments show long-term delivery of relevant ions like calcium, phosphate, and silicon, which will support osteogenesis while degradation of the scaffold. Good biocompatibility was proven via cytotoxicity and cell adhesion tests with dental pulp stem cells. In vivo experiments in an observation chamber model confirmed a fast vascularization of the scaffolds in contrast to the control group.

Conclusion
The combination of nano- and macroporous bioactive glass replicas coated with the elastic PGS and its phosphate derivative proved to be promising candidates for effective bone replacement. Especially, the newly developed phosphate derivative of PGS showed promising results in all investigations and will now be tested in terms of its osteogenic differentiation capability.

Acknowledgement
Thanks to the Institute for Technical Chemistry, and the Laboratory of Nano- and Quantum Engineering (LNQE) Leibniz University of Hannover for access to their characterization methods.
**synthesis procedure**
Scheme and microscopic images of the consecutive synthesis stages of the composite scaffolds

**References**
Electrospun composite scaffolds with PHBV and HA particles for bone tissue regeneration

Joanna E. Karbowniczek, Lukasz Kaniuk, Krzysztof Berniak, Adam Gruszczyński, Urszula Stachewicz

AGH University of Science and Technology, Faculty of Metals Engineering and Industrial Computer Science, Krakow, PL

Introduction

Biomimetic approaches in designing scaffolds for tissue engineering are gaining more and more attention. To address the requirements of each tissue the right composition, structure and properties of supportive materials are needed. Bone is a natural composite build of collagen fibers and hydroxyapatite (HA) crystals. Therefore, in this study scaffolds combining poly(3-hydroxybutyric acid-co-3-hydrovaleric acid) (PHBV) and HA nanoparticles were prepared by blend electrospinning [1].

Experimental Methods

Two solutions: 8% PHBV and 8% PHBV+1% HA in solvents mixture: chloroform and dimethylformamide (DMF) (9:1, v/v) were prepared by stirring for 4 hours. Both solutions were electrospun using an EC-DIG device (IME Technologies) by applying 17kV to the needle distanced at 20 cm to the collector. Fibers morphology was studied by scanning electron microscopy (SEM, Merlin, Zeiss) followed by focused ion beam (FIB) sectioning to expose fibers interior. Additionally, energy dispersive X-ray spectroscopy (EDS, Brucker) was used to confirm HA particles incorporation into PHBV fibers. In vitro studies with MG-63 cells were performed up to 7 days of incubation. Cell proliferation was evaluated using Cell Titer-Blue® Assay and cell morphology was studied by confocal and SEM imaging.

Results and Discussion

Randomly oriented smooth fibers with an average diameter of 2.92 ± 0.28 μm were obtained from electrospinning of PHBV solution. Addition of HA particles caused formation of porous fibers (Fig. 1 A, B), with increased diameter of 3.76 ± 0.37 μm. Ceramic particles were present both inside fibers as well as exposed on their surface. Based on proliferation test and confocal imaging results, the higher cell number was observed on composite PHBV+HA scaffolds compared with solely PHBV fibers. Especially significant boost in cell proliferation was observed between 4th and 7th day of incubation. Microscopy imaging enabled to visualize cells elongation, filopodia formation and penetration in depth on both type of scaffolds. Importantly, PHBV+HA fibers due to particles on their surface provided more anchoring points for cells spreading, therefore, higher cell number with longer filopodia were observed for composite scaffolds (Fig. 1 C and D).

Conclusion

In this study we showed that by simple approach of blend electrospinning combining PHBV and HA we could obtain scaffolds improving bone cells proliferation and growth. Therefore, we proved that such composite scaffolds are a very promising material for bone tissue regeneration.

Acknowledgement

This study was financially supported by the grant PRELUDIUM 15, No. 2018/29/N/ST8/02032, financed by the National Science Center in Poland.
Figure 1. SEM micrographs: (A) electrospun PHBV+HA fibers, (B) rough and porous morphology of PHBV+HA fibers (red arrows indicating HA particles), (C and D) MG-63 cell growing in between PHBV+HA fibers after 7 days of incubation.

References
Influence of pectins and other polymer additives on the properties of biomicroconcrete type bone substitute materials

Aneta Zima, Piotr Pańtak, Joanna Czechowska, Ewelina Cichoń, Anna Ślósarczyk

AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Krakow, PL

Introduction
The continuous efforts of scientists to create new and improved implants for the regeneration of bone tissue and the increased demand for bone replacement biomaterials lead to many innovative materials solutions. An interesting concept is the development of biomicroconcretes [1]. This type of materials consists of granules that are embedded in the biocompatible bone cement matrix. Surgically handy biomicroconcretes could be a new solution for tissue engineering allowing for easy adjustment of the material to the complex shape of bone defect. They can act as an alternative to commonly used sintered ceramic implants of predefined shape and size.

In this study, new hybrid biomicroconcretes with high surgical handiness were developed. Solid phase of the investigated materials composed of highly reactive α-tricalcium phosphate powder (α-TCP) and hybrid hydroxyapatite-chitosan (HAp/CTS) materials (in the form of granules of 300–400 μm and powders). The new idea of using the citrus pectin solution (5 wt%) as the cement liquid phase was applied. Moreover, the obtained biomicroconcretes were modified by adding into the solid phase polymer additives such as sodium alginate (SA) or hydroxypropyl methylcellulose (HPMC).

Experimental Methods
The initial highly reactive α-tricalcium phosphate (α-TCP) powder was synthesized by the wet chemical method according to the procedure described by Zima et al. [2]. The hybrid hydroxyapatite-chitosan granules were prepared after 48h freezing of hybrid hydroxyapatite-chitosan precipitate according to the previously developed method by Zima et al. [1]. To prepare granules, the obtained precipitate was sieved and dried at 40 °C to receive granules of 300-400μm. Biomicroconcretes were prepared by mixing α-TCP powder with hybrid hydroxyapatite-chitosan material (with 15, 20 and 25% wt.% of chitosan) in the forms of a precipitate, powder and granules. To the powder phase of biomicroconcretes also polymeric additives i.e. sodium alginate and hydroxypropyl methylcellulose were added in the amount of 2 or 4 wt.%. In order to prepare cement pastes, the citrus pectin solution was added into the well-mixed compounds of the solid phase and hybrid precipitate (L/P= 0.8). Then, the physicochemical and biological properties of the obtained biomicroconcretes were determined.

Results and Discussion
The new generation of fully injectable biomicroconcrete-type biomaterials combining α-TCP powder, hybrid hydroxyapatite-chitosan materials in the form of powder and granules and polymeric additives in the form of gel (pectin) and powder (sodium alginate or HPMC) were successfully obtained and investigated. After 7 days of setting and hardening in air, materials possessed two crystalline phases which were HAp and α-TCP. FTIR analysis of the studied materials confirmed the presence of functional groups characteristic for hydroxyapatite and polysaccharides such as chitosan or pectin (figure 1). The compressive strength (5.38 up to 17.2 MPa after 7-days after setting and hardening, and 1.56 up to 13.23 MPa after 7-days of incubation in SBF) was similar to that of human cancellous bone and allowed for low-load bearing applications [3]. The SEM observations revealed that obtained biomicroconcretes are characterised by the presence of numerous polymeric bridges between granules and the matrix which can suggest the occurrence of the system of various bonds in their structure (figure 2). Results showed that developed materials were chemically stable in SBF.

Page 1610 of 2028
Conclusion
Studies have shown that obtained biomicroconcretes have unique physicochemical properties due to the dual setting system originated from hydrolysis of α-TCP and gelation of pectin. Polymeric additives have shown the promising potential of using them as modifiers of developed hybrid materials. The properties of the manufactured biomicroconcretes result directly from the characteristics of the starting components as well as the presence of various bonds due to interactions between the biopolymers. Developed innovative biomicroconcretes based on hybrid HAp/CTS granules, highly reactive α-TCP powder and the citrus pectin can be prospective injectable materials for regenerative medicine.

Acknowledgement
This work was supported by the National Science Centre, Poland Grant No. 2017/27/B/ST8/01173 and by the Faculty of Materials Science and Ceramics AGH UST - University of Science and Technology, Kraków, Poland, Project No. 16.16.160.557 (2021).
AZ has been partly supported by the EU Project POWR.03.03.00-IP.08-00-P13/18 - PROM NAWA

Figure 1
FTIR spectra of the biomicroconcretes.

Figure 2
SEM microphotographs of obtained biomicroconcretes with polymeric additives in form of sodium alginate or hydroxypropyl methylcellulose. Polymeric bridges marked by arrows

References
The efficiency of bone and cartilage decellularization by means of High Hydrostatic Pressure

Christopher D. Pohl1, Charlotte Koppe1, Nico Brandt1, Janine Waletzko-Hellwig2, Friederike Poosch3, Daniel Strüder3, Michael Dau2, Rainer Bader4, Michael Schlosser1

1 University Medical Center Greifswald, Department of General Surgery, Visceral, Thoracic and Vascular Surgery, Greifswald, DE; 2 University Medical Center Rostock, Department of Oral, Maxillofacial Plastic Surgery, Rostock, DE; 3 University Medical Center Rostock, Department of Oto-Rhino-Laryngology, Head and Neck Surgery, Rostock, DE; 4 University Medical Center Rostock, Department of Orthopaedics, Rostock, DE

Introduction
The tissue replacement by allogenic materials is of increasing importance in clinical practice. To ensure optimal transplantation outcome, it is of utmost importance to reduce immunogenicity to minimize adverse inflammatory effects in recipients. The established decellularization methods are chemical treatment and/or β- and γ radiation. However, these methods also affect matrix integrity. Therefore, High Hydrostatic Pressure (HHP) is currently under investigation for the decellularization/devitalization of allotransplantation tissue. It utilizes pressure levels up to 500 MPa to induce apoptosis in cells, while preserving matrix integrity and biomechanical features. Especially for supportive tissue such as bone and cartilage, structural integrity is important for transplant functionality and host integration. Yet important aspects of this promising method remain unknown including proof for a full inactivation of tissue specific cells prior to implantation. Though cell devitalization is well documented, matrix compartments and cell debris could still pose an immunological/inflammatory risk, which might also effects the matrix integrity. Therefore, this in vitro study investigated different HHP treatment protocols with and without additional washing steps on bone- and cartilage tissue regarding decellularization by means of quantitative histological imaging.

Experimental Methods
Tissue samples were extracted post mortem from organ donors in the Institute of Anatomy of the University Medical Center Rostock with the apprroval of the local ethical committee (case number A 2016-0083). Following extraction, tissue samples were kept in PBS and HHP treated with either 250 mPa (Bone) or 450 mPa (cartilage) for 10 minutes. Additionally, selected samples received treatment with ultrasound in a washing chamber, to wash out debris created by the HHP. After treatment, all samples were fixated for 24 h with buffered 4% formaldehyde-solution at room temperature followed by a washing step of 2 hours in tap water. Bone tissue was further decalcified via aqueous EDTA-ammonia solution up to 14 days. Pre-treated tissue samples were placed in an embedding medium consisting of TissueTek®/PBS solution and shock frozen in liquid nitrogen, followed by preparation of cryosections of 10 µm thickness with a Leica CM 3050 S Cryotome. Hematoxylin and Eosin (H&E) staining was performed according to standard protocols. For morphometric analysis of the sections, pictures were taken with a Olympus 20 camera (Olympus K.K., Tokyo, Japan) and analysed by the ImageJ software. To compare different treatments of tissues, quantitative data were analysed by Mann-Whitney-Test (Cartilage) or a one way ANOVA (Bone) using the GraphPad Prism software.

Results and Discussion
Bone and cartilage revealed no macroscopic differences in structure and general appearance after HHP treatment (Figure 1). Both showed a significant reduction in cell number after treatment with HHP (Figure 2). For bone tissue HHP treatment resulted in an up to 10-fold reduction of median cell number per mm² (33.97, interquartile range IQR 25.66-49.82) in comparison to the untreated control (312.2, IQR 245.0-397; p<0.0001). The additional ultrasound treatment had no effect (257.7, IQR 183.8-351.9). The combined treatment by HHP and ultrasound, however,
resulted in a significant decrease up to 43.51 cells per mm² (IQR 34.55-112.7). For cartilage, HHD treatment also resulted in a significant (p<0.0001) up to three-fold reduction of cells per mm² in comparison to untreated controls (25.11 (IQR 14.8-32.38) vs. 76.47 (IQR 63.93-91.25), respectively).

Conclusion
Morphometric histological analysis demonstrates a significant, up to 10-fold reduction of cell number in bone and a three-fold reduction in cartilage caused by HHP treatment and in combination with ultrasound also for bone tissue. Since the ultrasound treatment alone showed no significant reduction in cell numbers, the main decellularization is most likely caused by HHD. No effect on the matrix structure as well as the general tissue appearance could be seen. Thus, the results indicate that HHD treatment is suitable method for a specific decellularization, however further efforts on the protocols, especially for the combination with more intense ultrasound procedures is needed.

Acknowledgement
This study was supported by the European Social Fund, HOGEMA project P4 (grant no. ESF/14-BM-A55-0015/18).
Apatitic bone cements with effective antibacterial and antibiofilm ability

Massimiliano Dapporto1, Francesca Carella1, Marta Tavoni1, Elisa Restivo2, Livia Visai2, Laura Mercatali3, Toni Ibrahim3, Anna Tampieri1, Michele Iafisco1, Simone Sprio1, This work was supported by the Italian Ministry of Health (Bando Ricerca Finalizzata 2016, no. GR-2016-02364704

1 National Research Council of Italy, Institute of Science and Technology for Ceramics, Faenza, IT; 2 University of Pavia, Molecular Medicine Department, UdR INSTM, Pavia, IT; 3 IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST), Osteoncology and Rare Tumors Center, Meldola, IT

Introduction
Self-hardening pastes and cements based on calcium phosphates are widely used biomaterials for several clinical applications, including spinal fusion, vertebroplasty and periodontal surgery. Their preparation involves the mixing of solid powder and liquid components, which then self-harden in vivo and transform into nanostructured, nanocrystalline apatitic phases. However, the occurrence of bone infections, i.e. osteomyelitis or bone tuberculosis, still represents a major responsible of therapy failure in orthopaedics. If bone infections occur, the clinical protocols usually include the long-term oral administration of antibiotics, which however is characterized by limited bioavailability of the drug at the healing site and, often, provoking the occurrence of systemic adverse side effects. A further drawback is represented by the bacterial resistance to antibiotics, which is widely considered as a major threat in medicine and particularly in orthopaedics in the incoming years. In this scenario, the development of biomaterials with regenerative ability and effective antibacterial and antibiofilm properties is a goal of ever-increasing relevance. Key aspects are the ability of biomaterials to prevent bacterial adhesion and proliferation, thanks to bioactive composition and surface texture, as well as the ability to act as carrier able to release drugs in situ in a sustained and therapeutically effective manner. The present work describes a novel approach to prepare osteointegrative Sr-doped apatitic bone cements featuring nanostructured architecture and capable of controlled release of tetracycline (TC), intended as broad-spectrum antibiotic, but also chemically close to anthracyclines, another wide group of drugs commonly used as first-line chemotherapeutic treatment of osteosarcoma and other solid tumors as breast cancer.

Experimental Methods
Firstly, the synthesis of nanocrystalline biomimetic apatites was performed by wet chemistry, followed by surface adsorption of TC. Then, drug-loaded bone cements were obtained by mixing proper amounts of TC-loaded nanoparticles (TC-NPs) to Sr-doped α-tricalcium phosphate powders and aqueous solutions enriched with sodium alginate. The antibacterial and antibiofilm effectiveness of native and TC-loaded cements was evaluated by microbiological tests assessing the viability of Staphylococcus aureus and Escherichia coli, among the most diffuse bacterial strains in nosocomial infections, proving substantial bacteriostatic and bactericidal properties in the native cement and complete eradication of bacterial cultures in the TC-loaded device.

Results and Discussion
The injectability, setting times and drug release profile of these final pastes were optimized according to the clinical need. A possible application is the treatment of cancer patients with implantation of self-hardening pastes and cements during surgery or radiological interventions. In order to achieve an antitumoral effect, specific concentrations of anthracyclins should be delivered. Clinical pharmacokinetic profiles of the drug administered systemically and locally are used to identify the correct concentration to be delivered in order to optimise the therapeutic effect and avoid toxicity. Chemotherapeutics concentration were setted at 0.6-1.2 ug/ml with a delivery duration within three four days. An extensive physico-chemical characterization of the cements was performed. The presence of TC-loaded apatites allowed to modulate the drug release profile in physiological conditions, if compared with the formulation...
without NPs. This work proved the ability of apatitic cements to contrast bacterial infections only by virtue of their composition and nanostructure and, furthermore, to act as a drug carrier capable of effective and controlled release, thanks to the possibility to modulate the release profiles by implementing the cement with apatite nanoparticles able to chemically link the drugs.

**Conclusion**

These results are relevant in the view of design and development of new devices for bone regeneration, more effective to improve the patient safety and clinical outcomes, and potentially able to limit or even prevent the use of drugs, thus aiding to contrast the steady increase of bacterial resistance to antibiotics.

**Acknowledgement**

This work was supported by the Italian Ministry of Health (Bando Ricerca Finalizzata 2016, no. GR-2016-02364704)
Dental implants surface roughness can influence bacterial site infection? A clinical in vivo prospective clinical study on 109 patients, preliminary results on radiologic evaluation

Nicola De Angelis, Luca Solimei, Alberto Lagazzo, Fabrizio Barberis

University of Genova, Genova, IT

Introduction
Dental implants are intended to provide long-term reliable dental restorations. Limited data exist on the comparison between different implant surfaces. This study aims to investigate if the surface topography and roughness might increase the bacterial adhesion and intensify the possibility of implant failure in patients with and without a previous history of periodontal disease.

Surface topography and roughness are some of the aspects that can be easily manipulated by resorting to post-production surface treatments and that play an important role in the determination of cellular response, influencing adhesion, adsorption and differentiation. Bacterial activity and colonization is faster where the surface shows deep irregularities, thus in patients with high risk of periodontal disease and Gram negative anaerobic presence, different surface treatments might generate early failures and onset/recurrence of inflammatory periimplant diseases.

Some of the frequently used treatment techniques include sandblasting, acid etching and electropolishing; each one imprinting unique topographic features on the treated surfaces. By projecting pressurized particles, the sandblasting treatment delivers titanium surfaces with roughness values highly superior relative to the ones in a controlled polishing technique, and is responsible for the introduction of contaminants into the surface. Regarding the acid etching, the treatment with strong acids cleans the metal substrate and delivers homogeneous roughness attributes throughout the entire surface. In turn, electropolishing is a electrochemical process that delivers titanium surfaces with a bright, clean and smooth appearance, through the removal of a thin top layer of the material.

Experimental Methods
109 patients were included in the study. After comprehensive evaluation, including non-surgical and surgical therapy for the treatment of periodontal disease, 59 implants with sand-blasted surface (Straumann Basel Switzerland – SLA surface) and 50 implants double etched surface (Biomet 3i Palm Beach FL USA - Osseotite®) were placed according to the guidelines, provided by the two different manufacturers. Implants were received directly from the companies and surface treatments were declared as mentioned above. Before implant installation a profilometric analysis was done randomly on both groups. Each treatment site was subject to X-Ray examination at 3 to 6 months after the final coronal restorations were placed. Patients were enrolled in the follow up maintenance program and radiological evaluation was carried out at 5 and 10 years.

Results and Discussion
A total of 109 SLA and Osseotite® were placed at the beginning of the study. Data recorded from 91 patients who completed the final T2 follow-up were included in the analysis (SLA, n=50 and Osseotite®, n=41). At the 10-year interval, in periodontally compromised patients the DIB (Distance Implant-Bone) between SLA and Osseotite® was significantly different (p = 0.001, 95% CI of mean difference: 0.55, 1.89 mm). Mean (SD) DIB for SLA implants was 2.1 mm (1.1 mm) compared to 0.9 mm (2.1 mm) for Osseotite® Implants. The overall survival rate of SLA and Osseotite® implant surfaces is high over the period of observation.

Conclusion
In conclusion, only non statistical differences were found in terms of bone loss around the two different surfaces in healthy patients. In periodontally compromised population failures were noticed in both groups and bone loss was evident and higher than the acceptable values but not probably attributable to the different roughness. Thus the history of previous periodontal disease plays an important role in the incidence of complications, regardless of the type of surface. This investigation will be continued with the profilometric analysis of the explanted and failed implants, in order to assess whether the surface shows signs of wear over the period of permanence in the oral cavity.

FIGURE 1
SEM image 2000x of Double Etched Surface

FIGURE 2
SEM image 2000x of Sand Blasted ( SLA) surface

References


Bioresorbable and Biomimetic Adhesives for Bone Repair and Regeneration

Antzela Tzagiollari1,2, Gerard Insley2, David Kelly2, Philip Procter3, Helen O. McCarthy4, Tanya J. Levingstone5, Nicholas Dunne1

1 Dublin City University, School of Mechanical and Manufacturing Engineering, Dublin, IE; 2 PBC Biomed, Shannon, IE; 3 GP Bio Ltd, Rathkeale, IE; 4 Queen’s University Belfast, Belfast, GB

Introduction
Complex bone fractures present significant challenges for orthopaedic surgeons [1]. Current surgical procedures involve the reconstruction and mechanical stabilisation of complex fractures using invasive techniques that often lead to poor clinical outcomes. An injectable, biocompatible, biodegradable bone adhesive that could glue bone fragments back together and at the same time meet clinical requirements presents a highly attractive solution [2]. This study aims to develop a new bioresorbable and biomimetic bone adhesive, comprised of alpha-tricalcium phosphate (alpha-TCP), phosphoserine and deionised (DI) water, with bone regenerative properties. Specifically, a phosphoserine-modified calcium phosphate cement (PM-CPC) will be developed and through analysis of chemical and biomechanical properties using Design of Experiment (DoE), the formulations that provide suitable setting and adhesive properties for application in bone repair will be identified.

Experimental Methods
The alpha-TCP powder was prepared by mixing calcium phosphate (CaHPO4) and calcium carbonate (CaCO3) at a 2:1 molar ratio (both from Sigma Aldrich) [3]. The powders were turbo-blended and heat-treated in a furnace (Elite BRF1600®, Elite Thermal Systems Ltd., UK) for 6 h at 1400 °C. Following rapid quenching using compressed air, the powder was subjected to particle attrition using a planetary mill (Pulverisette 6, Frisch, Germany) at a rotating speed of 600 ± 5 RPM for 5 min periods over 2, 6, 10, 11, 13 and 15 cycles. The physicochemical properties of the alpha-TCP powder were characterised in terms of particle size (laser diffraction particle analysis), morphology (SEM), phase purity (XRD) and chemical composition (FTIR spectroscopy). Alpha-TCP and phosphoserine (Flamma, S.p.A. Italy) at different percentages (10, 25, 40 wt.%) were combined with deionised water at liquid:powder ratio (LPR) ranging from 0.2, 0.35, 0.5 mL/g. the setting characteristics and biomechanical (compression and shear) properties were measured, analysed and optimised through DoE analysis.

Results and Discussion
XRD refinement technique confirmed the alpha-TCP demonstrated a phase purity of 99.9% (Figure 1a), which was corroborated by FTIR analysis. The particle size of the alpha-TCP powder reduced as a function of the number of attrition cycles (Figure 1b) with a D50 of 5.4±1.25 μm following 15 cycles. SEM analysis (Figure 1c) demonstrated the TCP-powder was non-spherical in morphology and also demonstrated a negative correlation (R² = 0.90) between the particle size and number of attrition cycle. The zeta potential of the alpha-TCP had an unstable range of -22.3 ± 0.36 mV. This difference was a function of the D50 value for the powder and agreed with findings of Valentim et al. [3]. The initial (t1) and final (t4) setting time of the PM-CPC increased as a function of particle size (Figure 2a), which agrees with previous studies [4]. Compression and shear properties were significantly increased as a function of phosphoserine, enhancing the adhesive/cohesive strength (Figure 2b, c).

Conclusion
Various compositions of PM-CPC have been successfully prepared. Initial results indicate the important role that particle size of the alpha-TCP powder plays in the setting properties. Measured biomechanical and adhesion properties were within the required clinical range [5]. These findings offer the potential to tailor the requirements of
the proposed bone adhesive for the treatment of bone fractures. Future work will optimise the rheological and biodegradation properties and also the *in vitro* and *in vivo* biocompatibility of the PM-CPC based bone adhesive.

**Acknowledgement**
The authors are grateful to Irish Research Council for funding this research number of grand (GOIPG/2020/371) and PBC Biomed for their support.

**Figure 1: Chemical and Physical Properties**
XRD (a), Particle size (b) and SEM (c), where the red and black arrows indicate the main peaks of alpha-TCP phase, as function of particle attrition.

**Figure 2: Setting and Mechanical Properties**
Setting time (a) as function of attrition cycles and compressive (b) and shear (c) strength as per phosphoserine amount.

**References**


Chondroprotective effects of ibuprofen-loaded PLGA nanoparticles in a pro-inflammatory condition

Cecília J. Alves, Alexandra Freitas, Marina Couto, Catarina L. Pereira, Daniela P. Vasconcelos, Beatriz Sousa, Bruno Sarmento, Raquel Gonçalves, Meriem Lamghari

Instituto de Investigação e Inovação em Saúde, NeuroSkeletal Circuits group, Porto, PT

Introduction
The inflammatory response initiated as a consequence of traumatic chondral lesion imposes a major challenge in cartilage repair. The released pro-inflammatory factors induce a shift in chondrocytes activity towards a catabolic metabolism and promote further cartilage degradation [1-2]. Several anti-inflammatory drugs have been proved to mitigate the deleterious effects of inflammation on the cartilage [2], and we propose the use of Poly(lactic-co-glycolic acid) (PLGA) nanoparticles loaded with ibuprofen as a strategy to deliver locally an anti-inflammatory treatment.

Experimental Methods
In this work, we aimed to evaluate the chondroprotective and chondrogenic effects of the ibuprofen-loaded PLGA nanoparticles. To achieve this, human chondrocytes cultured as 3D pellets under an inflammatory condition (stimulation with 100ng/mL of IL-1b), were treated with PLGA nanoparticles loaded with 15 or 30 mg/mL of ibuprofen. Effects on the chondrocyte activity were assessed on day 3 of exposure.

Results and Discussion
The results showed that the PLGA nanoparticles loaded with 30 mg/mL of ibuprofen induced, under inflammatory conditions, a decrease in the mRNA expression levels of metalloproteases, such as MMP-1 and MMP-9 and MMP13. Moreover, data from the immunohistochemical analysis showed that the treatment with PLGA-Ibuprofen 30μg/mL NPs induced the recovery of collagen II expression, and the expression of aggrecan also recovered after treatment with PLGA-Ibuprofen 15μg/mL.

Conclusion
Altogether, the obtained data suggests that treatment with ibuprofen-loaded PLGA nanoparticles mitigates the chondrocytes’ extracellular matrix degradation activity induced in an inflammation scenario.

Acknowledgement
This study was supported by European Union’s Horizon 2020 research and innovation program under grant agreement number 814558 project RESTORE.

References
Acrylate plasma polymerised 3D scaffolds for bone regeneration

Vineetha Jayawarna\textsuperscript{1}, Jane McLaren\textsuperscript{2}, Felicity Rose\textsuperscript{2}, Matthew Dalby\textsuperscript{1}, Manuel Salmeron-Sanchez\textsuperscript{1}

\textsuperscript{1} University of Glasgow, School of Engineering, 1Centre for the Cellular Microenvironment, Glasgow, GB; \textsuperscript{2} University of Nottingham, School of Pharmacy, Faculty of Science, Nottingham, GB

Introduction

Worldwide, more than 2.5 million bone grafts are carried out annually at a cost of approximately $3.4 billion, being the second most commonly transplanted tissue after blood\textsuperscript{1}. Bone grafts are used to provide stability, to replace missing bone from trauma, congenital defects, cancer etc., or to stimulate osteogenesis. However, current bone grafts, which mainly include autograft and allograft, are associated with several drawbacks such as limited availability, donor-site morbidity and pain, inflammation, risk of infectious disease transmission and immune rejection. Tissue engineered bone grafts have emerged as an effective solution to regenerate bone defects while addressing these shortcomings\textsuperscript{1}. In particular, three dimensional (3D) porous synthetic scaffolds made from bio-degradable materials with favourable osteogenic ability and mechanical properties produced using additive manufacturing offers promising conditions for bone repair and regeneration. Efficient presentation of proteins and growth factors (GFs) is however a major challenge when using synthetic materials for tissue engineering. Fibronectin (FN), an extracellular matrix protein that regulates cell adhesion, binds GFs via specific structural domains, offering synergistic signalling effects on mammalian cells. Here we used an inductively coupled plasma system to modify 3D printed polycaprolactone (PCL) scaffolds with a nanometer-thick coating of poly (ethyl acrylate) (PEA), a polymer known to induce FN organisation into a nanonetwork exposing its integrin and GF binding domains\textsuperscript{2,3}. Bone morphogenetic protein-2 (BMP-2) was used to promote osteogenic differentiation, osteointegration and bone regeneration. Human mesenchymal stem cell (hMSC) adhesion and differentiation were assessed in different scaffold geometries at the protein-GF interface to determine the overall efficiency of the system. The chick chorioallantoic membrane (CAM) assay was used to explore the angiogenic potential of the scaffolds. In addition, the coated scaffolds were subcutaneously implanted into rats to assess early-stage osteogenesis and vascularization.

Experimental Methods

Five different scaffold geometries (0/30°, 0/45°, 0/60°, 0/90°, 0/45/90/135°), utilizing various stack angles were designed using an extrusion-based fused filament deposition 3D bioprinter. A custom-built inductively coupled plasma chamber was used to deposit PEA onto printed scaffolds via plasma polymerisation. The efficiency of plasma polymerization, as well as FN adsorption and interaction with BMP-2, on PEA coated PCL scaffolds was evaluated. We then studied the \textit{in vitro} ability of PEA/protein coated PCL scaffolds to facilitate cell proliferation and osteoblast differentiation. The two optimal modified PCL scaffolds were then implanted into rat subcutaneous pouches, for a duration of 12 weeks, with micro computed tomography and histology used to evaluate ectopic bone formation and early-stage vasculature.

Results and Discussion

Porous PCL scaffolds with different angles were printed successfully. X-ray photoelectron spectroscopy (XPS) results confirmed the deposition of PEA coating throughout the entire 3D structure of scaffolds. ELISA results confirmed significantly higher availability of integrin and GF binding domains on PEA-coated scaffolds. Scaffolds coated with PEA/FN/BMP-2 showed improved cell growth, matrix deposition and osteogenic differentiation compared to uncoated scaffolds. Scaffolds with 0/60° interlayer rotation angles significantly enhanced the cell growth and differentiation compared to the other scaffold geometries. \textit{In vivo}, both coated and non-coated implanted scaffolds
were fully infiltrated with highly vascularised soft tissue and low levels of calcium deposits were detected using von Kossa staining.

**Conclusion**

We propose that our functionalised 3D scaffolds which significantly enhance MSC osteogenesis hold great promise for bone tissue regeneration and this new GF delivery mechanism should maximise the clinical effectiveness and reduce the side effects of BMP-2. Further work will include the testing of the optimised scaffold within a larger animal bone defect model.

**Acknowledgement**

The authors thank Sir Bobby Charlton Foundation for providing financial support for this work.

**References**


PS2-04-162

Doxorubicin-loaded hydroxyapatite nanoparticles as trigger for anticancer bone cements

Marta Tavoni¹, Francesca Carella¹, Massimiliano Dapporto¹, Claudia Cocchi², Alessandro De Vita², Anna Tampieri¹, Michele Iafisco¹, Simone Sprio¹, This work was supported by the Italian Ministry of Health (Bando Ricerca Finalizzata 2016, no. GR-2016-02364704)

¹ National Research Council of Italy (CNR), Institute of Science and Technology for Ceramics (ISTEC), Faenza, IT; ² Osteoncology and Rare Tumors Center (IRCCS), Istituto Romagnolo per lo Studio dei Tumori (IRST), Mendola, IT

Introduction
Nanomedicine is an emerging field of biomedical research and is defined as the applications of nanomaterials for diagnosis, monitoring, control, prevention and treatment of diseases. One of the most promising field in nanomedicine is the development of nanosized drug carriers to deliver anticancer drugs. Nanoparticles are characterized by very high surface/volume ratio, thus promising to control the drug adsorption and release. Moreover, nanoparticles may be capable to protect the drug from premature degradation. The possibility to use calcium phosphate nanoparticles as carriers for local and controlled delivery of drugs is attractive. Traditionally, apatite-based materials are used for bone regeneration applications due to their properties such as osteoinductivity and bioactivity. Furthermore, they are provided with low-cost production, low toxicity, high biocompatibility, biodegradability, tuneable chemical-physical properties and pH-dependent dissolution profile. This latter property comes to be very useful in case of tumor tissue, generally associated with lower pH.

Experimental Methods
In this work, hydroxyapatite nanoparticles (NPs) were developed by wet chemistry at low temperature and characterized, followed by the surface adsorption of doxorubicin (DOX), a drug commonly used in cancer chemotherapy. Then, the as-obtained DOX-NPs were mixed with Sr-doped α-tricalcium phosphate and aqueous solutions enriched with natural polymers, in proper amounts to obtain calcium phosphate bone cements with setting times and drug release profile according to clinical need [1-5]. The biological performance of the DOX-loaded cements on triple negative breast cancer (TNBC) cell line MDA-MB-231 was also assessed by the mitochondrial reduction assay 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) which is used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity.

Results and Discussion
The drug release profiles from both DOX-NPs and DOX-loaded cements were performed at physiological pH in HEPES medium, showing concentrations according to the in vivo pharmacokinetics values. The biological results showed a significant decrease in the TNBC cell line proliferation rate cultured on DOX-NPs loaded cements compared to unloaded control.

Conclusion
The obtained results demonstrate the potential of DOX-NPs to be included in apatitic cements addressed to bone regeneration which can act as smart drug delivery systems, promising for more effective local treatment of bone cancer.

Acknowledgement
This work was supported by the Italian Ministry of Health (Bando Ricerca Finalizzata 2016, no. GR-2016-02364704)

References


Strontium functionalisation of diatom frustules for bone regeneration

Andrew Reid¹, Fraser Buchanan², Matthew Julius³, Pamela Walsh²

¹ Queen’s University Belfast, Chemistry & Chemical Engineering, Belfast, GB; ² Queen’s University Belfast, Mechanical & Aerospace Engineering, Belfast, GB; ³ St Cloud State University, School of Biological Sciences, St Cloud, US

Introduction

Diatoms are a class of eukaryotic microalgaes¹ primarily found in aquatic environments. They are unique in that their cell walls (frustules), are composed of biogenic silica and have a very intricate structure. This structure is highly porous, nanopatterned and has a large surface area (~10 to 300 m²g⁻¹), which is identically replicated from generation-to-generation within the same diatom species. Additionally, it has been shown that diatoms have the ability to incorporate material other than silica into the frustule, allowing for diatoms to be doped to add functionality³⁻⁸.

Within our research we plan to modify the frustule of Cyclotella meneghiniana with Magnesium²⁺ and Strontium²⁺ ions using an in vivo feeding method as this could have the potential to be further developed into a novel implantable (bone tissue regeneration) technology. Research from within our own group has already shown that “diatom biosilica is non-toxic and does not invoke a pro-inflammatory response”¹. Magnesium and strontium were chosen as they have been shown to be beneficial for bone formation & health. We will characterise the doped diatoms with a vast array of analytical techniques, including SEM, EDAX, AFM, ICP & TGA in order to determine the extent & location of the ion incorporation. It is well documented within the literature that an “in-vivo feeding” approach has been successful in incorporating various metals, such as Calcium³⁻⁷,⁸, Germanium⁴, Iron⁵ and Titanium⁶ into the frustules of different species of diatoms, which is good supporting evidence for the viability of our research.

Bone regeneration therapies and implantable materials will be in increasing demand due to the increasing diagnosis rates of bone degenerative diseases such as osteoarthritis and osteoporosis. These conditions are often overlooked however their rates of diagnosis are on the rise due to a generally aging population⁹.

Experimental Methods

The diatom selected for this study is C. meneghiniana (which was isolated from the Mississippi River, USA), which will be cultured in D.I. H₂O enriched with Guillard F/2 solution containing the Mg & Sr 2+ ions in the form of their chloride salts. Silicon depletion of the diatoms will be carried out similarly to Hildebrand (et al.)¹⁰, in order to increase metal uptake. Post culturing, samples were cleaned to remove the residual organic material¹. Initial characterisation of the diatoms was be carried out, using Spectroscopy, Scanning Electron Microscopy (SEM) and Energy-dispersive X-ray spectroscopy (EDAX).

Results and Discussion

SEM images in Fig 1 (a and b) show the morphology of the frustules of C. meneghiniana prior to doping with a typical particle size of 12 to 14 mm. In the doped samples preliminary results have shown that Sr is being taken up into the diatoms’ frustules. EDAX results confirmed the uptake of strontium (Fig. 2a) whereas no Sr was detected in the control (Fig 2b). The concentration of Sr in the doped samples was quantified to be 4.08(±0.93) atomic weight percentage (n=10).

Work is now underway to fully characterise the doped diatom frustules using AFM, EDAX, SEM, ICP & TGA in order to assess their potential in bone repair.

Conclusion
In conclusion we hope to present the functionalisation of diatom frustules with magnesium and strontium ions. This may provide a potential new biomaterial for bone regenerative medicine.

**Acknowledgement**

I would like to acknowledge the contributions, advice and support of my supervisors in this research.

References

[9] Reginster (et al.) Bone 38: 4-9, 2006;
The Effect of Needle Diameter on the Diameter of Electrospun PCL Microfibres

Moresche G. Bartley, Kathleen E. Tanner

Queen Mary University of London, School of Engineering and Materials Science, London, GB

Introduction
A successful Bone Tissue Engineering scaffold requires biocompatibility, mechanical properties, biodegradability or lack thereof and scaffold architecture. The attachment, migration and proliferation of cells are fundamental to the formation and regeneration of bone tissue (O’Brien et al., 2011). Electrospinning is a fabrication technique used to create fibrous scaffolds which can resemble the native structure of the extracellular matrix environment. In this study, we investigated the effect of needle diameter on the diameter of electrospun (Polycaprolactone) PCL microfibers.

Experimental Methods
15% w/v of PCL (80,000 Mw) in chloroform was used to prepare electrospun microfibers. Needle sizes 10, 12, 14, 16 and 18 gauge, giving internal diameters (ID) of 2.69mm, 2.16mm, 1.60mm, 1.19mm and 0.84mm respectively, were used. The electrospun fibres were collected on a rotating mandrel. Parameters such as flow rate (2ml/hr), voltage (19.2kV), humidity (50%), temperature (17°C), distance 13cm and the speed (350 rpm) of the rotating mandrel were optimised and then remained constant. Fibre mats were characterised using Scanning Electron Microscopy (SEM) at a magnification of x1,000 and the diameter of the microfibres and intra-fibre spaces, but not the nanofibres, were measured using ImageJ software.

Results and Discussion
The PCL fibres formed had microfibres diameters ranging from 0.8-27µm (Figure 1). The resulting electrospun fibres demonstrated that fibre diameter can be controlled by the ID of the electrospinning needle (Figure 2). Small needle diameters such as gauge 18 and 14 (0.84mm and 1.60mm ID respectively) produced thinner microfibres with minimal needle blockages during the electrospinning process. Furthermore, needles with large IDs such as gauges 10 and 12 (2.69mm, 2.16mm respectively) frequently developed needle blockages but resulted in larger fibre diameters. A mixture of micro and nanofibers were observed for needles with a small ID such as gauge 14 (1.60mm). The instability of the Taylor cones formed caused the electrified jets to form secondary branching, fabricating thinner fibres branched off from the main electrospinning jet.

Conclusion
The ID of an electrospinning needle influences the diameter of fibres within a scaffold. This is most likely controlled by the size of the jet released from the needle tip. Further work will focus on using stop motion imagery and high-speed photography to identify changes in the electrospinning jets and the resultant fibres.

Acknowledgement
This research is funded by Queen Mary University of London via PhD studentship to MGB.
SEM image of PCL Electrospun Fibres Using a Gauge 14, ID 1.60mm needle

Figure 1. Electrospun PCL fibres using a Gauge 14, ID 1.60mm needle showing the bi-modal fibre distribution produced (marker bar = 100µm).

Effect of Needle Diameter on Microfibre Diameter

Figure 2. Effect of needle diameter on microfibre diameter. Mean data plotted ± standard deviation represented as error bars. Statistical significance due to the relationship between needle gauge and average fibre diameter using one-way ANOVA (n=5). Post-hoc Tukey’s HSD shows levels of statistical difference denoted as *P<0.05, **P<0.01.

References
3D bioprinted silk-gelatin ink system for cartilage regeneration

Cristina Manferdini¹, Shikha Chawla², Giovanna Desando³, Elena Gabusi¹, Aarushi Sharma², Diego Trucco¹,4,5, Juhi Chakraborty², Mauro Petretta⁶,³, Gina Lisignoli¹, Sourabh Ghosh²

¹ Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, IRCCS Istituto Ortopedico Rizzoli, Bologna, IT; ² Regenerative Engineering Laboratory, Department of Textile and Fibre Engineering, Indian Institute of Technology, New Delhi, IN; ³ Laboratorio RAMSES, IRCCS Istituto Ortopedico Rizzoli, Bologna, IT; ⁴ The BioRobotics Institute, Scuola Superiore Sant’Anna, Pisa, IT; ⁵ Department of Excellence in Robotics & AI, Scuola Superiore Sant’Anna, Pisa, IT; ⁶ RegenHU LTD, Villaz-St-Pierre, CH

Introduction
Cartilage lesions have become a major health problem worldwide because of the limited regenerative capacity of articular cartilage following trauma or chronic joint disorders like osteoarthritis (OA)¹. Despite multiple efforts in addressing this clinical need, there are no efficient and long-lasting therapies available for promoting cartilage regeneration². Tissue engineering approaches, combining a range of biomaterials, cells, and growth factors, could provide prospecting alternatives³-⁴. Several unanswered questions and aspects of this technology remain to be elucidated to expand its clinical application. There is still a lack of understanding of the role of specific biomaterials in modulating molecular signaling pathways during in vitro chondrogenic differentiation. Previously we have reported silk fibroin-gelatin (SF-G) bioink using progenitor cells or mature articular chondrocytes to make phenotypically stable engineered articular cartilage⁵. Therefore, we aim to identify the role of SF-G bioink in modulating in vitro chondrogenic signaling pathways in human bone marrow-derived stromal cells (hMSCs) using detailed proteomics analysis.

Experimental Methods
Silk gelatin-based 3D bioprinted constructs, composed of 5% bombyx mori silk fibroin (SF) and gelatin (G) were encapsulated with hMSCs through the bioprinting platform (3D Discovery, RegenHU). SF-G constructs were cultured with D-MEM high glucose with and without chondrogenic factors (GFs). Cell viability assessment and chondrogenic differentiation through histology, gene expression analyses by Real Time PCR and protein by immunohistochemistry and PANTHER and DAVID analyses were carried out. Chondrogenic differentiation was monitored at 1, 14 and 28 days.

Results and Discussion
SF-G 3D bioprinted constructs resulted biocompatible ensuring high percentage of cell viability. Gene expression analyses displayed a high expression of COL2, SOX9, COMP, ACAN and low expression of COL1 and COL10 in chondrogenic 3D bioprinted SF-G-compared to control. The interaction between SF-G bioink and hMSCs augmented several chondrogenic pathways, including Wnt, HIF-1, and Notch as explored with PANTHER and DAVID analysis. We elucidated the debatable role of TGF-β signaling in vitro, by assessing the differential protein expression by hMSCs-laden bioprinted constructs in the presence and absence of TGF-β3. We revealed a direct role of TGF-β3 in generating stable chondrogenic differentiation. hMSCs-laden bioprinted constructs contained a large percentage of collagen type II and Filamin B, typical to the native articular cartilage. Hypertrophic markers were not identified following TGF-β3 addition.

Conclusion
This is the first study reporting such extensive proteome profiling of the in vitro silk based bioprinted constructs. The SF-G bioink positively modulated the expression of cartilage-specific ECM components by emphasizing the regenerative potential of this 3D bioprinted SF-G bioink system for cartilage regeneration.

Acknowledgement
Page 1630 of 2028
This work was jointly funded by the Department of Science and Technology, India, and the Italian Ministry of Foreign Affairs and International Cooperation.

References


Effect of silane-containing gas flame surface pretreatment on the flexural strength of veneered Y-TZP ceramic before and after \textit{in vitro} aging

Katia Sarafidou\textsuperscript{1}, Eleni Siarampi\textsuperscript{1}, Lambrini Papadopoulou\textsuperscript{2}, Nikolaos Kantiranis\textsuperscript{2}, Eleana Kontonasaki\textsuperscript{1}, Petros Koidis\textsuperscript{1}

\textsuperscript{1} Aristotle University of Thessaloniki, Department of Dentistry, Thessaloniki, GR; \textsuperscript{2} Aristotle University of Thessaloniki, Department of Geology, Thessaloniki, GR

Introduction
A common design in prosthodontics consists of a zirconia restoration veneered with a feldspathic layer. The chemical composition of the two components is different and thus bonding techniques between the two materials, as well as the behavior of the interface, may control the mechanical properties of the bilayered restoration. Pretreatment of the zirconia surface could possibly improve the bond between core and veneering layer and the quality of the interface. Therefore, various surface treatment methods have been applied such as surface grinding, air-abrasion, application of ceramic liners or laser etching \cite{1}. Silane-containing butane gas flame (SGF) has been proposed as a pretreatment method because it can be easily applied and creates a silica rich layer on the interface that may enhance the bond strength \cite{2}. However there is no data in literature on how this method can affect the flexural strength of veneered zirconia specimens.

The aim of the present \textit{in vitro} study was to investigate the flexural strength of bilayered zirconia ceramics after SGF pretreatment of the zirconia core, before and after artificial accelerating aging.

Experimental Methods
Sixty bar-shaped specimens were manufactured out of a dental Y-TZP material and divided in two groups: in the first group specimens were layered with a liner (LINER) before application of a feldspathic veneering ceramic, while in the second group, the zirconia surface underwent pretreatment with SGF with the Silano-Pen device (SGF) before layering. Half of the veneered specimens in each group (n=15) underwent artificial accelerating aging for 10h at 122°C with 2.5 bars pressure, simulating more than 30 years of clinical time. A 4-point bending test was performed in order to determine flexural strength. Specimens were further analyzed using Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD) and Fourier Transform Infrared (FTIR) Spectroscopy.

Results and Discussion
There was no statistically significant difference between the flexural strength for the LINER and SGF pretreated specimens before or after aging. However, qualitative analysis revealed that with the majority of the specimens pretreated with SGF, areas of the veneering material remained firmly attached to the zirconia core after flexural strength testing while the liner layer becomes completely detached following the veneering material after fracture. However, most of the specimens presented cohesive type of fracture. SEM analysis on the fractured surfaces revealed the presence of Zr and Y traces on the intaglio surface of the delaminated veneer suggesting that while fracture was occurring, the bond between core and veneer layer controlled the event and led to particles of the zirconia core to be detached and relocated on the veneering material. Cross sectional analysis revealed that even in cohesive failures, the veneering layer is detached from the zirconia surface suggesting a lower bond at the interface. Silane-containing gas flame coating may be beneficial for the veneer-zirconia bond strength since it affects the interface in a microscopic level. Artificial aging had a statistically significant impact on the flexural strength of the specimens, with values being higher after aging [LINER: t(28)=2.255, p=0.032, SGF: t(28)=3.362, p=0.002]. This may be explained by the fact that aging in an autoclave promotes an extensive tetragonal to monoclinic (t→m)
zirconia phase transformation. The $t \rightarrow m$ transformation may lead to a volumetric expansion around defects, which induces compressive stresses that consequently stop propagation of the microcracks [3].

**Conclusion**

The silica rich layer on the interface created after silane-containing gas flame pretreatment may enhance the bond strength between core and feldspathic layer, although it did not significantly increased the flexural strength of bilayered veneered specimens. Pretreatment of the zirconia surface may influence the mechanical behavior of all-ceramic restorations on the long-term. Thus, further clinical investigations are required in order to evaluate the mechanical properties after in situ function.

---

**Figure 1.**
Backscattered SEM micrographs of fractured specimens. Top: zirconia cores after ZL (a) and SGF (c) pretreatment. Down: the intaglio veneer surfaces ZL (b) and SGF (d). White spots on (b) and (d) micrographs correspond to zirconia and grey areas on (a) and (c) to veneer remnants attached to the zirconia core.

**Figure 2.**
FTIR spectra of fractured specimens after being subjected to the 4-point bending test before and after aging. Left: veneered after liner application. Right: veneered after SGF treatment. Black lines correspond to cores being treated with liner or SGF treatment before veneering layer application.
References


Periodontal Restoration Using Femtosecond Pulsed Lasers

Antonios Anastasiou¹, Animesh Jha²

¹ The University of Manchester, Chemical Engineering and Analytical Science, MANCHESTER, GB; ² University of Leeds, School of Chemical and Process Engineering, Leeds, GB

Introduction

Periodontitis is an inflammatory disease affecting the periodontium i.e., the tissues that surround and support the teeth. The pathogenesis of the disease begins when microorganisms adhere and grow on teeth surface forming microbial plaque. If left untreated, formation of pathological pockets around diseased teeth is observed which eventually can lead to tooth loss [1]. Many treatment strategies have been proposed so far but the most of them fail to achieve regeneration of the PD ligament or tissue reattachment mainly because of:

• The oral bacterial which are attached on the tooth surface and lead to contamination.
• The formation of a junctional epithelium which is not practically attached to the tooth surface. Here we propose the use femtosecond lasers in order to sinter, densify and bond a layer of ceramic biomaterial on the surface of the tooth and close to the periodontal pocket. Based on the properties of the new layer (regenerative potential and antibacterial properties) we aim to promote the regeneration of the periodontal tissues and to protect the new tissue from the formation of bacterial biofilm. To be successful, we need appropriate procedures for the irradiation and attachment of the new layer and to engineer suitable biomaterials with both antibacterial properties and enhanced regenerative potential.

Experimental Methods

All the calcium phosphate minerals used in this work have been synthesised through wet precipitation method at temperature of 37 °C and pH=5.4 for brushite and pH=8 for fluorapatite. To enhance the laser-biomaterials interaction, we doped the minerals with 10% Fe²⁺/Fe³⁺. We also introduced Sr²⁺ and Ce³⁺ into the crystal lattice in order to enhance the regenerative and antibacterial potential respectively. For laser irradiation experiments bovine enamel blocks have been coated with a mixture of chitosan solution and the corresponding mineral powder. Two different lasers have been utilised i.e. a femtosecond pulsed laser with repetition rate of 1 KHz and emission at 800 nm (System A: Ti:Shaphire, LIBRA) and an ultrafast femtosecond pulsed laser with repetition rate of 100 MHz and emission at 1040 nm (System B: Chromacity Ltd).

Results and Discussion

After experiments with the two lasers we managed to form compact layers of fluorapatite on the surface of dental enamel using system B. The thickness of the new layers is between 20 and 30 μm and after nano-indentation the mechanical properties found to be very close to that of dental enamel and natural bone. System A, because of the low repetition rate produces pulses of high energy causes ablation. After we identified the appropriate laser, our efforts were focused on optimising the laser energy. The minimum power where sintering is taking place is 0.2 W. Although there are alterations on the surface of the coating, the delivered energy is not sufficient for bonding the mineral to the enamel. Bonding can be achieved for average power higher than 0.45 W [2]. In this case there is strong attachment of our mineral with the enamel and after checking the cross sections of the samples we verified that the thermal damage is restricted in a zone of 5 μm below the enamel-coating interface. For higher energy considerable thermal damage to the enamel was observed.

In terms of materials we concluded that brushite isn't appropriate for this application. Upon heating, brushite losses two molecules of water and is transformed into monetite. Further heating results in the transformation to γ- and β-pyrophosphate. Similar transformations were also observed during the laser irradiation of the brushite coatings.
Because of the radical change in crystal structure, there was shrinkage of the sintered layers and formation of microporosity, leaving exposed the surface of the underlying enamel. On the other hand, fluorapatite, resulted in dense layers and thus we further investigated its biological properties.

Three types of fluoroapatites (undoped, Sr-doped and Ce-doped) were tested for regenerative potential and antibacterial properties. After experiments with four bacteria (i.e. *E. Coli*, *S. Aureus*, *B. Subtilis*, *B. Cereus*), it was found that the undoped and the Ce$^{3+}$ doped fluorapatites present better antibacterial response than the Sr$^{2+}$ doped material [3]. The synthesised minerals in powder form were then incorporated into chitosan scaffolds and tested with Dental Pulp Stem Cells (DPSCs) to check their regenerative potential. Both the scaffolds containing Ce$^{3+}$-doped and those with Sr$^{2+}$ doped fluoroapatite, presented high osteoconductivity leading to the differentiation of the DPSCs into osteoblasts [4]. Overall, it was shown that doping with Ce$^{3+}$ retains the good antibacterial profile of fluorapatite and enhances its regenerative potential, which makes it a promising option for the proposed application.

**Conclusion**

With this platform technology we aim to transform the tooth itself to the scaffold that will promote the regeneration of the PD ligaments and prevent the formation of bacterial biofilm.

**Acknowledgement**

The authors would like to acknowledge the sponsors of this work: Marie Curie IF Pre-Facto (660147).

---

**References**


Development of bioadhesive microcapsules as a new cellular treatment for the diffuse cartilage lesions

**Desiré V. Bustos**¹, Aurelio V. Castrillo², Matilde A. Rodrigo¹,³, José Carlos R. Cabello¹,³

¹ Universidad de Valladolid, CIBER-BBN, BIOFORGE (Group for Advanced Materials and Nanobiotechnology), 47011 Valladolid, ES; ² Hospital Clínico de Valladolid, Departamento de traumatología, 47003 Valladolid, ES; ³ Technical Proteins Nanobiotechnology S.L, 47011 Valladolid, ES

**Introduction**

Cartilage structures can be damaged causing two types of injuries: focal; the lesion is restricted to a concrete zone or diffuse; the lesion affects a broad area of the articular cartilage. This type of lesions appears due to the reduced self-healing capacity of articular cartilage. Diffuse damage is much more difficult to treat, usually restricted to palliative care or systemic anti-inflammatory treatments. As the damage progresses up to the point where the majority of the cartilage is lost, the only option available is the surgical replacement of the arthritic joint with a prosthesis. This type of diffuse lesions have a high prevalence as the common type of degenerative processes associated to aging and intense professional sport.

More recently, cell-based approaches using autologous chondrocytes or, specially, mesenchymal stem cells (MSCs) [1] have been tested and it can even be found in some clinics. However, the efficacy of such cell-based treatment is controversial (MSCs plays a palliative role more than a direct restoration of the lost cartilage) [2] where the main problem is the unconfinement of the cells used. In general terms, the applied cells stay in the injection site only for short periods of time, so their regenerative potential is greatly reduced. The most promising approach is the combination of such cells with a biomaterial-based carrier that can play that role.

The general objective of the project, in order to resolve the previous task, is the development of bioadhesive and injectable cell microcarriers with the ability to regenerate the articular cartilage. This aim is materialized in the fabrication of multibiofunctional capsules that are able to promote the cell cargo with selective adhesion and location on the articular surface (cartilage). To do so, special attention is paid to the cell-material interaction on both the inner and outer surface of the capsule by means of the inclusion of, on one hand, specific cell adhesion domains and, on the other hand, sequences that promote the adhesiveness to collagen II and chondroitin sulfate, two distinctive components of the hyaline cartilage [3].

The microcapsules are based on a novel kind of biomaterials, named Recombinamers that are polypeptide materials obtained by recombinant DNA technology. In particular, the core composition of the microcarrier is the Elastin-like Recombinamers (ELRs) [4].

**Experimental Methods**

Recombinant DNA techniques have proven to be very powerful tools for the development of novel protein-based biomaterials. This class includes ELRs, which are protein-based polypeptides that comprise repetitive units of the Val−Pro−Gly−X−Gly (VPGXG)n pentapeptide, in which X (guest residue) could be any amino acid except L-proline.

ELRs are inspired by elastin, showing excellent biocompatibility, and they exhibit thermo-responsiveness in aqueous media [5].

The cloning and molecular biology for gene construction were performed using standard genetic-engineering methods. Production was carried out by recombinant techniques using Escherichia coli as the cell system. Purification was performed by several cooling and heating purification cycles (Inverse Transition Cycling) following centrifugation; the ELRs obtained in this manner were dialyzed against MilliQ water and lyophilized.
The purity and molecular weight of the ELRs were verified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Amino acid composition analysis and infrared spectroscopy (FTIR) were also performed.

Results and Discussion
Genetic-engineering methods allowed the synthesis of the genetic construct capable of synthesizing the desired biomaterial. Biopolymer adhesion to the hyaline-cartilage matrix was demonstrated measuring the adhesion forces between our biomaterial and surfaces with collagen II and chondroitin-sulfate versus control surfaces. Tailored layer-by-layer (LbL) approaches allowed the encapsulation of cell spheroids of autologous chondrocytes and MSCs.

In vitro and ex vivo assays were performed using cartilage explants from patients surgically intervened, this type of analysis showed the derivatized ELR adhesion efficacy to the articular surface and the final liberation of cell spheroids encapsulated hoping to demonstrate the cartilage regeneration.

Conclusion
In conclusion, a novel protein-based biomaterial with adhesion to the hyaline-cartilage matrix was synthetized. Besides, cell spheroids were encapsulated by the polymer. Hence, in this study could be possible overcome the limitations of the current treatments used in diffuse cartilage lesions and suppose an advance in regenerative medicine.

Acknowledgement
The authors are grateful for the funding from the Spanish Government (PID2019-110709RB-100, RTI2018-096320-B-C22, RED2018-102417-T), Junta de Castilla y León (VA317P18, Infrared2018-UVA06), Interreg V España Portugal POCTEP (0624_2IQBIONEURO_6_E) and Centro en Red de Medicina Regenerativa y Terapia Celular de Castilla y León.

References
2:00 p.m. – 3:30 p.m.

Poster floor

**PS2-05 | Biomaterials Development**
Polymeric Scaffolds reinforced with Graphene-Based Nanomaterials obtained by SLA: Preliminary study of the effect of nanofillers on curing and printability

Sara Lopez de Armentia¹, Silvia Fenández Villamarín¹, Juan C. del Real¹, Nicholas Dunne²,³, Eva Paz¹

¹ Comillas Pontifical University, Institute for Research in Technology, Madrid, ES; ² Dublin City University, Centre for Medical Engineering Research, Dublin, IE; ³ Dublin City University, School of Mechanical and Manufacturing Engineering, Dublin, IE; ⁴ Queen’s University of Belfast, School of Pharmacy, Belfast, GB; ⁵ Trinity College Dublin, Department of Mechanical and Manufacturing Engineering, Dublin, IE; ⁶ Dublin City University, Advanced Manufacturing Research Centre, Dublin, IE; ⁷ Trinity College Dublin, Advanced Materials and Bioengineering Research Centre, Dublin, IE; ⁸ Dublin City University, Advanced Processing Technology Research Centre, Dublin, IE; ⁹ Trinity College Dublin, Trinity Centre for Biomedical Engineering, Dublin, IE

Introduction

Despite the inherent capacity of the bone to regenerate and repair itself, when an irregular or a large defect appears, external solutions must be applied. Traditionally, autografts have been the gold standard; however, due to their drawbacks, like donor-site morbidity, alternative solutions have been explored. In this context, porous scaffolds hold great promise for bone tissue regeneration. They act as extracellular matrix that supports cell growth, promoting cell adhesion and proliferation. For an adequate performance, they must meet some requirements: e.g. biocompatibility, interconnected porosity, mechanical properties and biodegradability. Conventionally, different methods have been used for their manufacturing (e.g. electrospinning and freeze-drying). However, these methods do not allow an adequate control of geometry and porosity of the scaffolds.

The development of additive manufacturing techniques offers a promising solution since it allows the complete customisation and control of scaffolds geometry and porosity. To allow cells to adhere and proliferate on its surface, the material used to manufacture the scaffold must have a proper wettability. In general, polymers show a hydrophobic behaviour, which hinders cell adhesion. It has been proved that the incorporation of graphene (G)-based nanomaterials, like graphene oxide (GO) [1], improves hydrophilicity of different polymers. Furthermore, G-based nanomaterials present osteoconductivity and antimicrobial effect [2], besides, they reinforce the matrix from a mechanical point of view. It makes them suitable for being used as nanofillers to improve the properties of scaffolds. Amongst the different additive manufacturing technologies, stereolithography (SLA) shows many advantages: high accuracy, anisotropy, and liquid raw material, which favours the addition of nanofillers. However, when nanofillers are added to the resin, there is a competition in terms of light absorption between the photoinitiator and the nanofiller. Nanofillers may influence the UV curing process due to modifications in optical properties, which results in changes in absorbance or transmittance of the resin. They also can act as points for light scattering and shielding [3]. Besides, polymerisation may be influenced by nanofillers if they act as chain transfer agent, inhibiting the growth of polymer chain [4], or as free radical scavengers, cutting off the polymerisation process [5].

Therefore, the aim of this work is to study the effect of G-based nanofillers on the curing of an acrylic photocurable polymer.

Experimental Methods

As matrix, a commercial photocurable acrylic resin was used (Clear Resin Formlabs, USA). The matrix was reinforced with different G-based nanomaterials: G, GO and graphite nanoplatelets (GOxNP). Nanocomposites were prepared by ultrasonication with a load of 0.1 wt.% and viscosity was measured. Differential Scanning Calorimetry (DSC), Fourier-Transformed Infrared Spectroscopy (FTIR), hardness measurements and the extent of curing degree at
different ultraviolet exposure times were conducted. Finally, the printability using a SLA printer (Form 2, Formlabs) and dimensional stability of the different nanocomposites were examined.

**Results and Discussion**

An increase in the viscosity of nanocomposites was recorded for all nanocomposites investigated with the exception of G. The latter showed a reduction in the viscosity. This decrease in viscosity could be related with a poor dispersion of G within the resin.

The results demonstrated that nanofillers affects the curing of the matrix (R) to a different degree depending on the nanofiller type (Figure 1). Specifically, GO did not significantly affect the degree of curing, whilst GOxNP influence curing at low exposure times and G for all UV exposure times studied, which hampered the UV-curing of the resin. FTIR analysis and hardness test corroborated this trend, with a notable retardation in the UV-curing of the resin with the presence of G.

Printability tests were completed in accordance with curing study. G composite did not cure sufficiently to create the part, whilst the other composites did (Figure 2).

In terms of dimensional stability, it was found that the addition of GOxNP provided an improvement. However, the incorporation of GO did not affect dimensional stability.

**Conclusion**

It was found that the addition of GO and GOxNP to an acrylic resin do not affect the curing process or printability. Conversely, the incorporation of G affected both, probably due to the darker colour of this nanofillers, which absorbs more light and reduces the performance of the photoinitiator, negatively affecting the printability. Besides, GOxNP improved the dimensional stability of printed parts.

![Figure 1. Curing degree vs UV exposure time of resin and its nanocomposites](image-url)
Figure 2. Printed cubes with R (a), R+G (b), R+GO (c) and R+GOxNP (d)

References


Development and surface modification of poly(ester urethane)s for the production of fused deposition modeling printed scaffolds

Arianna Grivet Brancot\textsuperscript{1,2}, Monica Boffito\textsuperscript{1}, Gianluca Ciardelli\textsuperscript{1}

\textsuperscript{1} Politecnico di Torino, Department of Mechanical and Aerospace Engineering, Turin, IT; \textsuperscript{2} Università di Torino, Department of Surgical Sciences, Turin, IT

Introduction
Additive manufacturing and 3D printing are gaining an ever-growing importance in the biomedical field, due to the possibility to obtain personalized constructs in a cost-effective way when compared to traditional techniques. For this reason, optimized materials are needed in tissue engineering to combine the requirements of the printing processes with the bioactivity necessary for the native tissue repair and regeneration. Thanks to their general biocompatibility, non-toxicity and biodegradability, aliphatic poly(ester)s are widely used in this field; however, the stiffness of these materials is compatible only with the harder tissues present in the body (e.g., bone). In this sense, poly(ester urethane)s can offer an interesting solution. Indeed, their building blocks can be ad-hoc selected to tailor the mechanical and degradation properties of the resulting polymers, thus creating a plethora of materials suitable to replace also softer tissues (e.g., muscle and cardiac tissues).

In this work, a library of poly(ester urethane)s (PURs) based on poly(ε-caprolactone) and 1,6-hexamethylene diisocyanate has been developed. The effect of aliphatic chain extenders with different chain length (i.e., 1,4-butanediol, 1,8-octanediol, 1,12-dodecanediol) has also been analyzed. A surface modification protocol of the synthesized PURs has also been optimized to obtain more hydrophilic and cell-friendly substrates.

Experimental Methods
All PURs have been synthesized adapting a previously developed procedure [1]. The success of the process has been verified through Size Exclusion Chromatography (SEC) and Attenuated Total Reflectance Fourier Transformed Infrared (ATR-FTIR) spectroscopy. Thermogravimetric analysis (TGA), Differential Scanning Calorimetry (DSC) and rheological characterizations were also performed to study the suitability of these materials for Fused Deposition Modeling (FDM) printing applications. Mechanical characterization was also conducted through tensile tests on PUR films fabricated by solvent casting. To improve the hydrophilicity of the material surface, a plasma treatment in the presence of acrylic acid as monomer was optimized to expose -COOH groups. Wettability measurements were then performed on the treated samples and compared to the native PURs. Moreover, to obtain better biocompatibility and improve cell adhesion, the exposed groups were used to graft proteins and amino acids through carbodiimide chemistry. The grafting was verified through ATR-FTIR, \textsuperscript{1}H Nuclear Magnetic Resonance (\textsuperscript{1}H NMR) spectroscopy and colorimetric tests.

Results and Discussion
The PURs synthesized with the three different chain extenders exhibited a number average molecular weight comprised between 30 and 40 kDa. TGA, DSC and rheology characterizations showed that thermal and thermomechanical properties were in all cases suitable for processing through common FDM printers to obtain scaffolds for different tissue engineering applications. Mechanical testing showed that the use of 1,8-octanediol as chain extender resulted in a more elastomeric behavior of the final PUR, while 1,4-butanediol and 1,12-dodecanediol produced stiffer and more brittle materials. Surface modification through plasma treatment was successfully conducted on the PUR films, resulting in a relevant decrease in the contact angle values compared to non-treated samples, due to the introduction of carboxylic acid groups on the surface (Figure 1A and B). Gelatin was then effectively grafted on the plasma treated PURs, as verified...
by the presence of the gelatin peaks in the ATR-FTIR (Figure 1C) and $^1$H NMR spectra and indirectly by the bicinchoninic acid assay (BCA).

**Conclusion**

All the PURs synthesized in this work showed properties suitable for FDM printing processes. The different chain extenders used permitted to obtain differences in the mechanical properties of the materials, which can therefore be used to develop scaffolds for different types of tissues. The possibility to perform a post-processing surface modification of the PURs has also been demonstrated. The plasma treatment successfully exposed carboxylic acid groups, significantly improving the surface wettability and allowing for an easy grafting of biomolecules. In this way, the biocompatibility of the scaffolds produced with these PURs can be further enhanced. Indeed, PURs with similar composition and analogous modification have already demonstrated good interaction with cardiac primitive cells [2]. Moreover, the versatility of the carbodiimide chemistry used to graft proteins permits to ad-hoc select the most suitable biological cues (i.e. protein, amino acid, peptide) for the targeted application.

![Figure 1](image)

**Figure 1**

A) Contact angle measurements made on the three PURs before and after plasma treatment; B) Images of the drop formation on the butanediol-PUR films before and after plasma treatment; C) ATR-FTIR spectra of gelatin and PURs before and after protein grafting. The highlighted sections demonstrate the success of the functionalization.

**References**


Exploiting the network topology of photo-crosslinkable PCL as tool towards improved light-based engineered tissue constructs

Quinten Thijssen, Sandra V. Vlierberghe

Ghent University, Department of organic and macromolecular chemistry, 9000, BE

Introduction
Poly(ε-caprolactone) (PCL) has been the subject of extensive research due to its biocompatibility, biodegradability and excellent rheological and mechanical properties. Especially its suitability for extrusion-based 3D-printing in order to obtain patient-specific implants has attracted significant attention. However, its application in light-based 3D-printing techniques remains scarce, mainly due to the brittleness associated with acrylate-mediated photo-crosslinking. Nevertheless, light-based 3D-printing techniques such as digital light processing offer distinct benefits in terms of throughput and resolution. Therefore, in order to overcome the drawbacks associated with acrylate-mediated crosslinking, the network topology of photo-crosslinked PCL is reported herein as tool to control the mechanical properties. Via the optimization of the structure of the oligomeric precursor as well as the thiol-based crosslinker, networks with various topologies have been designed and the influence thereof on the material properties was elucidated. Finally, the biocompatibility and light-based printability of the designed materials was elucidated.

Experimental Methods
Structure elucidation was performed through 1H-NMR spectroscopy and Fourier-transform infrared spectroscopy (FTIR). The thermal properties were studied by thermogravimetric analysis (TA instruments Q50) and differential scanning calorimetry (TA instruments Q2000). Degrees of swelling and gel fractions were calculated using the following formulas: Gelfraction= m(dry)/m(initial) × 100 (%), Degreeofswelling= (m(swollen)-m(dry))/m(initial). Tensile experiments were performed using a Tinius Olsen Model 1 ST apparatus. Photo-rheology was performed using an Anton Paar Physica MCR 301 rheometer equipped with a UV light-source (365 nm, 3500 mW/cm²) with a parallel plate set-up. DMA experiments were performed in tension using rectangular samples.

Results and Discussion
With the aim to develop thiol-ene photo-crosslinked PCL-based networks with different network topologies, PCL-diol (Mn = 2000, 4000, 6000 and 8000 g.mol⁻¹) was end-functionalized with alkene functionalities (i.e. allyl alcohol, allyl isocyanate or pentenoyl chloride) via urethane coupling chemistry or via the addition elimination reaction with acid chlorides. Successful modification was confirmed via 1H-NMR (nuclear magnetic resonance) spectroscopy and FTIR (Fourier transformed infra-red) spectroscopy. Furthermore, substitution degrees exceeding 80% were retrieved for all network precursors. The ene-terminated PCL was subsequently combined with a multifunctional thiol (i.e. pentaerythritol tetakis(3-mercaptopropionate) or trimethylolpropane tris(3-mercaptopropionate)) in order to obtain thiol-ene photo-crosslinked networks. Network formation accompanied by excellent network integrity upon UV irradiation was confirmed via photo-rheology as well as via gel fraction and swelling experiments. Photo-crosslinkable PCL-based oligomers with increasing molar masses of the oligomer and varying amounts of urethane functionalities (i.e. 0, 2 and 4 per chain) have been designed via this approach. Subsequently, the influence of the functionality of the thiol crosslinker was investigated. Finally, based on the designed network, the influence of the network topology on the material properties was assessed.

In-depth characterization of the thermal, mechanical and biological properties of the networks was performed through thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), tensile testing and cytotoxicity testing. As a result of the step growth polymerization mechanism inherent to thiol-ene crosslinking, homogeneous networks with improved mechanical properties were obtained, as compared to networks resulting from acrylate-mediated crosslinking.
crosslinking (elongation at break extended from 100 up to 700% and ultimate strength increased from 10 up to 21 MPa). Furthermore, a distinct influence of the hydrogen bonding as well as the crosslink density was established, characterized by an increased elongation at break and ultimate strength. Finally, their application in digital light processing, a light-based 3D-printing technique, was successful for several of the synthesized precursors. Throughout the study, acrylate-terminated PCL was applied as reference. The 3D-printed scaffolds are currently being evaluated for their potential to support cell growth of fibroblasts. The key findings will be presented at the conference.

**Conclusion**

Thiol-ene photo-crosslinked PCL-based networks offer distinct benefits over conventional acrylate-based crosslinking. Furthermore, control of the network topology via rational design of the photo-reactive oligomers and functionality of the thiol-based crosslinker was proven to be an effective approach to control the mechanical properties of resorbable PCL-based networks. As a result, the proposed materials have great potential to be exploited as starting materials for light-based 3D-printing to serve biomedical applications.

**Acknowledgement**

FWO-SB (1SA2321N) and 3DMED (interreg2seas) funding acknowledged

**References**


Design of polymeric structures based on chitin-glucan complex and biocompatible ionic liquids for biomedical applications

Inês Ferreira¹,², Diana Araujo¹, Andreia Rosatella³, Carlos Afonso³, Filomena Freitas², Luisa Neves¹

¹ LAQV/REQUIMTE, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Almada, PT; ² UCIBIO/REQUIMTE, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Almada, PT; ³ Instituto de Investigação do Medicamento (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, PT

Introduction
This work aims to merge material design based on a microbial biopolymer - chitin-glucan complex (CGC) - with an innovative ionic liquid (IL) chemistry to develop polymeric structures in the form of films or hydrogels for biomedical applications.

Chitin-glucan complex (CGC) is a polysaccharide found in the cell wall of most fungi and yeasts, including, for example, Komagataella pastoris, Aspergillus niger, Schizophyllum commune, Gongronella butleri, Armillariella mellea and Saccharomyces cerevisiae. This natural co-polymer comprises a chitin moiety (N-acetyl-D-glucosamine units), which is covalently linked to a β-glucans moiety (glucose units), being biocompatible and possesses intrinsic wound healing and antimicrobial activity. However, its use is not widespread due to its insolubility in safe and biocompatible solvents.

In the last decades, ionic liquids (ILs) have been used as alternative solvents for the dissolution of natural biopolymers known for their intractability, such as chitin, cellulose, lignin and starch. ILs are compounds consisting entirely of ions, with an organic cation and an inorganic or organic anion. These solvents possess nonflammability, negligible volatility and their chemical and physical properties can be tuned by changing the cation or the anion in their structure, making them very attractive for a variety of applications, including as solvents for dissolution of biopolymers.

Experimental Methods
The novelty of dissolving chitin-glucan complex (CGC), from two fungal strains, Komagataella pastoris (CGCP) and Aspergillus niger (CGCKZ), using biocompatible ionic liquids (ILs) was explored in this work. Three cholinium-based ILs were tested, choline acetate, choline propionate and choline hexanoate.

Results and Discussion
Although all tested ILs resulted in the dissolution of the co-polymer at a concentration of 5 % (w/w), distinct polymeric structures, films or gels, were obtained from CGCP and CGCKZ, respectively. CGCP films were dense, flexible and elastic, with high swelling capacity (> 200 %). The IL anion alkyl chain length influenced the polymeric structures’ properties, namely, the CGCP films elongation at break and swelling degree. CGCKZ resulted in weak gels. For both polymeric structures, exposure to the ILs under the dissolution conditions caused significant changes in the co-polymers’ chemical structure, namely, reduction of their glucan moiety and reduction of the degree of acetylation, thus yielding chitosan-glucan complexes (ChGC) enriched in glucosamine (53.4 ± 0.3–60.8 ± 0.3 %).

Conclusion
Due to their biocompatibility and also tailorable physical and chemical properties by changing the IL used for CGC dissolution, both biopolymetric structures (films and gels) may be considered promising materials for biomedical applications.

Acknowledgement
This work was supported by the Associated Laboratory for Green Chemistry – LAQV, Applied Molecular Biosciences Unit – UCIBIO and Linking Landscape, Environment, Agriculture and Food research unit, which are financed by national funds from FCT/MCTES (UIDB/50006/ 2020, UIDB/04378/2020 and PEst-OE/AGR/UI0245/2020, respec-...
tively). Funding from the projects PTDC/CTM-CTM/29869/2017 and UID/DTP/04138/2019 funded by FCT/MCTS are also acknowledged. Inês C. Ferreira and Diana Araújo acknowledge FCT/ MCTES for financial support through PhD fellowships SFRH/BD/137636/2018 and SFRH/ BD/140829/2018, respectively.

References
Novel bioinspired GO-COOH decorated hybrid scaffolds based on natural polymers with potential application in tissue engineering

Jana Ghitman1, Elena I. Biru1, Gratiela Gradisteau Pircalabioru2, Horia Iovu1

1 University Politehnica of Bucharest, Advanced Polymer Materials Group, Bucharest, RO; 2 University of Bucharest, Microbiology Immunology Department, Bucharest, RO

Introduction

Bioinspired polymer-based scaffolds with nanofibrous architecture owing to their biomimetic microenvironments, appropriate guidance and mechanical support for cells adhesion and proliferation until hosts cells can repopulate and resynthesize a new natural matrix, are lately of particular importance to tissue engineering and regenerative medicine [1, 2]. The current research study deals with the design and investigation of novel bioinspired and biocompatible GO-COOH decorated hybrid polymeric scaffolds with nanofibrous architecture as biomaterials with highly proper features for functional restoration of damaged tissue. Gelatin (Gel) and alginate (Alg), two biobased - polymers with excellent biocompatibility, high microenvironment biomimicry and ability for proper guidance of cells development in combination with carboxylated graphene oxide (GO-COOH), embody the matrix of electrospun hybrid scaffolds (HAGS).

Experimental Methods

Following the design and characterization of GO-COOH decorated electrospun monocom-/bicomponent hybrid scaffolds based on Gel (GS), Alg (AS) or Alg/Gel (AGS), precursor mixtures consisting from Gel, Alg/polyethylene oxide (PEO) and Alg/PEO/Gel with two GO-COOH concentrations were prepared (Table 1) and subjected to electrospinning process.

Table 1. The detailed composition of all electrospun scaffolds

<table>
<thead>
<tr>
<th>Sample</th>
<th>Prec. syst.(mL)</th>
<th>Alg/PEO/Gel ratio (v/v/v)</th>
<th>wt. % GO-COOH</th>
<th>wt. % Triton-X</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS</td>
<td>5</td>
<td>0/0/5</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>HGS-0.1</td>
<td>5</td>
<td>0/0/5</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>HGS-0.2</td>
<td>5</td>
<td>0/0/5</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>AS</td>
<td>5</td>
<td>3.5/1.5/0</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>HAS-0.1</td>
<td>5</td>
<td>3.5/1.5/0</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>HAS-0.2</td>
<td>5</td>
<td>3.5/1.5/0</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>AGS</td>
<td>5</td>
<td>2/1/2</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>HAGS-0.1</td>
<td>5</td>
<td>2/1/2</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>HAGS-0.2</td>
<td>5</td>
<td>2/1/2</td>
<td>0.2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The stability of electrospun biomaterials was achieved by crosslinking, using glutaraldehyde (GA) vapour to crosslink the monocomponent protein-based biomaterials; Ca2+ ions to mediate the ionic gelation of polysaccharide-based scaffolds, while for the bi-component hybrid scaffolds, the two-step crosslinking process was employed, generating the crosslinked scaffolds with interpenetrated hybrid network.

Results and Discussion

The nanometric fibrous architecture of scaffolds along with the presence of GO-COOH are established by SEM (Fig. 1, a). The structural investigations performed by FTIR, Raman and XPS spectrometry emphasized the occurrence of different non-covalent interactions (e.g., H-bonding) as well the formation of new chemical bonds between the functionalities of the system ‘components. The interpenetrated network of bicomponent structures determines a 10-
folds increase of Young’s modulus as compared to monocomponent counterparts, while the dispersion of GO-COOH significantly increases the elasticity of materials. The increasing trend in elasticity of designed hybrid nanofibrous scaffolds beyond the amount of dispersed GO-COOH might be correlated with the hindering effect graphene lattices which decreased the extend of interactions among the polymer chains accompanied with some re-aggregation phenomena which occurred during the crosslinking process. The in vitro biological assessments performed on NTC fibroblast highlight the highest biocompatibility and proliferation ability of HAGS-0.1 (MTT assay) (Fig. 1, c) as well the low cytotoxic potential of GO-COOH decorated hybrid nanofibrous scaffolds (LDH test) as well its potency support the cells adhesion, growth and proliferation in a certain concentration.

**Figure 1.** (a) – Schematic illustration of the main components and underlying principle in designing bioinspired GO-COOH decorated hybrid scaffolds; (b) - SEM micrographs of all electrospun mono-/bicomponent and GO-COOH decorated hybrid scaffolds with nanofibrous architecture; (c) - Viability and proliferation of NTC fibroblast seeded with nanofibrous scaffolds determined by MTT assay after 2 and 6 days in standard culture conditions.

**Conclusion**

In this work we have designed and characterized novel bioinspired GO-COOH decorated bicomponent hybrid scaffolds with nanofibrous architecture based on alginate/gelatin using electrospinning approach. The preliminary physico-chemical, mechanical and in vitro biological investigations suggest that various types of interactions that occur between the functionalities of the system’s entities and their synergy along with rational nanofibrous architecture might be the driving factors in designing bioinspired scaffolds with highly proper features for personalized medicine. Further studies concerning in vitro and then in vivo screening of biological behavior of the designed biomaterials to ensure their safety and potency beyond the academic/laboratory investigations as biomaterials with highly proper features and performances for tissue engineering, are mantadory.

**Acknowledgement**

This work was funded by a grant of Ministry of Research and Innovation, CNCS-UEFISCDI, project number PN-III-P1-1.1-PD-2019-0205 (contract no. PD 83/2020) within PNCDI III and European Regional Development Fund through Competitiveness Operational Program 2014-2020, Priority axis 1, ID P_36_611, MySMIS code 107066, INOVABIOMED.

**References**


Silk fibroin interaction with other polymers and hydroxyapatite for novel biomaterial development

Arita Dubnika\textsuperscript{1,2}, Karina Egle\textsuperscript{1,2}, Andra Grava\textsuperscript{1,2}

\textsuperscript{1} Riga Technical University, Institute of General Chemical Engineering, Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre, Riga, LV; \textsuperscript{2} Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, LV

Introduction
Silk fibroin (SF) is a fibrous protein derived from Bombyx mori cocoons, that can form natural hydrogels with good biocompatibility, mechanical strength, and relatively slow degradation time \cite{1}. In tissue engineering, SF is often used in combination with other biomaterials. Composite materials can be formed with bioactive ceramics, glass and polymers - synthetic or naturally occurring \cite{2}, such as hyaluronic acid (HA) - a glycosaminoglycan that is used for the production of potential carriers for cell transplantation \cite{3} and hydroxyapatite (HAp) - a naturally occurring mineral that is biocompatible and osteoconductive. The aim of this study was to investigate the ability of silk fibroin to form novel biomatertials with other polymers and HAp.

Experimental Methods
Bombyx mori silkworm cocoons and silk fiber were obtained from China (natural origin). SF solution was obtained by previously described extraction and purification method \cite{4} ensuring concentration of 13.4 ± 1.5 weight %. Interaction of various concentrations of hyaluronic acid (HA), chitosan (CHI) and gelatin (G) was evaluated. Samples were prepared with various ratios of HAp weight % (30-90 %). Different concentrations (0.5 - 3.3\%) of crosslinking agents - glutaraldehyde (GTA) and sodium tripolyphosphate (TPP) were used for preparation of hydrogels. Crosslinking was done at 37°C and 60°C temperatures. Swelling ratio, gel fraction, microstructure and physicochemical properties were analyzed.

Results and Discussion
The highest swelling ratio was observed for SF/HA samples crosslinked with GTA at 37°C temperature compared to physically crosslinked SF/HA hydrogels with no HAp addition. With addition of HAp, CHI or G, swelling ratio and gel fraction is dependent on the crosslinking method, especially order of TPP and ethanol treatment.

Conclusion
Crosslinking with GTA increased the swelling ratio of SF/HA hydrogels compared to physically crosslinked SF samples at all tested temperatures. When HAp and gelatin or chitosan is introduced in SF based samples, crosslinking with TPP and ethanol can be iterated to achieve desirable properties.

Acknowledgement
This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the grant agreement No 857287.

References
\cite{1} N. R. Raia, B. P. Partlow, M. McGill, et.al., 2017, Biomaterials, 131, 58-67.
\cite{2} P. Bhattacharjee et al., 2017, Acta Biomater., 63, 1-17.
\cite{3} X. Hu et al., 2010, Biomacromolecules, 11(11), 3178-3188.
High-strength and light hydroxyapatite-titanium composite fabricated at physiological-temperature

Adelia Kashimbetova¹, Carolina Oliver-Urrutia¹, Mariano Casas-Luna¹, Karel Slámečka¹, Aída Mata-Leija¹, Serhii Tkachenko¹, Karel Dvořák², Jozef Kaiser¹, Ladislav Čelko¹, Edgar B. Montufar¹

¹ Brno University of Technology, Central European Institute of Technology, Brno, CZ; ² Brno University of Technology, Faculty of Civil Engineering, Brno, CZ

Introduction
The possibility of combining the mechanical strength of titanium (Ti) with the bioactivity of hydroxyapatite (HA) is attractive to develop high-strength and low-weight composite materials for the treatment of bone defects. However, the chemical reactions occurring during sintering of Ti in contact with HA have limited usefulness of such composites [1]. In this work, a new concept of achieving the goal is proposed based on the biomimetic precipitation of calcium deficient HA (CDHA) at physiological temperature to avoid the decomposition of both components. The load bearing robocast Ti scaffold was infiltrated with a self-setting CDHA foam and the structure, mechanical, and biological properties were studied. Moreover, to increase the adhesion strength with CDHA, and in turn strengthen the resulting composite, the functionalization of the Ti surface was explored.

Experimental Methods
Ti scaffolds were produced by robotic assisted deposition of an ink that was prepared by mixing of commercially pure Ti powder (ASTM Gd1 20-63 μm) with an organic binder, gelatine. A post-printing thermal treatment was optimized to eliminate the binder and maintain the purity during sintering. Scaffolds were sintered at 1300 and 1400 °C for 3 and 10 h in argon atmosphere using a tubular furnace. Part of the Ti scaffolds was functionalized with gelatine before the infiltration process. CDHA foams were produced by mechanical foaming of an alpha tricalcium phosphate self-setting paste [2], using either polysorbate 80 or gelatine as foaming agents. The obtained injectable foams were infiltrated into the sintered Ti scaffolds and allowed to set in water at 37 ºC for 10 days. The phases in the composites were evaluated by XRD and EDX analysis, additionally XPS and FTIR were used to characterize the functionalization of the Ti surface. The microstructural characterization was performed by optical microscopy, SEM, and μCT. Compression test and indentation adhesion test were used for the mechanical evaluation of the composites. The cytocompatibility of the Ti scaffolds was evaluated by the culture of SaOS-2 osteoblast like cells.

Results and Discussion
Debinding at 350 °C for 12 h was selected as optimal due to the maximum removal of the organic binder and the least thermal oxidation of Ti. The correlation between the consolidation conditions and the mechanical performance of the scaffolds was established. Sintering at 1400 °C for 10 h resulted in the strongest scaffolds, having a yield compressive strength of 61 MPa and a Young’s modulus of 1.2 GPa at 70 % of porosity. The Ti scaffolds showed an equiaxial grain microstructure of α-phase grains with the size of 75 μm (Fig. 1d) and had less than 2 wt.% of C and O. Normally, functionalization of Ti is performed on flat disks [3]. This work shows that functionalization can be done in scaffolds as the surface chemical analysis confirmed the incorporation of Si and N coming from silane and gelatine, respectively. Moreover, improved hydrophilicity was visually observed. The CDHA foams demonstrated the ability to retain a porous structure after infiltration (Fig. 1b, c) with the pore size of about 350 and 120 μm for polysorbate and gelatine, respectively. The crystalline composition of the composites after setting was hexagonal Ti and HA, and no secondary phases neither decomposition were observed by XRD, metallography, and EDX. CDHA had a nano-plate like microstructure (Fig. 1e). μCT reconstruction showed an interpenetrating phase composite of Ti, CDHA, and a pore network, all located continuously along the material (Fig. 1b). SaOS-2 cells growth and matured in the Ti scaffold. Page 1652 of 2028
scaffolds producing a lining layer on the strands and mineral deposits. Currently, the analyses of the CDHA/Ti interface, the mechanical strength, and cytocompatibility of the composites are ongoing.

**Conclusion**

Physiological temperature processing of CDHA/Ti composites without undesirable secondary phases is possible by the biomimetic setting of CDHA inside of Ti scaffolds. The composites exhibited a network of open pores with the size in the range optimal for bone regeneration and with mechanical strength suitable for moderate load bearing applications.

**Acknowledgement**

This work was supported by the Czech Science Foundation (grant 19-22662S). A.K. acknowledges the Brno Ph.D. Talent scholarship funded by the Brno City Municipality and BUT project CEITEC-VUT/FAST-J-21-7305. C.O.U. acknowledges the CONACYT grant 2020-000021-01EXTV-00235. A special acknowledgment to CzechNanoLab Research Infrastructure supported by MEYS CR (LM2018110).

![Figure 1. Structure of the CDHA/Ti composite.](image)

**References**


**Cellulose-Derived Injectable Biocomposites For Bone Tissue Regeneration**

Andrea Fiorati¹², Camilla Galante¹, Cristina Linciano¹, Lina Altomare¹²

¹ Politecnico di Milano, Dipartimento di Chimica, Materiali Ing Chimica ‘G. Natta’, Milan, IT ² INSTM, Firenze, IT

**Introduction**

Among natural polymers, cellulose represents a low cost, abundant and renewable polysaccharide with great versatility. This long chain biopolymer can be found in organized in hierarchical structures composed by nanofibers with high aspect ratio (3-4 nm wide and hundreds μm length). Among all the different methods to obtain cellulose nanofibers (CNFs) proposed in literature, TEMPO-mediated oxidation represents one of the most diffused. [1] From TEMPO-oxidized CNFs aqueous dispersions it is possible to obtain physically crosslinked hydrogels by means of the divalent cation addition. The realization of biocomposite hydrogels through inorganic nanoparticles incorporation can help in overcoming their lack in mechanical properties, mixing the softness of the gel and the hardness of the inorganic component. Moreover, the presence on inorganic components, such as calcium phosphates, can improve not only their mechanical properties but also the bioactivity of the gels. GO has also been tested as an effective treatment for cancer as it can specifically target cancer cells with a significant cytotoxic effect against osteosarcoma. [2,3]

The aim of this work is to design and characterize a TEMPO-oxidized cellulose nanofibers (TOCNFs) injectable hydrogel embedded with inorganic nanoparticles composed by calcium phosphates (CaP) and calcium phosphate and graphene oxide (CaP-GO) whose final application is the bone tissue regeneration.

**Experimental Methods**

TOCNFs aqueous dispersions were by oxidation of cotton linters cellulose, exploiting the NaClO/KBr/TEMPO oxidation system, followed by an ultrasonication step, as widely reported in literature. [1,4] Biocomposites were prepared by embedding the nanoparticles, synthetized at the IPCB (CNR; Naples), into the so obtained TOCNFs dispersion. The optimized final hydrogel composition was: TOCNFs with dry weight of 4% w/w on the final composite and CaP or CaP-GO with dry weight of 1% w/w on the final composite.

Gels were observed by SEM microscopy and the composition was evaluated by means of TGA, FT-IR and XRD analysis. Rheological characterization was performed by means of strain, frequency and shear rate sweep. The injectability of the hydrogels was evaluated by loading it into a 5 mL syringe mounted on a custom experimental setup and extruding it at 0.6 mm/sec for 15 mm through 18 G needle. [X]

Stability tests were performed by immersion in Dulbecco's Modified Eagle Medium (DMEM) up to 28 days. Mineralization tests were carried out in 1.5X simulated body fluid (SBF), incubated at 37°C under continuous shaking.

**Results and Discussion**

The embedding of CaP or CaP-GO in the gel proved that the nanoparticles act as physical crosslinkers interacting with the cellulose fibers, as proven by the rheological characterization, which reported an increase in the mechanical properties for the biocomposites and confirmed the thixotropic and shear-thinning behavior (Figure 1 a). The average load value registered in injection tests was in the range 1.5 - 4.4 N, far below 30 N, which is considered a reasonable injection force upper limit (Figure 1 b).

Stability assays in DMEM, results in an increasing growth in weight in the first 15 minutes; all the specimens registered the maximum increase (around 60%) after one day of immersion and were stable up to 28 days.
SEM and XRD analysis performed on the specimens before and after the mineralization tests suggested that gels incorporating CaP and CaP-GO nanoparticles show a greater bioactivity than TOCNF gels, accelerating the mineralization process and enhancing the formation of the apatite layer on the gel surface (Figure 2 a-d).

**Conclusion**

With the extensive characterization, this work demonstrated that the physico-chemical properties of TOCNF based dispersions could be enhanced through the addition of the inorganic phases within the polymer matrix. This yields a hydrogel that is easily injectable, with bioactive properties that promote mineralization of the hydrogel affordable for bone tissue regeneration. These properties can be easily modulated by modifying the amount of inorganic components depending on the final application.

**Acknowledgement**

AF and LA thank PRIN2017 ACTION program for founding.

---

![Figure 1]

**Figure 1**

a) Example of rheological characterization, strain sweep. b) Results of injection test.

![Figure 2]

**Figure 2**

a) Evolution in time of XRD patterns of TOCNF hydrogels exposed to SBF. b) Evolution in time of XRD patterns of TOCNF hydrogels loaded with CaP nanoparticles exposed to SBF. SEM images of TOCNF hydrogels loaded with CaP nanoparticles before the mineralization step (c) and after 28 days (d).
References
Degradable pHEMA hydrogels containing graphene-based materials for blood-contact applications: from soft inert to strong biodegradable materials

Duarte Moura¹,²,³, Andreia T. Pereira¹, Helena Ferreira¹,²,⁴, Cristina C. Barrias¹,²,⁴, Fernão D. Magalhães⁵, Inês C. Gonçalves¹,²

¹ i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Rua Alfredo Allen, 208, 4200-180, Porto, PT; ² INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Rua Alfredo Allen, 208, 4200-180, Porto, PT; ³ FEUP - Faculdade de Engenharia, Departamento de Engenharia Metalúrgica e de Materiais, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465, Porto, PT; ⁴ ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, 4050-313, Porto, PT; ⁵ LEPABE - Laboratório para Processo, Environment, Biotechnology and Energy, Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465, Porto, PT

Introduction
Blood contacting devices (BCDs) are daily used for the treatment of cardiovascular diseases. Currently used biomaterials still lead to complications such as thrombosis and infection, demanding improvements in this field [1, 2]. New insights have arisen when hydrogels with high-strength and stiffness were obtained by incorporating graphene oxide (GO) [3]. However, when tissue engineering approaches are desired, biodegradable materials with suitable haemocompatible and mechanical properties and small degradation products are still a hurdle [4]. The present work aims to develop a new biodegradable poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogel to be used as a potential scaffold in a post-implantation tissue engineering approach. For that, a degradable crosslinking agent was incorporated to allow hydrogel degradation and oxidized graphene-based materials (GBM) with differing lateral size and thickness explored to potentiate mechanical reinforcement. The hydrogel performance regarding physical-chemical features and biological performance were evaluated.

Experimental Methods
Degradable pHEMA (d-pHEMA) films were produced by adding (0.25, 0.5, 0.75wt.%) of the cross-linking agent pentaerythritol tetra(is-3-mercaptopropionate), named as tetrakis, to the previously described formulation of pHEMA films [5]. d-pHEMA/GBM composites were produced by mixing with HEMA monomers 1% (w/v) of single-layer graphene oxide (GO) or few-layer GO with 5 or 15 µm lateral size (M5ox or M15ox). pHEMA polymerization was assessed by Fourier-transform infrared spectroscopy (FTIR), hydrophilicity of the films was evaluated by optical contact angle. Topography and porosity were analyzed by SEM and crioSEM, respectively, and the swelling behavior through water uptake capacity. Degradation studies were performed in vitro in PBS under 100 rpm over 6 months (ISO 10993:13) and in vivo using the chick chorioallantoic membrane (CAM) assays. Cytotoxicity of degradation products (24h and 6 months) was evaluated towards human umbilical vein endothelial cells (HUVECs), assessing cell metabolic activity (resazurin assay) and cell morphology (dapi/phalloidin staining and fluorescence microscopy). The non-fouling character of the hydrogels was evaluated by direct contact assay with HUVECs, platelets and bacteria. HUVECs (1x10⁴ cells/well) were incubated on top of the hydrogels, and after 24h and 7 days the cell metabolic activity and cell morphology assessed as described above. Platelets’ adhesion and activation (3×10⁸ platelet/mL) was assessed by SEM after incubation of hydrogels with human platelets for 1h (37 °C; 100 rpm). Methicillin-resistant *Staphylococcus aureus* (S. aureus) (ATCC MRSA 33591™) adhesion (1.3x10⁸ bacteria/ml) to hydrogels was visualized by SEM after incubation during 24h at 37 °C.

Results and Discussion

Page 1657 of 2028
Hydrogels polymerization was confirmed by FTIR, showing no evidence of unreacted HEMA monomers (C=C; 1635cm⁻¹). The use of tetrakis does not induce significant changes on swelling (≈80%) and hydrophilicity (≈40°) of the native pHEMA, maintaining its hydrogel-like behavior.

Independently of the amount, incorporation of Tetrakis in pHEMA allows full degradation of the hydrogels within 2 months. When GBMs are added, the stability of the hydrogels increase, degrading after 3 months for GO and 4 months for M5ox and M15ox. The 24h and 6 months degradation products revealed no cytotoxicity to over HUVECs. CAM assays confirmed lack of cytotoxicity and irritability in all hydrogels. Mechanically, incorporation of tetrakis decreased the Young’s modulus and tensile strength (UTS) of pHEMA in 50%. However, upon addition of GBMs, particularly M5ox, the UTS increased to 0.2 MPa, with an elasticity of 0.25 MPa, while keeping a strain of 20%, improving native pHEMA’s mechanical properties and reaching values similar to the ones from native small diameter blood vessels.

The non-fouling properties were confirmed, where no HUVECs adhered to d-pHEMA nor d-pHEMA/GBMs materials surface, after 1 and 7 days. Similarly, there is no platelets nor S. aureus adhesion to the surface of the hydrogels. Such features are promising when envisioning the production of a BCD, since thrombus formation and infection may be prevented.

Conclusion
Overall, this work has demonstrated that pHEMA can be turned into a degradable material by incorporation of Tetrakis, despite compromising the mechanical properties. The incorporation of GBMs can however counterbalance this, reestablishing the stiffness and strength, and simultaneously allowing the tuning of the degradation time. Furthermore, the intrinsic features of pHEMA were kept, such as its hydrophilic and non-fouling behavior. Hydrogels with 0.25% Tetrakis and 1% M5ox show suitable mechanical properties and degrade within 3 months, matching blood vessel remodeling. This new developed hydrogel can therefore be envisioned for applications in tissue engineering, namely as small diameter vascular grafts.

Acknowledgement
The authors would like to acknowledge Ricardo Vidal from Biointerfaces and Nanotechnology of i3S Scientific Platforms (UID/BIM/04293/2019) (FTIR and contact angle) and Marta T. Pinto from CAM assays Lab (IPATIMUP) (CAM assays). This work was financially supported by PhD grant SFRH/BD/1400006/2018 and by the projects PTDC/CTM-COM/32431/2017, UIDB/04293/2020 funded through Fundação para a Ciência e a Tecnologia (FCT) and Fundo Europeu de Desenvolvimento Regional.

References
PS2-05-194

Gel/HA hydrogels as 3D in vitro breast cancer models

Matteo Pitton¹, Christian Urzì²,¹, Arianna Bossi¹, Nicola Contessi Negrini³, Silvia Farè¹,⁴

¹ Politecnico di Milano, Chemistry, Materials and Chemical Engineering Department, Milan, IT; ² UKC Bern, Department of BioMedical Research, Bern, CH; ³ Imperial College London, Department of Bioengineering, London, GB; ⁴ National Interuniversity Consortium of Materials Science and Technology, Florence, IT

Introduction

Adipose tissue (AT) is a highly complex connective tissue directly involved in several human functions [1]. Among diseases that affect AT, breast cancer is nowadays the most widely diagnosed pathology in women [2]. 3D in vitro models are emerging as valid tools to deeply understand mechanisms of breast cancer origin and development, and to test novel therapies in micro/nano scale, thanks to their capability in replicating tissue in vivo condition, compared to 2D cultures or in vivo animal models. In particular, natural hydrogels are promising materials to mimic physiochemical, mechanical, and biological properties of AT [3], since they are characterized by porosity, swelling ability, and tuneable mechanical properties. However, finely tuning the mechanical properties of natural-based hydrogels is still challenging and it represents a crucial feature to mimic both physiological and pathological AT conditions. Here, we developed gelatin (Gel) and hyaluronic acid (HA) hydrogels to realize a 3D in vitro breast cancer model. Gel aimed to replicate the collagen component naturally present in AT extracellular matrix (ECM), while HA simulated glycosaminoglycans in AT ECM.

Experimental Methods

Gelatin (Gel) and hyaluronic acid (HA) were functionalized by methacrylate groups (i.e., GelMA and HAMA, respectively), and the degree of functionalization (DoF) was assessed by ¹H NMR spectroscopy. Hydrogels were prepared upon light irradiation of polymer solutions (10 % GelMA and 1 % HAMA w/v). Two different photocrosslinkers were investigated and hydrogels crosslinked either by using UV irradiation (i.e., photoinitiator Irgacure I2959) or visible light (i.e., photoinitiator complex Ruthenium Ru and Sodium Persulfate SPS). Crosslinked samples were characterized by physical (i.e., stability tests in physiological-like solution 0.02 % w/v NaNO₃ in dH₂O, T = 37 °C, up to 3 weeks, n = 5), mechanical (i.e., compressive test, T = 37 °C, strain ramp = 2.5 % min⁻¹ down to 30 % and 5 % min⁻¹ up to 1 %, n = 3) and biological tests (i.e., indirect cytotoxicity, n = 3 per time point, UNI EN ISO 10993-5). GelMA hydrogels (10 % w/v) were characterized by performing same tests.

Results and Discussion

GelMA/HAMA hydrogels appeared less transparent than samples prepared only with GelMA (Figure 1.a), both after UV and visible light photocrosslinking. Weight variations curves (ΔW %) showed a higher increase in weight for blend hydrogels in the first 48 h (Figure 1.b), compared to GelMA samples (p < 0.05). This trend was associated to HAMA presence in hydrogels, since hyaluronic acid resulted able to retain huge amounts of water in ECM. Stress-strain curves showed that HAMA addition in GelMA hydrogels photocrosslinked by visible light exposure led to an increase in mechanical properties, such as elastic modulus (Figure 1.c.ii) and stiffness (Figure 1.c.iii), which resulted higher than other blend samples and respective GelMA hydrogels (p < 0.05). Ru/SPS crosslinked GelMA/HAMA hydrogels were approximately 3 times stiffer than respective GelMA samples (K⁺VIS = 103.2 kPa, K⁻VIS = 38.9 kPa), simulating pathological condition of AT [4]. Most breast tumours (i.e., fibroadenoma, low grade ductal carcinoma) exhibit indeed a 3-6-fold increased stiffness than native AT, while high grade ductal carcinoma shows an increase up to 13 times compared to physiological condition [4]. Moreover, HAMA addition allowed to enhance hydrogels stiffness involving low levels of photoinitiators. Higher photoinitiator concentrations could indeed increase material mechanical properties as well [5] but might lead to potential cytotoxic residual accumulations. Contrarily, GelMA/HAMA hydrogels...
photocrosslinked with I2959 showed a decrease in mechanical properties ($p < 0.05$), in comparison with both other blend and respective GelMA hydrogels ($K_{\text{b,UV}} = 9.6$ kPa, $K_{\text{g,UV}} = 53.2$ kPa). This trend was associated to an incomplete crosslinking of the polymeric network, which could be optimized by increasing UV time exposure. In vitro indirect cytocompatibility was thus performed on preadipocytes 3T3-L1 (Figure 2), after 1, 3, 7 days of samples incubation ($T = 37^\circ \text{C}, \text{CO}_2 = 5\%$). All the investigated formulations did not show cytotoxic effects on seeded cells (i.e., cell viability > 70\%).

**Conclusion**

HAMA addition to GelMA hydrogels allowed to increase the mechanical properties of the prepared hydrogels. Visible light photocrosslinking of GelMA/HAMA hydrogels resulted a promising approach for 3D in vitro breast cancer model realization, since led to an increase in stiffness, comparable to pathological AT condition [4]. Since no cytotoxic effects resulted from biological tests, breast cancer cells encapsulation could be further performed on investigated GelMA/HAMA hydrogels.

**Figure 1**

a) GelMA/HAMA and GelMA hydrogels images acquired by stereomicroscope (scale bar: 5 mm). b) Stability tests on GelMA/HAMA and pure GelMA hydrogels, up to 3 weeks. c) Compressive mechanical tests. Stress-strain curves of tested samples (i), elastic modulus obtained values (ii) and stiffness obtained values (iii). Statistical analysis was performed between blend formulations and significance was reported as ◊ (◊◊◊◊: $p < 0.0001$), while significance between blend hydrogels and respective pure GelMA samples was signed as * (**: $p < 0.01$, ****: $p < 0.0001$).
Indirect cytotoxicity on 3T3-L1 preadipocytes cultured with eluates obtained from GelMA/HAMA and pure GelMA samples, incubated (T = 37 °C, CO₂ = 5 %) for 1, 3, 7 days. All the investigated formulations did not exhibit cytotoxic effects on preadipocytes since cell viability resulted higher than 70 %, as UNI EN ISO 10993-5 reported.

References
Phloroglucinol-enriched whey protein isolate hydrogels for bone tissue engineering

Varvara Platania¹,², Timothy E. Douglas³,⁴, Mikhajlo K. Zubko⁵, Danny Ward⁶, Krzysztof Pietryga⁷, Maria Chatzinikolaidou¹,²

¹ University of Crete, Department of Materials Science and Technology, Heraklion, GR; ² FORTH, IESL, Heraklion, GR; ³ Lancaster University, Engineering Department, Lancaster, GB; ⁴ Lancaster University, Materials Science Institute (MSI), Lancaster, GB; ⁵ Manchester Metropolitan University (MMU), Centre for Bioscience, Manchester, GB; ⁶ Lancaster University, Department of Biological and Life Sciences (BLS), Lancaster, GB; ⁷ AGH University of Science and Technology, Department of Biomaterials and Composites, Krakow, PL

Introduction

Aging populations in developed countries will increase the demand for implantable materials to support tissue regeneration. Protein-based eco-friendly biomaterials such as dairy proteins attracted great attention recently as substitutes for commercial materials for biomedical applications [1, 2]. Whey Protein Isolate (WPI), derived from dairy industry by-products, can be processed into hydrogels with the following desirable properties for applications in tissue engineering: (i) ability to support adhesion and growth of cells; (ii) ease of sterilization by autoclaving and (iii) ease of incorporation of poorly soluble drugs with antimicrobial activity, such as phloroglucinol (PG), the fundamental phenolic subunit of marine polyphenols. PG possesses antioxidative properties, which is expected to be beneficial for bone tissue engineering, as oxidative stress has been indicated to impede both the differentiation of osteoblasts and the resorption of bone by osteoclasts [3, 4].

In this study, WPI hydrogels have been enriched with PG at concentrations between 0 and 20% (w/v). PG solubilization in WPI hydrogels is far higher than in water. WPI-PG hydrogels supported the proliferation of and collagen production by human dental pulp cells (DPSCs), and less by osteosarcoma-derived MG63 cells. In summary, enrichment of WPI with PG may be a promising strategy to prevent microbial contamination while promoting cell attachment and growth of mesenchymal stem cells.

Experimental Methods

In this study 40% w/v WPI hydrogels modified with 0, 2.5, 5, 10, and 20% PG were prepared. DPSCs and MG-63 cells seeded on WPI hydrogels modified with different concentrations of PG were evaluated for their viability, proliferation, cell adhesion, morphology, potential to enhance extracellular collagen production and antimicrobial capacity. As this potential is expected to be different for cell lines and primary cells, physiological and cancer cell types, we performed a comparative in vitro biocompatibility study. Viability and proliferation of both cell types on the WPI hydrogels were quantified by means of the cell viability PrestoBlue™ assay, while cell morphology and attachment of both cell types on the modified hydrogels were assessed by means of scanning electron microscopy. Moreover, we determined the levels of collagen produced in culture supernatants as a marker of extracellular matrix (ECM) formation. Bacterial strains used in the study included Bacillus cereus, Enterococcus faecalis, Escherichia coli, Klebsiella oxytoca, K. pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, S. aureus (MRSA), S. epidermidis, Streptococcus pyogenes. For susceptibility testing, the bacterial strains were grown overnight on nutrient agar at 37 °C.

Results and Discussion

Higher PG concentration in WPI hydrogels increased proliferation of both DPSCs and MG-63 cells. Compared to DPSCs, MG-63 osteosarcoma cells displayed a decrease of metabolic activity from day 4 to day 7 in all samples, indicating a downregulated proliferation potential of osteosarcoma cells cultured on WPI-PG hydrogels. After 7 days
of incubation, DPSCs adhered strongly into the surface and pores of the 10 and 20% WPI-PG hydrogels, whereas MG-63 appeared round and flat with an obvious decrease in cell density at the lower PG concentrations. Quantification of extracellular collagen as an indicator of ECM formation revealed that DPSCs enhanced the production of collagen at all time points relative to the PG-free WPI control hydrogel. MG-63 osteosarcoma cells exhibit significantly lower levels of collagen production with three-fold lower values compared to the DPSCs, indicating that mesenchymal stem cells cultured on WPI-PG samples favor ECM formation.

**Conclusion**

WPI-PG hydrogels demonstrate an excellent biocompatibility, allowing DPSCs and MG-63 cells to adhere, survive, and proliferate, favoring an enhanced growth and ECM collagen production by DPSCs compared to MG-63 cultures. The enrichment of WPI with various concentrations of PG did not adversely affect the mechanical properties of the hydrogels, while endowing them with antibacterial properties.

**Acknowledgement**

The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the “First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant” project number HFRI-FM17-1999 and the N8 Pump Priming grant “Food2Bone” (T.E.L.D.)

**References**


Accelerated Bone Regeneration by Rational Design of Magnesium Phosphate Cements

Friederike Kaiser1, Lena Schröter2, Jan Weichhold1, Svenja Stein2, Anita Ignatius2, Uwe Gbureck1

1 University Hospital of Würzburg, Department for Functional Materials in Medicine and Dentistry, Würzburg, DE; 2 University Hospital of Ulm, Institute of Orthopaedic Research and Biomechanics, Ulm, DE

Introduction
Calcium phosphates, e.g. hydroxyapatite, are commonly used as synthetic bone replacement material due to their high biocompatibility and similarity to natural bone tissue.1 However, a complete resorption of hydroxyapatite and replacement by bone tissue is rarely observed.2 Magnesium phosphates such as struvite (MgNH4PO4·6H2O) or newberyite (MgHPO4·3H2O) are a promising alternative, due to their higher solubility.3 In vivo studies in a sheep model showed an almost complete degradation of a struvite forming cement with a struvite content of ≈40% after 10 months.4 In the current study we aimed to further increase degradation rate by either forming K-struvite (MgKPO4·6H2O) or a mixture of struvite and newberyite during cement setting by changing both the composition and amount of the cement liquid.

Experimental Methods
Magnesium phosphate cements with different powder liquid ratios (PLR) were produced by mixing farringtonite (Mg3(PO4)2) powder with ammonium hydrogen phosphate (struvite cement) or potassium hydrogen phosphate solution (K-struvite cement). Cement paste was filled into silicone rubber molds (12 x 6 x 6 mm) and samples were hardened for 24 h at 37 °C and 100% humidity. The cements were characterized regarding compressive strength and phase composition. Initial setting time was determined by Gilmore needle test and porosity by mercury intrusion. The cements with PLR 1 were stored for 18 days in phosphate buffered saline to investigate passive degradation and in vivo degradation was studied by implantation in a partly-loaded tibia defect in sheep model.

Results and Discussion
The material characterization revealed that the replacement of ammonium ions by potassium ions in the cement only slightly influences compressive strength (Figure 1E), but noticeably extends the initial setting time (Figure 1A). XRD measurements (Figure 1B-D) show that the cements consist of (K-)struvite, farringtonite and newberyite. Although the struvite content in the corresponding cement is significantly lower than the K-struvite content in the corresponding cement, a high amount of newberyte was formed which is thought to further improve cement degradation. Both cement compositions degraded in vivo and new bone tissue was formed around the implants. Macroscopic images of the implantation site indicate faster degradation of the K-struvite forming cement (Figure 2).

Conclusion
Due to their large content of high soluble magnesium phosphate phases, sufficient compressive strength for partially loaded bone defects and their rapid in vivo degradation, the investigated magnesium phosphate cements are promising candidates for a bone replacement material.

Acknowledgement
We gratefully acknowledge funding by the German Research Foundation (DFG GB 1/15-3 and IG 18/11-3).
Material characterization
Figure 1 A Initial setting time, B XRD measurement, C and D phase composition determined by Rietveld refinement, E compressive strength of struvite and K-struvite cements

In vivo characterization
Figure 2 Macroscopic images of tibia explants after two and four months implantation time

References
Extraction and characterization of native type I collagen from aquaponics Tilapia skin

Nunzia Gallo¹, Maria Lucia Natali², Chiara Brunetti², Alessandra Quarta³, Antonio Gaballo³, Alberta Terzi³, Teresa Sibillano⁴, Cinzia Giannini⁴, Giuseppe E. De Benedetto⁵, Federica S. Blasi¹, Angelo Corallo¹, Alessandro Sannino¹, Luca Salvatore¹,2

¹ University of Salento, Department of Engineering for Innovation, Lecce, IT; ² Typeone Biomaterials Srl, Lecce, IT; ³ CNR Nanotec, Institute of Nanotechnology, Lecce, IT; ⁴ CNR, Institute of Crystallography, Bari, IT; ⁵ University of Salento, Department of Cultural Heritage, Lecce, IT

Introduction

Collagen is the most abundant structural protein in humans [1]. Thanks to its properties, it is one of the most widely used biomaterials in health-related sectors [1]. The industrial production of collagen mostly relies on its extraction from mammals. However, immune reactions triggering, zoonosis transmission risks, cultural and religious issues limited its use [1,2]. In this perspective, in the last two decades marine organisms attracted interest as safe, abundant and alternative sources for collagen extraction [2]. Every year fish processing industry discards account for about 70-85% of the total catch weight and raise significant environmental pollution [2]. The possibility to valorize fish byproducts as collagen sources makes fish collagen eco-friendly and attractive in terms of profitability and cost effectiveness. Additionally, fish byproducts harvesting from eco-sustainable aquaponics systems allows to obtain fish biomass with controlled properties over time. In this work, the physical, chemical, structural and biological properties of type I collagen isolated from Nile Tilapia skin raised in a pilot aquaponic system were assessed and compared with those of a commercially available isoform.

Experimental Methods

Type I collagen (T) was extracted from the skin of Tilapia (Oreochromis niloticus) bred in the pilot aquaponics plant of the “Urban Farming Lab” of the Dept. of Innovation Engineering (University of Salento, Lecce, Italy) according to a method developed by Typeone Biomaterials Srl (Lecce, Italy). Briefly, Tilapia skin were cleaned up by flesh and scales, washed and cut. Skin pieces were then treated with NaOH to remove non-collagenous proteins, defatted with butanol, and incubated at acid pH. The viscous suspension obtained was centrifuged and the collected supernatant salted out. The resulting precipitate was then filtered, lyophilized and ground, in order to obtain type I collagen dry flakes. The extracted material was fully characterized in comparison with a commercially available type I collagen isoform (N) produced by Nippi Inc (Tokyo, Japan). The protein molecular weight was assessed by Polyacrylamide Gel Electrophoresis in presence of Sodium Dodecyl Sulfate (SDS-PAGE). The amino acid composition was investigated by Gas Chromatography - Mass Spectrometry (GC-MS). The structural integrity was examined by mean of Transmission Electron Microscopy (TEM), Fourier-Transform Infrared Spectroscopy (FT-IR), Wide- and Small-angle X-ray scattering (WAXS, SAXS). The material thermal properties and degradation resistance were investigated by Differential Scanning Calorimetry (DSC) and enzymatic degradation. Lastly, the cytocompatibility of T and N was preliminarily evaluated by assessing the morphology, the metabolic activity and the viability of NHI/3T3 murine fibroblast up to 6 days.

Results and Discussion

The protein identity and the preservation of its native structure were confirmed by all performed analysis. The electrophoretic pattern showed the presence of the two typical bands of type I collagen [3,4]. The absence of other bands indicated the purity of the material, suggesting how T was as pure as N. The identity of T was confirmed also by its amino acid composition that was found to be comparable to N and according to literature [3]. The presence of
the typical IR vibrations of collagen in T and N confirmed their structural integrity and the extraction procedure quality [3, 4]. Moreover, a high triple helical content for both and a slightly high native structure conservation in N were found. Accordingly, WAXS confirmed the retention of triple helical structure at molecular scale, however highlighting the directional orientation loss of molecules in their lateral packing in T than N. Furthermore, SAXS showed that its typical arrangement became less visible at the nanoscale. The fibrillar structure of collagen was confirmed by TEM, with the presence of fibrils of diameter of about 250 nm and length of ≈mm. The denaturation temperature was found to be of about 33°C [4]. Lastly, the viability and the morphology of NH1/3T3 cells on T and N confirmed their cytocompatibility since a stimulated cell growth and absence of significant death rates were observed. Additionally, cell imaging showed morphological diversity between T and N, suggesting that different dynamics of adhesion/proliferation occurred. Intriguingly, the development of 3D structures after 3 days on N and in a less extent and later on T deserved further investigation.

Conclusion

All analysis confirmed how the extraction process allowed to isolate type I collagen in native state from aquaponics-derived Tilapia skin. The slight differences observed between T and N may be due to different extraction procedures, resulting in changes in their bioactive conformational structure. The promising biocompatibility results confirmed how T could be used as an alternative biomaterial for the development of medical devices and how aquaponics could be a valid source of byproducts for collagen extraction.

Acknowledgement

This work was supported by the project "ISEPA - Improving Sustainability, Efficiency and Profitability of Large Scale Aquaponics" (CUP: B37H17004760007)

References


Program ǀ Poster Session 2
Wednesday, 8 Sept, 2021

PS2-05-202

Structural characterisation of a gelatin/silica hybrid material, containing calcium, strontium and boron.

José Carlos Almeida¹,², Paula Maria C. Torres¹, Susana Maria H. Olhero¹

¹ Universidade de Aveiro, CICECO, Aveiro, PT; ² Universidade de Aveiro, TEMA, Aveiro, PT

Introduction
During the last ten years an effort has been made to develop new hybrid materials capable to produce biodegradable scaffolds or films, targeted to tissue regeneration [1]. Among the various hybrid systems, the gelatin-silica system has presented interesting results, namely as a stand-alone material for scaffolds [2,3].

Experimental Methods
In the present work, a sol-gel preparation protocol was developed in a way to include, in the inorganic domains of the hybrid system, ions recognized for enhancing bioactivity, such as calcium, strontium and boron [4].

Results and Discussion
In the first phase of the work, the structures of the hybrid material were analysed by FTIR, ¹H MAS (magical angle spinning), ²⁹Si MAS, ²⁹Si-{¹H} CP-MAS (cross-polarization magical angle spinning) and ¹¹B HAHN-ECHO NMR. Hybrid materials in the gelatin–SiO₂–CaO–B₂O₃ system were successfully obtained using GPTMS (3-glycidoxypropyltrimetoxysilane) as a coupling agent between gelatin and silica.

Conclusion
The formation of hybrid bonds between the organic gelatin-GPTMS and the silica domains, as well as, the presence of borosiloxane bonds (B–O–Si) were confirmed. The presence of calcium and strontium acting as network modifiers changes the boron coordination from trigonal to tetrahedral.

Acknowledgement
This work was developed within the scope of the project MULBIOIMPLANT (FCT Ref. POCI-01-0145-FEDER-032486), and by the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when appropriate cofinanced by FEDER under the PT2020 Partnership Agreement. The NMR spectrometers are part of the National NMR Network (PTNMR) and are partially supported by Infrastructure Project No 022161 (co-financed by FEDER through COMPETE 2020, POCI and PORL and FCT through PIDDAC).

References
2:00 p.m. – 3:30 p.m.

Poster floor

**PS2-06 | Ceramic Biomaterials**

*With the kind support of MDPI - Ceramics*
PS2-06-204

Synthesis of amorphous calcium phosphate from different Ca source

Marika Mosina\textsuperscript{1,2}, Liga Stipniec\textsuperscript{1,2}, Janis Locs\textsuperscript{1,2}

\textsuperscript{1} Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga, LV; \textsuperscript{2} Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, LV

Introduction

Different calcium phosphates are widely used as biomaterials. Formation of hydroxyapatites in the human body occurs through an amorphous precursor phase such as amorphous calcium phosphate (ACP). Due to the fact that ACP is highly soluble and has good remineralization ability, it is ascribed with excellent properties and promising forthcoming applications in the development of bone graft materials. Materials properties such as specific surface area (SSA) and Ca/P molar ratio are attributed to the response of cells in bone regeneration process and different synthesis parameters can influence these properties of materials. The aim of the study was to investigate the effect of different Ca sources, synthesis initial Ca/P molar ratio and synthesis end pH on properties of ACP.

Experimental Methods

ACP was synthesized with a modified wet precipitation method [1]. Two different Ca\textsuperscript{2+} ion sources, calcium oxide (CaO) and calcium chloride (CaCl\textsubscript{2}) were used. Orthophosphoric acid (H\textsubscript{3}PO\textsubscript{4}) was added to the starting solution. The initial Ca/P molar ratio of reagents was 1.5, 1.58 and 1.67. From calcium and phosphorous ion rich solution, ACP precipitation occurs with the rapid addition of strong base (NaOH). The synthesis was performed at end pH 9.5 and 11.5. Obtained precipitates were centrifuged, washed with deionized water, and lyophilized. If calcium oxide was used as a source to obtain the calcium and phosphorous ion rich solution, suspension was dissolved by the addition of hydrochloric acid (HCl). Products were characterized with x-ray diffraction analysis (XRD), Fourier transform infrared spectroscopy (FT-IR), Brunauer–Emmet–Teller method (BET) and gas pycnometry. Gallium content in all the obtained ACP samples was detected by inductively coupled plasma mass spectrometry (ICP-MS).

Results and Discussion

Synthesized ACP from different Ca\textsuperscript{2+} ion sources displayed characteristic amorphous structure (wide bump around 30 ° 2Theta in XRD pattern and wide rounded peak in FT-IR spectrum were observed). The characteristic amorphous structure remain also in different synthesis conditions that were employed. Ca/P molar ratio of obtained ACP was in the range from 1.50 to 1.57 which is lower than the initially observed molar ratio (1.5-1.67). However, higher synthesis end pH (11.5) lead to the increased Ca/P molar ratio in the product.

Conclusion

The correlation between synthesis parameters (pH and initial Ca/P molar ratio) and ACP microstructure parameters (SSA, density, particle size) was not observed. This is due to the fact that other parameters of synthesis could have influenced the properties of ACP.

Acknowledgement

This research is funded by the EuroNanoMed\textsuperscript{III} project “NANO delivery system for one-shot regenerative therapy of peri-implantis” (ImlpantNano). This project has received funding from the European Union’s Horizon 2020 research and innovation program under the grant agreement No 857287.

References

**PS2-06-206**

**Understanding the Influence of Chemical Properties of Silicon-Substituted Hydroxyapatite on Angiogenesis**

*Else Ellermann, Ruth E. Cameron, Serena M. Best*

*University of Cambridge, Materials Science and Metallurgy, Cambridge, GB*

**Introduction**

Synthetic hydroxyapatite (HA) is used widely in orthopaedic surgery for bone tissue engineering due to its similarity in composition to the mineral component of bone. There has also been considerable interest in the modification of the chemistry of synthetic HA to optimise osseointegration and periosteal apposition *in-vivo* [1]. In particular, the substitution of silicate groups for phosphate groups in the HA lattice has been investigated. Over the years, silicon-substituted HA (SiHA) has been observed to induce enhanced osteoblast attachment and proliferation, promote bone mineralisation and increase the mechanical stability of calcium phosphates [2,3]. However, successful bone tissue engineering relies on both the osteogenic- and also the angiogenic potential of biomaterials and should, therefore, be considered as part of the biological performance.

To date, there have been few studies that have investigated the influence of SiHA on angiogenesis *in vitro*, and uncertainty remains about the mechanism by which silicon contributes to bioactivity. Since the addition of silicon does not only change the chemical composition of HA but also alters the physical properties, such as grain size and mechanical stability, it has been proven difficult to assess the direct effects of silicon on cell behaviour. The objective of this study is to establish the formation of vessel-like structures on SiHA and gain an understanding of the extent to which chemical properties contribute to the bioactivity of calcium phosphates.

**Experimental Methods**

Phase-pure HA and SiHA (1.5 wt%) powders were produced with a particle size of approximately 17 µm. Discs were prepared through uniaxial pressing at 124 MPa for 1 minute using powder calcined at 900°C, followed by sintering at 1250°C for 60 minutes. A co-culture of primary human osteoblasts (hOBs) and human dermal microvascular endothelial cells (HDMECs) was used. Discs were pre-coated with fibronectin for 2 hours, seeded sequentially at a hOB:HDMEC cell ratio of 70:30 and incubated for 8 Days. The formation of vessel-like structures was assessed via fluorescence microscopy using cell nuclei staining (DAPI) and HDMEC-specific cell markers (CD31 and vWF). To minimise the differences in surface topography, a number of discs were polished and subsequently scratched (PS) using a 30 µm lapping film to induce a controlled surface roughness required for cell attachment. Untreated and PS surfaces were visually inspected using Scanning Electron Microscopy (SEM). To assess the isolated effect of topography on cell behaviour, chemical properties of untreated and PS-treated samples were masked using an 8 nm thick gold-sputter coating (SC samples). Possible coating-induced topography modifications were examined using Atomic Force Microscopy (AFM).

**Results and Discussion**

AFM analysis showed that the layer of gold deposited sputter coating was sufficiently thin to avoid modification of the surface topography of the SiHA and HA discs. Visual inspection of SEM images revealed similar surface topographies between all PS samples, despite SiHA exhibiting a significantly smaller grain size compared to HA. Qualitative analysis showed vessel-like structure formation with multiple branching points on 2D SiHA and HA surfaces. The network obtained on the former material was observed to exhibit a higher level of complexity compared to the latter for both untreated and PS samples (Figure 1a). Quantitative analysis, presented in Figure 1b, showed that the number of tubular structures was significantly enhanced on SiHA compared to conventional HA. Although the differences in angiogenic potential observed between the two calcium phosphates reduced on PS discs, the vessel...
density remained significantly higher on PS-SiHA. Sputter coated (SC) and PS control samples showed no significant difference between the number of vessel-like structures indicating successful minimisation of the variability in surface topography between the samples.

**Conclusion**
The formation of vessel-like structures with multiple branching points was achieved on SiHA samples using a co-culture of hOBs and HDMECs. Topography was found to have a significant influence on the angiogenic potential of the calcium phosphates. The polishing and scratching method was found to significantly minimise the variability in surface topography as evidenced by similar cell behaviour on SC-PS samples. Although the difference in the density of vessel-like structures between SiHA and HA decreased on PS samples, a clear difference could still be observed, with a higher number of tubular structures observed on silicon-substituted samples. These findings indicate a direct influence of silicon on the biological performance of calcium phosphates.

**Acknowledgement**
This research was financially supported by the Engineering and Physical Sciences Research Council (EPSRC) and Geistlich Pharma AG.

---

**Vessel-like Network Formation on Calcium Phosphates**

**Fig. 1:** a) Fluorescent images of untreated and PS-treated SiHA and HA discs co-cultured with a hOB:HDMEC cell ratio of 70:30 (scale bar indicates 150 µm) b) Density of vessel-like structures on co-cultured SiHA and HA discs.
References


Protein functionalized nano- and microporous ceramic biomaterials to tailor bacterial and cellular interactions

Deepanjalee Dutta¹, Jana Markhoff², Kurosch Rezwan³, Dorothea Brüggemann⁴

¹ University of Bremen, Institute of Biophysics, Bremen, DE; ² University of Bremen, Institute of Biophysics, Bremen, DE; ³ University of Bremen, Advanced Ceramics, Bremen, DE; ⁴ University of Bremen, Institute of Biophysics, Bremen, DE

Introduction
Surface modification of manifold biomaterial surfaces is important for orchestrating cell-ligand interactions during multicellular tissue repair. In particular, for ceramics-based biomaterials to be applied in soft tissue engineering such functionalization strategies are necessary to provide biochemical signals. Two essential proteins involved in tissue regeneration, namely collagen and fibrinogen, have been extensively used for the design of biomaterials for soft tissue repair, specifically to promote wound repair. Therefore, the fundamental aim of this study is to understand how ceramic biomaterials with variable topography can be modified with collagen and fibrinogen to tailor cell behavior and to prevent bacterial penetration.

Experimental Methods
As a model system for wound healing, we analyzed how 3T3 fibroblasts, HaCaT keratinocytes, and E. Coli bacteria interacted with protein-modified alumina nanopores and microporous alumina textiles for a possible regenerative therapy of the human body's largest organ, the skin. Using our established self-assembly routines of pH- and salt-induced self-assembly, alumina nanopores and microporous alumina textiles were silanized followed by modification with self-assembled collagen [1,2] or fibrinogen nanofibers [3-4], respectively.

Results and Discussion
Interestingly, collagen-modified alumina nanopores were able to prevent bacterial penetration. E. Coli bacteria died as a result of their inability to migrate through the membranes to access the underlying food supply (agar).[2] A very different behavior was found in fibrinogen-modified alumina textiles, with the textile surfaces being capable of acting as bacteria capture agents. Therefore, despite their microporous scaffold architecture, these textiles prevented bacterial infiltration, which is very interesting for protein-modified ceramics to be used as porous wound dressing materials that can promote adequate gas exchange and nutrient supply.

Further on, the interaction of skin cells, such as 3T3 fibroblasts and HaCaT keratinocytes, with protein-modified ceramics revealed the ability of both cell types to grow on these ceramics up to a period of 72 h. On the alumina nanopores, keratinocytes tended to mature in cell clusters [2], while on the microporous alumina textiles, a distinct difference in morphology was observed. Fibroblasts adhered well to the ceramics as well without noticeable changes in their morphology in response to the different topographies.

Conclusion
In summary, our results suggest a good potential of protein-modified ceramics with different topographies to mimic the microenvironment of native skin and other soft tissues and to enable the growth of various cell types via tailored biochemical cues.

Acknowledgement
We acknowledge funding via the Emmy Noether Program (grant no BR5043/1-1) and the RTG MIMENIMA (GRK 1860) of the German Research Foundation.
Nano/microporous alumina modified with amino groups and collagen nanofibers

Figure 1. Modification of nano/microporous alumina with amino groups and protein nanofibers supported keratinocyte and fibroblast adhesion. *E. Coli* suspensions did not penetrate through the modified alumina nanopores. For Al2O3 textiles, *E. Coli* growth was absent on the agar but *E. Coli* bacteria were found to adhere to the alumina textile surface.

References
The Influence of Formulation on the Release Kinetics of the Antibiotics from Biocompatible Ceramics

Rajan Choudhary¹,²

¹ Riga Technical University, Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga, LV; ² Riga Technical University, Baltic Biomaterials Centre of Excellence, Riga, LV; ³ National University of Science and Technology “MISiS”, Center for Composite Materials, Riga, LV

Introduction
Bone tissue reconstruction is often associated with bone infections such as osteomyelitis, septic arthritis, and prosthetic joint infections. *Staphylococcus Aureus* is found to be the most causative microorganism for these infections [1]. The bone-bonding ability of ceramics made them a potential biomaterial for bone tissue engineering, restoring bone defects, being an implant material, or coating over the metallic implants [2]. The regenerative potential of ceramics can be enhanced by using growth factors or antibiotics [3]. Therefore, ceramics have been explored as a drug carrier to deliver therapeutic molecules and prevent bone infections. One of the key terms in drug delivery is the drug release rate. The release rate of a drug is controlled by diffusion, dissolution, swelling, affinity, and ion exchange [4]. Also, material parameters can influence the drug release rate, such as pore size, surface modifications [5]. The current report attempts to study the influence of different formulations on the drug delivery behavior of biocompatible ceramics.

Experimental Methods
The microcrystalline hydroxyapatite was synthesized by a cost-effective sol-gel combustion method. The synthesized hydroxyapatite and commercially purchased zirconia were utilized as a drug carrier either in pure form or as a composite with biocompatible ceramic containing different amounts of polyvinyl alcohol (PVA) as a binder. The model drugs selected for comparison are ciprofloxacin hydrochloride and levofloxacin.

Results and Discussion
Ciprofloxacin hydrochloride shows a sustained release whereas levofloxacin shows burst release from the ceramic drug carrier. The percentage of polyvinyl alcohol added to the drug played a vital role as a higher concentration of the polymer affects the stability of the drug carrier. Hydroxyapatite/zirconia composite shows sustained release (29%) when compared to pure hydroxyapatite (43%) and zirconia (37%) after 6 hours of immersion in simulated body fluid.

Conclusion
Release kinetics shows that the nature of the drug, concentration of the binder, and the ratio of ceramics in the composites decides the release kinetics of the drug from the drug delivery carrier.

Acknowledgement
The authors acknowledge financial support from the European Union’s Horizon 2020 research and innovation programme under the grant agreement No. 857287.
The release profile of ciprofloxacin from pure hydroxyapatite, zirconia and their composites

References
Amorphous Calcium Phosphate with Fluoride for Dental Restoration

Vita Zalite, Janis Locs

Riga Technical University, Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga, LV

Introduction
Amorphous calcium phosphate (ACP) has been used as filler in ionomer cements, as colloidal suspensions in toothpaste, as ingredient in mouthwash and chewing gum, thus proving ACP remineralization efficiency in therapeutic dentistry [1]. Also there are some studies about ACP containing fluoride [2,3] to enhance anti-caries and remineralizing potential. However, wider application of ACP materials are limited due to thermal instability of this phase. Recently cold sintering process have been introduced [4] giving opportunity to sinter powdered materials without high temperature. This technique or its modification could be a key factor to produce ACP ceramic materials for dental hard tissue restoration. In order to obtain adequate mechanical strength and intensify anti-caries effect of the material we offer to incorporate fluoride into ACP structure (ACPF). The aim of this study is to produce ACPF ceramic as potential material for application in restorative dentistry.

Experimental Methods
ACPF was synthesized trough reprecipitation method, where CaO and H₃PO₄ were used as Ca²⁺ and PO₄³⁻ ion sources to precipitate CaP salt. 3M HCl was added to completely dissolve CaP salt and then 3M NaOH was used for reprecipitation of ACPF. The product was subjected to freeze drying. The ceramic ACPF tablets were obtained by uniaxial pressing (Ø 13 mm, 198 kN, 10 min), thus initiating cold sintering. To characterize obtained materials XRD, FTIR, picnometry was used, also bulk density and relative density were determined.

Results and Discussion
XRD pattern of synthesized ACPF powder showed only two humps characteristic of ACP phase. FTIR analysis revealed absorption bands attributed to [PO₄] group vibration in the range of 550-1000 cm⁻¹. [CO₃] group presence in the range of 1400-1520 cm⁻¹ and [HPO₄] group presence at 875 cm⁻¹ was detected. XRD data showed a very small amount of nanocrystalline phase presence in ACPF ceramic after sintering. There can be distinguished one peak with very low intensity at 28.6 2θ, that was strongly overlapped. The true density of the powder was 2.51±0.04 g/cm³. The bulk density of the ACPF ceramic tablets was 2.37±0.05 g/cm³ after pressing procedure and calculated relative density was 94.3%±0.6%.

Conclusion
In summary, by applying uniaxial pressure (1500 MPa) it is possible to obtain ACPF ceramic with relative density close to 100%. The preliminary results look very promising. However, mechanical testing should be performed in further study.

Acknowledgement
This work has been supported by the European Regional Development Fund within the Activity 1.1.1.2 “Post-doctoral Research Aid” of the Specific Aid Objective 1.1.1 “To increase the research and innovative capacity of scientific institutions of Latvia and the ability to attract external financing, investing in human resources and infrastructure” of the Operational Programme “Growth and Employment” (No.1.1.1.2/VIAA/3/19/459)

References

Biomimetic amorphous calcium phosphate biomaterials for in vivo guided biosynthesis of biomimetic hydroxyapatite

Jana Vecstaudza¹, Marika Mosina¹, Valentina Stepanova¹, Janis Locs¹,²

¹ Riga Technical University, Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga, LV; ² Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, LV

Introduction

Number of patients encountering musculoskeletal system traumas, bone related diseases and pathologies increases worldwide, therefore the need for effective biomaterials is still persistent. Such effective materials should copy both physical and chemical structure of the inorganic part of the bone tissue. Recently our group has developed synthesis route [1] for obtaining of amorphous calcium phosphate (ACP) with physical structure that is similar to the biological calcium phosphate (CaP) particles within the bone tissue. The similarity is attained by the nanostructures, that can be expressed as increased specific surface area (SSA) of the synthesized ACP (133-150 m²/g). The SSA of this ACP is much closer to SSA of the bone CaP particles (up to 240 m²/g [2]) as of other ACP materials reported in the literature. Further, to achieve the similarity of the chemical composition of the bone mineral, the ACP should be enriched chemically. Therefore, aim of the study is to obtain and characterize ACP with biomimetic chemical composition, where the biomimetic ions are carbonates, magnesium, strontium, sodium, potassium, chloride, fluorine and zinc. Introduction of such biomimetic ACP biomaterials into the body would ensure biosynthesis of biomimetic calcium phosphate, e.g., biomimetic hydroxyapatite, by dissolution and subsequent reprecipitation route.

Experimental Methods

ACP was synthesized using precipitation method [1], that was modified to enrich the composition with biomimetic ions. In brief, CaO was dissolved in hydrochloric acid and transparent solution was obtained. Then reagents containing biomimetic ions were added. Later being: MgO, SrCl₂, KOH, NaF and Zn(NO₃)₂. Next, under vigorous stirring stoichiometric amount of NaOH was added, thus inducing precipitation of biomimetic ACP. The precipitated product was rinsed with deionized water and dried at 80 °C temperature. The as-synthesized ACP was characterized using XRD, FT-IR in ATR mode, SEM-EDS, BET and DTA methods. Heat treatment (1100 °C/1h) was performed to determine quantitative phase composition and further use it to calculate Ca/P molar ratio of the biomimetic ACP. Furthermore, stability of the ACP phase in air atmosphere was studied in the course of 12 months using XRD. Stability in wet environment was studied as well in comparison with pure ACP. Parallelly three samples were obtained and characterized.

Results and Discussion

The modified precipitation method was successful for obtaining ionically enriched ACP. XRD patterns of respective samples demonstrated two wide humps characteristic to ACP (see Fig. 1). The FT-IR spectra confirmed presence of the carbonate ions. Next, the SEM-EDS confirmed the presence of the rest of the biomimetic ions: Mg, Sr, K, Na, F and Zn. The SSA of biomimetic ACP was 86±10 m²/g, which was slightly smaller than that of pure ACP obtained by the same method (133-150 m²/g [1]), however reason behind this is still unclear. Heat treatment produced biphasic mixture of HAp/β-TCP with ratio of 10/90 wt%, thus the calculated Ca/P molar ratio of the biomimetic ACP was 1.52. Crystallization temperature was determined with DTA; it was lower for the biomimetic ACP than for pure ACP: 620 °C and 650 °C, respectively. Regarding the ACP phase stability in air - it was stable for 12 months (study is ongoing). Further, it was found that
presence of biomimetic ions stabilizes ACP phase in water environment when compared to pure ACP. Pure ACP started to crystallize after 8-9 h, while for the biomimetic ACP crystallization was detected at 48 h (see Fig. 2).

**Conclusion**
- Biomimetic ACP with intended chemical composition was successfully obtained by a modified precipitation method.
- Addition of biomimetic ions to the ACP lowered its specific surface area and crystallization temperature, however specific surface area remained relatively high: \(86 \pm 10 \text{ m}^2/\text{g}\).
- At the same time stability in water environment for the biomimetic ACP was increased more than five times comparing to the pure one.
- Further studies include ACP phase stability tests in simulated body solutions and *in vitro* cell tests.

**Acknowledgement**
This research is funded by the Latvian Council of Science, project “Future of synthetic bone graft materials - *in vivo* guided biosynthesis of biomimetic hydroxyapatite”, project No. Izp-2018/1-0238.

---

*Fig. 1. XRD patterns of biomimetic ACP, where one pattern represents one parallel synthesis.*
Fig. 2. XRD patterns of pure ACP (A) and biomimetic ACP (B) in water at different time points.

References
Role of borosilicate bioactive glasses in bone regeneration

Nefeli Vaporidou, Azadeh Rezaei, Tim Schaefer, Joel Turner, Aldo Boccaccini, Gavin Jell

1 University College London, Division of Surgery and Interventional Science, London, GB; 2 University of Erlangen-Nuremberg, Institute of Biomaterials, Erlangen, DE

Introduction
An ageing population means an increasing need for materials that can stimulate bone regeneration in patients with impaired bone healing. Boron has been reported to affect bone metabolism and a lack of boron is associated with impaired bone development. Boron deficiency in animal models can also result in reduced chondrocytes density in the growth plate and decreased bone volume fraction. The incorporation of boron into the bioactive glass (BG) network has been shown to promote osteoblast metabolic activity and angiogenesis. However, there is still relatively little known about the cellular mechanism of boron. In this study, using an in vitro bone nodule formation model, the effect of boron and boron bioactive glasses (BBG) is investigated with the aim of developing tailored BGs with an optimised ion release profile for patients with underlying bone diseases.

Experimental Methods
Calvarial osteoblasts were isolated from 3-day-old Sprague-Dawley rats and cultured in α-MEM supplemented with 2 mM β-glycerophosphate, 10nM dexamethasone, and 50μg/mL ascorbate for 21 days. Osteoblasts were treated with dissolution products of boron bioactive glass (provided by Prof. A. Boccaccini with a composition in wt%: 37.5 SiO₂, 22.6 CaO, 5.9 Na₂O, 4.0 P₂O₅, 12.0 K₂O, 5.5 MgO, 12.5 B₂O₃) diluted to achieve 0.3, 0.7 and 1mM of boron (referred to B1, B2 and B3 respectively) as determined by ICP. 45S5 bioactive glass (similar Si level to B1) was used as control. The proliferation (total DNA quantification), angiogenic response (VEGF ELISA) and alkaline phosphatase activity of the cells were measured. Bone nodule formation in response to the BG dissolution products was characterised using Alizarin Red calcium staining and transmission electron microscopy (TEM). Raman spectroscopy and interferometry (for quantitative size analysis).

Results and Discussion
Boron was released from the BGs within biologically relevant ranges. Boron Bioactive Glass dissolution products (within the range of 0.3-1mM boron) did not affect total DNA or VEGF expression after 7 days of culture (Figure 1). For the first time, results will be presented on the effect of boron on bone nodule formation.

Conclusion
Understanding how boron interacts with osteoblasts in vitro will allow the development of improved bioactive glasses, that release different therapeutic ion concentrations to target different stages of healthy bone regeneration.
Figure 1. Osteoblast VEGF expression in response to Boron Bioactive glass (BBGs) Dissolution products.

BBGs did not affect VEGF expression (7-day culture).

References
2:00 p.m. – 3:30 p.m.

Poster floor

PS2-07 | Other Biomaterials Applications
3D bioplotting of neonatal porcine islets in a plasma-based bioink

Sarah Duin¹, Susann Lehmann², Elisabeth Kemter⁴, Eckhard Wolf⁴, Anja Lode¹, Michael Gelinsky¹, Barbara Ludwig²,³

¹ TU Dresden, Centre for Translational Bone, Joint and Soft Tissue Research, Dresden, DE; ² University Hospital Carl Gustav Carus, TU Dresden, Paul Langerhans Institute Dresden of Helmholtz Centre Munich and German Centre for Diabetes Research, Dresden, DE; ³ University Hospital Carl Gustav Carus, TU Dresden, Department of Medicine III, Dresden, DE; ⁴ Ludwig-Maximilians University Munich, Gene Center, München, DE

Introduction
Diabetes mellitus type 1 is characterized by insulin-deficiency due to autoimmune-derived destruction of the insulin-producing beta cells in the pancreas. The common therapy is treatment with exogenous insulin, however the majority of patients develop major complications such as kidney failure in the long-term. To avoid those it would be preferable to make islet transplantation available for a larger number of patients. Human islets for transplantation are sparse, but donor shortage could be solved through use of xenogeneic islets, whereby neonatal porcine islets are an attractive candidate [1]. Especially for xenogeneic islets separation from the host’s immune system is crucial, yet supply with oxygen and nutrients as well as the ability to sense blood-glucose should not be impaired, which requires short diffusion distances. For easy retrieval macroencapsulation devices are preferable over microencapsulation of islets, yet the diffusion distances in these are large.

In a proof-of-concept study we were able to show that it is possible to create macroporous scaffolds with encapsulated functional murine pancreatic islets by using extrusion-based 3D bioplotting [2] with a biologically inert hydrogel blend of alginate and methylcellulose [3]. Preliminary work of our group indicated that this approach is also a promising tool for the bioplotting of neonatal porcine islet-like cell clusters (NICC), yet the system was limited by a lower viability than had been observed in murine islets and a progressive loss of function over time. In this study, the biologically inert hydrogel was adapted for use with human platelet lysate to study the hypothesis that growth factors from platelets, e.g. insulin-like GF 1, and plasma components such as serum albumin support cell survival and function in plotted scaffolds.

Experimental Methods
The hydrogel used for bioplotting consisted of 3 % medical-grade alginate dissolved in PBS or in platelet lysate (PL), prepared from platelet concentrate obtained from a local blood bank (Deutsches Rotes Kreuz - Blutspedendedienst Nord-Ost, Dresden, Germany), and 9 % methylcellulose (algMC or PL-algMC). Viscosity of the cell-free blends was analysed using a plate rheometer (Rheotest RN 4).

NICC were incorporated into the gels prior to bioplotting carried out on a BioScaffold 3.1 (GeSiM, Germany). Scaffolds with NICC were crosslinked with SrCl₂ and cultured in supple-mented Ham’s F-10.

To investigate distribution and cell viability, NICC were stained with MTT or calcein AM/ethidium homodimer, and cryosections of islet-containing scaffolds were stained with DAPI and TUNEL. Presence of insulin was studied via dithizone staining of whole scaffolds, and of immunofluorescence-staining for insulin and glucagon inside the islets in cryosections. To study glucose stimulated insulin response, plotted islet scaffolds and free control islets were incubated in Krebs-Ringer buffer supplemented with either 3.3 or 16.4 mM glucose and a long-lasting glucagon-like peptide 1 (GLP1) analogue. Insulin content was analysed by quantification of secreted insulin with ELISA and normalization to the DNA content determined after cell lysis by QuantFluor assay.

Results and Discussion
By comparison to algMC we were able to show that PL-algMC has a similarly high viscosity, yet incorporation of NICC into either blend did not impact their morphology. For both blends, MTT-staining demonstrated an even distribution and metabolic activity of NICC inside the plotted scaffolds. Live/Dead as well as TUNEL stainings indicated viability of encapsulated NICC for as long as 28 days, whereby the viability of those exposed to PL was increased compared to NICC encapsulated in plain algMC. Dithizone and immuno-fluorescence stainings confirmed that insulin and glucagon are continuously produced and adequately located in encapsulated NICC. Our data also suggest that, while GLP-1 receptor agonists are required for functionality of NICC independent of the hydrogel used for encapsulation, functionality was improved in bioplotted constructs prepared with PL.

**Conclusion**

This study introduces a hydrogel blend with platelet lysate for increased biological support of neonatal porcine islet-like cells clusters in 3D bioplotted constructs.

**Acknowledgement**

The authors thank the Doktor Robert Pfleger Stiftung Bamberg (Germany) as well as the German Centre for Diabetes Research (DZD) for financial support, and the microscopy facility CFCl of the TU Dresden for providing equipment and support in cell imaging.

**References**


Development of biodegradable shape memory polymers towards minimally invasive, patient-specific breast reconstruction

Coralie Greant, Lana Van Damme, Sandra Van Vlierberghe

Ghent University, Polymer Chemistry & Biomaterials Group, Centre of Macromolecular Chemistry (CMaC), Department of Organic and Macromolecular Chemistry, Ghent, BE

Introduction
According to estimations of the Global Cancer Observatory, over 2.2 million women were diagnosed with breast cancer in 2020, worldwide. [1] While most often requiring surgical removal of the affected breast tissue, breast reconstruction following mastectomy remains a challenge. [2] Conventional reconstruction techniques, using synthetic prostheses or autologous tissue, are accompanied with risks and complications such as infection, prosthesis rupture, capsular contracture, donor site morbidity, uncontrollable resorption rates and the need for reinterventions. [3,4,5] Therefore, a strong research focus is directed towards regenerative adipose tissue engineering, shifting reconstruction strategies from tissue replacement to autologous tissue regeneration.

Experimental Methods
In the present work, a novel approach is introduced to enable minimally invasive, patient-specific, aesthetically elegant breast reconstruction. To this end, shape memory copolymers based on aliphatic polyesters were synthesized and functionalized into acrylate end-capped urethane-based polymers (AUPs) to enable chemical crosslinking. The materials were tuned by adjusting the ratio of building blocks and adapting the molar mass, in order to achieve a glass transition temperature below body temperature (30 - 35°C). The AUPs were characterized physico-chemically by nuclear magnetic resonance (NMR) spectroscopy, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and gel permeation chromatography (GPC). Subsequently, a photo-sensitive resin was developed based on the synthesized AUPs, rendering them suitable for digital light processing (DLP). By varying the molar mass of the polymer, the acrylate content of the AUPs and the concentration of photoinitiator and photoabsorber, the resin was optimized towards efficient photo-crosslinking, which was confirmed by means of photo-rheology. Furthermore, the gel fraction was assessed and swelling tests were performed, to study the crosslinking efficiency and network density. Once optimized, the photo-sensitive resin was processed into porous scaffolds by means of DLP, as an appropriate porous architecture will enable (adipose) tissue infiltration and regeneration within the scaffold. The morphology of the obtained scaffolds was investigated using scanning electron microscopy (SEM) and micro-CT. The results indicated that stable scaffolds can be realized, with pore sizes around 1000 μm.

Results and Discussion
Furthermore, in vitro biocompatibility assays were performed, to assure the absence of cytotoxic components. To this end, adipose-derived stem cells were seeded onto tissue culture plates. The cytotoxicity was tested through an indirect assay in which the crosslinked materials were submerged during 24h, 72h and 7 days in culture media. The culture media, containing any leached components, was placed on top of the seeded cells in order to assess biocompatibility through a live/dead staining and MTS assay. Finally, a crucial aspect in this project is the shape memory property of the final scaffold. Based on the glass transition temperature of the AUP, which is right below body temperature, a shift in shape can be induced - from a temporary reduced size into a permanent large, patient-specific volume - upon implantation in the body. This assures the minimally invasive approach of the developed strategy. The study of this shape memory effect, as evaluated by shape fixity and strain recovery, as well as the mechanical characterization of the scaffold, is currently ongoing.

Page 1689 of 2028
Additionally, future experiments include a profound study of the effect of shape memory characteristics on cell behavior, by injecting cell-encapsulating gelatin-based hydrogels in the porous scaffold.

**Conclusion**

As a result, the developed porous shape memory scaffolds can be considered promising candidates towards minimally invasive, patient-specific adipose tissue engineering.

**Acknowledgement**

The authors would like to acknowledge the Research Foundation Flanders (FWO) and the Interreg program MATMED for financial support under the form of the research grant G056219N and Interreg NWE 5B (2014-2020) project no. NWE764, respectively.

References


Conductive interpenetrating polymer network hydrogel for neural tissue engineering and 3D printing applications

Chiara Rinoldi1, Massimiliano Lanzi2, Roberto Fiorelli3, Paweł Nakielski1, Krzysztof Zembrzycki1, Tomasz Aleksander Kowalewski1, Olga Urbanek4, Valentina Grippo5, Katarzyna Jezierna-Woźniak6, Wojciech Maksymowicz6, Andrea Camposeo7, Renata Bilewicz5, Dario Pisignano7,8, Nader Sanai3, Filippo Pierini1

1 Institute of Fundamental Technological Research Polish Academy of Sciences, Department of Biosystems and Soft Matter, Warsaw, PL; 2 Alma Mater Studiorum University of Bologna, Department of Industrial Chemistry “Toso Montanari”, Bologna, IT; 3 Barrow Neurological Institute, Ivy Brain Tumor Center, Phoenix, US; 4 Institute of Fundamental Technological Research, Laboratory of Polymers and Biomaterials, Warsaw, PL; 5 University of Warsaw, Faculty of Chemistry, Warsaw, PL; 6 University of Warmia and Mazury in Olsztyn, Department of Neurology and Neurosurgery, Olsztyn, PL; 7 Istituto Nanoscienze CNR and Scuola Normale Superiore, NEST, Pisa, IT; 8 Università di Pisa, Dipartimento di Fisica, Pisa, IT

Introduction

Intrinsically conducting polymers (ICPs) have been largely applied to produce biomaterials. However, their use in neural tissue engineering is considerably restricted because of some intrinsic disadvantages, including their hydrophobicity and inadequate mechanical characteristics. In order to overcome these limitations, soft conductive polymer hydrogels (CPHs) have been explored, resulting in water-based platforms with optimal adhesion, biological and electrical features as well as comparable mechanical properties to the native tissues. Approaches providing the incorporation of ICPs as a conductive element into CPHs have been lately researched by synthesizing the hydrogel around ICP chains, resulting in an interpenetrating polymer network (IPN). Due to the efficient ICP integration into the hydrogel network, IPNs have shown some favorable properties including high chemical and mechanical stability, biocompatibility, electrical and electrochemical characteristics.

Experimental Methods

In this work, the interpenetrating polymer network hydrogel is designed and developed by the introduction of polythiophene (poly[6-(3-thienyl)hexanesulfonate], P3HT6S) into poly(N-isopropylacrylamide-co-N-isopropylmethacrylamide) (P(NIPAm-co-NIPMAm)) hydrogel network. The incorporation of P3HT6S as ICP aims to confer sufficient electrical characteristics to the final platform. In order to permit the production of the hybrid hydrogel in an aqueous medium, polythiophene monomers are modified and synthesized to guarantee the water-solubility. Upon dissolution of P3HT6S in water, NIPAm-co-NIPMAm constituents are added, resulting in the hydrogel precursor solution. The final system is established by synthesizing the P(NIPAm-co-NIPMAm) hydrogel interpenetrated with P3HT6S chains by UV light exposure in a cold environment, forming the IPN. Morphological, chemical, thermal, electrical, electrochemical, and mechanical characteristics of the IPN are examined to investigate the effect of different P3HT6S concentrations into the hydrogel formulation. Furthermore, human umbilical-derived mesenchymal stem cells and neural progenitor cells isolated from postnatal mouse brain sub-ventricular zone are selected to investigate the biological properties of P(NIPAm-co-NIPMAm)/P3HT6S hybrid hydrogel. Thus, the response of cells seeded and cultured on the conductive IPN is evaluated in terms of cell viability, proliferation, and neural differentiation. Finally, the suitability of the hybrid hydrogel as ink for 3D printing applications is assessed.

Results and Discussion

The influence of P3HT6S incorporation into the P(NIPAm-co-NIPMAm) hydrogel is evaluated in terms of morphological characteristics, evidencing similar properties of up to 2% P3HT6S concentrated hydrogels.
enhancements of electrical, electrochemical, and mechanical characteristics of the proposed P(NIPAm-co-NIPMAm)/P3HT6S hydrogel compared to the P(NIPAm-co-NIPMAm) pristine formulation are also reported. Hence, the suitability of the novel conductive hydrogel for promoting the conduction of neural electrical signals in physiological environments and inducing an optimal mechanical integration in the brain tissue is highlighted. Furthermore, the hydrogel's biocompatibility is proven by a high percentage of viable mesenchymal stem cells cultured onto the IPN platform. Additionally, the biological response of neural progenitor cells seeded onto P(NIPAm-co-NIPMAm)/P3HT6S reveals that the hybrid hydrogel supports the survival of neurons and astrocytes, as the two main cell types of the central nervous system (Figure 1). More in detail, it is evidenced that P(NIPAm-co-NIPMAm)/P3HT6S hydrogel can promote cell neural differentiation, as highlighted by the presence of a significantly higher quantity of neural cells on P(NIPAm-co-NIPMAm)/P3HT6S samples at late stages of culture. Lastly, the possibility to exploit the hydrogel solution as a printable ink for the fabrication of 3D biomaterials with tailored morphological and structural characteristics is illustrated.

Conclusion
In this study, a soft, low-impedance, and biocompatible P(NIPAm-co-NIPMAm)/P3HT6S hydrogel is designed and developed. Results reveal that the proposed platform is a suitable candidate for the design and production of 3D-printed conductive biomaterials, enlightening a promising potential for novel neural tissue engineering applications.

Acknowledgement
This study was supported by the First TEAM grant number POIR.04.04.00-00-5ED7/18-00 funded by the Foundation for Polish Science.

References
Nisin Z loaded wet-spun fibers: characterization and antibacterial features against *Staphylococcus aureus*

Natália C. Homem, Tânia D. Tavares, Catarina S. Miranda, Joana C. Antunes, Maria Teresa S.P. Amorim, Helena P. Felgueiras

*University of Minho, Centre for Textile Science and Technology (2C2T), Guimarães, PT*

**Introduction**

Microbial resistance against conventional antibiotics is being classified as a public health problem, with economic consequences worldwide. *Staphylococcus aureus* – a common opportunistic pathogen – induced infections are among the most prevalent, once *S. aureus* is resistant to multiple antibiotics. Thus, both the academia and the industry sectors have been devoting their efforts in finding solutions or alternatives to the widespread antibiotic resistance of *S. aureus*[1]. In this scenario, antimicrobial peptides (AMPs) are gaining more importance in pharmacology or biomedicine strategies as they can target multiple organisms with efficiency, are less likely to induce resistance, and are not just bacteriostatic but also bactericidal[2]. Nisin Z, an AMP which presents significant antibacterial activity against Gram-positive bacteria and low toxicity in humans, has been studied for food preservation applications, but very little research has been done to explore its potential in biomedical applications. In this context, the aim of this study was to engineer bio-based fibrous structures for the controlled release of Nisin Z, aiming to serve as platforms in the fight against *S. aureus*-derived infections. Single- and dual-polymer fibers, composed of sodium alginate (SA) and gelatin (GN), were produced via wet-spinning and subsequently loaded with Nisin Z. Loaded and unloaded fiber's controlled release kinetics and antimicrobial action was mapped, and their physical, chemical, and thermal properties were assessed. The long-term stability of the fibers was also evaluated.

**Experimental Methods**

Nisin Z peptide (Mw 3331.05, potency <38,000 IU/mg, Toku-E) was characterized via Fourier-transform Infrared Spectroscopy (FTIR) and its Minimum Inhibitory Concentration (MIC) against *S. aureus* was assessed by the broth microdilution method, in order to establish the loading concentration of peptide into fibers. SAGN spinning solutions were prepared by mixing SA (alginic acid sodium salt from brown algae, medium viscosity) and GN (type A from porcine skin, ≈ 300 bloom) under low stirring at a polymer ratio of 70:30 v/v (SA at 2 wt% and GN at ≈ 1wt% dissolved in distilled water). Then, SAGN fibers were produced by wet-spinning in a set-up composed of a syringe pump (NE-1600, New Era Pump Systems), a tray containing the coagulation bath (2wt% CaCl$_2$ aqueous solution at 21°C), and an 18-gauge (G) needle. The spinning was performed at a rate of 0.1 mL/min and room temperature, and the fibers were collected and stored in a cabinet desiccator. SA control fibers were produced in equal conditions, while SAGN-crosslinked and SA-free-crosslinked fibers (labeled as SAGNCL and GNCL, respectively) were produced via the removal of SA from SAGN fibers (immersion within PBS 0.25M solutions for 48h at 4°C) and subsequent crosslinking with glutaraldehyde (immersion in a concentrated PBS solution containing 2.5 wt% of glutaraldehyde for 1h at 4°C)[3]. The functionalization of SA, SAGN, SAGNCL and GNCL fibers with Nisin Z was achieved via adsorption (72h, 120rpm) at a loading concentration of 3xMIC, resulting in SAz, SAGNz, SAGNCLz and GNCLz fibers. The loading efficiency was confirmed by UV-VIS spectroscopy at 205 nm. The morphology (brightfield microscopy), chemical composition (FTIR), thermal resistance (Thermogravimetric analysis, TGA, and Differential Scanning Calorimetry, DSC) and degradation/release profile (SBF at pH 7.4, release via UV-VIS) of all fibers were assessed. In addition, the antibacterial activity against *S. aureus* of SA, GN, SAGN and Nisin Z solutions was acquired via the Kirby-Bauer method, while the unloaded and loaded fibers were evaluated via time-kill kinetics assay[4].

**Results and Discussion**

Page 1693 of 2028
FTIR results showed that SAGN fibers were successfully produced and that modifications to these fibers’ chemical stability and structure were introduced via PBS reactions, crosslinking with CaCl₂ and glutaraldehyde (GNCL and SAGNCL). Regardless of the chemical modification employed, all fibers were homogeneous both in size (≈ 243.55 µm) and shape (defect-free). SAGNCL fibers proved to be the most resistant regarding thermal resilience, assessed via TGA and DSC. Nisin Z was functionalized onto all fibers at an average concentration of 178 µg/mL. Nisin Z did not impact on the fiber’s morphology nor on their chemical/thermal stability. Its main effect was detected on the time-kill kinetics of the bacteria S. aureus. SAGNCLz and GNCLz were capable of progressively eliminating the bacteria, reaching an inhibition superior to 99% after 48h of culture. The peptide-modified SA and SAGN were not as effective, losing their antimicrobial action after 6h of incubation. Bacteria elimination was consistent with the release kinetics of Nisin Z from the fibers.

**Conclusion**

In general, data revealed the increased potential and durable effect of Nisin Z (significantly superior to its free, unloaded form) against S. aureus-induced infections, while loaded onto prospective biomedical wet-spun scaffolds.

**Acknowledgement**

This research received funding from the Portuguese Foundation for Science and Technology (FCT) under the scope of the projects PTDC/CTM-TEX/28074/2017 and UID/CTM/00264/2021. CSM also acknowledges FCT for PhD grant (2020.08547.BD).

**References**


PS2-07-228

Human-derived extracellular matrix solid foams for in vitro adipose tissue formation

Nerea Garcia Urkia¹, Iratxe Madarieta¹, Jose Ramon Pineda², Igor Irastorza², Gaskon Ibarretxe², Fernando Unda²

¹ Tecnalia, Basque Research and Technology Alliance (BRTA), Biomaterials, Donostia-San Sebastián, ES; ² Faculty of Medicine and Nursing, Basque Country University, Cell Biology and Histology, Leioa, ES

Introduction
Increased incidence of adipose-related diseases involves the need of in vitro models¹. The extracellular matrix (ECM) represents the suitable material for this purpose. In this work, we describe human-derived decellularized adipose tissue (hDAT)² processed as solid foams (SF) in combination with collagen type I (Col-I). The study is based on structural and human dental pulp stem cell (HDPSC)³ culture, as a cell type with the capacity to differentiate into adipocytes.

Experimental Methods
hDAT³ and Col-I-based SFs were obtained by freeze drying a 0,5 % (w/v) acid solutions (10 µl in an 4x 0,8 mm µ-slide) (Table 1). SFs microstructure (SEM), water absorption and HDPSC cultures⁴ (1.5 x 10⁴ cells/SF) were studied. After 3 days of culture, cell stained to determine viability (Calcein/Propodium Iodide) and proliferation (Ki67 antibody and Alexa 488) in the SF analyzed by fluorescent microscopy. The viable and proliferation rate was determined (positive cells/total cells) in 3 microscopic images of SF.

Table 1. Sample identification (ID) and hDAT/Col-I SF composition.

<table>
<thead>
<tr>
<th>% (w/v)</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SF1</td>
</tr>
<tr>
<td>hDAT</td>
<td>100</td>
</tr>
<tr>
<td>Col-I</td>
<td>0</td>
</tr>
</tbody>
</table>

Results and Discussion
All SF no showed significant differences in physically characterization. SFs showed an interconnected porous architecture (100µm pore, fig. 1A) with Young modulus between 2043 ± 400 Pa (SF1) and 5858 ±871 Pa (SF4), and water absorption 96-97 %. HDPSC viability and proliferation results are shown in Fig. 1 (B and C) and Fig 2. HDPCS cells maintained viable (>95%) and proliferate (>7%) on the SFs. The highest cell proliferation rate was detected with high hDAT quantity.

Conclusion
Freeze dried SFs obtained by various formulations of hDAT and Col-I ECM materials, shown optimal physical and biological properties for HDPSC culture. The best hDPSC viability and proliferation results were shown in SFs obtained by 100% and 75% hDAT in formulation which are currently being analyzed to determine the biologic capacity of the SFs to differentiate hDPSC in adipocytes and ideally form new in vitro adipose tissue model.

Acknowledgement
This research was supported by the Basque Government (ELKARTEK program 566 PLAKA KK-2019-00093)
Figure 1.
A-C: SF scanning electron microscopy (A, SEM), viable cells stained with calcein (green) (B), and proliferation cells (Ki67) with stained Alexa 488 (green) and nuclei with DAPI (blue) (C)
Figure 2
HSPSC viability and proliferation (%)

References
Green antibacterial thermosensitive hydrogels based on corn silk extract, hyaluronic acid and nanosilver for wound healing application

Assunta Borzacchiello¹, Mario di Gennaro¹,², Pooyan Makwandi³, Ghareib W Ali⁴, Francesca Della Sala¹, Wafa I. Abdel-Fattah⁴, Luigi Ambrosio¹

¹ National Research Council of Italy, Institute of Polymers, Composites and Biomaterials, Napoli, IT; ² Università degli Studi della Campania “Luigi Vanvitelli”, Caserta, IT; ³ Istituto Italiano di Tecnologia, Centre for Micro-BioRobotics, Pisa, IT; ⁴ National Research Centre, Inorganic Chemistry Division and Mineral Resources, Refractories and Ceramics Dept., Biomaterials Group, Cairo, EG

Introduction
Hydrogels have attracted research attention for wound healing applications since hydrogel dressings are an excellent source for providing moisture to a dry lesion, monitoring fluid exchange from within the wound surface, helping to cool down a wound, as well as provide temporary pain relief [1]. Thermosensitive amphiphilic block copolymers, polyethylene oxide-polypropylene oxide copolymers (Poloxamers or Pluronics, PPO-PEO-PPO), thanks to their ability to undergo thermal gelation, to their good tolerability, and low irritancy/toxicity, have been used in the biomedical field for instance for tissue engineering and drug delivery applications [2]. Hyaluronic acid (HA) is naturally occurring glycosaminoglycan, presents in mammalian connective tissues. It has been demonstrated that HA promotes dermal regeneration and for this, it is widely used as dermal fillers, as wound dressings and substrates for dermal engineering applications [3]. It has to be highlighted that infection is a crucial and generally unsolved issue in wound healing. Therefore, materials containing antimicrobial compounds, such as Ag nanoparticles (Ag NPs) have shown the capability to inhibit or decline infections. Corn silk extract (CSE), a waste material of the crop, has been used for AgNPs biosynthesis as both a reducing and stabilizing/capping agent [4]. In addition, CSE possesses excellent antioxidant capacity [5]. In this context, we propose novel and green thermosensitive nanocomposites hydrogels based on HA, Pluronics and AgNPs for wound healing applications.

Experimental Methods
The corn silk were heat extracted. AgNPs were synthesized within CSE by microwave. The hydrogels were prepared by dissolving different amounts of Pluronics F127 and F68 in silk extract with and without AgNPs. Subsequently HA was added. The hydrogels composition was optimised by rheological analysis. The hydrogels were characterised for the rheological properties. The formation of AgNPs was confirmed by UV-Vis surface plasmon band while their morphology was evaluated by DLS and HRTEM. Bacterial cell suspensions were prepared, for each tested Gram-positive and Gram-negative, the cell growth of the tested bacteria was determined at the end of the incubation period, based on the optical density measurements at a wavelength of 620 nm. In vitro biocompatibility was performed by Alamar Blue assay on L929 fibroblast cells culture. The wound healing potential of the realized formulations was assessed by wound healing assay on Human Dermal Fibroblast (HDF) cells after 24 and 48 h of thermosensitive hydrogels incubation. Wound healing assay was performed by scraping the cell monolayer in a straight line to create a “scratch area”. Wound area, was calculated using the ImageJ public domain software. The migration rate can be expressed as the percentage of wound area reduction or wound closure.

Results and Discussion
Separate Pluronic F127 and F68 solutions do not show an appropriate Tgel but by formulating Pluronic F127/F68 blends at specific concentrations, it was possible to obtain a medium with a Tgel close to Tb. The addition of HA slightly affects Tgel, but very interestingly, improved significantly the final gel viscoelastic properties. AgNPs were spherical with average diameter of 8 nm (Figure 1A-D). The presence of AgNPs did not alter the hydrogels rheological
properties. The AgNPs hydrogels showed an excellent bactericidal activity against the tested Gram-positive and Gram-negative bacterial strains. The results confirmed the bactericidal activity of the samples in comparison with control. It has been demonstrated the biocompatibility of the hydrogel on L929 cells and that the presence of Ag does not modify the cell viability. The in vitro wound healing assay of the realized thermosensitive corn silk extract-nanosilver hydrogels was performed on HDF cells; the results (Figure 2A-C) have been demonstrated that the wound surface area decreases with the increasing exposure times (24 to 48h) of the tested hydrogels compared to the controls. Accordingly, the wound closure percentage increases with the increasing time after exposure to the injectable hydrogels. The individual biomaterials used in this assay could affect positively the wound healing process.

**Conclusion**

We developed a Novel and green thermosensitive injectable hydrogels based on HA, CSE and Ag NPs. In-vitro model of wound healing revealed that the nanocomposites allow faster wound closure and repair, compared to the control. The obtained results highlight the potential application of these novel injectable hydrogels as wound dressing.

**Acknowledgement**

The authors acknowledge the research project "ADVISE DRUGS AND ANTI-TUMORAL VACCINES FROM THE SEA" - POR CAMPANIA FESR 2014-2020 AND WITH THE AXIS 1 OO.SS. 1.2.2 / 1.1 CUP B43D18000240007"
Figure 2

(A) Representative bright-field images show HDF cells migration after the scratch at time 0 and after 24 and 48 hrs. of thermosensitive hydrogels based on HA, Pluronics and AgNPs incubation. (B) Wound area expressed as the remaining area uncovered by the cells. The scratch area at time point 0 hrs. and after 24 and 48 hrs. of CSE, Silk Pluronics and HA (SPHA), Ag Silk Pluronics and HA (AgSPHA) incubation. (C) Wound closure expressed as the percentage of the closure of the scratched gap after 24 and 48 hrs. of CSE, SPHA, AgSPHA incubation. Results are the means of three measurements.

References
Development of vitamin C loaded plant-based microfibers for wound healing applications

Fabrizio Fiorentini¹,2, Giulia Suarato¹,3, Rosalia Bertorelli³, Athanassia Athanassiou¹

¹ Istituto Italiano di Tecnologia, Smart Materials, Genova, IT; ² Università di Genova, DIBRIS, Genova, IT; ³ Istituto Italiano di Tecnologia, Translational Pharmacology, Genova, IT

Introduction
Wound healing is an articulated process, which consists of several biological and chemical events. Tissue repair mechanisms can be improved by (1) stimulating cell proliferation through the employment of microfibrous scaffolds, which can effectively mimic the 3D conformation of the extracellular matrix, and (2) applying bioactive molecules able to boost the cell ability to restore the wound area. Moreover, materials derived from natural resources are becoming an optimal choice to create alternative wound dressing. The purpose of this work was to fabricate natural 3D microfiber scaffolds loaded with vitamin C for the treatment of skin lesions, by combining four plant-based molecules: zein, an alcohol-soluble protein derived from corn; low-methoxy pectin, the most abundant hydrophilic polysaccharide in the plant cell wall; soy lecithin, used as emulsifier agent; and vitamin C, the bioactive molecule able to exert antioxidant activity and stimulate collagen synthesis.

Experimental Methods
The polymeric microfibers were produced through vertical electrospinning of emulsion solutions, obtained by blending in aqueous 80 % v/v ethanol solution the four plant-based molecules described above. The optimized electrospinning parameters consisted in a voltage of 20 kV, a flow rate of 2 mL/hour and a distance of 25 cm between the tip of the needle and the collector. Five samples (labeled ZPC_0, 1, 2, 3, 4) containing different concentrations of vitamin C (0 – 10 mg/mL) were fabricated and their cross-linking was optimized, in order to enhance their water resistance. Two strategies of cross-linking were tested: (1) immerse in aqueous CaCl2 15 % (w/v) solution for 5 minutes at room temperature (RT); (2) trifluoracetic acid treatment for 3 minutes at RT in a closed environment. Morphological analysis was performed by SEM while the chemical characterization of the samples was carried out by FTIR spectroscopy. An in vitro drug release assay was set up by incubating the samples in 3 mL of PBS (pH 7.4) in an oven set at 37°C and replacing the buffer with fresh one at each time point until 24 hours. Swelling and degradation rate was monitored by immersing the matrices, before and after cross-linking, in PBS alone or with protease XIV from Streptomyces griseus inside at 37°C for a week. The biocompatibility was evaluated on Human Dermal Fibroblast adult (HDFa) cells by MTS assay.

Results and Discussion
SEM micrographs showed ribbon-like microfibers morphology without the formation of any beads (Figure 1a-j). The increase of vitamin C content in the samples resulted in an expansion in the fiber average diameter, from 0.84 to 1.19 μm (Figure 1k). The SEM imaging of the cross-linked samples demonstrates that TFA cross-linking was able to maintain better the microfibers structure compared to the CaCl2 cross-linking. In vitro drug release indicated a burst release of the vitamin C from the microfibers within 3 hours of immersion. Degradation assay showed a higher weight loss of the samples when immersed in PBS with protease, compared to the immersion in PBS alone. In vitro MTS assay on HDFa cells resulted in good biocompatibility for each sample of the study, except for the microfibers cross-linked through TFA treatment, which led to a decrease in cell viability compared to the control (Figure 2). Although this decline was recorded, biocompatibility improved with increasing concentrations of the vitamin C loaded into the fibers.

Conclusion
Page 1701 of 2028
So far, the results confirmed that our plant-based electrospun microfibrous patches represent a suitable biomaterial for wound healing applications. Ongoing experiments are carried out to test other physico-chemical properties of the matrices, such as their mechanical resistance and their radical scavenging ability towards DPPH• radicals.

Figure 1
SEM micrographs of ZPC_0 (a), ZAPC_1 (c), ZPC_2 (e), ZPC_3 (g) and ZPC_4 (i) and their respective higher magnification images (b, d, f, h, j). Analysis of the diameter (μm) average of the samples (k). A significance of p < 0.05 was considered.

Figure 2
Cell viability assay after 24 hours of HDFa cell growth in presence of the extraction medium of each sample before cross-linking and after cross-linking with CaCl2 solution or TFA vapor. A One-Way ANOVA test was conducted, considering a p-value < 0.05.

References
The Development of an Implantable Bioengineered Tracheal Scaffold for Respiratory Tissue Regeneration

Tehreem Khalid1,2, Sally-Ann Cryan1,3, Fergal O'Brien1,4, Cian O'Leary1,2

1 Royal college of Surgeons in Ireland, Tissue Engineering Research Group/School of Pharmacy and Biomolecular Sciences, Dublin, IE; 2 RCSI & Trinity College Dublin, Advanced Materials & Bioengineering Research (AMBER) Centre, Dublin, IE; 3 National University of Ireland, Centre for Research in Biomedical Devices (CÚRAM), Galway, IE; 4 Trinity College Dublin, Trinity Centre for Biomedical Engineering, Dublin, IE

Introduction

Attempted tracheal replacement efforts to date have had very little success, due to their inability to regenerate a protective layer of respiratory epithelium and often succumbing to stenosis within poorly-vascularised constructs [1, 2]. The major objective of this study was to optimise a bilayered collagen-hyaluronic acid scaffold (CHyA-B), which has shown to facilitate the growth of respiratory epithelial cells on its non-porous film layer and fibroblasts in its porous collagen sub-layer [3]. This redeveloped bilayered scaffold will be assessed for cellular biocompatibility, robust mechanical properties, and maximised submucosal blood vessel formation to produce an innovative tracheal medical device.

Experimental Methods

A biodegradable, thermoplastic polymer was used to 3D-print a tubular backbone which was integrated into the CHyA-B scaffold via lyophilisation. Construct design was optimised via multimodal mechanical assessment to assess compressive strength and scaffold flexibility. The composite scaffold’s vascularisation potential was determined by assessment of cellular viability and angiogenesis using a previously optimised delayed co-culture model of Human Umbilical Vein Endothelial Cells (HUVECs) and Human Mesenchymal Stem Cells (hMSCs) [4]. In vitro vessel formation and infiltration visualisation was assessed using confocal microscopy and angiogenic growth factor release was assessed via ELISA and qRT-PCR.

Results and Discussion

The 3D-printed backbone was successfully incorporated within the CHyA-B scaffold by lyophilisation, producing a composite scaffold with a mean pore size of 300μm, ideal for vascularisation. The construct mimicked native tracheal mechanics with a compressive modulus of 0.09 to 0.19 MPa and flexural moduli of 0.13 to 0.29 MPa. The composite scaffold enhanced cell growth, expressing 2-fold higher levels of double stranded DNA than control materials and similar levels of cellular metabolic activity compared to control. Notably, angiogenic growth factor release (VEGF and bFGF) and angiogenic gene upregulation (KDR, TEK-2 and ANG-1) was detected in composite scaffolds and remained sustainable up to 14 days. Additionally, the presence of vessel like structures were observed in samples stained with CD-31 antibody using confocal microscopy. Furthermore, scaffolds placed within chick chorioallantoic membrane (CAM) assay confirmed the infiltration of blood vessels was not hindered by the presence of the 3D-printed backbone via histological sectioning.

Conclusion

By addressing both the mechanical and vascularisation requirements of a tracheal scaffold, this work has begun to pave the way to solve the critical components required for clinically-successful, next-generation implants for tracheal regeneration. Ongoing experimental work is looking to optimise culture conditions for facilitating growth and differentiation of primary respiratory epithelial cells in addition of HUVECs and hMSCs to obtain an epithelialised and vascularised tracheal scaffold.

Acknowledgement

Page 1704 of 2028
This study is funded by the SFI-funded AMBER centre (Grant 17/RC-PhD/3477).

References
Nanocarriers delivering secretome of human umbilical cord mesenchymal stem cells for chronic wound healing

Barbara Blanco-Fernandez1,2, Cristina Alcoholado3, Antonio Villatoro3, Cristina Antúnez-Rodríguez4, Rick Visse3, José Becerra3, Elisabeth Engel5,2,1, Soledad Perez-Amodio2,1,5

1 CIBER en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Madrid, ES; 2 Institute for Bioengineering of Catalonia, Biomaterials for Regenerative Therapies, Barcelona, ES; 3 University of Malaga, Department of Cell Biology, Genetics and Physiology, IBIMA, Malaga, ES; 4 Center for Tissue and Cell Transfusion of Malaga (CTTC), Expansion and cell therapy unit, Malaga, ES; 5 Polytechnic University of Catalonia (UPC), Materials Science and Metallurgical Engineering, Barcelona, ES

Introduction
Chronic wounds are affecting more than 40 million people worldwide. Current treatments consist on wound debridement, infection control, compression and the use of wound dressings (gauzes, hydrogels, etc). In the last years, advanced wound dressings carrying active ingredients such as cytokines, antibacterial compounds or growth factors (GFs) have been developed. In this sense, mesenchymal stem cells (MSCs) secretome has shown its efficacy in wound healing [1]. Secretome is a dynamic combination of soluble molecules (GFs, cytokines, chemokines, hormones) and extracellular vesicles (transporting RNA, lipids, proteins, DNA, etc) released to the extracellular space. Secretome derived from MSCs has shown therapeutic effect in tissue engineering, having angiogenic, anti-apoptotic, anti-inflammatory, immunomodulatory and anti-fibrotic. Although secretome can be directly administered in the target site, its fast clearance is still challenging. Therefore, new efforts have been made to create delivery platforms that sustain secretome release and increase its retention time [2].

The aim of this work is to encapsulate human umbilical cord MSCs (hUcMSCs) secretome into nanoparticles (NPs) to achieve a sustained release and to evaluate their potential in chronic wound healing.

Experimental Methods
Secretome was obtained by culturing hUcMSCs under hypoxic conditions during 48h to increase the expression of proangiogenic molecules [2]. The content in angiogenic factors was determined using a protein array. Secretome was encapsulated in poly(lactic-co-glycolic) (PLGA) NPs by a double emulsification/evaporation method using two polymers concentrations (50 mg/mL PLGA50 and 25 mg/mL PLGA25). NPs size and zeta potential were measured using a Zetasizer. The loading content and release was evaluated using a microBCA. Secretome integrity was assessed by ELISA. NPs cellular cytocompatibility was studied using human dermal fibroblasts and keratinocytes (empty NPs were used as negative controls).

Results and Discussion
The most expressed proangiogenic molecules detected in the secretome were TIMP-2, MCP-1, IL-6 and RANTES; all involved in the healing process stimulation. NPs successfully encapsulated secretome, ranging from 7.2-10.2 µg/mg NPs. NPs have sizes of 300-400 nm and zeta potentials below -20 mV. NPs sustained the delivery of the cargo for 7 days, releasing from 2 to 4.0 µg/mg depending on the PLGA concentration used (Fig 1A). This allowed the modulation of the secretome release according to the therapeutics needs. Moreover, the released secretome maintained its integrity (Fig. 1B). NPs were biocompatible with skin cells at the range of the concentration tested.

Conclusion
The secretome composition evidenced its potential in wound healing. Secretome was successfully encapsulated in the NPs, showing a sustained release. Our results indicate that these nanoplatforms are promising chronic wound healing agents.
Acknowledgement

Work funded by Marie Skłodowska-Curie (712754), Severo Ochoa (SEV-2014-0425), CIBER-BBN (BBN19PIV03) and EuroNanoMed3 (PCI2019-103648) grants.

Fig. 1
Fig. 1. NPs encapsulating hUCMSCs secretome characterization. (A) Secretome release in phosphate buffer saline. (B) TIMP-2 integrity in PLGA NPs (measured by ELISA).

References
Functionalized hydrogels as therapeutic contact lenses for the controlled delivery of anti-inflammatory peptides

Nadia Toffoletto¹, Ana S. Silva-Herdade², Miguel A. R. B. Castanho², Benilde Saramago¹, Ana Paula Serro¹,³

¹ Instituto Superior Técnico, University of Lisbon, CQE, Lisbon, PT; ² University of Lisbon, Faculty of Medicine, Institute of Molecular Medicine, Lisbon, PT; ³ Instituto Universitário Egas Moniz, Centro de Investigação Interdisciplinar Egas Moniz, Caparica, PT

Introduction
Peptides have gained interest as drug candidates for the development of novel and safer medicines. They demonstrated to overcome most of the safety issues related to classic formulations, avoiding cytotoxicity and damage on microcirculation [1]. Kyotorphin (L-Tyr-L-Arg) is a small endogenous neuropeptide, which exhibited analgesic activity. To improve its membrane permeability and reduce enzymatic degradation, some derivatives (i.e. KTP-NH₂, Ibuprofen-KTP, Ibuprofen-KTP-NH₂) were previously designed. These derivatives also demonstrated to inhibit the liberation of pro-inflammatory cytokines in the inflammatory cascade [1,2]. The development of permeable drugs for topical application, able to provide a therapeutic effect in the back of the eye without need of intraocular injections, is a trending topic in ophthalmology. The use of KTP derivatives as anti-inflammatory and permeative peptides constitutes a novelty in the field. Herein, a KTP derivative (KTP-NH₂) was selected as a model peptide to investigate the possibility of its loading and release from therapeutic contact lenses (CLs), which could act as drug-reservoirs for the daily sustained release of the peptide.

Experimental Methods
Two CL materials (i.e. HEMA and HEMA-NVP-TRIS hydrogels) were investigated as potential CL backbone. HEMA is a hydrophilic monomer commonly used in commercial CLs, while HEMA-NVP-TRIS has been previously suggested as a hybrid CL material able to optimize oxygen permeability and water uptake [3]. Autodock software was used to model the molecular interactions between the monomers and the peptide, and identify potential functional monomers able to tune the release profile. Polymerization was performed at 60 °C for 24h. Then, hydrogels were washed to remove unreacted monomers and cut into 14 mm diameter discs to mimic the dimension of commercial CLs. Loading was performed by soaking dry discs (N=3) in 500 µL peptide solution (1 mg/mL) in PBS at 4 °C for 72 h. The concentration of the loading solution was measured over time to detect the amount of drug loaded in the hydrogel discs. Then, hydrogels were rinsed and immersed in 3 mL of PBS to investigate drug-release in vitro. At regular intervals (i.e. hourly for 8 h), 300 µL aliquots of the release medium were collected for quantification and replaced by fresh PBS. Peptide quantification was performed by UV-vis spectroscopy at 275 nm. To investigate their swelling behavior, discs (N=3) were weighted in dry state and after hydration in water or in the peptide solution (1 mg/mL). Light transmittance was assessed in the 200-700 nm range to prove the optical suitability of the materials.

Results and Discussion
Molecular docking with acrylic acid (AAc) and methacrylic acid (MAA) resulted in the lowest values of inhibition constant among the tested monomers (i.e. 10-13 mM) and the most negative values of the free energy (i.e. -2.6 Kcal/mol). These values indicate a predominance of the peptide-monomer complex over the two separate species and a strong interaction between the two. Therefore, AAc and MAA were incorporated in the prepolymer mixture as functional monomers. The presence of the functional monomers significantly increased the amount of peptide loaded during the soaking phase for both HEMA-based (i.e. from 1.54 µg/mg to 4.78 and 4.66 µg/mg of dry HEMA-AAc and HEMA-MAA discs, respectively) and HEMA-NVP-TRIS-based hydrogels (i.e. from 2.56 µg/mg to 4.72 and 4.57
µg/mg of dry HEMA-NVP-TRIS-AAc and HEMA-NVP-TRIS-MAA discs, respectively. HEMA-NVP-TRIS-based hydrogels released the loaded peptide within the first hour in vitro (Figure 1). HEMA-based hydrogels sustained the release for about 8h. The incorporation of functional monomers increased the amount of drug released over time. Despite the presence of the hydrophobic TRIS monomer, the swelling of HEMA-NVP-TRIS lenses (≈ 65%) was higher than for HEMA-based lenses (≈ 45%) due to the high water uptake of NVP [3]. The swelling of functionalized hydrogels increased in the peptide solution (i.e. ≈ 60% for AAc- or MAA-functionalized HEMA discs and ≈ 80% for AAc-functionalized HEMA-NVP-TRIS discs). All hydrogels exhibited a light transmittance higher than 90% at 500 nm, which is considered the minimum requirement for optical devices. Transmittance in the visible range was not affected by the presence of the functional monomers or by the loading with KTP-NH₂.

Conclusion

AAc and MAA, selected as functional monomers due to their high molecular affinity with KTP-NH₂ peptide, increased the amount of peptide loaded in both HEMA-based and HEMA-NVP-TRIS-based hydrogels. All designed hydrogels showed suitable physical properties (i.e. water uptake, light transmittance) for their use as contact lens materials. HEMA-based hydrogels sustained the release of KTP-NH₂ for about 8h in vitro, which is compatible with the use of daily therapeutic lenses for the treatment of inflammatory ocular conditions. Further investigation is needed to assess the peptide permeation to the back of the eye.

Acknowledgement

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement N° 813440 (ORBITAL—Ocular Research by Integrated Training And Learning).

References

Skin drug delivery bio-based polymeric structures using ionic liquids

Noemi Jordao, Joana Galamba, Luisa Neves

Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Departamento de Química, LAQV-REQUIMTE, Caparica, PT

Introduction
Nowadays, different efforts for the development of novel and innovative biomaterials, which can be used in several research areas, have been increased. Indeed, biomaterials derived from biopolymers, which are abundant and obtained from renewable sources, present outstanding properties such as biodegradability, biocompatibility and biological properties [1]. However, their application for medical field can be limited mostly due to their poor solubility in water and in most biocompatible organic solvents [1,2]. To overcome this limitation, ionic liquids (ILs) as low-melting organic salts appears as an interesting alternative for biopolymer valorization. In the last decade, different works that explores the ILs as alternative solvent for biopolymer dissolution have been reported [1-5]. In this context, the present work explores the use of functional ionic liquids, which are designed to be capable to disrupt hydrogen bonds but also have biological properties, to dissolve microcrystalline cellulose (MCC) and the development and characterization of valuable polymeric structures, in the form of films and gels. Different ILs composed by benzethonium and didecylidimethylammonium cations, which can exhibit anti-bacterial or anti-microbial properties, and short linear carboxylate anions have been developed.

Experimental Methods
The selected ILs were prepared by acid-base reaction, after previously preparation of the desired cation in their hydroxide form through adequate ion-exchange resin. All prepared ILs were analyzed by NMR (1H and 13C), ATR-FTIR spectroscopies, and elemental analysis to check their structure and relative purity levels. Also, thermal proprieties (DSC and TGA) were evaluated. The developed films and gels were analysed by ATR-FTIR spectroscopy, thermal analysis (TGA and DSC), and rheological studies.

Results and Discussion
Carboxylate ionic liquids (ILs) containing benzethonium (BE) and didecylidimethylammonium (DDA) as cations have been sucessful prepared to be used for the first time as dual functional solvent for microcrystalline cellulose (MCC) dissolution. The selected synthetic methodology to prepare the desired ILs consisted in two followed steps: i) preparation of hydroxide form of the desired cation, through an anion exchange resin method (Amberlite A26-OH form exchange resin). In general, all prepared ILs are liquid at room temperature, their structural and thermal properties have been evaluated. After optimized the dissolution conditions, it was observed that all tested ILs have been able to dissolve MCC up to a concentration of 4 % (w/w), resulting in different polymeric structures as gel or film depending on the type of IL and ratio between MCC and IL.

Conclusion
In conclusion, all tested ILs showed ability to dissolve MCC up to 4 % (w/w) of biopolymer concentration. In this sequence, different polymeric structures through phase inversion method as film or gels have been prepared, which can be correlated with the type of ionic liquid used as well as the ratio between MCC and IL. The developed films and gels were analysed by ATR-FTIR spectroscopy, thermal analysis (TGA and DSC), and rheological studies. Furthermore, according to ATR-FTIR spectroscopy, the obtained gels and films showed that some IL remains in the polymeric structures, enhance their application in biomedical field, namely for skin drug delivery.

Acknowledgement
This work was supported by the Associate Laboratory for Green Chemistry – LAQV, which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020) and the national project “PTDC/CTM-CTM/29869/2017”, which is financed by Fundação para a Ciência e a Tecnologia (FCT). The NMR spectrometers are part of The National NMR Facility, supported by FCT/MCTES (RECI/BBB-BQB/0230/2012).

References
[1] Brandt, A; Gräsvik, J; Halletta, J. P.; Welton, T; Green Chem, 2013, 15, 15550
Novel ternary organic/inorganic sol-gel derived hybrid biocomposite: A multifunctional platform towards bone cancer therapy and regeneration

Ashkan Bigham, Ines Fasolino, Alessandra Soriente, Luigi Ambrosio, Maria Grazia Raucci

National Research Council, Institute of Polymers, Composites and Biomaterials (IPCB-CNR), Naples, IT

Introduction

As a minimally invasive therapeutic approach, phototherapy is an effective substitute for traditional cancer therapies; in the exposure of light irradiation, a photocatalytic agent generates heat and reactive oxygen species locally culminating in the cancer cells death. Black phosphorus (BP) with excellent photothermal effect has recently drawn a considerable attention in bone cancer therapy. It is mainly composed of a single phosphorus giving BP a high degree of homology with natural bone. However, there are some bottlenecks revolving around BP—being highly prone to oxidation and a high aggregation tendency in the presence of salts; these issues, which limit the BP’s applicability and efficiency, are still the hotspots in the field.

Bioactive glass (BG)-based sol-gel derived hybrid materials are of particular interest in bone tissue regeneration. These materials are endowed with recapitulating the advantages of polymers and ceramics into a package. As calcium is vital for BG’s bioactivity and osteoblasts activities, its incorporation into the BG hybrids is still problematic. Due to lack of thermal treatment for organic/inorganic BGs, calcium ions cannot diffuse into glass structure and so it is a real challenge.

Considering the mentioned issues, we successfully gave birth to an innovative, stimuli-responsive, regenerative hybrid biocomposite composed of pluronic-F127/BG/BP (Figure 1). This hybrid is prepared in a non-aqueous solution in which the F127 molecules would self-assemble around BP nanoparticles followed by formation of a BG shell on the BP-encapsulated F127 liquid crystals. Putting the extraordinary regenerative properties of BG beside the photothermal effect of BP yielded a multifunctional platform for bone cancer therapy and regeneration.

Experimental Methods

The hybrid, which is composed of F127/BG/BP, is synthesized through evaporation-induced self-assembly sol-gel technique followed by being in the exposure of a microwave irradiation (Biotaage Initiator). The F127 is first dissolved in the mixture of ethanol/HCl followed by addition of 2D BP nanosheets with different concentrations. The mixture is then exposed to a two-step microwave irradiation. Finally, the solution is poured in a Petri dish to dry at room temperature. It is noteworthy that different calcium precursors (nitrate, chloride, and alkoxide) and microwave irradiation are applied to assess their effects on the physicochemical and biological properties of hybrid biocomposite. The samples without F127 and any calcium precursors are prepared to shed light on their effects as well. The XRD, Raman spectroscopy, FTIR, SEM equipped with EDS and elemental mapping, TEM, AFM, ICP, and thermal analysis are performed to characterize the physical and chemical properties of prepared hybrid. In the case of biological properties, the bioactivity in simulated body fluid (SBF), degradability in phosphate buffer saline (PBS), and the BP release at different temperatures (25 °C and 37 °C) are assessed in vitro. Moreover, the cytotoxicity was evaluated on murine fibroblast cell line L929 (ISO10993-5), meanwhile the in vitro anticancer activity on healthy osteoblast and osteosarcoma cells was also investigated.

Results and Discussion

The obtained results indicated that the F127 had a key role in homogenous distribution and controlled release of calcium ions from the hybrid. The samples without F127 induced toxicity when exposed to L929 due to burst release of calcium ions. The microwave irradiation had a stronger effect on the chemical stability and the samples treated with it showed a significant increase in the cell viability. The BP structure is turned out to be intact after the hybrid
preparation because of being encapsulated in F127 liquid crystals and no oxidation was observed attributed to synthesis environment. Regarding to thermo-responsivity of F-127, the encapsulated BPs were released faster when temperature raised to 37 °C. The bioactivity potential of hybrid with and without BP was assessed in SBF and the ternary hybrid outperformed the other one in deposition of newly formed calcium phosphate stemming from the released BP. The cell studies implied that the ternary hybrid selectively target cancerous cells without inducing toxicity towards healthy cells and also the encapsulated-BP exhibited a powerful photothermal effect when exposed to NIR against cancerous cells.

**Conclusion**

Two issues in the field are successfully addressed as follows: homogenous distribution of calcium ions in the sol-gel derived hybrid BG structure followed by the ions controlled release and *in situ* preparation of hybrid BG containing BP nanoparticles without being oxidized. Taken together, the thermo-responsive biocomposite can be exploited in simultaneous bone cancer therapy and regeneration.

**Acknowledgement**

This study was supported through funds provided by Progetto MIUR PRIN2017–ACTION, Grant N. 2017SZ5WZB.

![Figure 1](image)

**Figure 1**

A schematic showing the synthesis and applicability of ternary hybrid biocomposite towards bone cancer therapy.

**References**


**In vitro** biocompatibility and inflammation response of β-glucan based mixture extracted from barley for wound healing treatment

**Alessandra Soriente**¹, Maria Grazia Raucci¹, Giovanna Gomez d'Ayala¹, Donatella Duraccio², Hussman Razzaq³, Ines Fasolino¹, Luigi Ambrosio¹

¹ Institute of Polymers, Composites and Biomaterials, National Research Council, Naples, IT; ² Institute of Sciences and Technologies for Sustainable Energy and Mobility, National Council of Research, Turin, IT; ³ The New Zealand Institute for Plant & Food Research, Christchurch, NZ

**Introduction**

Over the past few years, β-glucans have met with great interest as potential multifunctional modulators of wound healing, alone and/or incorporated in a polymeric matrix [1]. β-glucans represent the major components of bacteria, fungi, yeast and cereals, mainly oat and barley’s cell walls, revealing a source-dependence of structural and biological properties. Among them, barley β-glucans are extensively studied as they have shown interesting antimicrobial and wound healing features [2]. In this study, β-glucan based mixtures (containing proteins, fats and other polysaccharides, such as starch) were extracted from barley at different pH, through a very simple and low cost extraction process [3]. The obtained mixtures were then employed for film preparation and the cell biocompatibility was studied. To this aim, different cell lines involved in wound healing, human dermal fibroblasts (HDF) and human umbilical vein endothelial cells (HUVEC), were used for **in vitro** biological studies. The results suggested that β-glucan films are biocompatible and support **in vitro** cell proliferation and migration. Moreover, these films are able to modulate the inflammation process in terms of pro-inflammatory and anti-inflammatory cytokines levels.

**Experimental Methods**

The β-glucan films were prepared and characterized to evaluate their biological properties. A simultaneous extraction of polysaccharides and proteins from barley flour was performed in alkaline medium, according to a procedure previously described [2]. The water soluble extract was recovered by centrifuging the extraction suspension at 7000 rpm for 20 minutes. Films (100 µm-thick) were obtained by casting at RT from a 5% (w/v) solution and subsequently conditioned at RT and 65% RH for 5 days, before analyses. Molecular mass of β-glucan was measured by MALD-MS.

In order to study the films effects on cell adhesion, proliferation and inflammation reaction, HDF and HUVEC were used. The viability and migration of fibroblast and endothelial cells were analysed by using the **in vitro** scratch wound healing assay. The cell–material interaction and spreading were determined by confocal laser scanning microscopy (CLSM) by staining the cells with cell tracker™ deep red dye, meanwhile Alamar blue assay was used as redox indicator to investigate the cell biocompatibility. Furthermore, the effect on inflammation response modulation, was carried out on a murine macrophage J774 cell line in order to assess the effects of β-glucan based mixtures on inflammatory and anti-inflammatory cytokines production (i.e. IL-6, IL-10, IL-1β), nitrites and reactive oxygen species (ROS) produced after cells stimulation by lipopolysaccharide (LPS).

**Results and Discussion**

**In vitro** biological studies showed that β-glucan films support cell survival, adhesion and migration. In particular, cell biocompatibility test evaluated through direct and indirect assays, demonstrated that β-glucan films are able to support fibroblast viability up to 72 hours of cell culture. In addition, these films promoted endothelial cell viability of HUVEC up to 48 hours. Furthermore, qualitative wound healing assay hinted β-glucan films’s capability of reducing the scratch area of 50% from quantitative point of view (within 24 hours of cell culture). As concerning morphological studies by confocal microscopy (cell tracker™ deep red dye), the β-glucan film treatment induces a cell spreading by...
reducing cellular shape. Finally, the materials are able to modulate inflammatory response in terms of ROS levels, IL-6, IL-10 and IL-1β secretion triggered by LPS in macrophages cell line.

Conclusion
The obtained results demonstrated a good biological properties of β-glucans based mixture as promising candidates for wound healing treatment.

Acknowledgement
The authors would like to thank Mrs. Cristina Del Barone of LAMEST laboratory for SEM investigations, Mariarosaria Bonetti for lab technical support & data elaboration and Dr. Roberta Marzella for support to project management.

References
Drug-eluting casein hydrogels for wound dressings

Leonor Garcia¹, Andreia S. Oliveira¹,²,³, Ana P. Serro¹,³, Benilde Saramago¹

¹ Instituto Superior Técnico, Universidade de Lisboa, CQE, Lisbon, PT; ² Instituto Superior Técnico, Universidade de Lisboa, IDMEC, Lisbon, PT; ³ Instituto Universitário Egas Moniz, CIIEM, Caparica, PT

Introduction

Chronic wounds represent a challenge to wound care professionals and are responsible for the consumption of numerous healthcare resources around the globe. In Europe wound treatment represents 2% to 4% of the medical expenses.

Drug loaded hydrogel dressings are considered promising candidates for the treatment of different types of wounds, since they present a 3D-structure that mimics the skin extracellular matrix (ECM), namely in what concerns the water content, being able to ensure a moist environment to the wound.

The present work aims to develop drug-eluting casein-based hydrogels for wound dressings. Due to their biocompatibility, mechanical properties, ability to interact with soft biological tissues, and presence of many functional groups susceptible to chemical modifications, casein hydrogels have been used in a vast range of biomedical applications, including wound dressings [1].

Hydrogels can be loaded with drugs which, if released in a controlled way, may contribute to the healing process. The release profile will depend on the hydrogel's characteristics such as the crosslinking degree, hydrophilicity, and affinity to the drug. In this work the produced hydrogels were loaded with octenidine dihydrochloride and polyhexanide, two antiseptics with a broad spectrum of effectiveness that represent an alternative to older substances such as chlorhexidine, polyvidone iodine or triclosan.

Experimental Methods

Casein hydrogels were prepared through free radical polymerization of acrylamide and coagulation of casein micelles. The pH of the casein solution was adjusted to 6 by the addition of NaOH. After casein dissolution, acrylamide and N,N’-methylenebisacrylamide were added. Ammonium persulfate and tetramethyleneethylenediamine were used, respectively, as radical initiator and crosslinking accelerator for acrylamide. The functional monomer N-(3-aminopropyl)methacrylamide hydrochloride was also added to the formulation to establish preferential interactions with octenidine dihydrochloride and improve the drug release profile. Subsequently, the solution was poured into a glass mould, exposed to UV for 4 h and then left for 22 h at 36 °C for casein gelation. After proper washing, the hydrogels were dried in the oven at 36 °C for 8 h.

The hydrogel's swelling was assessed in water and in both drug solutions. The mechanical behaviour was studied through tensile tests on hydrated samples. The hydrogels were soaked for 48 h in Octiset® (1 mg/mL of octenidine dihydrochloride and 20 mg/mL of 2-phenoxyethanol) and polyhexanide solution (0.5 mg/mL) at room temperature. After drug loading, the release kinetics was obtained in sink conditions in PBS at 34 °C and 180 rpm. Sterilization was carried out in autoclave at 121 °C for 20 minutes. Antimicrobial properties against S.aureus and P.aeruginosa were also studied.

Results and Discussion

The casein hydrogels showed high values of swelling, around 2100 % in water and 1038 % and 992 % in Octiset® and in polyhexanide solution, respectively. Tensile tests led to a Young module of 0.0156 MPa. Polyhexanide loaded samples were able to release the drug in a controlled way during 48 h (Figure 1), in contrast with the ones loaded with Octiset®, which led to a burst release. The drug-loaded hydrogels showed good antimicrobial properties.

Conclusion

Page 1716 of 2028
Further investigation in order to improve the release profiles of the Octiset® components (octenidine dihydrochloride and 2-phenoxyethanol) is under way.

Acknowledgement
The authors acknowledge to Fundação para a Ciência e a Tecnologia for the financial support (grant numbers: PD/BD/128140/2016 [A.S. Oliveira], UIDB/00100/2020 [CQE], UIDB/50022/2020 [IDMEC/LAETA], and UIDB/04585/2020 [CiiEM]).

Figure 1.
Cumulative drug release of polyhexanide from the produced casein-based hydrogels.

References
PS2-07-250

The effect of pNaSS grafting of knitted poly($\varepsilon$-caprolactone) synthetic ligaments on \textit{in vitro} mineralization and \textit{in vivo} bone tissue integration

Emeline Maurice$^{1,2}$, André Rangel$^3$, Thibault Godineau$^{1,2}$, Julie Vial$^{1,2}$, Amélie Leroux$^3$, Hanane El Hafci$^2$, Mathieu Manassero$^{1,2}$, Marie Vandesteene$^4$, Véronique Migonney$^3$, Véronique Viateau$^{1,2}$

$^1$ Ecole Nationale Vétérinaire d’Alfort, Service de chirurgie, Maisons-Alfort, FR; $^2$ Université Paris Diderot Paris 7, LB3OA UMR 7052, Paris, FR; $^3$ Université Sorbonne Paris Nord, LBPS/CSPBAT UMR CNRS 7244, Villetaneuse, FR; $^4$ MDB Texinov, Saint Didier de la Tour, FR

Introduction

Augmenting anterior cruciate ligament (ACL) repair using synthetic scaffolds to promote ligament healing is a new emerging surgical strategy for the treatment of ACL ruptures. With the increased interest for regenerative medicine, many studies are now focusing on the development of bioactive and biodegradable synthetic ligaments [1]. For this application, Polycaprolactone (PCL) is a well-known biocompatible and biodegradable polymer that presents the advantage of slow degradation depending on its molecular weight. Biointegration of such candidate scaffolds for ACL repair augmentation is a key property to address, as lack of osseointegration has been incriminated in long-term failures of ACL repair. PCL does not present any bioactive activity by itself, but its functionalization with poly(sodium styrene sulfonate) (pNaSS) has proven to enhance \textit{in vitro} cell adhesion and metabolic activity of fibroblasts [1-3]. The aim of this study was then to evaluate \textit{in vitro} and \textit{in vivo} the biocompatibility and osseointegration properties of PCL ligaments grafted with pNaSS.

Experimental Methods

\textbf{In vitro study:} The \textit{in vitro} osteoblast (MC3T3-E1 cells) response was evaluated on grafted and non-grafted PCL films and knitted portions of the PCL ligament. Cells viability and proliferation were evaluated, as well as the expression of alkaline phosphatase and the calcium deposition.

\textbf{In vivo study:} The osseointegration of knitted PCL ligaments was investigated \textit{in vivo} in a rat ACL repair augmentation model by histological evaluation. Following transection of the ACL, rats received either the pNaSS-grafted knitted PCL ligament (G group, N=12), the non-grafted knitted PCL ligament (NG group, N=12) or the flexor digitorum longus tendon autograft (AG group, N=12, control group). Three months after surgery, operated tibial bones were harvested and submitted to non-decalcified histological evaluation using a previously established modified ligament-to-bone core based on interface width, bone formation within the ligament, cellularity and inflammation [4]. Scoring was performed by two investigators blinded to the grafted or non-grafted status of the specimen.

Results and Discussion

\textbf{In vitro results:} Grafted knitted substrates showed statistically higher viability when compared with non-grafted analogs ($P < 0.05$), confirming the positive influence of pNaSS presence on the viability of the cells, as evidenced before on other polymeric surfaces [5]. Grafted knitted PCL fibers induced higher MC3T3-E1 cell proliferation (Fig.1), ALP activity and calcium deposits than their nongrafted counterparts and than grafted PCL films.

\textbf{In vivo results:} Three months after implantation, all groups showed histological evidence of osseointegration of the PCL ligaments or the autograft (Fig.2). Histological scores quantifying osseointegration of the PCL ligaments were higher in the G group compared to the NG group, arguing for a beneficial effect of pNaSS grafting on osseointegration of knitted-PCL ligaments into the tibial bone tunnels.

Conclusion
This small animal study is the first evaluation of the synthetic ligament performance. It provides encouraging in vitro and in vivo results on the effect of pNaSS grafting on bone formation and biointegration of knitted-PCL ligaments.

Acknowledgement
This work was funded as part of the “Future Investment Project” by the French Public Investment Bank and the French state – PSPC application – Liga2bio project.

Figure 1
Time curve of MC3T3-E1 proliferation on grafted and non-grafted (A) films and (B) knitted substrates tested. The highest cell proliferation on both substrates was observed on day 7 (P < 0.05).

Figure 2
Histological sections of the tibial bone tunnels showing osseointegration of the pNaSS-grafted knitted PCL-ligament after 3 months of implantation. Left: histological aspect of the PCL ligament at T0; Right: histological aspect of the PCL ligament at T0+3months. Cell nuclei are stained in blue (Stevenel’s blue) and bone tissue is stained in pink (Van Gieson picro-fuschin). GL : grafted ligament, B : bone, SH : screw hole.

References
Near-infrared-light responsive gold nanoparticle for HER-2 positive breast cancer therapy

Nora Bloise\textsuperscript{1,3}, Lorenzo De Vita\textsuperscript{2}, Mohammad Okkeh\textsuperscript{1,3}, Elisa Restivo\textsuperscript{1,3}, Cristina Volpini\textsuperscript{1,3}, Piersandro Pallavicini\textsuperscript{2}, Livia Visai\textsuperscript{1,3}

\textsuperscript{1} University of Pavia, Department of Molecular Medicine, V. Taramelli, 3B, 27100, Pavia, IT; \textsuperscript{2} University of Pavia, Department of Chemistry, V. Taramelli 12, 27100, Pavia, IT; \textsuperscript{3} Istituti Clinici Scientifici (ICS) Maugeri, IRCCS, Medicina Clinica-Specialistica - UOR 5 LABORATORIO DI NANOTECHNOLOGIE, Via Boezio, 28, 27100, Pavia, IT

Introduction
Breast cancer therapies have been constantly improving year by year, but a comprehensive, uniform cure for this disease with minimal side effects is still a long way off. Hence, there is a need to develop a more efficient therapeutic approach to improve breast cancer prognosis and enhance its survival rates. Gold nanoparticles (GNPs)-based delivery systems offer many advantages due to their unique chemical and physical properties, that make them biocompatible, easy to modify, and functionalize with cancer-specific biomolecules. GNPs can accumulate in tumor tissue either passively via the EPR effect or actively via their conjugation with a targeting molecule.\textsuperscript{[1]} Thus, GNPs conjugation with drugs can enhance drug uptake into cancer tissue. Among GNPs, gold nanostars (GNSs) are emerging as promising tools for cancer photothermal therapy (PTT).\textsuperscript{[2]} Due to their anisotropic spiked shape, they exhibit Near InfraRed (NIR) plasmon resonances with high thermal release efficiency as well as a high surface to volume ratio available for conjugation and functionalization. The study aimed to synthesize and characterize a novel Herceptin (H) conjugated polyethylene glycol-gold nanostars (H-PEG-GNSs) to be used as a selective PTT platform against HER2 (human epidermal growth factor receptor-2) expressing breast cancers.

Experimental Methods
GNPs were synthesized, pegylated, and characterized using Transmission electron microscopy (TEM), Dynamic Light Scattering (DLS), UV-Vis spectrophotometry, and Thermogravimetric analysis. The conjugation between PEG-GNSs and Herceptin was carried out using the previously reported EDC-NHS protocol.\textsuperscript{[3]} The conjugated GNSs were then characterized using UV-Vis spectroscopy absorption studies, DLS, Bicinchoninic acid (BCA) analysis, and dot-blot assay. Viability (MTT assay) and uptake studies were carried out to verify the interaction of Herceptin conjugated PEG-GNSs with the target breast cancer cells.

Results and Discussion
PEG-GNSs showed an intense LSPR (Localized Surface Plasmon Resonance) absorption with the maximum wavelength in the bio-transparent window (750-950 nm), making it a suitable tool that can be used for deep tissue PTT. Next, H-PEG-GNSs were prepared and characterized using DLS and UV-Vis spectroscopy: Herceptin conjugation resulted in particle size increase, less negative zeta potential, and UV-Vis absorption spectrum redshift of PEG-GNSs. The presence of Herceptin on the surface of GNSs also revealed by BCA and dot blot assays. The targeting and anti-proliferative properties of the nanosystems were observed by \textit{in vitro} studies against HER-2 overexpressing cells (SKBR-3 cell line) and compared to a healthy fibroblast cell line (NIH-3T3). Viability assays and uptake studies demonstrated an anti-proliferative efficacy of Herceptin conjugated to GNSs and internalization into target cancer cells, which were both evidence of its active function in recognizing the tumor cells.

Conclusion
Although preliminary, all data gathered showed the successful formation of Herceptin-PEG-GNSs conjugate. Currently, experiments are ongoing to explain other aspects related to the efficacy and the uptake mechanism of the
nanoconjugate systems in the target tumor cells. 3D cancer models and in vivo studies are required to assess the toxicity and efficacy of this PTT nanoplatform against HER-2 positive breast cancers.

Acknowledgement
This research was supported by a grant from the Italian Ministry of Education, University and Research (MIUR) to the Department of Molecular Medicine of the University of Pavia under the initiative “Dipartimenti di Eccellenza (2018–2022)” and Pavia University’s Crowdfunding on breast tumor studies (2015 and 2021 https://universitiamo.eu/en/campaigns/tumore-seno-diagnosi-curare-nanoparticelle-doro and https://universitiamo.eu/campaigns/tumore-al-seno-sconfiggerlo-con-nanosfere-doro-intelligenti-new-challenges/)

References
Nanostructured planar and sharp electrodes for neuronal electrophysiological studies

Ana Arché-Núñez¹, Beatriz L. Rodilla¹,², Ana Domínguez-Bajo³, Ankor González-Mayorga⁴, Ivo Calaresu⁵, Rossana Rauti⁶, Denis Scaini⁷, Julio Camarero¹,⁶, Rodolfo Miranda¹,⁶, Elisa López-Dolado⁴, Laura Ballerini⁵, Lucas Pérez¹,², María C. Serrano⁵, María T. González¹

¹ Instituto Madrileño de Estudios Avanzados (MDEA Nanociencia), Dpto. Nanomedicine, Madrid, ES; ² Universidad Complutense de Madrid (UCM), Dpto. Física de Materiales, Madrid, ES; ³ Instituto de Ciencia de Materiales de Madrid (ICMM-CSIC), Dpto. Materials for Medicine and Biotechnology, Madrid, ES; ⁴ Servicio de Salud de Castilla-La Mancha (SESCAM), Hospital Nacional de Parapléjicos, Toledo, ES; ⁵ International School for Advanced Studies (SISSA), Trieste, IT; ⁶ Universidad Autónoma de Madrid (UAM), Dpto. Física de la Materia Condensada-IFIMAC, Madrid, ES

Introduction

Current electrodes for electrical recording and stimulating cells generally exhibit high rigidity and harmful chemical composition. When used in implants, the tissue reaction to these characteristics can lead to glial encapsulation of the electrode, which will increase its overall impedance [1]. In addition, if the electrode material degrades, it might generate toxicity in surrounding cells and tissues, again negatively influencing the electrical signal transmission. Extracellular experiments and medical devices require planar electrodes to register or stimulate neural activity in the surrounding neurons. To improve current electrodes, we have developed low-disturbance and low-impedance, flexible, nanostructured planar electrodes based on vertical metallic nanowires standing over a flexible gold base [2] to obtain more efficient and biocompatible stimulation electrodes. Alternatively, to register single neuron activity, intracellular in vitro recordings can be performed by using the patch clump technique [3]. To decrease the damage produced to cells by the micropipette in this technique, we are developing conic shaped electrodes with a nanometric apex based on vertical metallic nanowires that will stand over the surface, minimizing cell invasion and reducing the membrane cell perforation size and the medium exchange.

Experimental Methods

Fabrication of either planar or sharp electrodes is based on the anodization process and the template-assisted electrodeposition technique. We use the first to oxidize the surface of the material and obtain an ordered nanoporous template. We have chosen aluminium as initial material to generate the template because of the highly ordered pore arrangement that it acquires once oxidized. Afterwards, the template pores are filled to obtain the vertical metallic standing nanowires on the electrode substrate. To do so, we use the template-assisted electrodeposition technique. Subsequently, nanowires are released from the membrane. The electrodes are then characterized by cyclovoltammetry and electrical impedance spectroscopy. Finally, the electrodes are tested in vitro with neural progenitor cells from rat embryos cerebral cortices to determine their biocompatibility. Electrodes surface is functionalized with a poly-L-lysine (PLL) coating (30-70 KDa) and then cells are cultured up to 14 days to study their morphology, viability and neural differentiation.

Results and Discussion

For planar electrodes, we have grown gold-based nanowires electrodes. Results showed proper neural cell adhesion, growth and differentiation on our gold-exposed electrodes. When compared with equivalent flat gold electrodes, we observed a more intimate contact of the nanostructured ones with the neuron axons. We also verified the lower impedance of the nanostructured electrodes compared with flat ones.

Regarding the sharp electrodes for intracellular recordings, we proposed a completely new electrode architecture which is still under study, preliminary experiments show proper neural cell adhesion, growth and differentiation.
Conclusion

While the cell survival and differentiation of neural cultures grown over nanostructured and flat electrodes is equivalent, a more intimate contact with neurons and a lower density of glial cell was observed for nanostructure electrodes, which also present a lower impedance.

Acknowledgement

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 737116 (ByAxon), and the I+D+I project DPI2017-90058-R (BiSURE) from the Spanish MICINN.

Figure:
Vertical metallic nanowires for planar electrodes.

References

Multi-targeted therapeutic single-patch for intestinal wound healing

Marco Araújo¹, João Silveira¹,²,³, Aureliana Sousa¹,², Mafalda Bessa-Gonçalves¹,²,⁴, Susana G. Santos¹,²,⁴, Cristina C. Barrias¹,²,⁴

¹ Instituto de Investigação e Inovação em Saúde, Porto, PT; ² Instituto Nacional Engenharia Biomédica, Porto, PT; ³ Faculdade de Engenharia Universidade do Porto, Porto, PT; ⁴ Instituto de Ciências Biomédicas Abel Salazar, Porto, PT

Introduction
Wound healing is a highly regulated complex process comprising several overlapping phases: hemostasis, inflammation, neo-tissue formation/remodelling [1]. Chronic wounds result from an abnormal WH process dominated by a persistent inflammatory condition. They can occur either as external or internal ulcers, the latter commonly associated to the stomach and intestine. In the past years, particular attention has been given to inflammatory bowel disease (IBD), which emerges as a global disease affecting ~10M people worldwide, with special incidence in young people (15-35 yrs old), bearing substantial costs to the health care system and society [2]. IBD, including ulcerative colitis and Crohn’s disease, are chronic, disabling, and progressive disorders characterized by a persistent inflammatory condition that leads to damage of the bowel wall. Current IBD therapies reduce inflammatory symptoms and prevent relapse episodes, but do not promote mucosal restitution, which should be key for effective healing. Herein, we propose a multi-targeted therapeutic strategy, based on a bioinspired multifunctional hydrogel-based patch combining both anti-inflammatory and pro-regenerative potential, as a disruptive approach for chronic intestinal wound healing.

Experimental Methods
Multifunctional hydrogel-based scaffolds were obtained from a pre-gel solution containing functionalized alginate, Irgacure 2959 and biopolymeric nanoparticles (BNP). The pre-gel solution was transferred to a glass slide containing 250 µm spacers and samples were irradiated using a BlueWave® 200 curing spot lamp for the desired time periods, inducing polymerization and entrapment of the BNP. Scaffolds were analysed for their mechanical properties by rheology, whereas swelling and degradation behaviour were investigated in PBS and in a cocktail of collagenases (Collagenase type II + Collagenase type IV). Their anti-inflammatory potential was evaluated in-vitro by analysing the conditioned media (CM) from Lipopolysaccharide (LPS)-stimulated macrophages, and the pro-regenerative ability was assessed in the presence of two relevant intestinal cell types (Caco-2 cells and human intestinal fibroblasts - HIF).

Results and Discussion
We achieved to produce softer-to-stiffer hydrogel matrices, with stiffness between G’= 145 – 3300 Pa. Importantly, the viscoelastic properties were maintained upon freeze-drying and rehydration, highlighting the versatility of the material to be applied either in the form of hydrated or freeze-dried hydrogel. This is an attractive feature regarding a clinical application, since the scaffold can be easily stored and rehydrated in situ upon implantation. Although the entrapped BNP reduced scaffolds hydrophilicity, the 3D sponges still exhibited a high swelling ratio, confirming their ability to be rehydrated under physiological conditions. The scaffolds were able to maintain 75% of their initial weight after 14 days incubation in a Collagenase mixture that mimics the enzymatic activity in inflamed tissue of patients suffering from IBD, suggesting their suitable application as therapeutic patches for IBD. The scaffolds were able to normalize the CM from LPS-stimulated macrophages, reverting the inflammatory condition to levels similar to the non-stimulated control. Importantly, the same scaffold could be reused up to 3 times while maintaining this regulatory performance, which is advantageous in an IBD scenario, where disease relapse frequently results in an...
accentuated increase of inflammatory response. HIF seeded on the top of the scaffolds migrated through the pores to their interior and produced ECM components, namely fibronectin, increasing deposition along time. Caco-2 cells seeded on top of the matrices were also able to adhere, grow and migrate into the macropores.

Conclusion
This work highlights the development of an innovative multifunctional therapeutic platform that targets two important processes of wound healing: inflammation and tissue regeneration. The developed biomaterial demonstrated ability to normalize the inflammatory environment promoted by LPS-stimulated macrophages and supported invasion, proliferation and de novo extracellular matrix deposition by intestinal fibroblasts and epithelial cells. Our results suggest that this multi-targeted therapeutic platform may find potential application as an innovative treatment for IBD, disrupting with current symptom-driven therapies.

Acknowledgement
This work was performed in the framework of IBEROS project (0245_IBEROS_1_E) - funded by POCTEP 2014-2020 and FEDER, and ANGIONICHE - funded by FCT. MBG acknowledges the FCT through the BiotechHealth Programme (reference PD/BI/128355/2017) and the PhD studentship PD/BD/135489/2018.

References
2:00 p.m. – 3:30 p.m.

Poster floor

PS2-08 | Hydrogels and Smart Biomaterials
Differently Modified Nanoporous Titania Coatings as a Material for Smart Drug Delivery Systems

Saskia Zailskas¹, Philipp Abendroth¹, Hannah Christmann¹, Katharina Doli², Henning Hartwig², Meike Stiesch², Peter Behrens¹

¹ Leibniz Universität Hannover, Institut für Anorganische Chemie, Hannover, DE; ² Medizinische Hochschule Hannover, Klinik für Zahnärztliche Prothetik und Biomedizinische Werkstoffe, Zentrum Zahn-, Mund- und Kieferheilkunde, Hannover, DE

Introduction
Although tooth implants are a common treatment in dentistry nowadays, peri-implantitis remains a major health issue. Due to late infections bacteria may form biofilms on the implants’ surface which unfortunately leads to high implant failure rates (>10%).[1] To cure these undesirable infections at an early state, occurring bacteria have to be effectively eliminated. A controlled drug delivery system directly located on the implants’ surface offers a promising opportunity to achieve the required antibacterial effect. A stimuli-response system is desired in order to ensure the release of an antibacterial drug not directly after the implantation but only when an infection occurs. For this purpose, a nanoporous titania coating can be attached to the implant surface. The nanopores functions as a depot for local drug release and the surface offers the possibility for chemical modification to construct a stimuli-response release system. Considering that bacterial biofilms often produce an acidic milieu, a pH-responsive polymer can be used to generate a stimulated release. A corresponding system has already been accomplished for nanoporous silica nanoparticles.[2]

Experimental Methods
The first step to transfer the system to titania surfaces is the establishment of nanoporous titania films by utilizing an adjusted cathodic electrodeposition method of Hu et al.[3] In a further step, the surface is modified with phosphonic acid derivates, which form strong chemical bonds to titania and can therefore introduce functional groups.[4] The attachment of a pH-responding polymer is carried out using a reaction route described by Menzel and co-workers.[5] Cell culture investigations are performed in regards to cytocompatibility and antibacterial behavior.

Results and Discussion
The resulting titania coatings were characterized as porous materials; layer thicknesses could be adjusted by the number of cycles during the deposition. We were able to successfully attach different phosphonic acids to these electrodeposited titania coatings. First studies with the antiseptic chlorhexidine show that the release kinetics could be adjusted by surface modification. Cell culture investigations indicate a high potential for future usage in dental implants.

Conclusion
Nanoporous titania coatings modified with different phosphonic acid derivates show a high potential to design smart drug delivery systems for dental applications.

Acknowledgement
This work was part of the research association BioFabrication for NIFE.

References
From supramolecular polymers to bioprinting of tough hydrogels

Matthew Baker

Maastricht University, MERLN Institute; Complex Tissue Regeneration Department, Maastricht, NL

Introduction
Reengineering the cell’s extracellular matrix (ECM) and the 3D spatial positioning of cells in tissue are necessary in order to create complex tissue constructs. While materials scientists have made significant progress in uncovering and recapitulating the complexities of the ECM, engineers have created sophisticated techniques which allow for complex 3D living structures to be fabricated. Supramolecular hydrogels provide an opportunity to mimic the fibrous structure and the self-assembled construction of the native ECM. Synthetic supramolecular hydrogels provide the opportunity to systematically control the mechanical properties (stress relaxation, stiffness) and self-assembly of the matrix; however, the control of the hydrogel properties and their translation to advanced fabrication technologies has remained a challenge.

Experimental Methods
Recently, we have explored molecular engineering of hydrogels based on the supramolecular polymerization of 1,3,5-benzenetricarboxamide (BTA) macromers. Via new synthetic pathways, and characterization of the supramolecular macromers, we can create a library of rationally designed structures. By characterizing the self-assembly of these structures (cryo-EM, Nile Red encapsulation, microscopy) and the resultant materials properties of the hydrogels (rheology, self-healing, tensile) we can begin to create structure/property relationships. In addition by exploring the effect of the materials properties on cell viability, cell aggregation, and printing performance, we can begin to create property/function relationships. In this talk, I will focus on the creation and optimization of the dynamics of these supramolecular polymers for use in cell culture and bioinks.

Results and Discussion
All target compounds were able to be synthesized and isolated in good yield and purity, showing the power of our new synthetic desymmetrization strategy. We observed that the amount of hydrophobics on the exterior of the macromer dictated the dynamic rheological properties (stress relaxation) over 4 orders of magnitude without significantly affecting the stiffness. Furthermore, we could engineer toughness and stretchability into the materials with select molecular units. All materials were cytocompatible and some showed excellent performance as bioinks.

Conclusion
By tuning of the dynamic and viscoelastic properties of the hydrogels we can closely tune the properties and create viable bioinks, even very tough and elastic hydrogels. With careful control over the (supra)molecular structure, we can move from soft, liquid-like supramolecular polymers to highly functional materials for tissue engineering.

Acknowledgement
Portions of this research have been made possible with via support of NWO (Innovation Fund Chemistry, project “DynAM” under project agreement 731.016.202), the Dutch Ministry of Economic Affairs, the Province of Limburg, and the European Research Commission.
Supramolecular BTA polymers allow control of viscoelastic hydrogel properties and 3D printing.
Tough fibrous mats prepared by electrospinning and photo-crosslinking mixtures of methacrylated poly(trimethylene carbonate) and methacrylated gelatin

Jia Liang1, Honglin Chen2, Dirk W. Grijpma1, André A. Poot1

1 University of Twente, BST, Enschede, NL; 2 South China University of Technology, Institute for Life Sciences, School of Medicine, Guangzhou, CN

Introduction
Materials prepared from combinations of synthetic and natural polymers are interesting for use in tissue engineering applications. Poly(trimethylene carbonate) (PTMC) is a synthetic non-crystallizing flexible polymer that degrades in vivo by surface erosion. Crosslinked PTMC networks are flexible, elastic and tough. Gelatin is a natural material that is widely used in biomedical engineering. It has excellent biological properties, but lacks mechanical resilience. By preparing hybrid networks from methacrylated PTMC and methacrylated gelatin (GelMA), materials that combine the advantageous properties of both can be prepared [1]. The aim of the current work is to prepare tough fibrous mats by electrospinning and photo-crosslinking mixtures of methacrylate-functionalized PTMC and GelMA.

Experimental Methods
Three-armed PTMC (20 kg/mol) and gelatin (50-100 kg/mol) were functionalized by reaction with methacrylic anhydride. The degrees of functionalization of PTMC-trimethacrylate (PTMC-tMA) and gelatin-methacrylate (GelMA) were 98% and 90%, respectively. PTMC-tMA and GelMA were dissolved in HFIP/AcOH (20/1, v/v) at different ratios (10/0, 8/2, 6/4, 3/7, 1/9 and 0/10, wt/wt). The mixtures (12.5%, wt/v) were stirred overnight at RT. Irgacure 2959 (5 wt% relative to the macromer) was added to the mixture and stirring was continued for 30 min before electrospinning. The mixture was transferred to a syringe equipped with a blunted 27G stainless-steel needle. Electrospinning was performed using a syringe pump (Multi-Phaser, Model NE-1000) at a rate of 1.0 mL/h and an applied voltage of 16 kV. The working distance from the tip of the spinneret to an aluminium collector plate was set at 15 cm. Two UV light bulbs (365 nm, 0.32 mW/cm²) were positioned at a distance of 10 cm from the fibers, which were continuously irradiated during spinning (2.5 hours). The collected mesh was subsequently immersed in ethanol containing Irgacure 2959 (3%, wt/v) for 1.5 hours and then post-cured in a UV box (365 nm, 7.0 mW/cm²) at 5 cm distance to the lamp for 40 min. The mechanical properties of the PTMC-tMA/GelMA electrospun mats were investigated by tensile testing and the proliferation of human smooth muscle cells (hSMCs) on the fibrous mats was evaluated by use of the CyQuant assay.

Results and Discussion
The tensile properties of crosslinked PTMC-tMA/GelMA and GelMA electrospun fibrous mats are shown in Table 1. Fibrous mats made from 8/2, 6/4, 3/7, and 1/9 PTMC-tMA/GelMA mixtures showed an outstanding toughness of 1023 ± 59, 539 ± 43, 33 ± 6, and 15 ± 4 N/mm², respectively. These values were significantly higher than that of a fibrous mat made from 100% GelMA (3 ± 1 N/mm²). Porosities of the 8/2 – 0/10 PTMC-tMA/GelMA fibrous mats ranged from 88 – 94%. After 7 days of culturing hSMCs on the fibrous mats, cell numbers on the 6/4, 3/7 and 1/9 PTMC-tMA/GelMA fibrous mats were similar as those on the 100% GelMA mats, and significantly higher than on the 100% PTMC-tMA specimens (shown in Figure 1).

Table 1. Elastic modulus, strain at break, maximum strength and toughness of GelMA and PTMC-tMA/GelMA electrospun fibrous mats in the hydrated state.
<table>
<thead>
<tr>
<th>Substrate</th>
<th>$E_{mod}$ MPa</th>
<th>$\varepsilon_b$ %</th>
<th>$F_{max}$ MPa</th>
<th>Toughness N/mm$^2$</th>
</tr>
</thead>
</table>
| PTMC-tMA/GelMA   | 0.643(0.021)  | 445(20)           | 4.127(0.248) | 1023(59)***
| 8/2              | 0.450(0.015)  | 375(5)            | 2.373(0.015) | 539(43)**
| 6/4              | 0.279(0.021)  | 110(10)           | 0.512(0.017) | 33(6)*
| 3/7              | 0.216(0.013)  | 96(2)             | 0.381(0.020) | 15(4)*
| 1/9              | 0.121(0.010)  | 66(4)             | 0.111(0.005) | 3(1)

Significant difference compared to the 100% GelMA fibrous mat: *p<0.01; **p<0.0001; ***p<0.00001

**Conclusion**

We have demonstrated the preparation of tough electrospun fibrous mats from homogeneous PTMC-tMA and GelMA solutions with a wide range of compositions. The addition of GelMA to PTMC-tMA yields stable photo-crosslinked hybrid structures upon electrospinning and enhances the proliferation of hSMCs on the fibrous meshes. Such structures have potential to be used in vascular tissue engineering.

**Acknowledgement**

The authors would like to thank the China Scholarship Council for financial support.

---

References

Retinol-crosslinked Supramolecular Hydrogels for Tissue Regeneration and Antioxidant Delivery

Antonio Feliciano, Matthew Baker

Maastricht University, MERLN, Maastricht, NL

Introduction
Bioengineered corneas have been fabricated using a variety of synthetic or natural materials to transplant corneal keratocytes. However, control of keratocyte phenotype remains a challenge. If a regenerative construct activates a fibroblast-like phenotype then unfavorable ECM akin to scar tissue is produced which will adversely affect transparency and ultimately impair vision. All trans-retinoic acid (RA), as a supplement, has been shown to promote favorable non-scarring phenotype and keratocyte proliferation. We hypothesize that supramolecular materials formed via host-guest inclusion of retinol and cyclodextrin can recapitulate dynamics of the ECM but also act as an injectable drug-delivery scaffold. Herein, we confirm the complexation between retinol and beta/gamma cyclodextrins and proceed to use this interaction to build a supramolecular system with cyclodextrin functionalized alginate.

Experimental Methods
Alginate was functionalized with cyclodextrins by EDC/NHS and characterized by NMR, GPC, and rheology. Complexation between cyclodextrins and retinol was characterized by phase solubility diagrams and association constants were calculated. Complexation was also confirmed by NOSY NMR. Material properties after the complexation were analyzed by UV-VIS, rheology (oscillatory and shear tests), and drug release of retinol.

Results and Discussion
We present host-guest complex characterization, synthesis of functionalized alginate, tunable stiffness of hydrogel, and cumulative release of retinol. In addition, we show supramolecular hydrogel behavior for example self-healing and shear thinning character from this interaction through rheological tests.

Conclusion
A supramolecular environment built with bioactive molecules like retinol which form a 2:1 association could be used in a variety of injectable tissue engineering applications. In addition, the release of retinol could enhance keratocyte proliferation in hydrogel constructs that deliver a sustained release of retinol.
References
Enzymatic crosslinked hydrogels based on gelatin loaded with maleoyl-chitosan/poly (aspartic acid) nanocarriers as potential therapeutic scaffolds for tissue engineering

Alina G. Rusu1, Aurica Chiriac1, Loredana E. Nita1, Liliana Tartau-Mititelu2

1 Institute of Macromolecular Chemistry "Petru Poni" Iasi, Natural Polymers, Bioactive and Biocompatible Materials Laboratory, Iasi, RO; 2 "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, RO

Introduction
In the field of tissue engineering and regenerative medicine, researchers’ attention has been focused on the developing ideal biomaterials that mimic the architecture and composition of the extracellular matrix (ECM), and their further use as therapeutic scaffolds. In this regard, hydrogels have known a substantial expansion due to their unique properties, being developed for utilization in many technological and medical applications like scaffolds for tissue engineering. Owing to their excellent compatibility hydrogels can embed different nanoparticle systems and thus creating new materials with unique properties, such as hierarchically porous structure, high surface area, and tunable water affinity and biodegradability. Therefore, in this study, enzymatic crosslinked hydrogels based on gelatin loaded with maleoyl-chitosan/poly (aspartic acid) (MAC5/PAS) nanogels containing an antimicrobial drug, namely amoxicillin, were prepared and characterized from the point of view of their structural, morphological and biological properties (swelling, biodegradability and in vitro cytotoxicity).

Experimental Methods
For the preparation of the hydrogels, two transglutaminase (TGase) concentrations related to gelatin (2U/g protein and 5U/g protein) were used. Also, in these types of enzymatic crosslinked hydrogels, nanogels based on MAC5 and PAS were incorporated to function as building blocks and nanocarrier of bioactive compounds (antimicrobial amoxicillin). The enzymatic crosslinking reaction and the interactions between nanogels and gelatin were investigated by FT-IR spectroscopy. SEM analysis was utilized to assess and compare the morphology of the hydrogels based on gelatin with the one of the materials enzymatic crosslinked loaded with MAC5/PAS nanogels. Additionally, the swelling capacity, biodegradability and in vitro cell viability were evaluated.

Results and Discussion
Crosslinking by TGase and addition of MAC5/PAS nanocarriers reduced the solubility of gelatin hydrogels as compared to the conventional protein systems. The gels also exhibited controlled biodegradability and in vitro biocompatibility. In general, degradation products from all investigated systems based on gelatin and nanogels did not show cytotoxicity; Specifically, drug-loaded hydrogels showed the highest cell viability, further confirming the suitability of these systems as a biocompatible cell matrix in tissue engineering.

Conclusion
The overall results showed that the nanostructured hydrogels are promising therapeutic scaffolds for tissue engineering with controlled degradability and drug delivery capacity.

Acknowledgement
This work was funded by a grant of the Romanian Ministry of Education and Research, CNCS-UEFISCDI, through the research project for postdoctoral research No. PN-III-P1-1.1-PD-2019-0271 “Engineered glycopeptide-based micro/nanomotors for anti-tumoral co-drug release”, within PNCDI III.
PS2-08-270

Analysis of reproducibility in free radical polymerisation of thermosensitive pNIPAm nanogels

Alena Vdovchenko, Karin Somby, Marina Resmini

Queen Mary University of London, Department of Chemistry, London, GB

Introduction
Thermo-sensitive nanogels based on poly(N-isopropylacrylamide) (pNIPAm) are widely investigate for potential applications in many fields such as drug delivery, sensing, tissue engineering or emulsification systems. [1] The most common approach for the preparation of nanogels is free radical polymerization of NIPAm and methylenebisacrylamide (MBA) which serves as a crosslinker of the polymer network. [2-5] The random character of the radical polymerization raises questions on the reproducibility of the synthetic methodology, and subsequently on the potential variability in the physico-chemical properties of the resulting nanomaterials. An interesting question is related to the crosslinker, and whether its concentration in the feeding solution has an impact on the overall monomer conversions, chemical yields and thermo-responsive behaviour. The potential application of pNIPAm nanogels in medicine requires these material to have well defined characteristics and the degree of reproducibility of the synthetic method is a priority.

Experimental Methods
The following study is focused on the statistical analysis of experimental data obtained in our research group by several researchers. The experimental data studied include chemical yields, monomer conversions obtained by 1H NMR, particle size determined by dynamic light scattering and volume phase transition temperature calculated though UV spectroscopy characterization.

Results and Discussion
The data analysis suggests the existence of a strong correlation between the amount of the crosslinker used during polymerization and the variability of the properties of nanomaterials.

Conclusion
Overall, the following study reveals that the statistical analysis of the reproducibility of the synthetic methodology is essential in the case of random polymerization, which is widely used in the preparation of nanomaterials for medical applications.

References
Electron Beam Mediated Synthesis and Material Properties of Gelatin/PEGDA Hydrogels with and w/o Ag Nanoparticles for Wound Dressings

Tugce Sener Raman, Catharina Krömmelbein, Stefanie Riedel, Stefan Mayr, Bernd Abel, Agnes Schulze

Introduction
Hydrogels are three-dimensional networks made of hydrophilic polymer chains that can retain a large amount of water or biological fluids in their swollen state [1]. They can be applied especially in biomedical applications, such as contact lenses, drug delivery systems, tissue engineering, and dressings for burn wounds [2]. Considering various merits, such as nontoxicity, ability to absorb exudates from wounds, [3] and ability to provide a moist environment good for wound healing, they have optimal properties to be used as wound dressings [4].

Hydrogels can be classified into natural hydrogels and synthetic hydrogels based on their source. Whereas natural hydrogels such as gelatin are highly desirable biomaterials for application in drug delivery, biosensors, bio actuators, and extracellular matrix components due to strong biocompatibility and biodegradability, insufficient mechanical properties are disadvantages of these natural materials [5]. However, hybrid hydrogels made from synthetic and biological polymers possess both suitable mechanical properties, and biocompatibility [6].

Nowadays silver is the most adopted antibacterial agent on various supports, due to its high biocompatibility, excellent resistance to sterilization conditions, effectiveness on different bacteria, and long-term durability of its antibacterial effect. Silver nanoparticles (AgNPs) in combination with hydrophilic, biocompatible polymers can be the basis of the new generation of antimicrobial materials [7-8].

Experimental Methods
In this study, Gelatin/PEGDA hybrid hydrogels and Gelatin/PEGDA hybrid hydrogels with Ag nanoparticles in the various concentrations were synthesized to provide biocompatibility, high mechanical strength, and sterilization required for biomedical applications (especially wound dressing). They were synthesized by electron beam polymerization since electron irradiation promises high efficiency as well as precise and fast crosslinking while not inducing cytotoxicity [9]. Also, the mechanical properties of hydrogels that are irradiated at different doses have been investigated, since crosslinking density can be easily adjusted by applying different irradiation doses. Gelatin/PEGDA hybrid hydrogels and Gelatin/PEGDA hybrid hydrogels with Ag nanoparticles composed of different concentrations which were applied to electron beam irradiation at various doses were synthesized to study the resulting mechanical properties of these hydrogels. The swelling ratio, rheology, and crosslinking ratio were investigated to determine the mechanics of the hybrid hydrogel.

Results and Discussion
The results of this study, the degree of crosslinking of the hydrogels was found to increase with an increasing irradiation dose. Additionally, although the hydrogels with AgNPs and the hydrogel without AgNPs have been prepared equally regarding concentration and irradiation, the hydrogels with AgNPs possess a different crosslinking density compared to hydrogels without AgNPs was adsorbed to reduce Ag(I) to AgNPs, therefore, the hydrogels...
without AgNPs were polymerized with a larger irradiation dose, which created an increased crosslinking density of the hydrogels.

**Conclusion**

In conclusion, it was determined that increased gelatin and decreased PEGDA concentration of hydrogels with AgNPs and without AgNPs affect the crosslinking density and swelling ratio of hydrogels. Electron irradiation demonstrated the ability to precisely tune the hybrid hydrogel properties by the selection of dose and concentration.

**Acknowledgement**

T. Sener Raman is grateful for a scholarship from the Republic of Turkey, Ministry of National Education.
References


Formation of macroporous cell-laden hydrogels with thermosensitive chitosan-salts mixtures

Phuong Anh Dang\textsuperscript{1,2}, Carla Palomino-Durand\textsuperscript{2}, Pierre Marquaille\textsuperscript{1}, Etienne Decencière\textsuperscript{3}, Sophie Norvez\textsuperscript{1}, Emmanuel Pauthe\textsuperscript{2}, Laurent Corté\textsuperscript{1,4}

\textsuperscript{1} ESPCI Paris, PSL University, C3M laboratory, Paris, FR; \textsuperscript{2} CY Cergy Paris University, ERRMECe, Neuville sur Oise, FR; \textsuperscript{3} Mines ParisTech, PSL University, Centre for Mathematical Morphology, Fontainebleau, FR; \textsuperscript{4} Mines ParisTech, PSL University, Centre des Matériaux, Evry, FR

Introduction

Cell-laden thermosensitive hydrogels are highly valuable encapsulation matrices for 3D cell culture, bioprinting or cell delivery. In such materials, the interconnectivity and the size of the pores are key parameters that govern migration, proliferation and potential colonization of encapsulated cells [1]. Despite many efforts and progress, elaborating systems that remain liquid at room temperature and form a macroporous matrix in the presence of cells at body temperature is still a challenge [2]. Here, we explore the potential of thermosensitive mixtures composed of chitosan (CS) and phosphate salts such as beta-glycerophosphate (βGP) [3] and ammonium hydrogen phosphate (AHP) [4]. These systems can be held liquid at room temperature and neutral pH while exhibiting a fast sol/gel transition at body temperature. Most interestingly, this gelation results from a phase separation which spontaneously produces concentration fluctuations over several tens of micrometers. We hypothesize that such mechanism could be advantageously used to make cell-laden thermosensitive hydrogels having the macroporosity required for cell activity.

In our approach, CS/βGP/AHP mixtures were prepared, which pH, osmolarity and gelation kinetics can be finely tuned to be suitable with cell encapsulation. Cell behavior was assessed by in vitro encapsulation for two cellular models: pre-osteoblast MC3T3 and primary human gingival fibroblast HGF. Using a quantitative image analysis based on confocal microscopy (LSCM), we characterized the macroporosity as a function of the CS concentration. Furthermore, observations in cell-laden formulations provided a way to address key questions such as the homogeneity of the cell distribution and their possible influence on the pore formation.

Experimental Methods

Thermosensitive CS/βGP/AHP solutions: Ratios of βGP and AHP were adjusted to satisfy pH = 7.4 and osmolarity of 300 mOsm.L\textsuperscript{-1} for CS concentrations ranging from 0.5 to 0.8 wt%. Each CS solution was chilled in an ice bath for 5 min, βGP and AHP solutions were added and the mixture was vortexed for 30 s between each step. Fluorescently labelled CS with AlexaFluor488 was synthetized and mixed with regular CS before mixing with salts.

Cell staining and encapsulation: (1) Cells were stained with MemBright560 and mixed with CS/βGP/AHP solutions at a final concentration of 10\textsuperscript{6} cells. mL\textsuperscript{-1}; (2) Solutions with or without cells were dispensed into cell culture plates and incubated at 37°C and 5% CO\textsubscript{2} for 1 h to allow gelation; (3) After 1 h of incubation, culture medium was added. The viability and metabolic activity of cells were evaluated by Live/Dead and AlamarBlue assays.

LSCM analysis: Images were acquired using lasers at 488 nm and 561 nm with x40 magnification (LSM 710, Zeiss) at 1 h and 24 h post-encapsulation. Stacks of images were deconvoluted and binarized to determine the porosity as the ratio of white and black pixels. 2D images were analyzed with the morphological sieve technique to estimate the pore size distribution [5].

Results and Discussion

Formulations having physiological pH and osmolarity were produced with CS concentrations varying from 0.5 to 0.8 wt%. They all remained liquid at 21°C for more than 2 h and formed a gel within 2 min at 37 °C. Macroporous
hydrogels are obtained with porosities ranging from 63 ± 2 % to 75 ± 5 % (Figure 1a-b). Within this concentration range, large distributions in characteristic pore size were measured with maximum pore size up to 30 µm with mean values of 5-8 µm (Figure 1c for hydrogel prepared with 0.8 wt% of CS).

Encapsulation studies of MC3T3 and HGF cells showed good viability of at least 80% up to 24 h post-encapsulation. Cells were distributed homogeneously along the observable depth and can be localized within the microstructure (Figure 1c). We found that the formation of the macroporous structure is barely affected by the presence of cells. Both the porosity and the distribution in pore size are the same as for the hydrogels formed without cells (Figure 1d).

Conclusion
Thermosensitive CS/βGP/AHP mixtures were developed having cytocompatible pH and osmolarity. By varying CS concentration, we show that these systems form a gel within minutes and develop a network of interconnected macropores having sizes of tens of micrometers. Cells distribute themselves homogeneously within these gels without altering the formation and the morphology of the macropore network. Such results and methods bring a valuable insight into the complex cell-matrix interactions occurring during the gelation process. Furthermore, these systems provide an interesting platform to design thermosensitive scaffolds for 3D cell culture and cell delivery.

Acknowledgement
The authors would like to thank Lamia El-Guermah and Remy Agniel (ERRMECe, CY University) for technical support. Funding by DIM RESPORIE is acknowledged.
A comparative study of AUP-based hydrogel wound dressings and commercial dressings for the treatment of exuding wounds

Manon Minsart¹, Arn Mignon², Aysu Arslan¹, Iain U. Allan³, Sandra Van Vlierberghe¹, Peter Dubruel¹

¹ Ghent University, Polymer Chemistry and Biomaterials Research Group, Department of Organic and Macromolecular Chemistry, Ghent, BE; ² KU Leuven, Surface and Interface Engineered Materials, Campus Group T, Leuven, BE; ³ University of Brighton, School of Pharmacy and Biomolecular Sciences, Brighton, GB

Introduction
Wounds are a global medical concern [1]. The international advanced wound care market is expected to exceed €18 billion by 2024 [2]. To date, a wide range of wound dressings is commercially available with hydrogel-based dressings being the most relevant for exuding wounds due to their strong exudate uptake capacity. Unfortunately, hydrogels often suffer from inferior mechanical strength.

To this end, our research focuses on the development of a novel hydrogel-based wound dressing with tunable mechanical strength and high exudate absorption capacity using acrylate-endcapped urethane-based precursors (AUPs). The selection of a dressing is of paramount importance as inappropriate wound management and dressing selection can delay the wound healing process. Not only can this be distressing for the patient, but it can also contribute to complications such as maceration and (subsequent) infection. Malodorous wounds, which typically display high levels of exudate, can be especially distressing and thus negatively influence the patients’ wellbeing.

The novelty of our work is situated on two levels. On the one hand, we propose the application of a novel hydrogel-based wound dressing. The potential to apply the AUPs together with activated carbon into wound dressings for malodorous wounds was also assessed. Secondly, we realized an in-depth benchmarking of our newly developed materials relative to the most commonly applied commercial dressings.

Experimental Methods
Acrylate-endcapped urethane-based precursors (AUPs) [3] with a varying poly(ethylene glycol) backbone molar mass (2–20 kg/mol) and (multi-)acrylate endcaps were selected. The syntheses of the different AUP polymers were performed according to standard in-house protocols [4]. The precursors were processed both into UV-cured sheets and electrospun membranes. Both synthesized and commercial dressings were mainly characterized in terms of exudate absorption capacity and mechanical properties. The materials containing activated carbon (AC) were further assessed in terms of sorption potential using colorimetry with crystal violet as model compound. The morphology of each material was studied with scanning electron microscopy (SEM).

Results and Discussion
The results can be subdivided according to the applied processing technique. The swollen AUP UV-cured hydrogel sheets (30 wt%) elegantly combined mechanical strength (up to 0.63 MPa) and high exudate uptake capacities (up to 34.9 g_{water}/g_{material}) along with high gel fractions (> 85%). The commercial dressings exhibited inferior swelling capacities (up to 24 g_{water}/g_{material}). However, several dressings revealed a higher mechanical strength given their multi-layered character and/or hydrophobic nature. Interestingly, upon processing the hydrogel precursors through electrospinning, SEM indicated a homogeneous fiber morphology with fiber sizes in the 0.7–1.5 μm range. The fibrous commercial dressings had diameters ranging between 10 and 25 μm. The hydrogel sheets containing 0.5 wt % AC showed a strong sorption of the model compound crystal violet (> 90% after 24 hours). Finally, indirect cell tests with human dermal fibroblasts confirmed the in vitro biocompatibility of all materials.

Conclusion
The present work offers a profound overview, characterization and comparison of various essential properties of the synthesized and commercial wound dressings. The tested commercial dressings exhibit either a high strength or a high swelling, suggesting the strong potential of dressings combining both a high strength and a high swelling capacity. In this respect, the herein developed AUP materials offer great potential with a superior swelling capacity as compared to the commercial dressings and tunable mechanical properties.

Acknowledgement
The authors Manon Minsart and Arn Mignon have received funding from Research Foundation Flanders (FWO) (SB PhD fellow at FWO: Development of a smart diagnostic antimicrobial hydrogel-based wound dressing, No. 3SB5619, and Co-extrusion electrospinning as novel tool for the next generation wound dressings: taking ultimate control over the dressing mechanical and release properties. No. 12Z2918N).

Processing of the AUPs into UV-cured sheets and electrospun membranes for wound healing purpose
This research focuses on the development of a novel hydrogel-based wound dressing with tunable mechanical strength and high exudate absorption capacity using acrylate-endcapped urethane-based precursors (AUPs). In this work, AUP-based hydrogels were synthesized with a varying backbone (poly(ethylene glycol) molar mass and (multi-acrylate) endcaps and processed into UV-cured sheets and electrospun membranes.

References
Production and characterization of dual-stimuli nanofibrous membranes

Adriana M.L. Gonçalves, Filipe V. Almeida, Paula I.P. Soares, João P. Borges

CENIMAT | i3N, Department of Materials Science, Caparica, PT

Introduction
Cancer is one of the main causes of death worldwide and its occurrence has been increasing over the years. Due to the therapeutic inefficiency and severe side effects of conventional cancer treatments, the development of new cancer treatment options has been one of the most studied research areas. The development of new multifunctional systems that deliver therapeutic agents locally to the tumor site allows a more personalized and effective treatment. The present work focuses on the development a dual-stimuli responsive device composed of Fe₃O₄ magnetic nanoparticles and PNIPAAm microgels embedded in electrospun polymeric fibers capable of providing a simultaneous and local combinatory cancer treatment: chemotherapy and magnetic hyperthermia. The incorporation of microgels and nanoparticles in electrospun nanofibers allows the localization of these systems in the tumor region in a sufficient concentration to enable a more effective treatment [1].

Experimental Methods
Fe₃O₄ nanoparticles (mNPs) were synthesized by chemical co-precipitation technique and later stabilized with dimercaptosuccinic acid (DMSA) and oleic acid (OA) [2]. Thermoresponsive PNIPAAm microgels were obtained by surfactant-free emulsion polymerization (SFEP) [3]. Poly(vinyl alcohol) (PVA) was used as fiber template and 10 wt. % of PNIPAAm microgels and 8 and 10 wt. % of mNPs were incorporated in the fibers through colloidal electrospinning technique to produce dual-stimuli nanofibrous membranes. The membranes were characterized and their mechanical properties, swelling ability, and heating ability were evaluated.

Results and Discussion
Fe₃O₄ nanoparticles with an average diameter of 8 nm were synthesized and successfully stabilized with DMSA and OA. Thermoresponsive PNIPAAm microgels were also successfully synthesized. At room temperature, the microgels are in a swollen state with a hydrodynamic diameter of around 1 μm. Above 32 ºC, their hydrodynamic diameter decreases and at higher temperatures the collapsed state of the microgels takes place, confirming their negative temperature response and Lower Critical Solution Temperature [4]. PVA fibers with a mean diameter of 179 ± 14 nm were obtained and both mNPs and PNIPAAm microgels were successfully incorporated. A bead-on-a-string morphology was observed, as shown in Figure 1. The presence of PNIPAAm microgels and Fe₃O₄ nanoparticles in the electrospun fibers decreases the swelling ratio of the membrane and increases the material stiffness, raising its Young's modulus when compared to the plain PVA membrane. A higher concentration of nanoparticles in the membrane also leads to an increase in this parameter. Also, DMSA coated nanoparticles appear to have a slight impact in the rise of rigidity of the membrane when compared to the OA coated nanoparticles. Magnetic hyperthermia assays show that a higher concentration of nanoparticles leads to a higher heating ability, as expected. The composite membrane with the most promising results is the one incorporated with 10 wt. % DMSA coated mNPs, since it shows the highest temperature variation, 5.1 ºC. If we consider a body temperature of 37 ºC, a temperature variation of 5.1 ºC is enough to reach the desired 42 ºC in magnetic hyperthermia treatment making this membrane a viable option in this type of cancer treatment. In order to evaluate the cytotoxic effect of PNIPAAm microgels and mNPs incorporated in PVA membranes, cytotoxicity assays were performed. All assays reveal that PVA membranes incorporated with PNIPAAm microgels and mNPs do not present any type of cytotoxicity and therefore could be used in biomedical applications.
Conclusion
In the present work, a dual-stimuli responsive system was successfully developed. This system is composed of magnetic nanoparticles and thermoresponsive microgels, which in turn were incorporated into PVA electrospun fibers. Results show the potential of this multifunctional device in magnetic hyperthermia applications as an alternative cancer treatment. In addition, the developed system also presents a special interest in drug delivery applications in which the device can be remotely triggered by an external stimulus like temperature variation. Drug delivery assays will be performed in the future.

Acknowledgement
This work is funded by FEDER funds through the COMPETE 2020 Program and National Funds through FCT—Portuguese Foundation for Science and Technology under the project POCI-01-0145-FEDER-007688 (Reference UID/CTM/50025) and PTDC/CTMCTM/30623/2017 (DREaMM).

References
In vitro simulation of extracellular vesicles delivery from thermosensitive nanocomposite hydrogel for regenerative medicine

Maria A. Grimaudo, Giada Bassi, Arianna Rossi, Monica Montesi, Silvia Panseri, Franco Furlani

National Research Council of Italy, Institute of Science and Technology for Ceramics, Faenza, IT

Introduction
Extracellular vesicles (EVs) have recently emerged as promising pleiotropic tools in regenerative medicine. EVs play a key role in cell-to-cell immunomodulation and, it has been recently demonstrated in preclinical neurotrauma models (e.g. spinal cord injury and traumatic brain injury), their role in functional recovery and neurovascular plasticity. For this reason, their delivery in situ will be beneficial for the recovery of damaged neural tissues. The aim of this work was to design an injectable thermosensitive nanocomposite hydrogel for the controlled delivery of a synthetic model of EVs.

Experimental Methods
Synthetic model of EVs was prepared by injecting 0.1 ml of organic phase containing 25 µl of 20 mg/ml cholesterol ethanolic solution, 12.5 µl of 40 mg/ml α-phosphatidylcholine ethanolic solution and 62.5 µl of pure ethanol into 1 ml of sterile water. Mimetic vesicles were then encapsulated into polymeric nanogels obtained by mixing 0.25 ml of 1.5 mg/ml sodium tripolyphosphate aqueous solution and 1 ml of 1 mg/ml chitosan (base, low MW) acid solution (0.1% w/v acetic acid aqueous solution). Encapsulated mimetic vesicles were prepared substituting the aqueous phase with 1.5 mg/ml sodium tripolyphosphate aqueous solution. Obtained systems were physically characterized by dynamic light scattering, while cells internalization assays were carried out by fluorescent labeling (Topfluor® PC, 3.6 µg/ml) or doxorubicin encapsulation into systems. In vitro release patterns from mimetic vesicles and hybrid systems were studied by dialysis membranes. Thermosensitive hydrogels were prepared by mixing 25 mg/ml chitosan (base, low MW) acid solution (1% w/v acetic acid solution) and 470 mg/ml disodium β-glycerophosphate salt hydrate (β-GP) aqueous solution (1:1 vol/vol ratio) at 4°C. Hydrogels were then casted overnight at 37°C. The addition of sodium hyaluronanate (1.6 MDa) to thermosensitive hydrogel was also evaluated. Polymeric mixtures were studied to evaluate gelation temperature by frequency sweep test (1 Hz, 0.01 strain, 1°C/min, from 5°C to 37°C), and swelling behaviour examined in vitro by hydrogel immersion in cells culture complete medium.

Results and Discussion
Synthetic EVs showed 133±25 nm, 0.149±0.021 and -21±6 mV, as dimensions, polydispersity index and net charge, respectively. EVs encapsulation into polymeric nanogels led to hybrid systems of 235±43 nm, 0.327±0.045 and +33±3 mV as size, polydispersity index and net charge, respectively.

Gelation temperature of thermosensitive hydrogel composed of chitosan and β-GP was found 36.6°C, while casted hydrogels showed the capability to swell up to 100% (weight ratio) after 6 h full immersion in cells culture complete medium. Successfully, cells assays demonstrated that labeled synthetic EVs were efficiently internalized by cells either in free form or encapsulated in polymeric nanogels. In vitro release studies revealed that doxorubicin, used as biomolecule model, was completely released from isolated synthetic EVs and hybrid systems in 24 h. Interestingly, uptake efficiency of doxorubicin in MG63 cells was superior for loaded hybrid systems in comparison to loaded synthetic EVs, while loaded chitosan coated EVs showed an intermediate behavior.

Conclusion
This study demonstrated the possibility to wrap EVs in a nanocomposite hydrogel that will work as EVs reservoir, and the chance to release EVs in a controlled manner, ascribable to the degradation kinetic of the nanohydrogel
itself. These data addresses the smart delivery of EVs as natural therapeutics useful in regenerative medicine applications.
Optimisation of a Thermoresponsive, Shear-Thinning Collagen-Hydroxyapatite Scaffold as a Platform for Structurally Complex Bone Repair

Christopher R. Simpson1, Silvia T. Paiva1,3, Helena M. Kelly1,4, Ciara M. Murphy1,2,3

1 Royal College of Surgeons Ireland (RCSI), Tissue Engineering Research Group (TERG), Dublin, IE; 2 Trinity College Dublin (TCD), Trinity Centre for Bioengineering, Dublin, IE; 3 RCSI and TCD, Advanced Materials and Bioengineering Research Centre (AMBER), Dublin, IE; 4 Royal College of Surgeons Ireland, School of Pharmacy and Biomolecular Sciences, Dublin, IE

Introduction
The most severe clinical consequences of osteoporosis are fragility fractures, with an estimated occurrence of one every 3 seconds[1], with a significant portion of these occurring in the vertebrae. To alleviate pain and immobility associated with osteoporotic vertebral fractures, kyphoplasty and vertebroplasty structurally reinforce fractured vertebrae using a bone cement, however peripheral vertebrae are still weakened and remain at a 5 times greater risk of secondary fracture[2]. The intricate geometry of osteoporotic bone, pathological reduction in bone production and delicate nature of vertebral bone make a minimally invasive tissue engineering approach an attractive solution. Smart/responsive hydrogel based biomaterials offer the potential to conform to complex geometries found in osteoporotic bone, and provide surrogate ECM environments to promote cellular infiltration and proliferation[3]. Furthermore, these biomaterials can be functionalized with materials and cell signals to direct endogenous cells to repair local tissue[4]. Herein, we report the development and characterisation of a thermoresponsive methylcellulose collagen-hydroxyapatite(Ha) hydrogel as a minimally invasive delivery platform for spatially complex bone repair. Specifically, the objectives were to 1) investigate the effect of Ha particle size and concentration on the rheological and thermoresponsive properties of formulated hydrogels, 2) determine the ability of the hydrogels to support osteoblast viability and osteogenesis.

Experimental Methods

Hydrogel Fabrication: Collagen-Ha slurries were mixed with high viscosity methylcellulose (MC) (4000cP) to yield gels of 2.5% w/v MC and 0.1% w/v collagen with either nano or micro Ha content ranging from 50-200%w/collagen. The samples were lyophilized at -40°C for 25 hours, sterilised by ethylene oxide, before being rehydrated in a 5.6 w/v β-glycerophosphate solution adjusted to physiological pH (~7.2) using 0.1M HCL.

Rheological analysis: Gelation temperatures were assessed by measuring the storage(G’) and loss modulus(G”) while increasing temperature from 20-40°C, applying 1Pa of stress at a frequency of 1Hz. The samples were given 1 min to thermally equilibrate before measurement. The point of gelation was defined as when the ratio of G”/G’ =1 or Tan δ= 1.0. Gelation behaviour was also observed using G’ at 37°C over 30 minutes. Injectability of the hydrogels was demonstrated by measuring force (N) required to extrude scaffolds through a 25G Luer Lock needle.

Cell Viability: MC3T3s were cultured in MEMα containing 10% FBS, 1% Pen/Strep and 1% l-glutamine. 1ml of each hydrogel was pipetted to cover the bottom of a 24 well plate, followed by gelation at 37°C for 30 minutes. MC3T3s were seeded on top of each hydrogel at a density of 70,000 cells per well with a total media volume of 1ml per well. At days 1, 3, 7 & 14, well cultures were lysed and dsDNA was quantified using Quant-iT™ PicoGreen™ dsDNA Kit (Invitrogen™) according to the manufacturer’s protocol.

Results and Discussion
A range of thermoresponsive collagen-Ha hydrogels were successfully developed. Addition[CM(1)] of nHa and mHa resulted in a concentration dependent reduction of gelation temperature with the exception of nHa 200%(Fig 1.A).
This reduction was not significant and all sol-gel transitions occurred between room temperature and body temperature (at~30°C), indicating appropriate thermoresponsive properties for in situ application. At room temperature shear thinning properties enabled easy hydrogel extrusion through a 25G needle (Fig 1.B) demonstrating Ha content will not impede minimally invasive application. Upon exposure to 37°C for 30 minutes all hydrogels displayed increasing elastic modulus meaning physical crosslinking continues beyond sol-gel transition point (Fig 1.C). The speed and extent of hydrophobic interaction is reduced by higher Ha content with the exception of mHa 50%. Interactions responsible for gelation (Fig 2.B) between methylcellulose polymer chains could be interrupted by nano Ha interference. Hence we observe that larger Ha particles and lower Ha concentrations allow stronger gelated stiffness.

Our recent work includes successful cell culture of MC3T3s on hydrogel surfaces showing biocompatibility with no reduction in dsDNA observed (data not shown) over 7 days. Future work will involve further exploring cellular interaction and osteogenic potential of the hydrogels. Moreover, in anticipation of further improving osteogenesis we will investigate drug loading and release potential of anabolic agents for restoring bone structure and strength.

**Conclusion**

Overall, the hydrogels’ properties indicated appropriate characteristics to serve as a platform for minimally invasive and spatially complex bone application. The results suggest the ceramic element of these gels can be tuned in terms of particle size and concentration while retaining minimally invasive character and in situ fixation which could be significant for drug loading and cellular interaction.

**Figure 1**

A) Sol-Gel transition temperatures of hydrogels, B) Max force (N) of extrusion required for expulsion via 25G needle, C) Scaffold elastic modulus profiles over 30 minutes at 37°C.
Figure 2
A) Inversion test showing sol-gel of mHa 200% hydrogel when heated to 37°C B) Schematic representation of methylcellulose thermoresponsive mechanism of gelation

References
Drug-eluting PCU hydrogels reinforced with cellulose acetate for cartilage replacement

Inês Ferreira\textsuperscript{1,2}, Ana C. Branco\textsuperscript{1,2,3}, Andreia Oliveira\textsuperscript{1,4}, Carolina Costa\textsuperscript{1}, Pedro Nolasco\textsuperscript{1}, Rogério Colaço\textsuperscript{4}, Célio G. Figueiredo-Pina\textsuperscript{3,5,2}, Ana P. Serro\textsuperscript{1,2}

\textsuperscript{1} Instituto Superior Técnico, CQE, Lisbon, PT; \textsuperscript{2} Instituto Universitário Egas Moniz, CiiEM, Monte da Caparica, PT; \textsuperscript{3} Instituto Politécnico de Setúbal, CDP2T, Setúbal, PT; \textsuperscript{4} Instituto Superior Técnico, IDMEC, Lisbon, PT; \textsuperscript{5} Instituto Superior Técnico, CeFEMA, Lisbon, PT

Introduction
Osteoarthritis (OA) is a chronic joint pathology characterized by the degradation of articular cartilage, which incidence has been increasing over the last decades due to the population aging and the increasing prevalence of obesity. One of the most common approaches in severe stages of cartilage degradation is the total or partial removal of the damaged tissue and its replacement by synthetic materials. Hydrogels are very promising replacement materials for this purpose since they present similar structure and properties to articular cartilage. Besides, their high capacity to absorb water and dissolved substances turn them potential platforms for the vehiculation of drugs and other therapeutic agents. Polycarbonate urethane (PCU) has been used in several applications in the orthopedic field due to its high toughness and flexibility, long-term biocompatibility and bio-durability [1,2]. Some works demonstrated that cellulose acetate (CA) may be added as a reinforcement material to improve the mechanical properties of other polymeric materials [3]. This work aims to develop PCU hydrogels reinforced with cellulose acetate able to release diclofenac in a controlled way, in order to replace damaged articular cartilage and minimize the inflammatory postsurgical reactions.

Experimental Methods
PCU samples with 10\%, 15\% and 25\% of CA were produced by solvent exchange and compared with non-reinforced PCU, concerning the equilibrium water content and mechanical behaviour (compressive and tensile tests). The best performing material was further characterized in terms of wettability (captive bubble method), morphology (scanning electron microscopy) and friction coefficient (pin-on-plate tests against stainless steel 316L in phosphate buffered solution with hyaluronic acid (3 mg/mL)). Finally, the hydrogel was loaded by soaking with diclofenac and its release ability was compared to that of non-reinforced PCU, and the hydrogel's cytotoxicity was studied.

Results and Discussion
The water content of all the materials was similar, falling in the range of 62-67\% which mimics natural cartilage (water content < 80\% [4]). Regarding mechanical properties, the best results were obtained for PCU+15\%CA, with a compressive and tensile elastic modulus increase of 240\% and 12.5\% relatively to PCU. Besides, the ultimate tensile strength became approximately 20\% higher compared to PCU. PCU+15\%CA revealed to be quite hydrophilic, presenting a porous structure, with pores of different sizes heterogeneously distributed along the materials' structure. Although the friction coefficient (COF) slightly increased relatively to PCU, the values were of the same order of magnitude, being lower than 0.25. It was also observed that COF changed with the applied load: the higher the load, the higher the COF. Regarding the drug release behavior, PCU+15\%CA released a higher amount of the anti-inflammatory than PCU and led to a more controlled release kinetics, ensuring a sustained release for at least 48h (Figure 1). Finally, the cytotoxicity assays demonstrated that PCU+15\%CA is a biocompatible material, with a cellular viability close to 100\%.

Conclusion
Overall, the results showed that it is possible to produce PCU-based hydrogels for cartilage replacement with suitable mechanical properties, that should be able to control diclofenac release during at least the 48h.

Acknowledgement
To Fundação para a Ciência e a Tecnologia (FCT) for funding through the project PTDC/CTM-CTM/29593/2017 (CartHeal) and the unit projects UIDB/00100/2020 (CQE), UIDB/04585/2020 (CiiEM), UID/CTM/04540/2020 (CeFEMA) and UIDB/50022/2020 (IDMEC/LAETA), and for the PhD grant of A.S. Oliveira (PD/BD/128140/2016) and A.C. Branco (SFRH/BD/145423/2019).

References
In situ sequential co-delivery microparticle/hydrogel system for drug delivery in cancer therapeutics

Henrique Carrêlo, Maria T. Cidade, João P. Borges, Paula Soares

NOVA/FCT, Departamento de Ciências dos Materiais, Lisbon, PT

Introduction
Systemic delivery of cytotoxic chemotherapeutic agents is known to cause many negative side effects that diminish the patient’s quality of life. Local delivery of chemotherapeutic drugs has been the topic of many studies in recent years. This study focuses on the development of a thermoresponsive injectable microparticle/hydrogel for chemotherapeutic drugs co-delivery system for in situ injection. The embedding of microparticles within a hydrogel matrix will allow a more localized drug delivery with a sustainable and prolonged release [1].

Experimental Methods
Composite microparticles of gellan gum and sodium alginate were produced with a coaxial air flow technique. The precursor solution was extruded to an ionic crosslinking bath with calcium ions. Afterwards, microparticles were embedded in a Pluronic hydrogel to produce a thermoresponsive composite system. Mechanical properties, swelling, gelation time and rheological properties were determined by comparing the composite system with the hydrogel alone.

Results and Discussion
Air flow had a statistically significant impact on the size of the particles. A higher ratio of gellan gum diminished the particle size distribution most likely due to an increase in the viscosity of the precursor solution. Injectable hydrogels sensitive to temperature allow in vivo administration, through a minimally invasive procedure, and can serve as excellent support for the particles [2].

Conclusion
This system was designed to serve as a sequential drug co-release system with a potential synergetic effect [3]. In the near future, it is expected to load the hydrogel and microparticles with drugs suitable for cancer treatments, followed by its drug load and drug release characterization. Drugs loaded directly in the hydrogel will have an earlier release compared to the ones loaded within the microparticles. The different release patterns allow a sequential co-release of drugs that will increase the efficiency of the treatment. The proposed composite system is a promising composite for in situ drug delivery to tumours.

References


PS2-08-290

Investigation of Parameters Influencing Tubular-Shaped Chitosan-Hydroxyapatite Layer Electrodeposition

Mariusz Mąkiewicz¹, Katarzyna Nawrotek¹, Radosław A. Wach²

¹ Lodz University of Technology, Department of Environmental Engineering, Łódź, PL; ² Lodz University of Technology, Institute of Applied Radiation Chemistry, Łódź, PL

Introduction
Recent developments have introduced electrodeposition as a method for obtaining chitosan-based tubular deposits. In this process, the action of electric current on chitosan solution results in deposit formation on the electrode. Although the literature is lacking in explanation of the electrodeposition phenomenon, properties of chitosan-based deposits obtained in electrodeposition are widely described. Chitosan is a biomaterial widely studied in tissue engineering applications. Tubular shape chitosan-based deposits can be employed as implants in peripheral nerve injuries treatment. The aim of this work is to investigate parameters influencing the electrodeposition process from chitosan-hydroxyapatite colloidal solution and characterise the obtained tubular deposits.

Experimental Methods
Electrodeposition from chitosan, hydroxyapatite and chitosan-hydroxyapatite colloidal solutions containing lactic acid was conducted in a cylindrical stainless steel reactor with an inner rod serving as a cathode. In order to investigate the process, the following parameters: amperage, volume of evolved gas and gain in deposit thickness were recorded over time for different values of set voltage. Properties of applied colloidal solutions, e.g average size and zeta potential of particles were determined by dynamic laser scattering (DLS) and electrophoretic light scattering (ELS). The structure of deposits was assessed using scanning electron microscope (SEM) and Fourier transform infrared spectroscopy (FTIR).

Results and Discussion
Among the three investigated solutions, only electrodeposition in chitosan-hydroxyapatite solution results in tubular deposit formation on the cathode. Hydroxide ions, resulting from water electrochemical reduction, deprotonate –NH₃⁺ groups of chitosan and may react with calcium ions derived from hydroxyapatite which are coordinated by deprotonated chitosan chains. Changes of amperage recorded during the process reveal intricate character of investigated phenomenon. A possible explanation for these results is hydrogel deposition over time as well as gas evolution, which constantly changes resistance of the system. Rate of gain in deposit thickness and gas evolution is determined by set voltage. However, it does not influence deposit thickness for the process lasting over 20 minutes. The obtained results of properties of colloidal solutions indicate that the average size of particles and zeta potential values in chitosan-hydroxyapatite solution are higher than in chitosan and hydroxyapatite solutions. It suggests formation of new interactions between chitosan chains and hydroxyapatite. Morphology assessment of deposit surface via SEM indicates high surface irregularity for samples received at higher set voltage. Additionally, FTIR spectra present molecular differences between inner and outer side of the received tubular deposit. Obtained data indicates heterogeneous structure of the deposit. Outer and inner side of deposit is characterised by bands of chitosan chains and hydroxyapatite-derived species. However, bands assigned to chitosan chains are more pronounced for the outer side.

Conclusion
Electrodeposition from chitosan-hydroxyapatite solution is based on electrochemical reduction of water and formation of interactions between calcium ions coordinated and chitosan chains. As a result, the deposit is created on the cathode. Set voltage of the process has influenced the ratio of gas evolution and gain in deposit thickness.
Consequently, surface morphology of deposit is determined by set voltage. Reported results may serve as a valuable tool for manufacturing of biocompatible implants dedicated to treatment of peripheral nerve injuries.

**Acknowledgement**

This research was funded by the National Centre for Research and Development, Poland, grant LIDER/18/0116/L-10/18/NCBR/2019.
A silated hyaluronic acid hydrogels platform with bioinspired properties for skeletal tissue engineering

Killian Flegeau\textsuperscript{1,2}, Hélène Gautier\textsuperscript{1}, Gildas Rethore\textsuperscript{1}, Anthony Bresin\textsuperscript{2}, Pascal Bordat\textsuperscript{2}, Pierre Weiss\textsuperscript{1}

\textsuperscript{1} Université de Nantes, RMES U 1229, Nantes, FR; \textsuperscript{2} HTL Biotech, Javené, FR

Introduction

Tissue engineering is a promising approach to regenerate damaged skeletal tissues. In particular, the use of injectable hydrogels alleviates common issues of poor cell viability and engraftment. However, uncontrolled cell fate, resulting from unphysiological environments and degradation rates, still remain a hurdle and impedes tissue healing. We thus aim at developing a new platform of injectable hyaluronic acid (HA) hydrogels with a large panel of properties (stiffness, degradation...) matching those of skeletal tissues. For bone regeneration, Biphasic calcium phosphate (BCP) granules are osteoconductive biomaterials used in clinics to favor bone reconstruction. We incorporated BCP granules into in situ forming silanized hyaluronic acid (Si-HA) hydrogels.

Experimental Methods

Si-HA is prepared by amidation of the carboxylic acid functions of HA \cite{1}. To improve osteoconduction properties, calcium phosphate ceramics particles (40-80 µm) (Biphasic Calcium Phosphate; BCP\textsuperscript{®}, Biomatlante) has been added to the high pH (12.4) viscous Si-HA solution. Si-HA-BCP has been adjust to a final concentration of 3% w/v Si-HA in a 4:1 volume ratio with sterile HEPES buffer (pH 3) using Luer-lock syringes. Subsequently, different HA were functionalized with silylated moieties and formed hydrogels upon injection through a sol-gel chemistry occurring at physiological pH.

Results and Discussion

Upon solubilization and pH adjustment, the low-viscosity precursor solutions are easily injectable through fine-gauge needles prior to in situ gelation. Rheological characterization demonstrated that these hydrogels set within 5 to 20 minutes at 37°C. Tunable mechanical properties (stiffness from 1 to 40 kPa) and associated tunable degradability (from 4 days to more than 3 weeks in vivo) are obtained by varying the degree ofsilanization (from 4.3% to 57.7%) and molecular weight (120 and 267 kDa) of the hyaluronic acid component. Following cell encapsulation, high cell viability (>80%) is obtained for at least 7 days. Finally, the in vivo biocompatibility of silanized hyaluronic acid gels is verified in a subcutaneous mouse model and a relationship between the inflammatory response and the crosslink density is observed.

We have developed hybrid silated HA/biphasic calcium phosphate granules hydrogels and demonstrated a mechanical reinforcement (E = 0.1 MPa) while maintaining similar degradation rates. In vivo implantation in a rabbit femur model finally assessed their efficacy as bone substitutes (Figure 1) in comparaison to an silated HPMC composite.

Conclusion

Collectively, these results suggest that these hydrogels with a large panel of ECM-like properties offer promising outcomes for skeletal tissue engineering.

Acknowledgement

We acknowledge the Association Nationale de la Recherche et de la Technologie and the HTL company for funding this work (ANRT N°2015-1080). We acknowledge the ONIRIS Department of Experimental Surgery, MicroPiCell imaging facility, and SC3M Histology facility of the SFR Santé F Bonamy (UMS INSERM 016/CNRS 3556) (Nantes, France) for their technical support.
Tunable silated HA with in vivo results

References
**PS2-08-294**

**Fabrication of Bovine Hair Keratin/ Halloysite Bionanocomposite Hydrogels**

**Ezgi Ismar, Oguzhan Polat, Aysegul Uzuner Demir, Aykut Sancakli**

*Kazlicesme R&D Center and Test Laboratories, Istanbul, TR*

**Introduction**

During the conventional leather process, bovine hair is come out as a waste due to the de-hairing process. Bovine hair is destroyed with acidic treatment which makes it difficult to uptake from the wastewater. Thus, the enzymatic process for de-hairing the bovine leather is followed to obtain hairs from the discharged wastewater. Waste valorization in means of keratin is possible after obtaining the hair from the stream, the first removal of fat and other impurities should be followed with the addition of surfactant and obtained keratins are dried. Halloysite is a natural clay which’s chemical formula is $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$. It has a tubular shape with an outer part siloxane and aluminol inner part and it is a naturally occurred nanotube [1],[2],[3].

**Experimental Methods**

After de-hairing process of the leather, hair is collected from the wastewater and treated with SDS, and dried. To create the composite hydrogel structure cross-linking is added to keratin solution different halloysite ratio was studied to find the optimum ratio [4]. After the reaction process hydrogel is lyophilized and stored [5].

**Results and Discussion**

TEM is used to observe the halloysite distribution inside the hydrogel, SEM is used to characterize the sponge-like structure of the hydrogel, and FTIR and SDS-page are used to characterize the properties of the keratin. Thermal properties of the bionanocomposites are investigated via TGA and DSC. Swelling properties of the hydrogels were recorded for different pH environments.

**Conclusion**

Waste valorization of keratin is successfully applied and Keratin/ Halloysite bionanocomposite hydrogels are obtained. They can be a good candidate for biomedical applications.

**References**


Introducing external triggers to bioinspired hydrogels design for flexible cell encapsulation

Minye Jin¹², Alisa Gläser¹, Supun Mohotti¹, Julieta I. Paez³

¹ INM-Leibniz Institute for New Materials, Dynamic Biomaterials, Saarbrücken, DE; ² Saarland University, Chemistry Department, Saarbrücken, DE; ³ University of Twente, Developmental BioEngineering, Enschede, NL

Introduction

Hydrogels are used as extracellular matrix mimics in biomaterials field, including 3D cell culture, drug delivery and tissue engineering. Recently, our group developed a bioinspired covalent crosslinking strategy for hydrogel formation in the presence of living cells, via a condensation reaction between 2-cyanobenzothiazole (CB) and aminothiol (AT) groups, rendering stable adducts. The resulting CB-AT hydrogels showed rapid and tunable gelation kinetics, were mechanically stable, homogeneous at the microscale and cytocompatible.¹ To achieve fine tuning of gelation onset and kinetics, implementing other external cues to trigger the CB-AT gelation on demand is desirable. The aim of the present work was to explore variations of the CB-AT design and equip this system with redox and enzymatic triggers that enable fine tuning of the gelation onset and kinetics. Our strategy involved the use of “masked” AT precursors that can be unmasked under mild conditions by action of a reducing agent or a protease, thus triggering the CB-AT condensation on demand (Fig. 1a). Inspired by previously reported masked AT groups,²³ here we present the synthesis of novel protected AT polymer precursors and the investigation of condition reactions to trigger the CB-AT gelation with convenient rate (seconds to minutes) and adjustable mechanical strength under redox and enzymatic regulation, while keeping good cytocompatibility.

Experimental Methods

4-arm, 10 and 20 kDa poly(ethylenglycol) (PEG) macromers carrying either CB groups, or thiol-masked (R= SEt and SBu), or amine-masked (R' = furin or caspase-3 labile peptides) AT groups were synthesized in high yields and purity. Solutions of CB and masked-AT precursors (2-10 wt% in 20 mM HEPES buffer) were prepared and used for gel formulation under physiological conditions. Triggering of gelation was investigated in the presence of diverse reducing agents (TCEP, glutathione, DTT) or enzymes (furin, caspase-3), under variable polymer concentration (3.3-5 wt%), diverse trigger concentration and working pH (7-8). Gelation kinetics and mechanical properties of the resulting hydrogels were characterized by shear oscillatory rheology, to determine the effect of molecular design and working media parameters on materials properties. Swelling ratio of the gels was determined following established protocols.⁴

Results and Discussion

With redox triggering, PEG hydrogels via the CB-AT condensation reaction were successfully prepared at working pH range of 7.0 to 8.0. Hydrogels at 5 wt% polymer concentration showed gelation kinetics from seconds to minutes depending on the thiol-masking groups of AT precursors (R, Figure. 1b). The gelation rate trend observed was R=SEt > R= StBu at both pH 7.0 and 8.0, as revealed by in-situ rheology. This reflects the relative rate of reductive cleavage of disulfide bonds. The gelation kinetics was also tuned by varying pH, regardless from the molecular structure of masking group (Figure 1b). The pH change from 8.0 to 7.0 enabled a slower gelation process from a few seconds to minutes, in agreement with obtained results before.¹

In addition, the influence of reductant type on gelation kinetics was investigated (Figure 1c). The resulting CB-AT(SEt) gels were formed at 3.3 wt% concentration in 20 mM HEPES buffer at pH 8.0 using variable reducing agents (i.e. TCEP, DTT and GSH), keeping a constant (1:1) reductant: SR ratio. The use of TCEP produced a faster gelation time in seconds range while the use of GSH and DTT resulted in slower gelation times of 5 min and 15 min, respectively.
respectively. However, the final mechanical strength of the gels did not differ significantly. This indicates that the variation of reducing agent in this system allows tuning of gelation time between seconds to minutes without altering final gel mechanics.

Enzymatically triggered experiments were performed with peptide-masked AT precursors in the presence of furin and caspase-3 proteases in corresponding buffers. Unexpectedly, no gel formation was observed after 1 day. This is probably due to the steric hindrance of the PEG-peptide conjugate, which impeded adequate enzymatic recognition at the active site.

**Conclusion**

Two strategies to externally trigger CB-AT crosslinking were explored under physiological conditions. Redox triggering of the gelation was achieved successfully, gel formation was efficient and gelation kinetics could be fine-tuned from seconds to minutes range by varying molecular structure of masking groups of AT precursors, pH, polymer concentration and reductant type. Attempts to achieve enzymatic triggering proved unsuccessful under tested conditions, probably because of steric hindrance of the PEG-peptide conjugate that impeded adequate enzymatic recognition at the active site. In the future, the redox-triggerable CB-AT hydrogels presented here will be further developed as injectable and printable formulations for applications in tissue engineering and regenerative medicine.

**Acknowledgement**

This project was funded by the Deutsche Forschungsgemeinschaft (DFG), Project number 422041745.

References

Boosting Nanostructured Lipid Carriers performance against *Helicobacter pylori* by developing a pH-responsive system

**Sandra Gomes**$^{1,2,3}$, Rute Chitas$^{2,3,4}$, Cláudia Nunes$^{5}$, Joaquim M. Oliveira$^{6,7}$, Rui L. Reis$^{6,7}$, M. Cristina L. Martins$^{2,3,4}$, Paula Parreira$^{2,3}$

$^1$ FEUP, Faculty of Engineering, University of Porto, Porto, PT; $^2$ i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, PT; $^3$ INEB, Instituto Nacional de Engenharia Biomédica, Universidade do Porto, Porto, PT; $^4$ ICBAS, Abel Salazar Biomedical Sciences Institute, University of Porto, Porto, PT; $^5$ LAQV, REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, PT; $^6$ 3B’s Research Group, I3Bs - Research Institute on Biomaterials, Biodegradables and Biomimetics of University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Avepark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, PT; $^7$ ICVS/3B’s – PT Government Associate Laboratory, Braga/Guimarães, PT

**Introduction**

*Helicobacter pylori* is a Gram-negative bacterium that colonizes the stomach of nearly 4 billion people, i.e., half of the worldwide population [1]. Chronic infection with this bacterium can lead to chronic gastritis, peptic ulcers and, in some cases, gastric carcinoma. In fact, *H. pylori* infection accounts for 89% of all diagnosed gastric cancers [1], the 5th most common and 4th deadliest [2]. Although there is an antibiotic-based treatment, it fails in 20%-40% of the cases, prompting the need for newer therapeutic strategies [3]. Previously, our team showed that nanostructured lipid carriers (NLC) are efficient in eradicating *H. pylori* [4]. Still, to achieve complete bacterial clearance, NLC bioavailability must be improved, namely by increasing their gastric retention time. For that, development of a pH-responsive system to act as carrier appears as a good strategy to boost NLC performance.

**Experimental Methods**

Herein, low acyl gellan gum (LAGG) hydrogels with different polymer concentrations (1%, 1.5% & 2% w/v) were screened as possible NLC carriers. Hydrogels production was adapted from Silva-Correia et al. [5]. Briefly, after LAGG powder dissolution in deionized water, at 90°C, PBS (10% v/v) was added. Then, the solution was transferred to a Petri dish where the gelation process occurred. Finally, small discs were cut with a 9 mm diameter puncher. The mechanical behaviour of LAGG hydrogels and LAGG hydrogels with NLC (10% v/v) (NLC-LAGG) were analysed by dynamic mechanical analysis, using a compression test for 30 minutes. The hydrogels degradability was evaluated in different pH (7.4, 6.0, 4.0, 2.6 and 1.2) over 24h, at 37°C and 150 rpm, by tracking changes in weight and diameter of both LAGG and NLC-LAGG hydrogels. *In vitro* release of NLC was studied using LAGG hydrogels with NLC marked with coumarin-6 (cNLC-LAGG), which were incubated under the same settings used for the degradability test. At different time points, supernatants were collected and fluorescence readings were performed using a wavelength of 420 nm for excitation and 490 nm for emission. Confocal microscopy was used to evaluate NLC distribution on the cNLC-LAGG hydrogels. Finally, 1.5% and 2% NLC-LAGG hydrogels were immersed in pH 2.6 and 7.4 and, at different timepoints (24, 48 and 72h), extracts were collected. Then, extracts’ antimicrobial activity was evaluated against *H. pylori* J99 (highly pathogenic strain) by incubating with the bacteria for 24h. Afterwards, plating was performed and the viable bacteria was determined by colony forming unit (CFU) counting. The quantification of the number of released NLC from these hydrogels was performed by nanoparticle tracking analysis.

**Results and Discussion**

LAGG 1% hydrogels did not have mechanical properties to allow its handling and undergo further testing. Results from compression tests showed that both LAGG 1.5% & LAGG 2% hydrogels had similar elastic behaviour. A slight decrease was observed in both storage modulus and loss modulus when the polymer concentration decreased. The Page 1760 of 2028
ratio between loss and storage moduli remained on the same range. The incorporation of NLC into the structure of the hydrogels (10% v/v) did not alter its mechanical properties. Degradability tests showed that both LAGG 1.5% & LAGG 2% hydrogels, either with or without NLC, had similar mass losses, except for pH 1.2, where less concentrated hydrogels had higher mass loss. Regarding the different pH tested, higher mass loss was achieved in pH 1.2 and pH 2.6, indicating different behaviour according to the pH, as expected. However, no significant variations were observed in the hydrogel’s diameter. NLC release study showed that, after 24h, around 4% of the nanoparticles were released from the cNLC-LAGG 2% hydrogels whereas in the cNLC-LAGG 1.5% only around 2% of NLC were released on all the pH’s tested. Confocal images showed that NLC were widely distributed throughout the structure of both cNLC-LAGG 1.5% & cNLC-LAGG 2% hydrogels. Antimicrobial studies showed that the amount of NLC released from the hydrogels after 72h of incubation was not enough to kill the bacteria since less than 2% of the nanoparticles were released from both NLC-LAGG 1.5% & NLC-LAGG 2% hydrogels. Therefore, further optimization of the NLC releasing profile needs to be done by tuning the acylation degree of the gellan gum.

**Conclusion**

These results highlight LAGG hydrogels as a good strategy to further pursue in order to deliver NLC on gastric settings and boost their efficiency for *H. pylori* eradication.

**Acknowledgement**

The authors acknowledge FCT for CEECIND/01210/2018, NORTE-01-0247-FEDER-033399.

**References**


Design and development of bio-hybrid hydrogels containing the system of pH/thermosensitive nanocarrier - salicylic acid

Katarzyna Bialik-Wąs¹, Dagmara Malina², Klaudia Pluta²

¹ Cracow University of Technology, Faculty of Chemical Engineering and Technology, Department of Organic Chemistry and Technology, Kraków, PL; ² Cracow University of Technology, Faculty of Chemical Engineering and Technology, Department of Chemical Technology and Environmental Analytics, Kraków, PL

Introduction
Nowadays, hybrid biomaterials become more and more popular, because they can combine the features of inorganic, organic as well as polymeric components. Then the final product can be characterized by multitasking properties, which are interesting from the point of view of medical and biomedical applications [1-3]. Moreover, hydrogel materials can be used as modern wound dressings, especially in the treatment of long-healing skin disorders, such as bedsores, venous and diabetic ulcers [4,5].

Experimental Methods
Here, studies were focused on the design and development of bio-hybrid hydrogels based on sodium alginate (SA), poly(vinyl alcohol) (PVA), glycerin, and Aloe vera solution (AV). Additionally, salicylic acid - pH/thermosensitive nanocarrier were incorporated into SA/PVA/AV hydrogel matrix. This combination of active ingredients of natural and synthetic origin can improve the therapeutic efficacy of the product. The main goal of our research is the preparation of innovative dressings for the treatment of Psoriasis. Bio-hybrid hydrogels were obtained through the chemical crosslinking method using poly(ethylene glycol) diacrylate (PEGDA, Mₙ = 700 g/mol) as a crosslinking agent. First, the gel fractions of bio-hybrid hydrogels containing the system of pH/thermosensitive nanocarrier - salicylic acid were determined and their swelling behavior in distilled water and simulated body fluids was tested. Additionally, the degradation tests were carried out. Subsequently, the following properties of the modified materials were studied: structural (FT-IR spectra analysis), morphological (SEM analysis), and mechanical (tensile strength, elongation at break, and hardness). Finally, the cytotoxicity tests using in vitro method were conducted.

To identify the chemical structure of the bio-hybrid hydrogels as well as perform an analysis of the materials after degradation, infrared spectroscopy was done with a Thermo Scientific Nicolet iS5 FT-IR spectrometer equipped with an iD7 ATR accessory in the range of 4000 cm⁻¹ - 400 cm⁻¹. The hydrogel morphology was observed by a means of an SEM (Scanning Electron Microscope) using a Tescan Mira 3 instrument equipped with a FEG Schottky electron emission source at an acceleration voltage of 3.0 kV. The samples were sputter-coated with a thin film of gold for 90 sec. The maximum tensile strength and the elongation degree to break tests were conducted on the hydrogels using an MTS Bionix machine with a tensile loading rate of 0.2 mm/s. All specimens were cut into a specific dumbbell shape (75 mm long, 4 mm at the middle, and 25 mm of measuring segment).

Results and Discussion
The content of the gel fraction of bio-hybrid hydrogels reaches the value of about 70% and it turned out that the type of nanocarriers does not influence this parameter. Moreover, the basic SA/PVA/AV hydrogel matrix has a slightly lower result, which means that the addition of drug-carrier improved the degree of cross-linking. For all samples, the swelling abilities in PBS are at the same level about 200-250%. In the case of water, these values are more varied and they are about 200-340%. SEM images exhibited that the surface of hydrogels is crosslinked densely whereas the cross-section presented that the internal structure is more irregular and porous. The chemical structure of bio-hybrid hydrogels before and after degradation was confirmed based on the FT-IR analysis. In the range of 3500-
3000 cm$^{-1}$, it was observed some changes in the case of samples after degradation tests, which suggests that salicylic acid was released. Additionally, positive results from mechanical research were obtained.

**Conclusion**

On the basis of results, we can conclude that the presence of the system of pH/thermosensitive nanocarrier - salicylic acid does not significantly influence on different properties of the SA/PVA/AV hydrogel matrix. This is very important information because it confirms that it was developed a proper preparation method of bio-hybrid hydrogels is a new class of wound dressings. Moreover, the introduction of salicylic acid in the form of a pH/thermosensitive nanocarrier into the hydrogel matrix allows obtaining a double system of the active substance release. Due to the time release of drug is prolonged significantly.

**Acknowledgement**

This research was financially supported by The National Centre for Research and Development — project LIDER/41/0146/L-9/17/NCBR/2018.

![SEM images of bio-hybrid hydrogels.](image)

**References**


Bioengineered injectable hydrogels based on ELRs with PLA encapsulated for cardiac regeneration

Mercedes Santos¹,², Julio Fernández¹,², Jesús Carbajo¹, J. Carlos Rodríguez-Cabello¹,², Soledad Pérez-Amodio³,⁴,⁵, Elisabeth Engel³,⁴,⁵, Matilde Alonso¹,²

¹ University of Valladolid, BIOFORGE (Group for Advanced Materials and Nanobiotechnology), Valladolid, ES; ² CIBER en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Valladolid, ES; ³ The Barcelona Institute of Science and Technology, Biomaterials for Regenerative Therapies group, Institute for Bioengineering of Catalonia (IBEC), Barcelona, ES; ⁴ CIBER en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Barcelona, ES; ⁵ Technical University of Catalonia (UPC), IMEM-BRT group. Department of Materials Science and Metallurgical Engineering, EEBE Campus, Barcelona, ES

Introduction

Adult cardiovascular tissue exhibits an inability to repair itself after injury, due to the limited regenerative capacity of mature cardiomyocytes (CMs)¹. Previous in vitro tests have shown a higher rate of cardiomyocytes proliferation in the presence of lactate. Elastin-like recombinamers, ELRs, are a class of recombinant polymeric material whose composition is bioinspired in natural elastin and characterized by their biocompatibility and stimuli responsive. Our objective is to prepare an injectable “click” chemical hydrogel based on ELRs² with polylactic acid (PLA) encapsulated, able to modulate the release of lactate to the medium. Lactate from both partially hydrolyzed PLA, after oxygen plasma treatment, and once PLA hydrolyzed at physiological conditions, will be released in a sustained manner as an energy source for cardiomyocytes. ELRs can include in their structure bioactive sequences such as cell adhesion and protease-sensitive domains³ so that biocompatible, bioactive and biodegradable ELRs hydrogels could prove to be very useful scaffolds for the regeneration of infarcted tissue and the consequent recovery of cardiac function.

Experimental Methods

Two different ELRs have been bioproduced and subsequently functionalized to bear azide and ciclooctine groups, respectively, by NHS-ester methodology. L-PLA (MW 10000, PDI < 1.1) has been subjected to oxygen plasma treatment and some ester bonds have been hydrolyzed, giving rise to lactic acid and low molecular weight (PLA)s as determined by ¹H-NMR and FTIR-ATR⁴. The covalent crosslinking of both modified-ELRs, in the presence of treated PLA, allowed the formation of the hydrogel with embedded PLA. An in vitro kinetic release study was performed to explore the delivery of lactic acid and the release profile was obtained by measurement of lactic acid by “Enzymatic UV method”.

Results and Discussion

We have obtained “click” ELRs hydrogels with embedded PLA as a sustained lactic acid drug delivery device. The hydrogel is formed by interchain crosslinking based on click chemistry methodology from two tailored designed ELRs, one of which bearing RGD adhesion domain and the other one bearing arginine enriched domain, to retain lactic acetate by ionic interactions between negatively charged carboxylic group and the positively charged arginine. PLA oxygen plasma treatment provides partially hydrolyzed PLA as well as lactic acid and lactic acid oligomers, as could be observed by FTIR-ATR and ¹H-NMR in which the corresponding new signals of smaller molecules appeared. Release tests carried out with PLA-embedded gels will show a continuous and sustained release of lactic acid. Previous studies have shown that gels embedded with lactate released all of it in less than 10 hours. Therefore, different release kinetics will be produced and, in a first stage, the lactic acid generated by PLA plasma treatment will be released and, in a sustained manner, lactate will be released as the PLA is hydrolyzed at physiological conditions.
These injectable biomaterials based on ELRs will help cardiac regeneration acting as lactate molecules releasing devices which favors the survival and integration of the cells.

**Conclusion**

In the face of classic treatments for heart disease, such as myocardial infarctions, by means of drugs or transplantation, tissue engineering is committed to repairing the affected tissues. In this regard, lactate-bearing cardiac scaffolds, such as injectable chemical hydrogels of ELRs, will be used to stimulate the regeneration of cardiac tissue.

**Acknowledgement**

The authors are grateful for the funding from the Spanish Government (PID2019-110709RB-100, RTI2018-096320-B-C22, RED2018-102417-T), Junta de Castilla y León (VA317P18, Infrared2018-UVA06), Interreg V España Portugal POCTEP (0624_2IQBIONEURO_6_E) and Centro en Red de Medicina Regenerativa y Terapia Celular de Castilla y León. Financial support was received from MICIU (MAT2015-62725-ERC and RTI2018-096320-B-C21) and EUIN73. Red TERCEL.

**Figure 1**

Schematic overview of lactate delivery from ELR-hydrogel. Different release kinetics will occur due to the presence of lactic acid from the hydrolysis of PLA treated with oxygen plasma, and that produced by PLA hydrolysis under physiological conditions.

**References**


2:00 p.m. – 3:30 p.m.

Poster floor

**PS2-09 | Biomaterials for Cardiovascular Applications**
Development of drug-loaded, 3D printed PLLA/PLCL bioresorbable stents: characterization and influence of sterilization

Victor Chausse\textsuperscript{1,2}, Clàudia Iglesias\textsuperscript{1}, Cristina Canal\textsuperscript{1,2}, Marta Pegueroles\textsuperscript{1,2}

\textsuperscript{1} Technical University of Catalonia (UPC), Biomaterials, Biomechanics and Tissue Engineering Group, Department of Materials Science and Metallurgical Engineering, Barcelona, ES; \textsuperscript{2} Technical University of Catalonia (UPC), Barcelona Research Center in Multiscale Science and Engineering, Barcelona, ES

Introduction
Bioresorbable stents (BRS) are designed to overcome the limitations of drug-eluting stents (DES) by providing temporary support to the vessel wall while simultaneously allowing for the release of an anti-proliferative drug to avoid neointimal hyperplasia. Since the stent resorbs, the cause of potential inflammation that can lead to late stent thrombosis (LST) may be avoided [1,2]. Besides, drug release from drug-loaded stents may also be driven by polymer degradation obtaining a sustainable delivery for longer periods of time. Nevertheless, BRS present a few limitations, such as the need for large strut thickness in order to achieve enough radial support to prevent vessel recoil, which in turn increases resorption time and the probability of late undesirable events [3].

The main aim of this work is the fabrication of 3D-printed polymeric BRS by solvent-cast direct-write (SC-DW) technique to characterize their degradation behavior and to study the effects of sterilization on stents’ physicochemical properties. To confer higher added value, modification of the inks with everolimus to prevent restenosis is further intended in order to manufacture drug-eluting bioresorbable stents and monitor its release.

Experimental Methods
Poly-L-lactic acid (PLLA) and poly(lactic-co-caprolactone) (PLCL) 3D-printed stents were obtained by using a modified commercial 3D printer (BCN 3D+, BCN 3D technologies) with a rotating mandrel, in order to print cylindrical structures. The ink consisted in a solution of high molecular weight PLLA (PL 65, Purac) or PLCL copolymer (PLC 9538, Purac) in chloroform at 10\% w/v and 12.5\% w/v, respectively. Stents were printed by SC-DW on mandrels with 3 mm in diameter using a 250 μm tip (Nordson). After fabrication, stents underwent a thermal treatment during 12 h at 80\°C.

An accelerated degradation assay was performed on printed stents by immersing them in 10 mL of media (0.1M NaOH solution) in sealed glass vials and maintained at 37\°C for 10 days [4]. At different time points, stents were taken from the media, dried and weighed to calculate mass loss. Stents were further characterized by SEM, DSC, GPC and compression tests.

Two sterilization methods were evaluated: ethylene oxide or γ-irradiation (8 kGy). Sterilization effects on stents' crystallinity, molecular weight and mechanical properties were analyzed with DSC, GPC and compression tests. Inks were further modified with the addition of antiproliferative drug everolimus (Sigma Aldrich) at 2 wt.\% and 4 wt.\% with respect to polymer content. Everolimus-loaded stents were printed by SC-DW (Figure 1) and characterized with SEM and compression tests. Everolimus release was evaluated by incubation of stents in 5 mL of release medium (0.7\% Triton X-405 in 0.01M potassium phosphate buffer pH 6 at 37\°C in 7\% ACN), which was replaced by fresh medium at selected time points [5]. The release of everolimus was quantified with High Performance Liquid Chromatography (HPLC, Prominence XR, Shimadzu). Statistical analysis was performed by non-parametric Mann-Whitney U-test using Minitab software.

Results and Discussion
PLLA and PLCL stents with 3 mm in diameter were obtained by means of SC-DW, with strut thickness in the range 130 to 150 µm. Chloroform residues were eliminated after thermal treatment and the crystallinity of the stents increased with respect to non-treated stents (i.e. PLLA: from 21% up to 30%).

Stents immersed in 0.1M NaOH solution showed significant degradation after 10 days, with mass loss over 60%. DSC results showed alterations in crystallinity over time and GPC confirmed a sustained decrease in molecular weight.

Stents were successfully sterilized by means of ethylene oxide or γ-irradiation (8 kGy) and changes were detected in terms of crystallinity, molecular weight and mechanical properties.

The addition of everolimus in the polymeric ink resulted in the manufacture of everolimus-loaded stents showing drug entrapment within stents’ struts. Everolimus release assays showed initial fast release due to surface-available drug followed by a highly desirable sustained release over 1 month (Figure 2). Release was higher for PLCL stents than for PLLA stents, although drug release was subjected to the polymer degradation time frame.

Conclusion

3D-printed polymeric PLLA and PLCL stents were successfully fabricated by SC-DW. Printed stents were fully characterized in terms of thermal and mechanical properties. The accelerated degradation assay in NaOH solution showed the degradation behavior for PLLA and PLCL, with changes regarding crystallinity degree and a marked reduction in molecular weight. Sterilization modified crystallinity, molecular weight and mechanical properties of printed stents. Ink modification with the addition of antiproliferative drug rendered everolimus-eluting stents. Everolimus release was found to be faster for PLCL stents than for PLLA stents.

Acknowledgement

Financial support was received from Spanish Government, MINECO/FEDER (RTI2018-098075-B-C21) and the Government of Catalonia (AGAUR 2017 SGR 1165 and FI scholarship for V. C.).
Figure 2
Everolimus release from PLLA and PLCL stents with 2 wt.% and 4 wt.% everolimus loading over 4 weeks.

References
Encapsulation of macrophages and umbilical cord-derived cells in a liquefied and compartmentalized environment aiming bone regeneration

Sara Nadine, Clara R. Correia, João F. Mano

CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, PT

Introduction

The immune system plays an indispensable regulatory role in the progress of bone regeneration, with special focuses on macrophage activity.[1] Inspired on the native dynamics between immune and skeletal systems, we propose an in vitro bioengineering system of a biomimetic bone niche that comprises liquefied and multilayered microcapsules co-encapsulating macrophages, mesenchymal stromal cells (MSCs), and endothelial cells (ECs). This encapsulation system, already tested in vivo [2], is composed by (i) a multilayered membrane obtained through the layer-by-layer assembly of three polyelectrolytes (ii) a liquefied alginate core, (iii) surface functionalized poly(ε-caprolactone) microparticles (µPCL), and (iv) cells. While the multilayered membrane wraps all the cargo contents and ensures permeability to essential molecules for cell survival, the liquefied core maximizes their diffusion through the entire 3D construct. Additionally, µPCL are loaded into the liquefied core to provide cell adhesion sites. Taking advantage of the liquefied core, microcapsules are dynamically cultured using spinner flasks, which enhance MSCs osteogenesis while better mimicking the environment of native tissues.[3] Microcapsules encapsulating only MSCs and ECs (CO microcapsules) or a tri-culture with macrophages (TRI microcapsules) were cultured up to 21 days in culture medium with (OSTEO medium) or without osteogenic differentiation factors (BASAL medium). The main goal is to promote a well-orchestrated cell-to-cell interaction enabling the evaluation of the bioperformance of macrophages toward bone tissue repair.

Experimental Methods

Alginate microgels are generated by electrohydrodynamic atomization (EHDA) technique (Figure 1-I). For that, under influence of electrical forces (10 kV), a liquid jet of alginate containing a dispersion of cells (5x10^6 cells/mL) and µPCL (30 mg/mL) breaks up into droplets. MSCs, ECs, and macrophages are isolated from the umbilical cord tissue and blood, respectively. After crosslinking in calcium chloride, microgels encapsulating cells and µPCL are obtained. Then, layer-by-layer is performed using poly(L-lysine), alginate, and chitosan as polyelectrolytes to produce the multilayered membrane surrounding the obtained microgels (Figure 1-II). The process is repeated until a 10-layered membrane is created. Ultimately, the core is liquefied by chelation with EDTA (Figure 1-III). Afterwards, liquefied microcapsules are cultured up to 21 days in basal or osteogenic differentiation media. Microcapsules without macrophages are used as control. The proposed capsules were tested under dynamic culture conditions, using a rotary cell culture system.

Results and Discussion

Microtissues were successfully obtained inside the compartmentalized and controlled environment of capsules with an appropriate diffusion of essential molecules for the long survival of the encapsulated cells (Figure 2-A). The metabolic activity was increased in all microcapsules during time. Notably, the osteogenic osteocalcin marker was only found in microcapsules encapsulating macrophages (Figure 2-B). Nodules-like structures were observed by scanning electron microscope (SEM) in CO and TRI microcapsules cultured in OSTEO medium. The correspondent energy dispersive spectroscopy (EDS) mapping evidenced a phosphorus and calcium enriched extracellular matrix (Figure 2-D). Additionally, microcapsules encapsulating macrophages released significantly more osteoprotegerin...
(OPG), osteopontin (OPN), and vascular endothelial growth factor (VEGF), comparing with the CO microcapsules condition (Figure 2-C and 2-E).

**Conclusion**

Overall, the study demonstrated that the presence of macrophages promoted a well-orchestrated cell-to-cell interaction inside the microcapsules, allowing to recreate the bone repair process within a controlled and self-regulated “microengineered niche”. Accordingly, we intend to use the proposed system as hybrid devices implantable by minimally invasive procedures for bone tissue engineering applications.

**Acknowledgement**

The authors acknowledge the financial support given by the Portuguese Foundation for Science and Technology (FCT) with the doctoral grant of Sara Nadine (SFRH/BD/130194/2017), the project “CIRCUS” (PTDC/BTM-MAT/31064/2017), and the European Research Council for the project “ATLAS” (ERC-2014-AdG-669858). This work was developed within the scope of the project CICECO-Aveiro Institute of Materials (UIDB/50011/2020 & UIDP/50011/2020).

Figure 1

Schematic representation of the production method of liquified microcapsules. (I) Under influence of electrical forces, a liquid jet of alginate containing a dispersion of cells and surface modified polycaprolactone microparticles (μPCL) breaks up into droplets. (II) Layer-by-layer is subsequently performed using poly(L-lysine), alginate, and chitosan as polyelectrolytes. (III) The core is liquefied after immersion in ethylenediaminetetraacetic acid solution (EDTA). Ultimately, to better mimic the dynamic environment of native tissues, microcapsules are cultured in spinner flasks.
Figure 2
A. Live-dead fluorescence assay for 21 days of culture. B. Immunofluorescence of osteocalcin (pink) in CO and TRI microcapsules cultured in OSTEO medium after 21 days of culture. C. VEGF protein release measured by ELISA of CO and TRI microcapsules at 21 days of culture. D. SEM images of the encapsulated μPCL and cells (TRI microcapsules) cultured in OSTEO medium. Corresponding elemental analysis by chemical mapping of phosphorous (P, red) and calcium (Ca, green). E. Heat map of the bone cytokine profile of CO and TRI microcapsules at 21 days of culture.

References
PS2-09-308

High-throughput Screening to Mimic Tunica Media Smooth Muscle Cells Alignment in Porous Blood Vessel Scaffold

Klaudia M. Jurczak1, Ruichen Zhang1, Richte Schuurmann2, Henri G. Leuvenink3, Jan L. Hillebrands4, Ruud Bank4, Jean-Paul P. de Vries5, Patrick van Rijn1

1 University of Groningen, Department of Biomedical Engineering, Groningen, NL; 2 University of Groningen, Faculty of Medical Sciences, Groningen, NL; 3 University of Groningen, Department of Cardiology / Thoracic Surgery, Groningen, NL; 4 University of Groningen, Department of Pathology and Medical Biology, Groningen, NL; 5 University of Groningen, Department of Surgery, Groningen, NL

Introduction
The development of functional blood vessel engineering has become an urgent need for the treatment of cardiovascular disease and blood vessel disorders, such as aortic aneurysm. The ideal vascular scaffold should resemble the 3D geometry of native blood vessel and reconstitute its three layers including tunica intima, tunica media and tunica adventitia (1). The secondary medial layer consists of circumferentially oriented smooth muscle cells (SMCs) which are particularly important for the contractile function of blood vessel. Reproducing the adequate cellular orientation and facilitating SMCs colonization to enable standardized studies in CVD research still remains a challenge. We are fabricating vascular scaffolds from poly(trimethylene carbonate) (PTMC), the material that is flexible, biodegradable and biocompatible (2). For directing smooth muscle orientation, we are investigating the biophysical factors such as anisotropic topography using in house high throughput screening platform (HTS). The technology enables to identify optimum material parameters in a single cell experiment at the same time evading adverse effects of cell-material interactions (3).

Experimental Methods
Porous structures should facilitate cell proliferation and nutrient flow, therefore the porosity in PTMC is prepared by the means of salt leaching. To promote continuously varying surface parameter, PTMC is imprinted from stretched and plasma oxidized molds as described previously (4). The response of SMCs from porcine origin is further investigated.

Results and Discussion
Porosity of PTMC is evaluated by micro computed tomography (micro-CT). As previously published the pore size in range of 50-100 um should facilitate vascular permeability and support flow of oxygen and nutrients (5). PTMC anisotropic topography gradients are characterized via atomic force microscopy (AFM). Measurements of created wrinkles are taken along the surface area covered by the prism mask during plasma oxidation process. The wrinkle size increases from the least exposed side (close side of the mask) to the most exposed side (open side of the mask).

Conclusion
Combination of porous PTMC material with highly efficient screening technology of topography gradients can be a crucial tool for organized cell alignment and controlled phenotype of SMCs in tunica media. Development of such tissue engineered vascular scaffolds can provide a real benefit in pre-clinical testing of aortic aneurysm and other cardiovascular diseases.
Porous Vascular Scaffold with a Wrinkle Topography

Representation image of a porous tubular PTMC scaffold with incorporated wrinkle topography for smooth muscle cell orientation. Image inspired by Biorender.

References

Biosensitive, photosensitive materials dedicated to the manufacture one-piece miniature impeller for a blood pump

Roman Major¹, Marcin Surmiak³, Roman Kustosz², Justyna Więcek¹, Romana Schwarz⁵, Maciej Gawlikowski¹,², Juergen M. Lackner⁶

¹ Institute of Metallurgy and Materials Science, Polish Academy of Sciences, Reymonta Str. 25, 30-059 Cracow, Poland, Krakow, PL; ² Artificial Heart laboratory, Foundation of Cardiac Surgery Development, Wolnosci Str. 345, 41-800 Zabrze, Poland, Zabrze, PL; ³ Department of Medicine, Jagiellonian University Medical College, Skawinska Str. 8, Cracow, Poland, Kraków, PL; ⁴ Department of Biosensors and Processing of Biomedical Signals, Faculty of Biomedical Engineering, Silesian University of Technology, Roosevelt Str. 40, 41-800 Zabrze, Poland, Zabrze, PL; ⁵ Montanuniversität Leoben, Department Kunststofftechnik, Lehrstuhl für Chemie der Kunststoffe, Otto Glöckl-Straße 2/IV, 8700 Leoben, Leoben, AT; ⁶ Joanneum Research Forschungsges.m.b.H., Institute of Surface Technologies and Photonics, Functional Surfaces, Leobner Str. 94, Niklasdorf 8712, Austria; juergen.lackner@joanneum.at, Niklasdorf, AT

Introduction
The aim of this study was to increase the effectiveness of heart failure treatment, in particular to overcome the material and technological limitations of cardiac assist devices. The main medical problem of state-of-the-art cardiac assist devices is device-induced thrombus formation, resulting from insufficient blood flow dynamics in the blood pump rotor, due to the high limitations of conventional pump manufacturing processes: milling or casting.

Experimental Methods
Based on research and simulations, the stereolithography (SLA) method was selected as an incremental manufacturing technology for blood pump rotors with increased affinity with flowing blood and reduced risk of thrombus formation. Currently, the SLA method, which does not meet the necessary requirements for the manufacture of safe devices for blood contact, requires R&D work to achieve the product goals of the project, for which an international consortium has been organised in the areas of: (i) Photo-polymerizing resins: a novel polymer with thiol groups, UV-polymerized, biocompatible and mechanically stiffened by carbon nanotubes in the SLA process; (ii) SLA technology: advanced light beam steering process in SLA technology for a highly smooth surface as a condition for minimising thrombogenicity; (iii) Surface: haemocompatible thin films resistant to corrosion in a biological environment, applied homogeneously over the entire surface of the device in contact with blood, using a low-temperature ALD (atomic layer deposition) process. All biomaterial tests were performed under the same experimental conditions, as described in detail elsewhere. Blood was collected from the same donor, i.e. a healthy man taking no medications.

The blood was brought from the Center for Blood Donation and Blood Treatment immediately before the test, which took ~60 min. A vacuum collection system was used (BD Vacutainer Systems), observing the rules for testing platelets: lack of a tourniquet, using a 12 G needle, and rejection of the first blood tube. In total, 3 tubes of 4.5 mL blood were used for the experiment, containing sodium citrate (0.5 mL, 0.105 M, BD Vacutainer systems). Blood morphology was investigated using an automatic cell counter (Sysmex K-1000 cell counter; Sysmex Co.), calibrated before each experiment. The discs were subjected to a sterilization process using gas ethylene sterilization. The measurement of each material was made using a control, unmodified polyurethane disc (3 discs). In addition to the dynamic test, two blood samples were stored under static conditions: non-activated and activated by the addition of adenosine-di-phosphate (ADP, Sigma- Aldrich, final concentration 20 μM). The analysis of each surface was repeated at least three times.

Results and Discussion
Page 1775 of 2028
A controlled cellular response was achieved by inventing new photopolymer and application 3D additive manufacturing method. As a result, it was possible to control cell attachment in the scale corresponding to biological processes. The newly elaborated material ensured optimal flow of oxygen and nutrients within a biomaterial what was of a key importance for processes of adhesion and proliferation of cells. As a result an antiadhesive surfaces to blood were elaborated. This solution has its premise in the construction of pumps.

**Conclusion**

The above technologies will enable the achievement of the project objective in the form of biomimetic, dynamic rotor designs developed on the basis of numerical simulation studies and experimental research for modification of the implantable centrifugal pump for long-term left ventricular assist.

**Acknowledgement**

The work was financially supported by The Polish National Centre of Research and Development (Grant no. M-ERA.NET/2014/01/2016, “Nonthrombogenic metal–polymer composites with adaptable micro and macro flexibility for the next generation heart valves in artificial heart devices”).
Electrospun gelatin-lysozyme nanofibrils based patches for myocardial regeneration


Introduction

According to the World Health Organization, cardiovascular diseases, remarkably myocardial infarction, are the leading cause of death in the modern world. Currently, the only form of treatment is heart transplantation. Therefore, it is urgent to develop alternative and efficient therapies for the treatment of myocardial infarction. For instance, tissue engineering that combines cells with biomaterials, has the therapeutic purpose of regenerating damaged myocardial tissue, inducing cell growth and differentiation, while the implanted biomaterial is slowly bioabsorbed.

The results already obtained in this domain demonstrate the potentialities of this strategy in myocardial regeneration, however the development of biomaterials with adequate properties is still one of the main limitations.

Protein nanofibrils are self-assembled, highly organized, bio-based nanostructures, forming peculiar nanofibrillar structures. As a result of their biological nature and unique properties, arising from their remarkable mechanical performance, thermal stability, insolubility in aqueous media, and the ability to be formed in vitro from any protein or peptide, the protein nanofibrils are already being exploited in various applications, including in the design of scaffolds for regenerative medicine. However, the use of protein nanofibrils for the fabrication of biomaterials for the regeneration of an infarcted myocardium is an almost untouched field. In this context, this work aimed to study the effect of the addition of lysozyme nanofibrils (LNFs) to gelatin electrospun patches for application in myocardial regeneration.

Experimental Methods

The produced electrospun nanocomposite patches, with different contents of LNFs (0, 5 and 10% w/w), were produced, crosslinked, and characterized in terms of physicochemical and biological properties. Namely, the morphology, mechanical performance, and the antioxidant activity of the electrospun patches were evaluated, as well as their bioabsorbability, cytotoxicity and cell adhesion in vitro. Furthermore, a poorly water-soluble drug, curcumin, was incorporated into the electrospun patches to evaluate its release from the different produced materials.

Results and Discussion

The addition of the LNFs into the gelatin electrospun patches promoted an improvement of the mechanical properties of the scaffolds (Figure 1A), their bioabsorbability (Figure 1B), their antioxidant activity (Figure 1C), and the drug release ratio. All other properties of interest for the described application were maintained, namely the electrospun fibers morphology and thickness (ranging from 1.43 to 1.79 µm), non-cytotoxicity, cell adhesion and proliferation of both fibroblasts and cardiomyoblasts.

Conclusion

Considering the described results, LNFs revealed to be interesting functional nanostructures for the use on the development of innovative biomaterials for the regeneration of an infarcted myocardium.

Acknowledgement

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the Portuguese Foundation for Science and Technology.
(FCT)/MCTES. FCT is also acknowledged for the doctoral grant to T.C. (SFRH/BD/130458/2017) and the research contract under Scientific Employment Stimulus to C.V. (CEECIND/00263/2018). HiLIFE Research Funds, the Sigrid Jusélius Foundation, and the Academy of Finland (Grant No. 317042) are also acknowledged for financial support.

![Figure 1](image)

(A) Young's modulus, (B) Bioabsorbability ratio, and (C) antioxidant activity of the gelatin electrospun patches with different contents of LNFs. Samples containing 0, 5 and 10% are represented as orange, yellow and green, respectively.

References

[1] World Health Organization 2019 ‘Cardiovascular Diseases’, Place of publication: https://www.who.int/health-topics/cardiovascular-diseases#tab=tab_1

[2] Streeter, Benjamin W., Davis, Michael E. 2019, ‘Therapeutic Cardiac Patches for Repairing the Myocardium’, Advances in Experimental Medicine and Biology, 1144, 1, Place of publication: Springer, Cham


Cytokine expression in the early phase of small diameter vascular graft healing

Sabrina Rohringer\textsuperscript{1,2}, Pia Hager\textsuperscript{1,2}, Clemens Hahn\textsuperscript{1}, Katharina Ehrmann\textsuperscript{3,4}, Christian Grasl\textsuperscript{2,5}, Roman Lieber\textsuperscript{1}, Barbara Kapeller\textsuperscript{1}, Stefan Baudis\textsuperscript{3,4}, Bruno K. Podesser\textsuperscript{1,2,4}, Helga Bergmeister\textsuperscript{1,2,4}

\textsuperscript{1} Medical University of Vienna, Center for Biomedical Research, Vienna, AT; \textsuperscript{2} Ludwig Boltzmann Institute for Cardiovascular Research, Vienna, AT; \textsuperscript{3} Technical University of Vienna, Institute of Applied Synthetic Chemistry, Division of Macromolecular Chemistry, Vienna, AT; \textsuperscript{4} Austrian Cluster for Tissue Regeneration, Vienna, AT; \textsuperscript{5} Medical University of Vienna, Center for Medical Physics and Biomedical Engineering, Vienna, AT

Introduction
Cardiovascular diseases are the main cause of death worldwide. The substitution of diseased small diameter vessels (< 6 mm) is challenging due to the lack of applicable autologous grafts or eligible synthetic graft materials. The target of creating a small diameter vascular graft (SDVG) which has adequate mechanical properties and satisfying patency rates remains unmet so far. In this study, the cytokine expression of different graft materials during healing was observed to identify mechanisms which might help to improve vascular graft materials in future.

Experimental Methods
Autologous aortic grafts, biodegradable electrospun thermoplastic polyurethane (TPU) grafts and expanded polytetrafluorethylene (ePTFE) conduits were implanted in an infrarenal aorta replacement model in male Sprague Dawley rats. Grafts were retrieved after one week. Proteins from the anastomoses regions were isolated and analyzed with a Proteome Profiler Rat XL cytokine array. Furthermore, cells from the perivascular adipose tissue (PVAT) were isolated and characterized via immunofluorescence and 3D culture assays.

Results and Discussion
The comparison of different graft materials revealed that ePTFE grafts induce a higher expression of all cytokines. Significant differences were seen in the expression of pro-inflammatory CCL3, ICAM-1 and VCAM-1, anti-inflammatory IL-1RA and IL-13 as well as cytokines regulating tissue hemostasis. Autologous implants and TPU express higher levels of regulatory and proliferation inducing proteins IGF-1 and Galectin-3 whereas significant elevation of pro-inflammatory cytokines was absent. WISP-1, a molecule involved in smooth muscle cell proliferation and vascular calcification was significantly higher expressed in TPU graft condition compared to the native control. The isolation of cells from PVAT of anastomosis regions shows heterogenous cell populations consisting of adipocytes, endothelial cells, fibroblasts and mesenchymal stem cells.

Conclusion
Our findings give first insights into the graft healing mechanisms of different conduit materials and the possible influence of PVAT on the healing process. The selection of additional time-points for analyses and more detailed investigation of signaling pathways will follow. The observed expression profiles may help to identify potential reasons for graft failure and to select new therapeutic targets for improved graft healing.
Development and characterisation of a novel anti-inflammatory coronary stent coating

Daniel Longhorn¹, Susan Currie², Christopher McCormick¹

¹ University of Strathclyde, Department of Biomedical Engineering, Glasgow, GB; ² University of Strathclyde, Strathclyde Institute of Pharmacy and Biomedical Science, Glasgow, GB

Introduction
Coronary stenting triggers a cascade of inflammatory responses, leading to neointimal formation. The drugs used in current drug eluting stents (DES) inhibit smooth muscle cell proliferation, a key component in this cascade. However, these drugs also inhibit endothelial cell proliferation, thereby delaying recovery of a function endothelium [1]. Research activity is therefore focussed on the use of alternative drugs that inhibit neointimal formation without exerting deleterious effects on endothelial cells. Sodium salicylate is a drug with well characterised anti-inflammatory and anti-thrombotic effects [2], a profile of activity that makes it a particularly promising candidate for the next generation of DES.

The drug release profile is critical to success of a DES. A range of polymers have been used to provide sustained release of drugs from existing DES platforms, although their use has also been implicated in thrombosis and significant opportunities to use alternative coating technologies optimise drug release kinetics remain [3]. Electropolymerisation is used to generate polypyrrole (PPy) coatings and this may be utilised to develop a reliable and tuneable method of fabricating DES coatings. This study investigated the use of electropolymerisation for the production of PPy-Salicylate coatings. Characterisation of their drug-eluting kinetics and in-vitro biological compatibility with endothelial cells has allowed a series of optimised coatings to be identified for further evaluation.

Experimental Methods
PPy coatings were generated on 316L Stainless Steel wires (ø=200 µm) from a 0.1M pyrrole and 0.1M sodium salicylate solution by potentiostatic (+1.1 and 1.3 V vs. Ag/AgCl reference electrode) and galvanostatic (0.1, 0.5 and 1 mA) electropolymerisation for 10 minutes. The electropolymerisation reaction was initially characterised by monitoring the current and voltage during generation. PPy coatings were imaged using scanning electron microscopy (SEM) (15 kV, 400 and 2000x magnification) to assess surface topography, and further characterised using electrical impedance spectroscopy (EIS) in Phosphate Buffered Saline (PBS) (5 mV, 1Hz-100kHz). Separately, PPy coated samples were incubated in PBS for up to 7 days at 37°C and salicylate elution was determined using UV-spectroscopy (298 nm).

HUVECs were cultured on 316L SS strips (30 x 5 mm) coated by potentiostatic electropolymerisation. After 24 and 48h, the number of viable cells on each sample surface was assessed using MTT assays and a standard curve of known cell densities. Fluorescence microscopy (acridine orange) was also used to confirm cell attachment.

Results and Discussion
Current and voltage vs. time profiles identified potentiostatic (at both +1.1 and 1.3 V) and galvanostatic (at 0.5 mA) electropolymerisation as suitable methods for reliable generation of PPy coatings, which SEM imaging confirmed to have characteristic cauliflower topography. The impedance of 316L SS wires was significantly reduced by the presence of the PPy coating (1.3V, potentiostatic) at low frequencies (<50 Hz).

The total salicylate eluted from the coatings varied depending on the electropolymerisation conditions used, with potentiostatic (1.1V: 149±30, vs. 1.3V: 265±26 mg) and galvanostatic (0.1mA: 46±4, vs. 0.5mA: 257±32, vs. 1mA: 696±16 mg) methods generating coatings with significantly different salicylate elution characteristics (p<0.05, n=3,
one-way ANOVA and post-hoc Tukey. Salicylate eluted rapidly from all PPy coating, with >80% elution achieved at 24 h with all coatings except 0.5 mA galvanostatic, which exhibited slower release kinetics (>80% at 48 h).

After 24 h, the number of viable endothelial cells on PPy samples was comparable to 316L SS controls (316L: 9889±294, vs. 1.1V: 9556±676, vs. 1.3V: 8778±1528; p>0.05, n=3). After 48 h, there was however a significant increase in the number of viable cells on 316L (1433±2296) compared with the coated samples (1.3V: 8556±509; p<0.05, n=3, two-way ANOVA with post-hoc Fisher’s LSD). Imaging of stained cells confirmed the attachment of cells on all surfaces.

**Conclusion**

The data demonstrates the potential utility of electropolymerisation as a method for reliably generating PPy coatings with uniform surface characteristics. PPy coated samples exhibited lower impedance with Warburg characteristics suggesting the electrochemical behaviour of PPy is strongly influenced by its increased surface area. The difference in the mass of incorporated salicylate between conditions provides the means of generating tuneable elution characteristics. It is shown that endothelial cells attach to PPy surfaces and remain viable for 24 h, although further work is needed to understand reduced viability observed at 48 h before progression to in vivo evaluation is warranted.

**References**

Hybrid inorganic-organic borosilicate materials produced by non-hydrolytic sol-gel for tissue engineering applications.

Soraia Coelho, José Carlos Almeida, Isabel Margarida M. Salvado, Maria Helena V. Fernandes

Universidade de Aveiro, CICECO, Aveiro, PT

Introduction

Hybrid organic-inorganic materials based in the polydimethylsiloxane – silica (PDMS-SiO$_2$) system are known to be of great interest due to their wide range of possible applications, namely as biomaterials, anti-corrosion, anti-fouling, or anti-reflective coatings [1]. Recently, boron containing PDMS-SiO$_2$ hybrids were developed for biomedical applications in tissue engineering [2]. The incorporation of therapeutic ions such as boron has been recognized as an interesting way to stimulate bone regeneration and angiogenesis [3,4]. Angiogenesis is a process that consists in the growth of new blood vessels that allow the transport and exchange of oxygen, nutrients, growth factors and cells. An alternative to the use of vascular growth factors is the adoption of these inorganic angiogenic ions, due to its low cost, high stability and potentially greater safety [4].

Experimental Methods

The present work proposes a non-hydrolytic sol-gel protocol for the preparation of PDMS-borosilicate films incorporating therapeutic ions.

Results and Discussion

The structures of the hybrid materials were analyzed by FTIR, $^1$H MAS (magical angle spinning), $^{29}$Si MAS, $^{29}$Si-$^1$H CP-MAS (cross-polarization magical angle spinning) and $^{11}$B HAHN-ECHO NMR and are here discussed aiming to make a comprehensive relationship between structural features and rate of ions release from the fabricated films.

Conclusion

Acknowledgement

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM/50011/ 2013), financed by national funds through the FCT/MEC and when appropriate cofinanced by FEDER under the PT2020 Partnership Agreement. The NMR spectrometers are part of the National NMR Network (PTNMR) and are partially supported by Infrastructure Project No.022161 (co-financed by FEDER through COMPETE 2020, POCI and PORL and FCT through PIDDAC).

References


PS2-10 | Biomaterials for Drug Delivery

With the kind support of Biomaterials Science
Understanding and characterizing the drug sorption to Polyvinyl chloride and Polyethylene materials

Meriem Sahnoune, Nicolas Tokhadze, Philip Chennell, Julien Devémy, Alain Dequidt, Florent Goujon, Valérie Sautou, Patrice Malfreyt

Université Clermont Auvergne, CNRS, Sigma Clermont, ICCF, Clermont-Ferrand, FR

Introduction

Plastic materials such as polyvinylchloride (PVC) and polyethylene (PE) are widely used in various medical devices (MD)\(^1\). Two phenomena known as leaching and sorption lead to adverse effects for the patient. The leaching is defined by the migration of the plasticizer into the drug solution whereas the sorption relates to the adsorption into the polymer matrix\(^2\). Sorption can affect the efficacy of a drug product via loss of the active pharmaceutical ingredient (API) which makes difficult the control of the delivered drug concentration. Drug-polymer materials interactions represents a major issue for the pharmaceutical industry in the delivery of drugs. The understanding of the sorption requires experiments with concentration measurements, but also a microscopic description with an energetic view. This dual microscopic/energy approach can be carried out using molecular simulation methods. We investigate the adsorption of two drugs (paracetamol and diazepam) on PE and PVC materials. The diazepam is known to interact with medical tubings whereas the paracetamol did not show significant adsorption. We model the adsorption process of the drug on materials by calculating the potential of mean force (PMF) along the direction normal to the surface.

Experimental Methods

Experimental sorption studies were conducted to evaluate paracetamol and diazepam concentration variation after a static contact with pure PE or pure PVC (without additives) tubes. Paracetamol and diazepam were respectively diluted to 10 µg/ml and 20 µg/ml with sterile water. Initial API concentration was quantified just after tubes filling (T0), at day 1 (T1), 2 (T2), 4 (T4), 7 (T7) and 14 (T14). Quantification was performed by liquid chromatography with UV detection.

The molecular dynamics simulations were performed with the LAMMPS package on a system composed of a polymeric surface (PE or PVC), a drug (paracetamol or diazepam) and 4000 water molecules. In semi-crystalline PE, both crystalline and amorphous states coexist. In order to simplify the simulation, the amorphous and crystalline states have been considered independently. Thermodynamic properties were calculated over a period of 40ns. The potential of mean force (PMF) was calculated by using an extended version (eABF) of the Adaptative Biasing Force (ABF) method. In this study, the PMF profile corresponds to the Gibbs free energy profile \(G(z)\) along the \(z\)-coordinate between the \(z\)-positions of the center of mass of the polymer surface and drug molecule.

Results and Discussion

The evolution over time of the concentration of both drugs in contact with PVC and PE tubings is shown in Figure 1. For both materials, the paracetamol solution remained stable for 14 days. Diazepam concentration was also stable for 14 days in contact with PVC tubing, but when in contact with PE tubing, a drug loss was observed from day 1 and fell to 56% of the initial concentration at 14 days.

Figures 2 shows the profiles of the Gibbs free energy as a function of the \(z\)-separation distance between the centers of mass of the surface and the drug molecules. In any case, the adsorption is thermodynamically favored: all the PMF curves show a negative minimum of \(\Delta G\) close to the surface. The values of the free energy minimum fall into a range of -35 to -15 kJ/mol. The Gibbs free energy of adsorption is minimum for the system diazepam/PE (around -33
kJ/mol). When comparing experimental results to simulations, it appears that no drug loss by sorption was observed when Gibbs free energy was comprised between 0 and -25 kJ/mol, which corresponds to a weak adsorption.

**Conclusion**

We have reported a combined approach of experiments and molecular simulations to characterize the sorption of paracetamol and diazepam to pure PVC and PE materials. The simulated Gibbs free energy of adsorption that are within the range of -35 to -15 kJ/mol establish a physical adsorption through van der Waals interactions. The most favorable adsorption is predicted with diazepam that adsorbs on PE. The experiments establish a drug loss of 56% as diazepam is in contact with PE tubings whereas no drug loss has been measured on PVC tubings and with paracetamol. This coupling between experiments and molecular simulation is able here to provide a threshold value of about -30 kJ/mol above which we did not detect any drug loss. This is an essential result that should enable molecular simulations to be used for the prediction of adsorption properties and further for the design of new polymeric surface with specific properties.

In this study, experiments and simulations have considered PVC materials without plasticizers. Yet, plasticized PVC medical tubings are widely used for the infusion of medications and the presence of plasticizer is also expected to increase adsorption mechanisms\(^3\). Future work will be to develop more realistic model plasticized PVC materials.

**Acknowledgement**

The authors acknowledge the financial support received from the Auvergne Rhônes Alpes regional council through the program “Pack Ambition Recherche” (MEDSIM-2019). The authors would like to thank colleagues of SimatLab for stimulating discussions about the results, and also CAIR LGL for their financial support for the discussion about the MEDSIM project and for providing the tubings used in this study.

---

**Figure 1**

Evolution of paracetamol and diazepam concentrations in static contact with PVC and PE tubings, compared to initial concentrations.
Gibbs free energy profiles $\Delta G(z_1)$ of the interaction of a) paracetamol and b) diazepam drugs with different surface geometries. $z_1$ is defined as the distance between the centers of mass of the drug and surface.

References


Peptide-modified nano-bioglass for growth factor immobilization and delivery

Matthias Schumacher, Pamela Habibovic, Sabine van Rijt
Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, NL

Introduction
Systemic administration of signalling molecules (growth factors, GF) that promote tissue regeneration bears a risk for off-site effects and, by applying supraphysiological concentrations, cancer development. Hence, targeted GF administration with the help of suitable carriers is considered a superior approach to stimulate different stages of tissue regeneration (precursor cell recruitment, angiogenesis, differentiation etc.) [1]. In the context of bone regeneration, a lack of appropriate carriers that allow delivery of GF with unimpaired biological activity and that co-act as potent bone graft substitutes limits clinical implementation of this therapeutic approach. (Porous) bioactive glasses (BGs) are promising carriers for GFs because of their high bone regeneration potential, degradability and bioactivity [2]. In most reported cases, GF immobilization on BGs is based on electrostatic interactions, which are poorly controllable in terms of adsorption and release kinetics. Moreover, such immobilization may deteriorate GF protein conformation and activity. To overcome these problems, we propose nano-sized BG particles decorated with peptides that bind a target GF with high specificity while maintaining its native state as a novel GF delivery system. In this pilot study, a peptide sequence derived from the transmembrane glycoprotein prominin-1 [3] which binds pro-angiogenic vascular endothelial growth factor (VEGF, [4]) with high selectivity and affinity was coupled to amine reactive groups grafted on nano-sized BG particles. The degree of functionalization, binding efficiency and selectivity for VEGF as well as biological activity of the bound GF were studied.

Experimental Methods
Nano-sized bioactive glass particles (nBG) were synthesized via the sol-gel route using tetraethyl-orthosilicate (TEOS), triethyl phosphate (TEP) and calcium nitrate (CN) as precursors. nBG composition was chosen to achieve high bioactivity based on earlier findings [5]. 3-Aminopropyltriethoxysilane (APTES) was used to surface-graft the obtained nBG with reactive amine groups. Subsequently, VEGF-binding peptide was covalently attached to the particle surface using EDC coupling chemistry. The materials were characterized using FTIR, TEM and elemental analysis (ICP-MS), and surface modification and peptide coupling were monitored using fluorescent labelling. VEGF binding to peptide-functionalized nBG particles from single- as well as multi-protein solutions (competitive binding) were quantified.

Results and Discussion
Amorphous nBG particles with a diameter around 188.3 ± 21.2 μm were obtained (Fig. 1a). The density of reactive amine groups on nBG surface could be controlled and did not alter particle morphology (Fig. 1a, b). Alike, the amount of peptide covalently coupled to the surface of nBG-NH₂ could be controlled, enabling us to design particles with different VEGF binding capacity (Fig. 1c). Binding experiments (Fig. 1d) revealed efficient immobilization of VEGF on the peptide-modified nBG particles that was stable over several washing steps. In contrast, the amount of non-specifically bound GF was significantly lower and was further reduced during repeated washing of the control group.

Conclusion
Taken together, the proposed peptide-functionalized nBG particles are a promising new strategy to immobilize and deliver biologically active GFs as exemplified here using the pro-angiogenic factor VEGF. Employing a highly biocompatible and bioactive synthetic bone graft material as carrier, this system could aid bone regeneration.

Acknowledgement
This work was financially supported by the Gravitation Program of the Netherlands Organisation for Scientific Research (NWO) (project ‘Materials-Driven Regeneration’; grant no. 024.003.013). MS gratefully acknowledges the partners of Regenerative Crossing Borders (www.regmedxb.com). Powered by Health-Holland, Top Sector Life Sciences & Health. PH gratefully acknowledges the NWO Vidi grant “Bone Microfactory” (grant no. 15604).

nBG characterization and functionalization. TEM micrographs of as-synthesized and amine-modified nBG particles (a, scale bar = 100 nm), quantification of surface-grafted NH₂ (b) and covalently immobilized peptide on nBG (c), specific (left) and unspecific (right) binding of VEGF to peptide-functionalized and blank nBG (d).

References
Optimized terpene-loaded poly(\(\varepsilon\)-caprolactone) (PCL)-based nanoparticles with anti-bacterial properties

Leonard I. Atanase\(^1\), Oana M. Daraba\(^1\), Daniela L. Ichim\(^1\), Delia M. Rata\(^1\), Anca N. Cadinoiu\(^1\), Christelle Delaite\(^2\)

\(^1\) Apollonia University, Biomaterials, Iasi, RO; \(^2\) University of Haute Alsace, LPIM, Mulhouse, FR

Introduction
Terpenes have attracted a great interest in the recent years as these bioactive compounds can be used for topical applications on skin. Moreover, they can increase the skin permeation and have anti-inflammatory, anti-bacterial and anti-tumoral properties. In order to avoid their chemical degradation and volatilization, terpenes can be encapsulated in polymeric nanocarriers which must have several features: high encapsulation and loading efficiencies, controlled drug release kinetics, a simple, reproducible, and scalable preparation process as well as biocompatibility and biodegradability. Polymeric nanoparticles (NPs) obtained from preformed polymers are among the much easiest to obtain nanocarriers.

The aim of the present study was to prepare a series of optimized terpene-loaded NPs based on poly(\(\varepsilon\)-caprolactone) (PCL) homopolymers. These NPs were obtained by a nanoemulsion/evaporation method in the presence of poly(vinyl acetate-co-vinyl alcohol) (PVA) copolymers as emulsion stabilizers. The schematic representation is provided in Fig 1:

Experimental Methods
The obtained NPs were characterized by physicochemical techniques, such as: DLS, electrophoresis, SEM, TEM, FTIR, DSC, and TGA. Moreover, the cellular viability of the optimized terpene-loaded NPs was assessed using human fibroblast cell lines. Finally, their anti-bacterial activity was compared on both positive and negative Gram bacteria strain lines.

Results and Discussion
The effect of several parameters, such as: the nature of the organic solvent, the molecular weight of PCL homopolymers, the concentration of the PCL in the organic solution, the concentration, hydrolysis degree and molar mass of the PVA stabilizers, the drug/polymer ratio. Two terpenes, camphor and thymol, were loaded in these NPs in order to compare their anti-bacterial activity.

As expected, the size of the NPs decreased by increasing the concentration of the PVA solution and with decreasing the ratio between organic and aqueous phase. The optimal sizes of the NPs, under 150 nm with low PDI, and a ZP value around \(-6\) mV were obtained in the presence of a 1 wt% solution PVA sample with a hydrolysis degree of 80%.

Conclusion
These drug-loaded NPs, with low PDI and sizes, were obtained by a simple and reproductible method and can be applied to several biomedical applications.

Acknowledgement
This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P1-1.1-TE-2019-0664, within PNCDI III.
Fig. 1: Schematic representation of the preparation method
Poly(DL-lactic acid) scaffolds adsorbed with minocycline and voriconazole: a new pathway towards infection containment

Miguel Zegre\textsuperscript{1,2}, Margarida Henriques\textsuperscript{1}, Inês Anjos\textsuperscript{1}, Catarina Santos\textsuperscript{3,4}, Isabel Ribeiro\textsuperscript{1}, Liliana Caetano\textsuperscript{1,2}, Lídia Gonçalves\textsuperscript{1}, Ana Bettencourt\textsuperscript{1}

\textsuperscript{1} Faculdade de Farmácia da Universidade de Lisboa (FFUL), iMed.ULisboa - Research Institute for Medicines, Lisboa, PT; \textsuperscript{2} Escola Superior de Tecnologia de Lisboa (ESTeSL) - Instituto Politécnico de Lisboa (IPL), H&TRC - Centro de Investigação em Saúde e Tecnologia, Lisboa, PT; \textsuperscript{3} Instituto Superior Técnico, Universidade de Lisboa, CQE - Centro de Química Estrutural, Instituto Superior Técnico, Lisboa, PT; \textsuperscript{4} Escola Superior de Tecnologia de Setúbal (EST) - Instituto Politécnico de Setúbal (IPS), CDP2T - Centro de Desenvolvimento de Produto e Transferência de Tecnologia, Setúbal, PT

Introduction
Bone infection or osteomyelitis remains a burden as a clinical complication of orthopaedic surgeries [1]. New approaches that hold great promise to treat and prevent these infections, are well acknowledged controlled antimicrobial release systems. These may consist in biomaterials based on porous scaffolds, granting the administration of large antimicrobial concentrations locally, without the systemic toxicity and for extended time. Targeting bone tissue engineering, synthesized scaffolds have combined bioresorbable polymers as poly(DL-lactic acid) (PDLLA) with bioactive bioglasses, presenting biodegradability, biosafety and can grant appropriate microenvironment, structure and surface chemistry to hold osteogenic differentiation and cell growth for bone regeneration [2]. Since co-encapsulation of drugs aimed at commonly associated diseases provides an advantageous means for administration [3], this research work presents a novel strategy directed to the co-delivery of two antimicrobials at the infection site (one antifungal agent and one antibacterial agent), regarding osteomyelitis treatment.

Experimental Methods
PDLLA scaffolds were prepared by solvent casting / particulate leaching techniques and then functionalized with bioglass. The manufactured scaffolds were adsorbed simultaneously with 0.1 mg/mL of minocycline (MH), a tetracycline analogue that exhibits important antibiotic properties, and 0.1 mg/mL of voriconazole (Vor), a potent second generation triazole, fungistatic against Candida species, leading to its decreased formation and growth restraining. Produced scaffolds were physicochemically characterized by attenuated total reflectance (FT-IR/ATR), scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS). Adsorption efficiency (AE, \%) and drug loading efficiency (DL, \%) were also determined. In vitro release studies in Hepes 10 mM solution at pH 7.4 (37°C, 7 days) have been completed. Finally, the antimicrobial activity (against S. aureus and C. albicans) was also evaluated using the agar diffusion method.

Results and Discussion
FT-IR analysis revealed that MH or Vor inclusion did not change the scaffold composition, as the obtained spectra can be considered analogous. SEM assays showed a rough and porous surface in all samples, where bioglass or salt particles cannot be noticed. In vitro release studies suggest that both antimicrobials were adsorbed to the polymer, maintaining an expected release profile. At the same time, results point to better AE and DL related to Vor, than the ones observed with MH. Lastly, microbiological appraisal suggests that the scaffold combining both antimicrobials has activity aginst C. albicans for 48 h and additionally, proposes a synergistic activity within their association.

Conclusion
The scaffolds presented physicochemical and pharmaceutical characteristics applicable for controlled antimicrobial release systems, administrated locally. Furthermore, the scaffolds displayed antimicrobial activity against S. aureus and C. albicans and a synergistic activity within the association is proposed. These scaffolds emerge as a promising drug co-delivery system for local antimicrobial therapy in osteomyelitis.

Acknowledgement
This work was supported by National Funds (Fundação para a Ciência e Tecnologia - FCT, Portugal) through iMed.ULisboa (UIDB/04138/2020, UIDP/04138/2020) and Principal Researcher grant CEECIND/03143/2017 (L. Gonçalves).

References


Effect of Calcium Interaction and Mineralization in the Transfection Efficiency of Polyplexes for Gene Therapy

Teo Atz Dick¹, Hasan Uludağ¹²

¹ University of Alberta, Department of Chemical and Materials Engineering, Edmonton, CA; ² University of Alberta, Faculty of Pharmacy and Pharmaceutical Sciences, Edmonton, CA; ³ University of Alberta, Department of Biomedical Engineering, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada, Edmonton, CA

Introduction
Mineralization is traditionally used to coat materials with calcium phosphate or calcium carbonate layers for cell delivery applications. Particulate systems can be mineralized to increased robustness and release of cargo triggered by endosomal pH intra-cellularly[1]. In nature, the mineralization process seems to give viruses the ability to survive harsh conditions outside the body and infect a host more efficiently through airways [2]. These properties seem to be translatable to therapeutic applications in gene therapy using viral particles [3]. In non-viral gene therapy using polyplexes, reports have been limited to the achievement of improved and sustained transfection levels by modification of polyplex physical properties [4]. However, emerging literature showing that a simple calcium incubation process can achieve similar improvements in transfection efficiency challenges the usefulness of mineralization [5]. Considering that calcium interaction with organic matrices is an essential step for mineralization, from the current status of the literature, it is difficult to ascertain if mineralization is needed for improvement of transfection efficiency or if calcium incubation by itself is enough. To answer this question, we developed negatively charged poly(aspartic acid) coated polyplexes capable of undergoing mineralization and performed an analysis of particle size, binding efficiency, and surface charge at the various steps of the particle fabrication and mineralization process as well as transfection and uptake studies in MC3T3-E1 mouse osteoblastic cells using calcium incubated polyplexes as positive controls to the mineralized polyplexes.

Experimental Methods
The polyplexes were fabricated by incubation of pDNA and a lipid-modified low molecular weight PEI (1.2 KDa) followed by coating by poly(aspartic acid) in pure water. Polyplexes were incubated with CaCl₂ or mineralized with Na₃PO₄ or Na₂CO₃ at various concentrations, and calcium incubated samples were used as positive controls to mineralized samples with the same Ca content. Particle characterization was done using gel electrophoresis of freshly prepared samples and samples submitted to heparin-induced dissociation, assessment of zeta potential and hydrodynamic size, transmission and scanning electron microscopy, and x-ray diffraction for phase identification. In vitro transfection and uptake efficiency of the calcium incubated and mineralized polyplexes in preosteoblastic MC3T3-E1 cells was assessed using a Flow Cytometer and fluorescence microscopy.

Results and Discussion
Calcium incubation and mineralization both increased polyplex robustness; however, only mineralization completely inhibited dissociation induced by heparin. Untreated polyplexes showed highly negative ζ-potential. Calcium interaction with polyplexes was identified by an increase in ζ-potential to close to neutral values, and mineralization was identified by a decrease in ζ-potential. Hydrodynamic size analysis by dynamic light scattering revealed that calcium incubation and mineralization were important factors in increasing the size of the polyplexes. TEM images revealed that untreated polyplexes were well dispersed, and calcium incubation triggered a noticeable increase in particle size and aggregation. Mineralization resulted in a heterogeneous morphology characteristic of mineralized
particles. Both uptake and transfection efficiency using MC3T3-E1 cells were improved by mineralization and calcium incubation.

**Conclusion**

Both calcium incubation and mineralization improved transfection efficiency and uptake of polyplexes through size increase, improved pDNA binding, and adjustment of zeta potential to less-negative values. We believe that in applications involving mineralization of polyplexes, the effect of calcium incubation should be assessed through the use of appropriate controls to avoid misinterpretation of the cause-effect mechanism. With calcium incubation being a more time-efficient process, we find that mineralization might not be necessary if improved transfection efficiency in vitro is the only effect desired. Other potential properties acquired through mineralization that are reported for other particle systems still need to be studied for the specific case of polyplexes, such as improved physical protection of cargo and superior efficiency when particles are administered through airways.

**Acknowledgement**

Teo Atz Dick would like to thank CAPES for a full PhD scholarship.

---

References


Protamine nanoparticles: a new attractive gene-delivery system for glioblastoma treatment

Sheila B. Esteban¹, Sonia R. Trotiño¹, Ruman Rahman², Cameron Alexander³, Marcos G. Fuentes¹, Noemi Csaba¹

¹ University of Santiago de Compostela-CIMUS, Pharmacology, pharmacy and pharmaceutical technology, Santiago de Compostela, ES; ² University of Nottingham, Biodiscovery Institute-School of Medicine, Nottingham, GB; ³ University of Nottingham, Boots Science Building-School of Pharmacy, Nottingham, GB

Introduction
Glioblastoma (GBM) is one of the most aggressive brain tumors and is classified as grade IV of malignancy by the WHO. Standard-of-care treatment combines surgical resection, radiotherapy, and chemotherapy, but such interventions result in severe side-effects and only offer a median survival of 15 months from diagnosis (1). Among the current treatments, gene therapy has become an attractive alternative due to its capacity to target specific pathways within glioblastoma cells by the introduction of exogenous tumor suppressor sequences that are rendered therapeutically effective by using delivery systems (2). Polymeric nanoparticles (NPs) have been explored as a gene-delivery system for the treatment of glioblastoma due to their intrinsic ability to encapsulate and protect DNA and RNA, high biocompatibility, and delivery efficacy (3).

Experimental Methods
Polymeric NPs were formulated by the ionic-complexation method using the ratio 4:1 of protamine:dextran (4:1 Pr:Dx NPs). The physicochemical and structural properties of the nanosystem were studied by Photon Correlation Spectroscopy, Laser Doppler Anemometry, and Transmission Electron Microscopy. The long and short-term stability was examined for one month under storage conditions and for 4 hours in biological media at 37°C, respectively. Moreover, the association of nucleic acids was studied by agarose gel electrophoresis using an excess of heparin as ionic competitor. Cell-viability studies were initially optimized in U87MG monolayers and spheroids, followed by assessment in three primary patient-derived glioblastoma cell lines (GIN-8, GIN-28 and GCE-28). The 2D and 3D cellular uptake of 4:1 Pr:Dx NPs was studied using fluorescently labelled NPs by confocal microscopy and quantified by flow cytometry. Finally, the transfection efficiency was studied using different concentrations of a model plasmid encoding the enhanced Green Fluorescent and Luciferase Proteins (pEGFP-Luc), by flow cytometry and a Luciferase Reporter Gene assay, respectively.

Results and Discussion
The formulation consisted of a population of spherical NPs composed by protamine, a cationic cell-penetrating peptide with high capacity to condense DNA, combined with dextran, a natural anionic polysaccharide. These polymeric NPs had a size below 150 nm, positive surface charge and allowed the efficient encapsulation of different nucleic acids. In addition, the formulation presented a satisfactory physical and biological stability. Viability studies indicated low/non-toxicity in U87MG cells and spheroids, as well as, in glioblastoma patient-derived cell lines. Microscopy studies revealed an efficient internalization of 99% of the NPs in 2D and 3D glioblastoma models. Finally, the transfection assay showed an efficient capacity of the polymeric NPs to transfect glioma cells and spheroids for doses above 1 µg/well.

Conclusion
We have developed a formulation based on polymeric NPs with physicochemical properties suitable for the association and protection of different genetic cargos. Their low cellular toxicity, high internalization, and their...
Capacity to transfect different 2D and 3D glioblastoma models indicated that this formulation could be considered a promising gene nanocarrier for glioblastoma treatment by gene therapy.

Acknowledgement

Gobierno de España (Ministerio de Economía y Competitividad (SAF2016-79230R)), Xunta de Galicia (Consellería de Cultura, Educación e Ordenación Universitaria (2016-2019, ED431G/05)), European Regional Development Fund (ERDF) and BritishSpanish Society.

References


Biodegradable composites with antibiotics and growth factors for dual release kinetics

Julian Hess\textsuperscript{1}, Anke Bernstein\textsuperscript{1}, Hermann O. Mayr\textsuperscript{1}, Diana Voigt\textsuperscript{2}, Hagen Schmal\textsuperscript{1}, Michael Seidenstuecker\textsuperscript{1}

\textsuperscript{1} Albert-Ludwigs-University Freiburg, G.E.R.N. Center for Tissue Replacement, Regeneration and Neogenesis, Freiburg, DE; \textsuperscript{2} Research Institute of Leather and Plastic Sheeting (FILK gGmbH), Freiberg, DE

Introduction
Infections of the bone, whether acute or chronic, as a result of hematogenous spread or as a postoperative complication (e.g. implant-associated infections) are difficult to treat even according to the current state of medicine. Systemic and local antibiotic therapy using implantable PMMA (polymethyl methacrylate) chains are as much part of the standard therapy in today's guideline recommendations as radical surgical debridement (1). While systemic pharmacotherapy is easy to implement, the low degree of efficacy in situ is repeatedly demonstrated with nevertheless already very high dose applications. In contrast, PMMA cements which are provided with antibiotics release larger concentrations of pharmaceuticals on site, but must eventually be removed in a second operation. Biodegradable carrier systems such as calcium phosphates could therefore offer a suitable alternative, as they have already proven their biomechanical stability in dentistry and surgery. It has also been demonstrated in previous work that microporous b-tricalcium phosphate (b-TCP) ceramics impregnated with antibiotics (rifampicin, daptomycin) could ensure a high local release.

In this work, we now aim to demonstrate that antibiotic-impregnated hydrogels loaded into b-TCP scaffolds can release pharmaceuticals over a period of several weeks while maintaining high concentrations. In addition, Bone Morphogenetic Protein 2 (BMP2) was added to the gels showing positive effects on both bone and wound healing.

Experimental Methods
To prepare the hydrogels low viscosity plasma sterilized alginates were added to aqua bidest in solution along with BMP2 and antibiotics (either daptomycin, clindamycin or rifampicin) and homogenized for at least 12 hours. The gels were then loaded into microporous b-TCP ceramics using a patented flow-through chamber (EU patent PCT/EP2014/001935) and a directed vacuum, followed by crosslinking by a calcium chloride solution. Release experiments were then performed in aqua bidest after 1, 2, 3, 6, 9, 14, 21 and 28 days. Subsequently, quantitative analyses of the released antibiotics were performed by HPLC (high performance liquid chromatography) or of the growth factors by ELISA. For statistical analysis, mean values and standard deviations were determined via sampling time point and an analysis of variance was collected using ANOVA.

Finally, biocompatibility was investigated both qualitatively and quantitatively in several in vitro cell culture experiments (live/dead, LDH and WST) using the cell culture line MG63, and antimicrobial efficacy was demonstrated in microbiological fouling tests.

Results and Discussion
So far, parts of the HPLC analysis on the release of clindamycin are available as interim results (up to day 14). After only one day, over 80% of the antibiotic had been released. By the third day of collection (day 3), 89.5% of clindamycin had passed into the medium. Nevertheless, the released concentrations of pharmaceuticals remained above the minimum inhibitory concentration (MIC) of 5ng/mL (at 1.32µg/mL) up to day 14, while the detection limit was 0.76µg/mL.

During the production of the various hydrogels, different viscosities between the individual antibiotics were noticed, which may also be reflected in the releases.

Page 1797 of 2028
References
[1] AWMF Leitlinie „Akute und chronische exogene Osteomyelitis langer Röhrenknochen des Erwachsenen“, Leitlinien der Deutschen Gesellschaft für Unfallchirurgie AWMF-Nr. 012-033
Riboflavin-SPIONs for enhanced cellular internalization in breast cancer cells

Wid Mekseriwattana\textsuperscript{1,2}, Anna Roig\textsuperscript{2}, Kanlaya P. Katewongsa\textsuperscript{1,3}

\textsuperscript{1} Mahidol University, School of Materials Science and Innovation, Faculty of Science, Bangkok, TH; \textsuperscript{2} Universitat Autònoma de Barcelona, Institut de Ciència de Materials de Barcelona (ICMAB-CSIC), Bellaterra, ES; \textsuperscript{3} Mahidol University, Department of Biochemistry, Faculty of Science, Bangkok, TH

Introduction
Female breast cancer is the most prevalent type of cancer worldwide, according to a report in 2020 \cite{1}. Different types of riboflavin (Rf)-associated proteins were found to be overexpressed in breast cancer patients \cite{2, 3}; thus, several research efforts use Rf-vitamin and its derivatives as directing ligands to target drug delivery nanoparticles to the cancer cells. Although positive results have been reported in nanoparticle delivery, the uses of riboflavin are limited by its hydrophobicity, resulting in complex synthesis routes and unfavorable stabilizing properties which are crucial for nanoparticle formulation. Therefore, this work aims to develop a Rf-based ligand for superparamagnetic iron oxide nanoparticles (SPIONs) by a simple synthesis to provide stability to the Rf-SPIONs and demonstrate enhanced cellular uptake of SPIONs in breast cancer cells.

Experimental Methods
The ligand was made through conjugation of Rf with citric acid, followed by coating on the SPIONs through complexation of the citrate moieties and the Fe atoms. The stability of the Rf-SPIONs was evaluated in terms of aqueous hydrodynamic diameter and zeta-potential, in comparison with uncoated- and citrate-coated SPIONs. MCF-7 breast cancer cell line was used as an \textit{in vitro} model for cellular uptake assessments. Biocompatibility of Rf-SPIONs was ensured by performing MTT cell viability assays. Nanoparticle cellular uptake for the different systems were observed by Prussian blue staining and subsequently quantified by flow cytometry.

Results and Discussion
With the citrate-Rf ligand, Rf-SPIONs showed a sub-200 nm hydrodynamic size with a narrow polydispersity index, significantly lower than for the uncoated SPIONs. The Rf-SPIONs showed no toxicity toward the cells when treated with concentrations up to 200 µg mL\textsuperscript{-1}. Prussian blue staining and flow cytometry both showed a superior cellular internalization behavior of the Rf-SPIONs. At 4 °C, cellular uptake of the Rf-SPIONs drastically dropped as a consequence of the inhibition of cellular activity, suggesting an active transport cellular internalization pathway. The tests were also performed in MCF-10A normal breast cell line, and no significant differences in cellular uptake were observed among the three SPION systems studied.

Conclusion
The citrate-Rf has proven to be a potent ligand to functionalize SPIONs and activate their cellular internalization in breast cancer cells and in the future, could be applied to other nanoparticle platforms.

Acknowledgement
This research is supported by the Science Achievement Scholarship of Thailand (SAST).
Synthesis scheme of citrate-Rf ligand and surface structure of Rf-SPIONs

References
Lab-on-chip for studying Shwachman-Diamond syndrome mechanisms

Nora Selicato1, Eleonora De Vitis2,4, Francesca Gervaso2, Alessandro Polini2, Valentino Beizzeri3, Marco Cipolli1

1 Azienda Ospedaliera Universitaria Integrata, Cystic Fibrosis Center of Verona, Verona, IT; 2 CNR Nanotec, Institute of Nanotechnology, Lecce, IT; 3 Azienda Ospedaliero Universitaria Ospedali Riuniti di Ancona, Cystic Fibrosis Center, Ancona, IT; 4 Università del Salento, Dipartimento di Matematica e Fisica E. de Giorgi, Lecce, IT

Introduction

Shwachman-Diamond syndrome (SDS) is a rare inherited multisystemic syndrome (worldwide affects almost 1/75,000 live births) characterized by bone marrow failure, exocrine pancreatic insufficiency, skeletal dysplasia and failure to thrive. Similarly to other bone marrow failure syndromes, SDS is associated with juvenile myelodysplastic syndrome and high risk of leukemic transformation [1]. SDS is mainly caused by mutations in the Shwachman-Bodian-Diamond Syndrome (SBDS) gene (located on chromosome 7q11.22) encoding a protein involved in ribosomal biogenesis. Interestingly, three genes have recently been associated with an SDS-like phenotype: elongation factor-like 1 (EFL1), signal recognition particle 54 (SRP54) and eukaryotic elongation initiation factor 6 (eIF6). All these genes are involved in ribosome biogenesis, strengthening the postulate that SDS is a ribosomopathy [2].

Currently, there is no cure for SDS. The treatment is based on managing the symptoms. In order to prevent malignant transformation, hematopoietic stem cell transplantation is the unique available option. Promising new therapies aiming to correct the genetic defect have been supposed. Since most patients with SDS present nonsense mutations in the SBDS gene, recently it has been proposed the drug repositioning of the small nonsense suppressor molecule, namely ataluren [PTC124; 3-(5-(2-fluorophenyl)-(1,2,4)oxadiazol- 3-yl)-benzoic acid]. This drug has been already approved by the European Medicines Agency for the treatment of Duchenne muscular dystrophy. In previous studies, ataluren restored significantly SBDS protein expression in bone marrow progenitor cells from patients with SDS, promoting myelodysplastic syndrome and high risk of leukemic transformation [1].

Neutrophil chemotaxis plays a crucial role in human immune system. Unfortunately, neutrophil and monocyte chemotaxis is impaired in SDS [4]. The chemotaxis defects of SDS have been previously studied with traditional cell migration assays, which cannot provide the temporal and spatial control of the chemical gradients, neither study the single cell behaviour [5].

Experimental Methods

Lab-on-chips are a new research platform for studying chemotaxis and chemokinesis able to overcome the limitations of traditional chemotaxis assays due the advantage of visualization, precise control of the chemical gradient and small consumption of reagents. In this study, we developed a lab-on-chip platform and evaluated ataluren potential in restoring chemotaxis using a microfluidic device. We fabricated a flow-free microfluidic device with three different perfusable compartments with distinct inlets and outlets, interconnected through a series of parallel microchannels that can be used to control cell migration and cell differentiation on chip. The platform was fabricated by SU-8-based multi-level photolithography and PDMS replica molding. Gradient profiles were previously evaluated to ensure an adequate time to perform the chemotaxis experiments and a preliminary cell line was used to set up the device for the purpose of this study.

Results and Discussion

Firstly, we utilized the device to confirm the impaired SDS neutrophil chemotaxis. Primary neutrophils were obtained from patients carrying the c.183-184TA> CT nonsense mutation. A precise number of cells was seeded in the lowest...
compartment and the SDS neutrophil migration was evaluated in the presence and absence of a defined concentration of chemoattractants injected in the upper compartment of the device. Brightfield time-lapse images of cell migration were captured for maximum 1 hour and the cell trajectories analysed using Manual Tracking plugin ImageJ. Then, the data were further analysed to obtain the trajectory plots and the main output parameters for chemotaxis (Forward Migration Index, center of mass, velocities, Rayleigh test, distances, directness). Lastly, we tested ataluren potential in restoring the chemotaxis, treating primary mononuclear cells (MNCs) obtained from fresh peripheral blood or bone marrow specimens from SDS patients. At the seeding time, ataluren was added in the culturing medium at different concentrations and spontaneous growth of granulocyte-monocyte colony formation (CFU-GM) was determined at different time point of incubation. Then, the chemotaxis assay was performed and analysed as previously indicated.

References
**PS2-10-338**

**Clobetasol-loaded chitosan patches for the treatment of autoimmune diseases affecting the oral mucosa**

**Elena Maria Varoni**¹,², Lina Altomare²,³, Marcello Manfredi⁴, Andrea Cochis⁴, Lorenzo Bonetti²,³, Lia Rimondini⁴

¹ University of Milan, Dipartimento di Scienze Biomediche, Chirurgiche ed Odontoiatriche, Milano, IT; ² National Interuniversity Consortium of Materials Science and Technology (INSTM), Firenze, IT; ³ Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering “G. Natta”, Milano, IT; ⁴ Università del Piemonte Orientale UPO, Department of Translational Medicine, Center for Translational Research on Autoimmune and Allergic Diseases CAAD, Novara, IT

**Introduction**

The standard care for managing immune-mediated and autoimmune diseases affecting the oral mucosa includes the use of topical steroid therapy¹,². Clobetasol propionate (CP) is one of the most potent corticosteroids (Europe: class IV), used only for dermatological application due to its high lipophilicity¹. CP application in the oral cavity is challenging due to the salivary flow and the muscular activity, which easily displace the drug³. So far, no commercial CP formulation is available for the delivery of CP to oral mucosa³. Hence, we developed an innovative bilayer chitosan (CS)-based muco-adhesive patch, via electrophoretic deposition (EPD), loaded with CP, holding a porous architecture and showing an excellent swelling rate after re-hydration⁴. Here, we investigated the same patches for CP release under conditions similar to salivary environment, while an ex vivo porcine model of oral mucosa⁵ was used to evaluate CP absorption throughout the epithelial layers as well as mucoadhesion.

**Experimental Methods**

- **EPD of bilayer CS-CP patches**: CP (1g/L) has been mixed together with CS (1g/L) in a 30% water + 70% ethanol bath (pH = 4.8). Titanium plates (Ti, grade 2) were used in an electrophoretic deposition bath where conditions have been optimized to obtain CS-CP patches: square waves (100-75 V / Dc = 0.17 t = 5 min). Deposited patches were standardized as 2x2 cm² squares, freeze-dried and finally re-equilibrated at room temperature. CS bilayer patches were obtained by a double step deposition: a first deposition starting from a solution containing only CS and, after drying, a second layer was deposited starting from a CS solution with or without CP, namely CS-CP and CS respectively.

- **Morphological surface analysis**: Surface and cross-section morphology was visually investigated by SEM imaging.

- **CP in vitro release**: CP concentration within CS patches was determined by liquid chromatography mass spectrometry (LC-MS). CP release was performed in a dissolution medium (0.5M phosphate buffer saline and 0.5% sodium dodecyl sulphate, pH 6.8) at 37 °C. Specimens were incubated in 1 ml of solution, and fixed vertically in 2 ml tubes in agitation (100 rpm, 37°C) thus allowing CP release from both sides of the specimen. The solution was then collected and analyzed by LC-MS using a mass spectrometer coupled with an UHPLC.

- **Ex vivo CP release**: Porcine oral mucosa was surgically resected, standardized into 2 cm side-5mm thickness squares and maintained in DMEM/F12 medium at 37°C. Patches were adjusted onto mucosa samples at the air-liquid interface and allowed to spontaneously release CP for 0.5, 3 and 6 hours. At each time-point mucosa samples were separated from patches, mechanically homogenized and the CP amount determined by LC-MS.

- **Ex vivo mucoadhesion**: A tack –test was performed using Anton Paar MCR302 rheometer. Porcine oral mucosa 10 mm side-5mm thickness squares were deeply washed with PBS and frozen. After being re-equilibrated at RT, the mucosa was to a glass substrate and to the probe of the rheometer. Wet patches (CS or CS-CP) were similarly attached on the fixed side. Mucosa and patches were placed in contact; 1N force was applied for 10 sec. Mucosa
was moved in the opposite direction at 50mm/min until detachment. Release pressure (S=F/surface) and muco-
adhesion work (Wad=area under the curve) were calculated.

Results and Discussion
- **Morphological surface analysis**: SEM images (Figure 1 A-D) showed patches with a homogeneous and defined porous structure.
- **CP in vitro release**: CP was loaded in the patches at a concentration of 3458.8 ± 952.2 ng/mL. The amount of drug released was about 1245.7 ± 889.6 ng/mL/patch, as achieved after 40 min. Figure 2 A-B summarizes the release profile of CP from CS-CP patches.
- **CP ex vivo release**: After 6 hours ≈50% of the loaded CP was released in the tissue thus achieving an estimated drug amount of 1392.2 ± 708.3 ng/mL/patch. (Figure 2 C-D)
- **Ex vivo mucoadhesion**: No significant differences were observed between CS and CS-CP for both release pressure (557 ± 213 Pa and 448 ± 8 Pa, respectively) and Wad (745 ± 126 Pa*mm and 699 ± 128, respectively). The presence of the drug does not affect the mucoadhesive behavior of chitosan.

Conclusion
Bilayer CS-based patches can be successfully loaded using a highly lipophilic drug as CP, thus representing a promising drug delivery system for the oral mucosa. The CP drug release during time under in vitro condition similar to saliva is confirmed. The ex vivo porcine model of oral mucosa showed encouraging data about topical absorption and mucoadhesion.
Figure 2.
Clobetasol release and absorption in vitro (A-B) and in ex-vivo oral mucosa porcine model (C-D).

References
A Nanocomposite Coating for Implant-Associated Local Drug Delivery from Neuronal Electrodes: Nanoporous Silica Nanoparticles Embedded in Nanoporous Platinum

Mosaieb Habib1,3, Tim-Joshua Strauß1, Jennifer Harre2,3, Kim D. Kreisköther1, Saskia Zailskas1, Arne Schierz1,3, Hendrik A. Schulze1, Dawid P. Warwas1, Mandy Jahns1, Hans-Christoph Schwarz1, Thomas Lenarz2,3, Athanasia Warnecke2,3, Peter Behrens1,3

1 Leibniz University Hannover, Institute of Inorganic Chemistry, Hannover, DE; 2 Hannover Medical School, Department of Otorhinolaryngology, Hannover, DE; 3 Cluster of Excellence Hearing4all, Hannover, DE

Introduction
According to the WHO, 1 in 5 people worldwide live with hearing loss.[1] One type of hearing loss is sensorineural hearing loss, which is present in about 95% of deaf people. This condition results from damage to or loss of the actual sensory cells in the inner ear, the hair cells. The nerve cells, the spiral ganglia, are often still present but damaged. The cochlear implant, as an electronic stimulation prosthesis, can be used to restore hearing. Due to the previous disease as well as to the insertion trauma the biological balance in the inner ear is disturbed and the spiral ganglion neurons degenerate. With the help of neuroprotective substances, neuronal growth factors, anti-inflammatory drugs or antibiotics it is possible to restore the balance in the inner ear, to stabilize the spiral ganglion neurons and to furthermore induce the outgrowth of dendrites from these cells.[2] For optimal application, these drugs should be released locally, i.e. employing an implant-associated local drug delivery system. Due to the inertness of the materials from which the cochlea electrode is manufactured - platinum and silicone - the construction of such a system is not trivial.

Here, we present a novel nanocomposite material, composed of nanoporous platinum (NPPt) and nanoporous silica nanoparticles (NPSNPs) as a novel implant-associated local drug delivery system which is generated as a coating on the surface of the CI electrode.[3] NPPt exhibits high conductivity and favourable electrochemical properties. NPSNPs which are embodied in the pore system of the platinum offer a high specific surface area, large permanent porosity and a high versatility with regard to easily adjustable surface properties in order to accomplish a high drug loading.[4,5]

Experimental Methods
For the generation of the NPSNP@NPPt material on the surface of the electrode contacts, a hard template approach was used utilizing silica-polystyrene core-shell nanoparticles as template particles. Platinum was deposited electrolytically between the particles and the polystyrene was removed via extraction. The composite coating was characterized by means of scanning electron microscopy (SEM), krypton physisorption measurements, cyclic voltammetry and electrochemical impedance spectroscopy. Release experiments were performed with the dye methylene blue. Cell culture investigations with fibroblasts and spiral ganglion cells were conducted to investigate the biocompatibility of the novel material.

Results and Discussion
SEM investigations show the successful embedding of the NPSNPs in the pores of the NPPt (figure 1). The composite material exhibits good electrochemical properties and enhanced surface area due to the nanoporous silica nanoparticles. The release experiments indicate a higher loading and release of methylene blue. Cell culture experiments of the new material show good cytocompatibility.

Conclusion
The novel nanocomposite material combines the favourable properties of both nanoporous platinum and nanoporous silica nanoparticles, specifically the excellent electrochemical behaviour of NPPt and the high specific surface area, large pore volume and amenability to surface modifications of the NPSNPs. Cell culture experiments of the new material show high biocompatibility.

Acknowledgement

TEM measurements were performed at the Laboratory for Nano- and Quantumengineering (LNQE) Hannover. This work was funded within the Cluster of Excellence Hearing4all by the DFG under Germany’s Excellence Strategy – EXC 2177/1 – Project ID 390895286.

References

[1] https://www.who.int/health-topics/hearing-loss#tab=tab_1, accessed on 13.05.2021
Long-acting injectable formulations for the sustained delivery of Aripiprazole based on poly(lactic acid)/poly(butylene adipate) block copolymers

Nikos Bikiaris¹, Evi Christodoulou¹, Alexandra Zamboulis¹, Vasiliki Karava², Aggeliki Siamidi², Marilena Vlachou², Margaritis Kostoglou³, Eleni Gounari⁴, Panagiotis Barbalexis⁶

¹ Aristotle University of Thessaloniki, Department of Chemistry, Laboratory of Polymer Chemistry and Technology, Thessaloniki, GR; ² National and Kapodistrian University of Athens, Zografou Campus, Department of Pharmacy, Section of Pharmaceutical Technology, Athens, GR; ³ Aristotle University of Thessaloniki, Laboratory of Chemical and Environmental Technology, Thessaloniki, GR; ⁴ Biohellenika Biotechnology Company, Leoforos Georgikis Scholis 65, Thessaloniki, GR; ⁵ Aristotle University of Thessaloniki, Department of Biochemistry, School of Medicine, Faculty of Health Sciences, Thessaloniki, GR; ⁶ Aristotle University of Thessaloniki, Department of Pharmaceutical Technology, Thessaloniki, GR

Introduction
In the past few decades synthetic polymers that degrade under physiological conditions (i.e., biodegradable polymers) have become increasingly common in medical and pharmaceutical applications. Especially, in the case of particulate drug formulations (such as nano- or micro-particles) an increasing number of polymeric materials, and especially polyesters, have been introduced and implemented for drug delivery. Among them, the preparation of long-acting injectable (LAI) formulations is probably the most intensively studied application for such polyester-based systems.

The present study evaluates the use of newly synthesized poly(l-lactic acid)-co-poly(butylene adipate) (PLA/PBAd) block copolymers as microcarriers for the preparation of drug-loaded long acting injectable (LAI) formulations. Aripiprazole (ARI), a second-generation antipsychotic drug, was used as a model drug.

Experimental Methods
PBAd and PLLA/PBAd, corresponding to a final copolymer weight ratio of 95/5, 90/10, 75/25 and 50/50 w/w PLA to PBAd, copolymers were prepared. PBAd was synthesized via a typical two-stage esterification and polycondensation of adipic acid and 1,4-butanediol in a 1/1.1 molar ratio, in the presence of catalyst tetrabutyl titanate. PLLA/PBAd copolymers were afterwards prepared via ring opening polymerization of L-lactide using Tin(II) 2-ethylhexanoate (TEH) catalyst. Aripiprazole (ARI) MPs were finally fabricated using PLA and PBAd polymers, as well as their copolymers via an emulsification/solvent evaporation method.

Results and Discussion
The prepared PLA/PBAd block copolymers were thoroughly characterized so as to evaluate their use as matrix/carriers for the preparation of ARI long-acting injectables depot delivery systems. FT-IR spectroscopy and XRD patterns were used to confirm their successful synthesis and their crystalline structure, respectively. FT-IR was also used to evaluate the molecular interactions among the copolymers. Their thermal properties were assessed by DSC, whereas monthly degradation tests exhibited slow polymer erosion, a key factor for slow drug-release (Fig.1). Cytotoxicity studies, both in vitro and in cell cultures, confirmed their good biocompatibility and low toxicity.

Regarding the fabricated microparticles, SEM images showed the formation of well-shaped spherical MPs, mostly in the range 15-60 μm, with smooth exterior surface and no particle’s agglomeration (Fig. 2), while DSC and pXRD data revealed that the presence of PBAd in the copolymers favors the amorphization of ARI. The effect of the newly synthesized block copolymers on the in vitro dissolution characteristics of ARI were finally evaluated. Results showed
that the prepared MPs followed a biphasic release profile in all cases, and highly tunable extended-release profiles for the API, which may be controlled for up to 30 days.

Conclusion

In the present study PLA/PBA-d-based ARI-loaded LAI MPs were successfully prepared for the first time. Results regarding the highly tunable enzymatic hydrolysis profile and the low cytotoxicity of the new copolymers, amplified the previously made suggestions that these new copolymers can be considered as a quite promising candidate for the preparation of drug sustained release formulations.

Acknowledgement

We would like to thank Pharmathen S.A for funding our research.

Figure 1

In vitro drug % release vs time of pure ARI and encapsulated ARI in PLA/PBA-d MPs, during the 30 (a) and the 0.5 (b) days of the experiment.

Figure 2

SEM images of PLA/PBA-d 75/25 microparticles erosion process after 30 days of dissolution.
Determination of salicylic acid release profile from polymeric nanocarriers

Małgorzata Miastkowska, Katarzyna Bialik-Wąs

Cracow University of Technology, Department of Chemical Engineering and Technology, Kraków, PL

Introduction
In the preparation of controlled delivery system of active substance, the selection of an appropriate drug carrier is the most important. Currently, it can be used synthetic and natural biodegradable polymers, which occur in the nanometric scale. Moreover, polymeric materials which react to specific external factors, such as: temperature, pH, ionic strength, electric or magnetic field, light, and other chemical and biological stimuli, are particularly interesting. Due to they can be used in different area of medicine and pharmacy [1-4].

Experimental Methods
In this studies pH sensitive (poly(acrylic acid-co-methyl methacrylate)) and temperature sensitive (N-isopropylacrylamide) polymeric nanocarriers were obtained by radical polymerization and the initiator of the reaction was ammonium persulfate (APS), while poly(ethylene glycol) diacrylate (PEGDA, Mn = 575 g/mol) or N, N'-methylenebisacrylamide were used as the crosslinking agent, respectively. After that, the encapsulation of the model active substance – salicylic acid, was carried out. The salicylic acid belongs to nonsteroidal anti-inflammatory drugs with antiseptic and analgesic properties. After, the encapsulation efficiency was assessed and the average particle size of the carrier – drug system was determined. In addition, various studies were carried out using the following research techniques: SEM, DLS and FT-IR, which allowed to analyze both the carrier and the carrier-drug system. Finally, the release of drug (salicylic acid) was conducted using USP4 method (DZF II Flow-Through System, Erweka GmbH, Langen, Germany). The equipment incorporated seven in-line flow-through diffusion cells. The release study of salicylic acid was carried out using a regenerated cellulose membrane. The assays were performed in buffer solution at pH 7.4, at 37°C. The released concentration of salicylic acid in the receptor solution was analyzed by means of UV-Vis spectroscopy (Perkin Elmer Company), at the wavelength of 300 nm.

Results and Discussion
On the basis of obtained DLS histograms, the average size of the thermosensitive carriers was about 118 nm and for pH – sensitive was below 500 nm. The encapsulation was in the range of 70-80%. The chemical structure both carriers and carrier-drug systems was confirmed based on FT-IR spectra and it exhibited the hydrogen interaction between components. Additionally, SEM analysis showed that the polymeric carriers are in the spherical form as well as cylindrical and after the encapsulation the surface is more irregular.

The release profile of salicylic acid form pH and T-sensitive polymeric nanocarriers is shown at figure 1 and 2. In the case of salicylic acid, we observe pulsatile release from both carriers (Fig. 1 and 2). The significant difference is that in the case of a pH-sensitive carrier, the release takes place after a lag time of 5 h, while in the case of a thermally-sensitive carrier, we still observe the effect of rapid release, i.e. quick release caused by a weak carrier-drug bond [5].

Conclusion
This part of research constitutes the basic system of drug – thermo/pH-sensitive carrier for further research on the bio-hybrid hydrogel materials for the treatment of Psoriasis. In the next stage of studies, it is necessary to introduce a nanocarrier to hydrogel matrix, which will reduce the "burst effect" and result in prolonged drug release.

Acknowledgement
This research was financially supported by The National Centre for Research and Development — project LIDER/41/0146/L-9/17/NCBR/2018.

**Figure 1.** The release profile of salicylic acid from pH-sensitive nanocarrier.

**Figure 2.** The release profile of salicylic acid from thermosensitive nanocarrier.

**References**


**PS2-10-346**

**Implementing RNAi Therapy Targeting FLT3-ITD Mutation in Acute Myeloid Leukemia (AML)**

**Aysha Ansari, Remant Kc, Hasan Uludag**

*University of Alberta, Edmonton, CA*

**Introduction**

Acute myeloid leukemia (AML) is a highly heterogeneous disease with considerable diversity in molecular pathogenesis and clinical outcomes. Crosstalk between different genetic pathways adds to the complexity of AML pathogenesis as well as treatment [1]. Almost 25% of newly diagnosed AML patients display an internal tandem duplication (ITD) in the *fms-like tyrosine kinase 3* (*FLT3*) gene. The FLT3-ITD mutation is an early driver of leukemogenesis and its presence is accompanied by a high leukemic burden and poor prognosis [2]. Although both multi-targeted and FLT3 specific tyrosine kinase inhibitors (TKIs) are being utilized for clinical therapy, drug resistance and high relapse rates are challenges that still need to be tackled [3]. RNA interference (RNAi), mediated by short interfering RNA (siRNA) presents a novel therapeutic modality with the potential of personalization due to its sequence-driven mechanism of action. The success of RNAi therapy to a great extent is dictated by the siRNA delivery vehicle employed. This study explored the use of lipid-substituted low molecular weight polyethyleneimine (lipopolymer) for delivering FLT3 siRNA in FLT3-ITD positive AML cells.

**Experimental Methods**

MV4;11 cells were utilized as an in vitro FLT3-ITD positive AML model for all studies. Low molecular weight (1.2 and 2 kDa) polyethyleneimine (PEI) polymers were modified with aliphatic lipids to yield lipopolymers which self-assembled into nanocomplexes with siRNA. Three siRNAs targeting different regions of the *FLT3* gene (FLT3 siRNA1, FLT3 siRNA2, and FLT3 siRNA3) and scrambled negative control siRNA (NC siRNA) were employed. Cell viability was assessed by the MTT assay. Cell proliferation studies were executed by performing cell counts (with trypan blue) daily over four days after treatment to generate the cell growth curves. RT-qPCR was conducted to quantify relative mRNA transcript levels and FLT3 protein levels were determined by flow cytometry through immunostaining. The percentage of cells undergoing apoptosis were evaluated by FITC-Annexin V/propidium iodide (PI) staining. The colony forming cell (CFC) assay was performed to determine effectiveness of FLT3 silencing in eliminating leukemia cells. Results are reported as mean ± standard deviation of three independent experiments and analyzed for statistical significance by Student’s two-tailed t-test (assuming unequal variance).

**Results and Discussion**

Treatment with lipopolymer/FLT3 siRNA nanocomplexes resulted in significant reduction in cell viability. Quantitative analysis revealed downregulation of *FLT3* gene transcript and FLT3 protein levels after treatment. This decrease in FLT3 mRNA and protein levels was manifested as a marked decline in cell proliferation rates (Figure 1) as well as induction of apoptosis, with a significant increase in percentage of cells in the late apoptosis stage. Furthermore, a notable drop in the ability to form colonies in methylcellulose post FLT3 silencing was observed. Combining the lipopolymer/FLT3 siRNA nanocomplexes treatment with current chemotherapy, specifically daunorubicin and midostaurin, showed enhancement of apoptosis as well as further depletion in AML stem cell burden through reduced colony formation ability.

**Conclusion**

The biological response to the decrease in *FLT3* gene and protein levels was seen in the significantly reduced cell numbers after treatment as well as increase in percentage of cells undergoing apoptosis. The decrease in the ability to form colonies post FLT3 silencing demonstrates the potential of RNAi by FLT3 siRNA to curtail the self-renewal ability.
capacity of AML progenitor cells. Furthermore, combining the RNAi approach with current chemotherapy showed potentiation of anti-leukemic activity. RNAi implemented with lipopolymer nanocomplexes, thus, bears promising potential for AML molecular therapy as well as prospectively serve as an addition to the treatment modalities available to target the heterogeneity prevalent in AML.

Acknowledgement

Uludag Lab members
NSERC (Natural Sciences and Engineering Research Council of Canada)
CIHR (Canadian Institutes of Health Research)
Alberta Innovates Technology Futures

![Cell growth curves](image)

**Figure 1**
Cell growth curves obtained by counting cell numbers (with trypan blue) daily over four days after treatment with lipopolymer/siRNA nanocomplexes. NC siRNA - negative control siRNA.

References

2:00 p.m. – 3:30 p.m.

Poster floor

**PS2-11 | Antibacterial Biomaterials**
Pyridinium-decorated PVC surfaces for the prevention of ventilator-associated pneumonia

Matthew P. Wylie, Nicola J. Irwin, Louise Carson, David S. Jones, Colin P. McCoy

Queen's University Belfast, School of Pharmacy, Belfast, GB

Introduction
Ventilator-associated pneumonia (VAP) is the most common hospital-acquired infection in intensive care units and is associated with a significant risk of patient mortality \(^1\). Endotracheal tubes (ETT) are crucial in facilitating mechanical ventilation, but a significant drawback of these devices is their role in the development of VAP with bacteria capable of developing biofilms on ETT surfaces which can be displaced into the deep lung by air turbulence allowing the development of pneumonia. The use of antimicrobial-eluting surfaces to prevent VAP are often unsuccessful and encourage the development of antibiotic resistance as well as the risk of downstream toxicity \(^2\).

Non-leaching, durable antimicrobial surfaces are an attractive solution to medical device-related infection as they can avoid bacterial exposure to sub-lethal concentrations of antimicrobials. Quaternary ammonium compounds (QACs) are well described broad-spectrum antimicrobials which can be chemically modified without detriment to their activity. Modification of QACs with a thiol group can facilitate their permanent attachment to PVC via nucleophilic substitution to produce a long-lasting antibacterial surface which can be directly applied to PVC endotracheal tubes.

Experimental Methods
PVC was modified with 4-mercaptopyridine in a DMSO:H\(_2\)O cosolvent system and subsequently quaternised with haloalkanes to produce QAC-decorated surfaces. FTIR-ATR was used to confirm and quantify the attachment of 4-mercaptopyridine to PVC. QAC-surfaces were characterised using water contact angle analysis, thermal analysis, tensile strength and QAC leaching studies. The ability to prevent protein adherence was assessed using bovine serum albumin. Antimicrobial performance against methicillin-resistant Staphylococcus aureus (ATCC 33593), Pseudomonas aeruginosa (LMG679) was evaluated using anti-adherence assays, disk diffusion assays and fluorescent staining of biofilms.

Results and Discussion
FTIR-ATR analysis confirmed the covalent attachment of 4-mercaptopyridine to PVC with an average graft density of \(\sim 18\) µg cm\(^{-2}\). Attachment of a pyridine ring was confirmed by the appearance of a strong peak at 1574 cm\(^{-1}\). Subsequent quaternisation of PVC-pyridine surfaces with bromoalkanes was confirmed with FTIR-analysis by the loss of the peak at 1575 cm\(^{-1}\) and the emergence of a new peak at 1626 cm\(^{-1}\) as shown in Figure 1. Covalent bonding of QAC structures led to increased hydrophobicity of PVC surfaces from 75° for unmodified PVC to 85-89° for modified surfaces. A decrease in thermal stability of modified PVC films was observed but all QAC-films were thermally stable to at least 160°C confirming their compatibility with standard heat sterilisation techniques. Leaching studies over 14 days detected no release of pyridinium salts demonstrating the stability of their covalent attachment to PVC.

Reductions in bacterial adherence to PVC modified with QACs compared to control were observed as shown in Figure 2. Surfaces displayed significant inhibition of S. aureus adherence compared to P. aeruginosa with maximum reductions of 99.4% and 75.5%, respectively after exposure to \(10^6\) CFU/mL for 24 h. These initial results demonstrate the potential of covalent attachment of cationic moieties to PVC as a preventative method against VAP development. The reduced efficacy against Gram negative bacteria is likely caused by differences in the ability of tethered pyridinium structures to effectively disrupt the bacterial cell membranes of Gram positive and Gram negative bacteria.
Further studies will aim to optimise the performance against Gram negative bacterial biofilms through introduction of more complex cationic structures on the PVC surface.

**Conclusion**

QAC-tethered PVC surfaces display broad-spectrum resistance to biofilm formation. The surface modification technique can be directly applied to PVC device surfaces without adversely affecting bulk thermomechanical properties and could be useful for the prevention of medical device-releated infections, such as VAP. Further studies on biocompatibility will be performed to fully determine their applicability to VAP prevention.

**Acknowledgement**

The authors would like to acknowledge the Engineering and Physical Sciences Research Council (EPSRC) for funding this project.

---

**Figure 1**

Infrared absorbance spectra of PVC surfaces functionalised with pyridine and pyridinium moieties. Black line indicates the presence of a pyridine ring and the red line indicates the presence of a quaternised pyridinium structure.

**Figure 2**

Percentage reduction in adherence, compared to unmodified PVC, when challenged with $10^6$ CFU/mL of *S. aureus* and *P. aeruginosa* for 24 h at 37°C.

---

**References**


Nanofibers functionalized with lignocellulosic-based nanoparticles to fight prevalent pathogens colonizing diabetic foot ulcers

Joana Domingues1,2, Maria O. Pereira2, Helena P. Felgueiras1, Joana C. Antunes1

1 University of Minho, Centre for Textile Science and Technology (2C2T), University of Minho, Campus de Azurém 4800-058 Guimarães, Portugal, Guimarães, PT; 2 University of Minho, Centre of Biological Engineering (CEB), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal., Braga, PT

Introduction
Non-healing diabetic foot ulcers (DFUs) are a common and costly complication of diabetes, leading to high limb amputation prevalence worldwide. Infection hinders wound healing initiation, particularly in severe and refractory cases, therefore effective solutions are greatly needed. Persistent pathogens such as Staphylococcus aureus and Pseudomonas aeruginosa are the main microbial inhabitants of infected DFUs, often gaining antimicrobial resistance to treatment [1]. Nanoparticle (NP)-mediated therapies may overcome this problem, as they are able to carry and protect loads from biodegradation, be internalized by the cell, and release the load(s) in a controlled manner [2,3]. As payloads, plant-derived essential oils (EOs) exert quick and strong bactericidal action. To achieve a more localized and sustained delivery of EOs, NPs can also be incorporated onto nanofibrous structures to act as wound dressings, topically controlling infections and accelerating wound healing. Hence, this work proposes EO-encapsulation into versatile and easily scaled-up polyelectrolyte complexed (PEC) NPs, produced from natural, renewable, and bactericidal polymers [quaternized cellulose (QC) and carboxymethyl lignin (CML)] and their incorporation onto electrospun nanofibrous mats, for effective treatment of infected chronic wounds such as DFUs.

Experimental Methods
The antimicrobial activity of laurel, oregano and dill EOs was screened for the first time against reference strains of S. aureus and P. aeruginosa, by minimum inhibitory concentration (MIC), following the broth microdilution method, and time-kill kinetics [1,4]. Glycidyltrimethylammonium chloride was added to microcrystalline cellulose to obtain QC. CML was obtained through reaction with monochloroacetic acid to softwood kraft lignin and served as anionic counterpart. EOs were added to the anionic polymer before complexation, then added to polycation and ultrasonicated to form EO-loaded QC/CML PEC NPs. Dialysis and condensation purified NP dispersions, and the release profile of loaded EOs was monitored by UV-Visible spectroscopy (UV-Vis). NPs were characterized through dynamic and electrophoretic light scattering (DLS and ELS), and transmission electron microscopy (TEM). Antimicrobial activity of loaded PEC NPs was confirmed through the determination of agar diffusion and time-kill kinetics assays, against S. aureus (ATCC 6538) and P. aeruginosa (ATCC 25853) [1,5]. Free EOs, polymer derivatives and unloaded PECs were also examined (controls). Polyvinyl alcohol (PVA) and polycaprolactone (PCL) solutions were prepared individually in acetic acid/chloroform and dimethylformamide (DMF), respectively, and blended at 3:1 ratio. These polymeric solutions were then processed by electrospinning at 21 cm distance of collector, using 18G needles, at potential 15-30 kV, and 0.4-3.0 mL/h of feeding rate. PVA/PCL electrospun mats were characterized by scanning electron microscopy (SEM), and Fourier-transform infrared spectroscopy (FTIR). Mats were loaded with EO-PEC NPs at 2x and 5xMIC for 72h, and the presence of the NPs was confirmed along the mats trough UV-Vis and SEM/TEM. EOs release profile in simulated body fluid was also monitored via UV-Vis, up to 24h. Thermal behavior of loaded mats was analyzed through thermogravimetry (TGA) and differential scanning calorimetry (DSC) [1,4]. The antimicrobial activity of EO-PEC NPs-loaded mats was tested by agar-well diffusion assay (halo formations) and time-kill kinetics studies up to 24 h of culture [4].

Results and Discussion
Page 1817 of 2028
PEC NPs nano-scaled size, narrow polydispersity index and positive zeta potential were verified by DLS and ELS, with round shaped morphology confirmed through TEM. EO encapsulation efficiency and loading content, NP stability (after freeze-drying), and load release were monitored by UV-Vis, DLS and ELS. The antimicrobial activity of EO-loaded PEC NPs was confirmed through time-kill kinetics and agar-well diffusion assays against *S. aureus* and *P. aeruginosa*. Electrospinning conditions and solvent concentrations were optimized to obtain uniform, continuous, bead-free nanofibers, with a flexible structure. The presence of the polymers in the produced electrospun mats was confirmed by FTIR [1,4]. Also, bright field and SEM were used to attest the uniformity of a nanofibrous polymeric structure. The presence of PEC NPs was verified along the mats through SEM/TEM. The sustained and controlled release of the entrapped EOs was also established in physiological media. TGA and DSC data attested the thermal stability of the loaded mats [1,4]. EO-loaded PEC NPs nanofibers showed promising antimicrobial activity against the aforementioned bacterial strains, but with less impact as expected since access to the wound is limited by nanofibers.

**Conclusion**

Preliminary data pointed out the potential of EO-loaded PEC NP-containing nanofibers to work as an alternative wound dressing to fight some of the pathogens colonizing DFUs.

**Acknowledgement**

Authors acknowledge the Portuguese Foundation for Science and Technology (FCT) for supporting the projects with reference PTDC/CTM-TEX/28074/2017 and UID/CTM/00264/2021. JD is also grateful for the FCT PhD grant 2020.07387.BD.

**References**


Use of piezoelectric Poly-L-lactic acid (PLLA) film to fight bacteria growth

Lea Gazvoda (Udovč)¹², Matjaž Spreitzer¹, Marija Vukomanović¹

¹ Jožef Stefan Institute, Advanced materials Department, Ljubljana, SI; ² Jožef Stefan International Postgraduate School, Ljubljana, SI; ³ Jožef Stefan International Postgraduate School/ Jožef Stefan Institute, Advanced materials department, Ljubljana, SI

Introduction

Bacteria developing resistance to antibiotics motivate the research into finding new approaches to fight against it. One alternative method is to exploit piezoelectric property of soft polymer film, which is generally directed towards the wound healing process and cell stimulation growth. The need for electrostimulation of the wounded tissue also brings the possibility of infection when implants are inserted. It is important to access the issue of bacteria present, therefore determining the effect that electrostimulation can provide on present bacteria.

Our group is preparing biodegradable piezoelectric PLLA film, which could enhance the healing of the wound and at the same time prevent bacteria growth. A simple method like “template wetting” can be used to prepare nano-texture polymer films with stretched and crystalline nanotubes [1], which is a requirement for a polymer to exhibit piezoelectricity [2], with improved cell adhesion when compared to smooth films.

Experimental Methods

Anodised aluminium oxide plate (AAO) with well-determined nano-sized pores is used as a template, in which polymer is imprinted from the melt state. Collected nano-texture film is amorphous (no-piezoelectric) and is afterward annealed to crystalize and express piezoelectric properties.

Antibacterial test was performed in saline solution with bacteria and nano-texture film (crystalline and amorphous) in contact for 24h. Dilutions were transferred on agar plate for colony counting to compare bacterial survival with or without US stimulation (30 min at start).

Results and Discussion

Cellular weight can activate piezoelectric properties on nano-texture film [3], however to increase electric stimulation, US was used to enhance piezoelectricity of the film. Different behaviours were observed, depending on the bacteria strain used. We observed a high antibacterial effect (6 log reduction) with E. coli bacteria when in contact with piezoelectric nano-texture films when piezoelectricity was activated using US. Smaller but significant effect was observed for S. epidermidis, where 3 log reduction was confirmed. However, with one important difference, only when no US was used on piezoelectric nano-texture films, the drop in bacteria count was evident. This also suggests increased bacteria growth for S. epidermidis when US is used (higher electric stimulation), therefore some undamaged bacteria were observed with SEM. When bacteria were put into the growth medium after the test, a delay in bacteria growth was evident, which is in agreement with CFU count results.

Conclusion

By exploiting the piezoelectric properties, a charge on the surface is generated, which plays a key role in the healing process of injured tissues and also influences the bacteria growth. Therefore, we expect that prepared nano-textured PLLA films are piezoelectric and can stimulate human cell growth while providing protection against bacteria.
Nano-texture film preparation and antibacterial properties.
(a) Nano-texture film: concept of preparing from AAO template with optical and SEM images; SEM images and counted bacteria colonies after 24h with nano-textured films from (b) E. coli bacteria and (c) S. epidermidis bacteria.

References
**Antibacterial effect of Ti6Al4V modified with Zn-incorporated or Cu-incorporated TiO$_2$ nanotube arrays**

**Bruno Ribeiro**$^1$, Ruben Offoiach$^2$, Claudia Moneiro$^{3,4}$, Elisa Salatin$^1$, M. Cristina L. Martins$^{3,4}$, Ana P. Pêgo$^4$, Lorenzo Fedrizzi$^2$, Maria Lekka$^5$

$^1$ Lima Corporate, S.p.A, Research & Innovation Department, San Daniele del Friuli, IT; $^2$ University of Udine, Polytechnic Department of Engineering and Architecture, Udine, IT; $^3$ University of Porto, i3s - Instituto de Investigacao e Inovacao em Saude, Porto, PT; $^4$ University of Porto, INEB - Instituto Nacional de Engenharia Biomedica, Porto, PT; $^5$ CIDETEC, Surface Engineering, Parque Científico y Tecnológico de Gipuzkoa, ES

**Introduction**

Surface modification of Ti6Al4V (ASTM grade 5), through the fabrication of vertically oriented TiO$_2$ nanotubes, has received an increasing attention in the field of medical implants. Ti gr.5 has been widely used in both orthopedic and dental implants for over 5 decades, mostly due to its mechanical properties, corrosion resistance and overall biocompatibility. Nevertheless, it is a bioinert material and thus, even though it does not trigger an immediate immune rejection or any remarkable toxic effect in the body, it is also not able to actively drive osteointegration or prevent bacterial colonization by its own. On the other hand, vertically oriented, hollow nanotubular structures of TiO$_2$ render the surface of Ti grade 5 more attractive to cell attachment and tissue growth while simultaneously, can act as reservoirs that allow a controlled local release of antimicrobial agents. In this regard, the electrochemical anodization of Ti6Al4V is a simple and effective surface treatment that has the potential to improve both osteointegration and antimicrobial activity.

The purpose of this work is to develop an antibacterial surface modification on Ti gr.5, through the fabrication of TiO$_2$ nanotubes followed by incorporation of Zn or Cu into the nanotubular structures. These inorganic compounds are highly regarded due to their broad-spectrum antimicrobial action and osteogenic properties, therefore, being of interest for orthopedic applications.

**Experimental Methods**

TiO$_2$ nanotube arrays were grown on the surface of Ti6Al4V alloy through an electrochemical anodization in a non-aqueous, ethylene glycol-based electrolyte containing 0.5 wt.% of NH$_4$F and 2.5% V H$_2$O. In a first step, the anodization process has been optimized in order to obtain well-defined nanotubular structures with specific morphological features. A post-anodizing heat treatment was then performed so that a crystalline anatase oxide layer was achieved, with a higher mechanical stability. Following these treatments, Zn or Cu were incorporated into the nanotubular structures through an electrodeposition process. The morphology of the obtained surfaces was analysed through FESEM and the composition through EDXS.

The antimicrobial performance of the obtained surfaces was assessed against *Staphylococcus Epidermidis*, a gram-negative bacteria common in implant-related infections. Bacterial viability and adhesion were determined in phosphate-buffered saline through the determination of colony forming units.

**Results and Discussion**

Zn-incorporated nanotubes led to a significant reduction of both bacterial viability in the surrounding media and bacterial adhesion to the sample surface while Cu-incorporated nanotubes led to 100% loss of both bacteria viability and adhesion.

**Conclusion**

TiO$_2$ nanotubes have the potential to act as vectors for local drug release. Zn or Cu-incorporated nanotubes are effective active antibacterial surface modification towards *S. Epidermidis*. 

Page 1821 of 2028
Acknowledgement
This work has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 794977.

Figure 1
(A) bacterial viability (log (CFU ml⁻¹)) and (B) bacterial adhesion (log (CFU cm⁻²)) obtained on non-anodized, mirror-like polished titanium (Ti), anodized titanium (TNT), Zn-incorporated TNT (Zn) and Cu-incorporated TNT (Cu). Statistical significant differences are indicated, against Ti samples, as *p<0.1, *** p<0.001 and **** p<0.0001.
Development of novel peptides and methods for the functionalisation of polymer, metal and ceramic medical surfaces

Klaudia O. Sobczak1, Pietro Riccio2, Diana Gomes2, Mohadeseh Zare2, Laura Colomina3, Edwige Meurice1, Anne Leriche1, Artemis Stamboulis2, Antonella Bandiera3, On behalf of Horizon 2020 Marie Sklodowska Curie ITN - Antimicrobial Integrated Methodologies for Orthopaedic Applications under the grant agreement 861138

1 Université Polytechnique Hauts-de-France, LMCPA, Cambrai, FR; 2 University of Birmingham, School of Metallurgy and Materials, Birmingham, GB; 3 University of Trieste, Life Sciences Department, Trieste, IT

Introduction
The post-operative complications related to orthopaedic surgeries are constantly increasing due to implant-associated infections, especially those associated with antibiotic resistance. This has resulted in the need for revision surgeries, causing an increase in health care costs and higher morbidity and mortality rates. Moreover, the ageing population leads to an increase in orthopaedic procedures, raising the probability of post-operative complications. Hence, there is an urgent need to develop antibacterial agents incorporated directly into the implants and maintain antibiotic resistance. The Horizon 2020 Marie Sklodowska Curie ITN Antimicrobial Integrated Methodologies for orthopaedic applications (AIMed) project brings together a network of 12 universities and six industrial and academic partners, the aim of which is to develop novel biomaterials for use as orthopaedic implants to target the problems mentioned above and train 15 Early Stage Researchers in antimicrobial methodologies.

Experimental Methods
The project will focus on developing novel peptide sequences based on human defensin domains, which are part of the human innate immune system. They exhibit a broad antibacterial activity and are less prone to induce resistance behaviour. One of the main aspects of AIMed is a cost-effective large-scale production of new biomaterials to be used by the healthcare sector and readily available to the end-users. For this purpose, the human defensin-like peptides will be designed based on computational models to optimise their biological activities. They will be produced via chemical synthesis and molecular biology procedures to establish the most efficient method that results in the highest purity end product.

The new defensin-like peptides will be fused with the human elastin-like polypeptide and bacterial collagen to form new biopolymers, which will mimic the natural extracellular matrix. These novel molecules will be grafted on implant materials to increase their biocompatibility and bioactivity. The resulting peptides will also be immobilised on the metallic, ceramic and polymeric implants using plasma surface functionalisation with primary amines. These amine groups act as anchor points to covalently graft biomolecules.

Results and Discussion
The resulting biomaterials will allow for the optimised and localised release of the antimicrobial agent, delivered directly to the infection site, without affecting the systemic microbiota. Different functionalisation methods will allow for implant integration with natural bone, enhancing bone tissue regeneration and antibacterial behaviour. The developed techniques will also take into consideration additive manufacturing applications.

Conclusion
This project intends to deliver novel materials suitable for future implant interventions, reducing the burden of post-operative infections and taking the pressure off the healthcare systems.

Acknowledgement
This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement AlMed No 861138
PS2-11-358

Development of novel copolymers to resist bacterial attachment to medical devices and surfaces.

Nicola McClelland¹, Colin McCoy¹, Louise Carson¹, Jessica Moore¹

¹ Queen’s University Belfast, School of Pharmacy, Belfast, GB; ² Engineering and Physical Sciences Research Council, EPSRC, Swindon, GB

Introduction
Polymeric biomaterials can be introduced into the body as part of an implanted medical device or used to replace an organ or bodily function and are increasingly ubiquitous in healthcare (1). The introduction of biomaterials into the body, however, brings with it a risk of infection from microorganisms (2). This infection represents a major risk to patients and financial burden to healthcare systems. With infection rates approaching 100% in some devices, there is an urgent need to develop ways to prevent bacterial biofilms forming on the surface of medical devices (3). Typically, infection involves initial attachment of bacteria to the surface of the biomaterial, followed by colonisation of the surface and development of a biofilm (2). This biofilm is highly resistant to treatment by antibiotics, and acts as a reservoir for further spread of infection in the body (4). This can lead to sepsis and death.

Using synthetic chemistry this research will develop polymers using new ‘building blocks’ that will provide a novel coating to current medical device materials. By selectively tuning the chemistry of these units, polymers can be engineered to inherently resist bacterial attachment (5). Herein, we report the anti-adherent properties of copolymers ethylene glycol di-cyclopentenyl ether acrylate-co-diethylene glycol methyl ether methacrylate (p(EGdPEA-co-DEGMA)) and ethylene glycol di-cyclopentenyl ether acrylate-co-2-hydroxyethyl methacrylate (p(EGdPEA-co-HEMA)). The ability of these materials to resist bacterial adherence, compared to a unplasticised polyvinyl chloride (UPVC) control, will be assessed.

Experimental Methods
Copolymer films were synthesized by mixing p(EGdPEA), p(DEGMA) and p(HEMA) to the appropriate ratio. The resulting solutions were injected into moulds and polymerised for 2 h at 90°C. Then, 1 cm diameter p(EGdPEA-co-DEGMA), p(EGdPEA-co-HEMA) and UPVC samples were cut and inoculated with 10⁶ CFU/mL Escherichia coli and Staphylococcus aureus suspensions, which were prepared in a 0.5% v/v tryptic soy broth-supplemented phosphate buffered saline solution. The inoculated samples were incubated at 37°C in an orbital incubator (100 rpm) for 4 and 24 h. Microbial adherence was quantified after 4 and 24 h.

Results and Discussion
S. aureus was more susceptible to the anti-adherent properties of the copolymers compared to E. coli with both p(EGdPEA-co-DEGMA) and p(EGdPEA-co-HEMA) reducing the number of adherent bacteria to below the detectable limit by 24 hours. In comparison, the p(EGdPEA-co-DEGMA) copolymer produced a 47% reduction; the p(EGdPEA-co-HEMA) copolymer produced an 88% reduction in adherent E. coli compared to UPVC at 24 hours.

Conclusion
Overall, the results confirm the strong capacity of novel copolymers to resist bacterial adherence over 24 h relative to control. These novel copolymers, with their inherent anti-adherent properties, have the potential to be further developed to potentiate their anti-infective properties.

Acknowledgement
The authors would like to acknowledge the Engineering and Physical Sciences Research Council (EPSRC).
Figure 1
Mean (± SD) microbial adherence (%) relative to control UPVC of *E. coli* on p(EGdPEA-co-DEGMA) and Ep(EGdPEA-co-HEMA) after 4 and 24 h incubation at 37°C in 0.5 % v/v TSB, (n=5).

Figure 2
Mean (± SD) microbial adherence (%) relative to control UPVC of *S. aureus* on p(EGdPEA-co-DEGMA) and p(EGdPEA-co-HEMA) after 4 and 24 h incubation at 37°C in 0.5 % v/v TSB, (n=5).

References
Antimicrobial coatings for the prevention of catheter-associated urinary tract infections

Jane Burns, Colin McCoy, Nicola Irwin

Queens University Belfast, School of Pharmacy, Belfast, GB

Introduction
Catheter-associated urinary tract infections (CAUTIs) attribute to almost 80% of nosocomial infections worldwide. One of the main difficulties associated with CAUTIs that make them problematic to treat is device encrustation. Encrustation occurs due to colonisation of the catheter surface by urease-producing bacteria, mainly Proteus mirabilis, and leads to catheter lumen blockage (1). Up to 50% of all long-term catheterised patients experience recurrent infections and blockages, resulting in urine retention, septicaemia and pyelonephritis (2). Furthermore, many uropathogens have shown resistance to common antibiotics (3). Weak organic acids (WOAs) have been used as naturally occurring preservatives for centuries and are reported to exert their antimicrobial activity by the flow of unionised molecules through bacterial cell membranes. The reduction in intracellular pH due to accumulation of acidic anions and hydrogen ions causes damage to enzymes, increase in turgor pressure and oxidative stress, thereby inhibiting metabolic processes (4). Previously, the WOAs (citric acid, mandelic acid, malic acid, propionic acid, lactic acid, hippuric acid, benzoic acid and pyruvic acid), antibacterial activity, effect on P. mirabilis ATCC 51286 crystalline biofilm formation and efficacy against catheter blockages was determined (2). Herein, the efficacy of HEMA:PMMA hydrogel coatings, loaded with citric acid, in resisting bacterial adherence and urinary catheter encrustation is investigated.

Experimental Methods
The antibacterial activity of WOA-loaded hydrogel coatings against common uropathogens, (P. mirabilis ATCC51286, Staphylococcus aureus ATCC 29213, Escherichia coli NSM59 and Pseudomonas aeruginosa679), was investigated through determination of zones of inhibition and adherence assays. In vitro bladder model assays and scanning electron microscopy were performed for determination of bacterial viability, urinary pH, time to catheter blockage and the extent of catheter encrustation.

Results and Discussion
Significant reductions in P. aeruginosa adherence of ~ 99% was observed on citric acid-loaded hydrogel coated silicone samples relative to uncoated silicone controls (Figure 1). Preliminary results show the rate of calcium and magnesium phosphate encrustation around the catheter eyeholes was reduced in all of the catheters coated with WOA-loaded hydrogels, resulting in ~ 1.5-fold increase of catheter time to blockage relative to control (Figure 2, 57.11 h and 41.22 h respectively).

Conclusion
The multi-mechanistic, infection-resistant hydrogel coatings identified present a new strategy for the prevention of P. mirabilis crystallisation and are anticipated to play an important role in the prevention of CAUTIs.
Figure 1
Adherence (%) of *P. aeruginosa* to hydrogel- and citric acid-loaded hydrogel coated silicone samples relative to uncoated silicone controls after 4 h and 24 h incubation at 37ºC. Error bars represent mean values ± standard deviation (n = 5).

Figure 2
The time taken for catheter blockage of uncoated control catheters and catheters coated with WOA-loaded hydrogel coating (HC), when supplied with *P. mirabilis*-infected artificial urine.

References
Exploring the effect of pH on the antimicrobial activity of weak organic acids against common uropathogens.

May A. Tayyem, Nicola Irwin, Colin McCoy, Louise Carson

Queen's University Belfast, School of Pharmacy, Belfast, GB

Introduction
Urinary catheters are the most commonly used medical devices around the world. Although these devices are deployed to treat several conditions, their use is often complicated by infection where the formation of biofilms on catheter surfaces is a key element. Therefore catheter-associated urinary tract infections (CAUTIs) are among the most common nosocomial infections [1]. Colonization with urease producing species such as Proteus mirabilis induces an increase in urine pH, precipitating struvite crystals and the formation of crystalline biofilms resulting in catheter encrustation. Several approaches have been developed to prevent CAUTIs including the modification of catheter surfaces to offset the formation of crystalline biofilms or modulating urinary pH [2]. Weak organic acids (WOAs) have been employed as preservatives in food manufacturing for centuries as antimicrobial and antifungal agents. WOAs are reported to exert their antimicrobial activity by the flow of unionized molecules by simple diffusion through bacterial cellular membranes [3]. The accumulation of acid into the cytosol results in reduced intracellular pH, due to the accumulation of ionised anions and protons causing inhibition of bacterial growth through the inhibition of bacterial enzymes and glycolysis, and metabolic processes. Moreover, oxidative stress causes disruption of the integrity of cellular membrane [4]. Since extensive dissociation of WOAs are affected by the pH, the aim of this study was to investigate the effect of pH on the activity of WOA to inform potential employment of these agents as novel alternatives to antibiotics for the prevention and treatment of CAUTIs.

Experimental Methods
The antimicrobial activity of WOAs (citric acid, lactic acid, pyruvic acid, hippuric acid, benzoic acid, mandelic acid, propionic acid and malic acid), against common uropathogens (Proteus mirabilis ATCC51286, Pseudomonas aeruginosa 679, Escherichia coli NSM59 and Staphylococcus aureus ATCC29213) was investigated through the determination of minimum inhibitory concentration (MIC) values and minimum bactericidal concentration (MBC) values at normal (pH 6) and infected (pH 9) urine pH levels in Mueller Hinton Broth (MHB). Time-kill assays were conducted to evaluate the antimicrobial properties of pyruvic and citric acids against P. mirabilis in in vivo relevant conditions, in MHB at pH 6 and 9 and in artificial urine media.

Results and Discussion
Reduced bactericidal activity was demonstrated for WOAs at elevated pH levels due to dissociation of the acid molecules. Nevertheless, pyruvic and citric acids exhibited rapid killing in MHB at pH 6, with approximately three-log reductions after 1 h and 3 h incubation, respectively. In artificial urine media pyruvic acid achieved three-log reductions of the viable count after 1 h in comparison to citric acid which demonstrated bacteriostatic activity against P. mirabilis over the 24 h incubation period. On the other hand, pyruvic acid needed more than 12 h to exert its bactericidal activity when added to previously infected artificial urine media with P. mirabilis. This can be explained by the relation between the antibacterial activity of WOAs and pH, as WOAs cross the cell-membrane in their undisassociated form, ionise in the cytoplasm, exerting oxidative stress and affecting bacterial growth [4, 5].

Conclusion
Based on the promising antibacterial activity of WOAs in artificial urine, these agents are anticipated to play an important role in the prevention and treatment of catheter-associated urinary tract infections, as an alternative to conventional antibiotics thus preventing the emergence of resistant bacteria. Future work will focus on the effect of
these agents against urinary pathogens under in vivo representative conditions in multispecies communities. This includes the determination of multispecies interaction under static and dynamic conditions and the susceptibility of multispecies communities to WOAs.

Acknowledgement

I gratefully acknowledge my group in the biomaterial-related infections team and all the staff of the prep room who helped in the preparation of media.

References


Biobased polymeric network designed for lavender oil encapsulation

Aurica P. Chiriac, Loredana E. Nita, Alina G. Rusu

Petru Poni Institute of Macromolecular Chemistry, Department of Natural Polymers, Bioactive and Biocompatible Materials, IASI, RO

Introduction
The present study is devoted to the preparation of an amphiphil polymeric network for the entrapment of an essential oil. Poly(ethylene brassylate-co-squaric acid) (PEBSA), a biobased compound, was synthesized through a reaction of ethylene brassylate macrolactone ring-opening and copolymerization with squaric acid. The copolymer was evaluated for hydrophilicity and confirmed a balance between hydrophilic and hydrophobic character. In this context, lavender essential oil was encapsulated in the polymer matrix.

Experimental Methods
The encapsulation of lavender bioactive compound into the PEBSA polymeric matrix was realized by a co-precipitation technique, realized by an inclusion complexation performed in DMSO by entrapping the essential oil into the amphiphilic PEBSA network. The encapsulation yield as well as the antimicrobial character was evaluated by specific methods.

Results and Discussion
The complex formation between PEBSA and lavender oil was confirmed by physicochemical characterization. Thus, spectroscopic analyses confirmed the chemical structure of the copolymer and of the new compound, and the microscopy analyses illustrated the homogeneous aspect of the copolymer network and as well of the bioactive structure. The antimicrobial activity of the complex was also investigated.

Conclusion
The study confirmed the preparation of PEBSA/lavender oil bioactive complex. The antimicrobial activity of the complex investigated against eight different reference strains, namely bacterial strains - *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25922, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 10031 and *Salmonella typhimurium* ATCC 14028, yeast strains represented by *Candida albicans* ATCC10231 and *Candida glabrata* ATCC 2001, and the fungal strain *Aspergillus brasiliensis* ATCC9642, proved an increased efficiency of the PEBSA/lavender oil complex against the bacterial strains represented by *S. aureus, E. coli, E. faecalis, K. pneumoniae*, and against the fungal strain represented by *A. brasiliensis*, and confirmed the advantage brought by using PEBSA matrix for coupling the essential oil.

Acknowledgement
This work was funded by a grant of the Romanian Ministry of Education and Research, CNCS-UEFISCDI, through the research project number 562 PED / 2020, PN-III-P2-2.1-PED-2019- 2743 “Advanced 3D intelligent magnetic supports for bone tissue engineering and regeneration”, within PNCDI III.

References
Nanoscale cerium oxide as a potential antibacterial agent

Neelam Iqbal¹, Antonios Anastasiou², Zabeada Aslam¹, Mostafa Raif³, Thuy Do³, Peter Giannoudis⁴, Animesh Jha¹

¹ University of Leeds, School of Chemical & Process Engineering, Leeds, GB; ² University of Manchester, Department of Chemical Engineering and Analytical Science, Manchester, GB; ³ University of Leeds, School of Dentistry, Leeds, GB; ⁴ University of Leeds, Academic Department of Trauma & Orthopaedic Surgery, School of Medicine, Leeds, GB

Introduction

Post-operative infections can lead to compromised bone healing and non-union if not managed properly. The treatment of infection is complex often involving parenteral or systemic antibiotic drug administration, and in extreme cases, bone and tissue debridement is required due to the compromised blood circulation [1]. Although the systemic delivery of antibiotics is common practice for treating bone-related infections, the broad overuse of antibiotics has led to multidrug-resistant bacterial strains [2, 3], which now require higher and prolonged doses of multiple antibiotics. To tackle this problem, we aim to design and fabricate scaffolds for localised delivery of antibacterial agents which will prevent the occurrence of infections and potentially accelerate the healing and remodelling of hard tissue. Due to the growing urgency to minimise the dependency on antibiotic drugs, alternative treatment strategies, including the use of nanoparticles, have attracted significant attention. Nanoparticles (NPs) are emerging as novel antibacterial agents which have proven their effectiveness for treating infectious diseases [4] as due to their large surface area to volume ratio they can exhibit special properties which their bulk counterparts do not. In the present work, cerium oxide nanoparticles (Ce⁴⁺ and Ce³⁺) have been selected due to their reported antibacterial potential and the potential to enhance vascularisation [5].

Experimental Methods

Cerium oxide nanoparticles were formed using a hydroxide mediated approach. A 0.3M aqueous solution of sodium hydroxide was added dropwise to 0.1M aqueous cerium nitrate hexahydrate solution at 25°C. The mixture was left under continuous stirring for 24 hrs. The nanoparticles were collected by filtration and washed several times with distilled water and ethanol. Type-A NPs were subsequently frozen at -80°C for 24 hrs and then placed into a freeze dryer for 24 hrs. Type-B NPs followed the same procedure, however, after the nanoparticles were filtered and washed they were placed into a furnace at 80°C for 24 hours to dry. The nanoparticles were calcined at/above the phase change temperatures 280°C, 385°C, and 815°C identified by the Simultaneous Thermal Analysis technique.

Results and Discussion

Synthesis of cerium oxide nanoparticles (4 nm to 53 nm) was confirmed by the XRD, BET surface area, and TEM analysis. Interestingly, the drying method (i.e., freeze-drying or furnace drying) and calcination temperature significantly affected the physicochemical properties, i.e. size, shape, agglomeration, and the oxidation ratio Ce⁴⁺:Ce³⁺. The increased calcination temperatures from 280°C to 815°C caused the size of the synthesised nanoparticles to increase; the average particle size grew by eight times 280°C to 815°C (C815) when compared with FRNP nanoparticles. The FRNP, C385 and C815 nanoparticles exhibited antibacterial properties against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus epidermis. The FRNP nanoparticles presented significant antibacterial efficacy as compared to the larger size C385 and C815 nanoparticles.

Conclusion

The antibacterial potential of cerium oxide nanoparticles is dependent on the particle size distribution and processing routes controlling the overall available oxygen vacancies in the fluorite crystal structure. The synthesised nanoparticles present a promising alternative to current treatments for bone-related infections.
nanoparticles consisted of particle sizes ranging from 4-53nm. The calcination temperature affected the agglomeration tendency, particle size distribution and the ratio of Ce$^{3+}$:Ce$^{4+}$ oxidation states. The antibacterial efficacy was characterised in the concentration range of 50-200µg/ml and tested against *Escherichia coli*, *Staphylococcus epidermis*, and *Pseudomonas aeruginosa* by determining the half-maximal inhibitory concentration (IC50). The freeze-dried nanoparticles exhibited 18.5±1.2%, 10.5±4.4%, and 13.8±5.8% increased antibacterial efficacy at concentrations of 200µg/ml compared to nanoparticles consisting solely of Ce$^{4+}$ ions.

**Acknowledgement**

The authors would like to acknowledge Mr Mohammed Javed, Dr Ben Douglas and Dr Simon Strafford for the laboratory support at the School of Chemical and Process Engineering, University of Leeds.
Normalized EELs spectra depicting the presence of dual oxidation states of freeze-dried cerium oxide nanoparticles.

References


2:00 p.m. – 3:30 p.m.

Poster floor

**PS2-12 | Nanobiomaterials**
PS2-12-368

Phosphatase Like Fullerene Nanostructures for Bone Regeneration

Gülcihan Gülseren

Konya Food and Agriculture University, Molecular Biology and Genetics, Konya, TR

Introduction
The phosphatase enzyme is a crucial marker for bone formation process and frequently used in bone regeneration studies because of its important role through process of osteogenesis. The model structures in our new design will form the basis for biomineralization as well as catalytic activity. The mineral induction capacity of the enzyme-like structure will be tested with the new design, since the alkaline phosphatase enzyme regulates biomineralization process beside its catalytic activity, and another function -biomineralization- will be provided by a single structure. In this context, osteoinductive and osteoconductive capability of the designed artificial mimic will be tested with in vitro studies following the kinetic demonstration of its biocatalytic activity. At present, artificial catalysis studies are only at the catalytic activity phase and the biological activities of these artificial catalysts were not tested in model cell applications. Our ultimate goal in this work is to develop a biomimetic enzyme structure that can be applied to living cells, mimicking not only the chemical function but also the biological functions of the enzyme. Thus, developed artificial model will be used as a potential alternative to natural enzymes for bone regeneration studies.

Experimental Methods
Fullerene synthesis was performed by conjugation of multiple bioactive sites. The Nuclear Magnetic Resonance, Thermogravimetry, Atomic Force Microscopy and nano Fourier Transform Spectroscopy analysis was used to chemical characterization of synthesized materials. Single molecule size and molecular assembly were shown by the Dynamic Light Scattering method. After particle characterization, the antimicrobial activity of synthesized nanoparticles was tested with enzyme kinetics study. Michaelis-Menten fittings were used for the calculation of kinetic parameters. Synthesis, characterization and kinetics experiments are followed by cell culture experiments for testing osteoregenerative potential of nanoparticles. Osteoprogenitor SaOS-2 cell line was used as a model for osteoregeneration. Biocompatibility of the fullerene-based biomaterial was checked with Alamar Blue viability tests. Alizarin Red Staining was applied for testing the biomineralization capability of the nanomaterial applied cell model. Lastly, osteoinduction was shown by RT-PCR, expression levels of Runx-2 and Col-1 were investigated to show osteogenic differentiation at the gene level.

Results and Discussion
Fullerene synthesis was performed by conjugation of multiple bioactive sites. The Nuclear Magnetic Resonance, Thermogravimetry, Atomic Force Microscopy and nano Fourier Transform Spectroscopy analysis was used to chemical characterization of synthesized materials. Single molecule size and molecular assembly were shown by the Dynamic Light Scattering method. After particle characterization, the phosphatase-like catalytic activity of synthesized nanoparticles was tested with enzyme kinetics study. Michaelis-Menten fittings were used for the calculation of kinetic parameters. Synthesis, characterization and kinetics experiments are followed by cell culture experiments for testing osteoregenerative potential of nanoparticles. Osteoprogenior SaOS-2 cell line was used as a model for osteoregeneration. Biocompatibility of the fullerene-based biomaterial was checked with Alamar Blue viability tests. Alizarin Red Staining was applied for testing the biomineralization capability of the nanomaterial applied cell model. Lastly, osteoinduction was shown by RT-PCR, expression levels of Runx-2 and Col-1 were investigated to show osteogenic differentiation at the gene level. The catalytic activity of phosphatase-like nanoparticles have been shown with the kinetics experiment. Fullerene based biocatalyst have exhibited significant activity in the presence of p-nitrophenyl phosphate which is a model substrate resembling the actual substrate of phosphatase. Biocompatibility

Page 1836 of 2028
was also shown with SaOS-2 cells, synthesized particles have found biocompatible. Fullerene biocatalyst also induced biomineralization of SaOS-2 cells, induced calcium deposition have shown with Alizarin Red Staining, osteoblast-like clusters were obtained by microscopy imaging. Osteoblast differentiation-specific gene expressions were also studied with RT-PCR.

**Conclusion**

In this study, a multi functionalized fullerene nanostructures have been used for bioregenerative application. This phosphatase inspired nanostructure can be employed for both catalytic purposes and osteoinductive/conductive purposes similar to its native counterpart. Carbon nanomaterials are more stable structures compared to biological macromolecules, hence the presented study will be inspirational for future biocatalyst studies with practical applications.

**Acknowledgement**

This project is funded by TUBITAK (The Scientific and Technological Research Council Of Turkey), 218Z021 Career Project

**References**

Detection of mesenchymal stromal cells osteogenic differentiation by SERS

Adrianna Milewska\textsuperscript{1,2}, Olafur E. Sigurjonsson\textsuperscript{3,4}, Kristjan Leosson\textsuperscript{1}

\textsuperscript{1} Innovation Center Iceland, Reykjavik, IS; \textsuperscript{2} University of Iceland, Reykjavik, IS; \textsuperscript{3} The Blood Bank, Landspitali University Hospit, Reykjavik, IS; \textsuperscript{4} Reykjavik University, Reykjavik, IS

Introduction
Mesenchymal stromal cells have been at the epicentre of regenerative medicine and therapeutic applications since their identification, due to their ability to differentiate into various cell types, such as osteoblasts. In vitro, osteogenic differentiation is induced by a cocktail of dexamethasone, ascorbic acid and β-glycerophosphate, however, it strongly depends on the donor-specific osteogenic differentiation potential. Since native MSCs demonstrate such variability, it is possible that their response to experimental manipulation may also vary, so it is of high importance to study cells with minimally invasive methods. The most commonly used procedures for following cell differentiation include e.g. immunofluorescence staining, qPCR, colorimetric assays. However, a label-free method that does not require the termination of the cell culture prior the analysis, is still lacking.

Experimental Methods
Here, we show the direct and label-free approach for studying the extracellular matrix and membrane changes during differentiation in mesenchymal stromal cells with surface enhanced Raman scattering (SERS). We have used SERS as a sensitive tool to study the structure of cellular compounds, providing comprehensive information on the molecules in the nm-scale proximity of gold nanoisland substrates. We fabricated the substrates by repeated gold deposition and thermal annealing, providing sufficient enhancement and a homogenous distribution of “hot spots” [1, 2]. In order to demonstrate their applicability as in-vitro sensing platforms for long-term cell proliferation, we cultured MSCs and recorded spectra of the cellular membrane at different timepoints during differentiation.

Results and Discussion
The results of biological assays indicate that the SERS platforms are biocompatible, suitable for long term cultivation of the cells and in principle do not induce cell death. Moreover, osteogenic differentiation on such substrates is not compromised, which was confirmed with Alizarin Red staining and Alkaline Phosphatase activity assays (Fig. 1). The SERS cell data generated with the use of our SERS-active platforms allowed to identify multiple characteristic molecular compounds of the cells in the close proximity of the gold nanoisland substrates and follow changes of extracellular matrix and calcium phosphate deposition during osteogenic differentiation (Fig. 2).

Conclusion
We have employed simple approach of SERS substrate fabrication. The substrates provide uniform and sufficiently high enhancement to enable molecular characterization at the single-cell level. In conclusion, the possibilities of SERS to follow biochemical and physiological changes in the cell membrane can be used to monitor the differentiation of the mesenchymal stromal cells into osteocytes.
Figure 1. Mineralization during osteogenic differentiation.
Mesenchymal stromal cells (MSCs) on SERS substrates (A-C) and glass controls (D-F) stained with Alizarin Red S on days 7 and 21 of osteogenic differentiation. MSCs cultured in expansion media (A, D) included as controls. Black arrows depict mineralized clusters. Scale bars: 100 µm (A-F). G-H magnified areas of osteogenic cell mineralization on SERS substrates for days 7 and 21. Scale bars: 20 µm (G-H).

Figure 2. Surface-enhanced Raman scattering (SERS) of mesenchymal stromal cells of differentiation
Representative SERS spectra of MSCs during different stages of osteogenic differentiation with highlighted peak at 960 cm\(^{-1}\) assigned to hydroxyapatite (laser excitation, 785 nm, intensity: 3 mW, acquisition time: 3 s, scale bar: 50 cps).

References
Design of microRNA-releasing lipoplexes for efficient reprogramming of adult human cardiac fibroblasts into induced cardiomyocytes in cardiac regeneration strategies

Letizia Nicoletti¹,², Camilla Paoletti¹,², Giulia Tarricone³, Carla Divieto⁴, Ilaria Andreana⁵, Barbara Stella⁵, Silvia Arpicco⁵, Clara Mattu¹,², Valeria Chiono¹,²

¹ Politecnico di Torino, Department of Mechanical and Aerospace Engineering, Turin, IT; ² Politecnico di Torino, POLITO BioMedLab, Turin, IT; ³ Italian Institute of Technology, IIT Central Research Labs Genoa, Genoa, IT; ⁴ National Institute of Metrological Research (INRIM), Division of Advanced Materials and Life Sciences, Turin, IT; ⁵ University of Turin, Department of Drug Science and Technology, Turin, IT

Introduction
Heart disease is the leading cause of death globally. Myocardial infarction (MI) causes a decrease or interruption of blood supply to the cardiac district, leading to a significant loss of contractile cardiomyocytes and tissue remodelling with the formation of a fibrotic scar. Currently, microRNAs (miRNAs) have arisen considerable interest as a therapeutic approach to induce cardiomyocyte proliferation or transdifferentiation of cardiac fibroblasts (CFs) into induced cardiomyocytes (iCMs) in the perspective of cardiac regeneration (1,2). In a recent work, Paoletti et al. reported direct reprogramming of human CFs into iCMs by transfection of CFs with a combination of four microRNAs (miR-1, 133, 208, 499) called miR-Combo using a commercial lipidic transfectant. However, tailored designed nanocarriers are required for safe and efficient administration of reprogramming agents in order to increase direct reprogramming efficiency.

In this work, new lipoplexes composed of a cationic and a helper lipid mixture were designed for efficient encapsulation and delivery of microRNAs to human CFs respect to a commercial agent, aimed at enhancing direct reprogramming efficiency.

Experimental Methods
New lipoplexes based on a mixture of a cationic and a helper lipid and loaded with negmiR, miR-1 or miRcombo were prepared at different N/P ratios changing the amino to phosphate groups ratio (N/P 3; 1.75; 0.7; 0.35) (Figure 1). Lipoplexes were characterized in terms of size, zeta potential and encapsulation efficiency. Physical stability in different media and miRNA release were also evaluated. Biocompatibility, cellular uptake and transfection efficiency were assessed using adult human CFs (AHCFs). Furthermore, miRcombo-loaded lipoplexes were characterized for their physicochemical properties and their influence in miRcombo-mediated trans-differentiation of AHCFs into iCMs based on the expression of cardiac markers, such as cardiac troponin T (cTnT). Lipoplex properties and their biological effects were compared to those of a commercially available transfection agent.

Results and Discussion
Designed lipoplexes displayed an average hydrodynamic diameter increasing from 372 nm to 876 nm and a Z-potential decreasing from + 40 mV to -26 mV with decreasing N/P ratios (3.0-0.35). Lipoplexes encapsulated a high amount of miRNA (99%) and allowed faster miRNAs release than the tested commercial agent. Moreover, they showed long-term stability under storage conditions. Based on stability experiments at different temperatures (4°C and 37°C), lipoplexes with N/P ratio of 3 were selected for further biological characterisations with AHCFs. They showed biocompatibility and significantly enhanced miR-1 expression compared to the tested commercial agent. Lipoplexes loaded with miRcombo significantly enhanced the expression of cardiac markers such as cTnT, compared to the commercial agent.

Conclusion
Page 1840 of 2028
Our results indicate that the new designed lipoplexes are promising for *in vitro* miRNA delivery, showing biocompatibility and efficient miR-1 release to human CFs. Furthermore they improved miRcombo-mediated direct reprogramming of AHCFs into iCMs compared to the control commercial agent suggesting their potential as efficient vectors for direct cardiac reprogramming.

**Acknowledgement**

This project is supported from European Research Council (ERC) under European Union’s Horizon 2020 research and innovation program (grant agreement No 772168).

---

**Figure 1**

Schematic representation of lipoplexes preparation by electrostatic interactions between the cationic lipid and the negatively-charged miRNA.

---

**References**


**PS2-12-374**

The Importance of Protein Corona when determining Nanoparticle-Biological Interactions: How the nanoparticle physicochemical properties change biological interactions in different biological environments

**Medina Guliyeva**, Oran Maguire, Charlie Brown, Amita Gupta, Saeed S. Motevallian, Joel Turner, Gavin Jell

**UCL, Department of Surgical Biotechnology, London, GB**

**Introduction**

Upon exposure to biological fluids a protein corona form around NPs. The formation of protein corona is dependent on the interplay between the physiochemical characteristics of NPs and the characteristics of biological environment (e.g. pH, temperature and the proteinous environment). The composition of the NP protein corona may be an important factor that determines cellular response, for example the concentration of proteins in serum has been shown to influence NP internalisation and active targeting success. In the last 5 years, 97% of in vitro tests concerning nanoparticle-cell interaction studies are performed using foetal bovine serum (FBS). Considering the known differences in the serum protein composition between foetal serum and adult bovine serum (BS) and human serum (HS), little is known about how this affects the cellular interactions or the accuracy of using NP-cellular interaction in vitro models. If the species of the serum influences NP-cellular interactions or the “biological identity”, this will help our understanding of the importance of the NP corona in determining biological interactions and improve the translation of nanomedicine by creating more accurate in vitro models.

**Experimental Methods**

**Size and composition analysis of protein corona.** Samples collected were then used for SDS-PAGE, DLS, LC-MS analysis. For SDS-PAGE analysis protocol used by Walkey et al was applied to investigate the NP protein corona composition. For DLS analysis samples were loaded into the chamber of disposable folded capillary cells (Malvern Instruments) and a Zetasizer Nano-ZS from Malvern Instruments (Zetasizer Nano ZSP, Malvern, England) was used to determine the particle sizes and zeta potentials. LC-MS analysis were performed using the protocol previously described in Lombardi et al. Analysis of cell uptake of nanoparticles. Qualitative analysis of NP uptake was performed using TEM. Human dermal fibroblasts (HDF; P10) and Bovine Dermal Fibroblasts (BDFs P10) were seeded at a cell density of 10^5 million cells/cm². HDFs were left to rest for 24hrs prior to washing with PBS and addition of DMEM (Gibco, high glucose), supplemented with 10% FBS, HS or BS. 1ml of AuNP (10^3 AuNPs/cell) were added to the media and plates were left overnight to allow uptake. Finally, the cells were washed thrice with PBS, fixed, and sectioned. Each section was placed onto a copper grid and imaged by TEM. Quantitative analysis of NP uptake was measured by ICP. HDFs BDFs were seeded at 10^5 million cells/cm². Cell growth medium was supplemented with 10% FBS, HS or BS and left to rest for 24hr. Cells were then treated with AuNPs (10^3 AuNPs/cell). Cell suspension were transferred to 25 ml glass bottles for Aqua Regia (1ml) digestion in the oven for 30 mins and left overnight in the fume cabinet for complete digestion. The final digests were then filtered and quantified using ICP.

**Results and Discussion**

Identification of proteins isolated from AuNP-protein corona formed in different sera. LC-MS analysis revealed that the top 10 abundant proteins isolated from protein corona complexes formed in HS, FBS and BS are...
dissimilar (Figure 2a). Serotransferrin and albumin are only proteins found in all sera (Figure 2a, 2b). Proteins isolated from corona formed in HS, FBS and BS contains 8, 4, and 4 distinct proteins respectively (Figure 2b).

**The uptake of AuNPs in different sera.** TEM and ICP revealed that the cell uptake of AuNPs (1000 NPs/cell) incubated in 10% HS by HDFs was increased by 3 folds compared to the uptake by HDFs which were incubated in 10% FBS (figure 3e). The uptake of AuNPs was also significantly decreased for HDFs incubated in 10% BS.

**The uptake of PEG-AuNPs in different sera.** Despite this lower uptake of NPs, species on sera still effected the biological indent and uptake. PEGylated NPs exposed to FBS had a significantly lower uptake compared to HS or BS (Figure. 4e). The different uptake of AuNPs in different serum conditions was also present in PEGylated NPs, emphasising the importance of selecting a suitable condition for nanoparticle-cell interaction assessments.

**Matching the species of serum to species of cells.** TEM images shows that the number of AuNPs in cells incubated in HS is higher than those incubated in FBS and BS (figure 5b-d). AuNPs internalised by cells in media supplemented by FBS appears ICP analysis revealed that the uptake of AuNPs by BDFs (figure 5e) incubated in HS is significantly higher than cells incubated in FBS (P<0.05). HDFs incubated in HS showed no significant change compared to those in BS (figure 5e). These results confirm the importance of selecting proper biological milieu and cell type for in vitro cellular interaction studies of nanoparticles.

**Conclusion**

Human serum better mimics the composition of the in vivo environment. Successful translation of NP targeting systems is reliant upon accurate modelling NP – cell interactions.

---

[Internalisation and quantification of the uptake of AuNP by HDFs (24 h exposure).]

- **a.** Control cells without nanoparticles. TEM nanographs showing the uptake by:  
  - b. Cells cultured in media containing 10% human serum  
  - c. cells cultured in media containing 10% foetal bovine serum,  
  - d. cells cultured in media containing 10% FBS.  
- **e.** ICP-AES analysis showed significantly increase the uptake of AuNPs by HDFs cultured in HS compared to FBS and BS. Values shown are means of three measurements ± SD. **** p ≤ 0.00005.
LC-MS results showing the composition of protein corona isolated from 16nm AuNP incubated in different conditions:

a) 4 out of 10 Top 10 proteins from AuNP-HS corona was identified to be immunoglobulin fragments. 
b) Venn Diagram revealed that only 2 of the Top 10 abundant proteins from the corona were shared between all three sera.
c) Top 20 proteins isolated from protein corona classified according to their physiological functions.

References
Hazelnut Extract-Loaded Nanostructured Lipid Carriers and Evaluation of Their Antioxidant Properties

Melis Emanet1,2, Ozlem Şen2, Gianni Ciofani2

1 Sabanci University Nanotechnology Research and Application Center (SUNUM), Istanbul, TR; 2 Istituto Italiano di Tecnologia, Smart Biointerfaces, Pontedera, IT

Introduction
In healthy physiological conditions, living organisms possess a variety of antioxidant mechanisms in order to scavenge over-produced reactive oxygen species (ROS). However, under pathological conditions, endogenous antioxidants may not be adequate to eliminate the excessive rate of oxidants [1]. In this case, a continuous exogenous antioxidant income is required, mainly from nutraceutical products, in particular derived from fruits, nuts, spices, vegetables, and mushrooms [2]. In this regard, extensive investigations on hazelnut extracts indicated as their high content in antioxidant compounds make them suitable as excellent nutraceutical agents, especially in neurodegenerative disorders [3].

Experimental Methods
NE-NLCs were prepared by hot homogenization technique using glyceryl dibehenate as solid lipid and oleic acid as liquid lipid, jointly to colloidal stabilizers (DSPE-PEG), surfactants (pooloxamer 188) and NE, as indicated in Figure 1B. The morphology of NE-NLCs has been assessed by transmission electron microscopy (TEM) imaging, while colloidal stability was analysed by dynamic light scattering and ζ-potential measurements. The antioxidant ability of NE-NLCs was investigated using a Total Antioxidant Capacity Assay Kit (Sigma-Aldrich).

Results and Discussion
TEM imaging of NE-NLCs indicates the uniformity of the structures, as shown in Figure 1A. The hydrodynamic size distribution of NE-NLCs resulted to be 210 ± 8 nm, while the ζ-potential -31.7 ± 0.6 mV. The antioxidant ability of NE-NLCs was found concentration-dependent, in the range of 12.1 – 149.4 μM of Trolox equivalent for concentrations in the range of 31 - 1000 μg/mL (Figure 1C).

Conclusion
All considered, the uniformity and the high stability of NE-NLCs in an aqueous environment indicate their suitability for biomedical applications. Besides the physical properties, an excellent concentration-dependent antioxidant ability of NE-NLCs suggests their exploitation in ROS scavenging treatments.
Representative TEM image (a) and schema (b) of NE-NLCs. Characterization of NE-NLCs antioxidant activity expressed as Trolox equivalent (c).

References
**PS2-12-378**

**Enhanced Magnetic Hyperthermia by Tunable Exchange Coupled Magnetic Nanoparticles**

**Valentin Nica, Andrea Desii, Alessio Carmignani, Gianni Ciofani**

*Istituto Italiano di Tecnologia, Center for Materials Interfaces, Pontedera, IT*

**Introduction**

Exchange coupled bi-magnetic nanoparticles have proved very interesting properties for inductive hyperthermia applications due to the quantum synergism between the magnetic core and a magnetically thin shell [1]. The heating ability of the particles is quantified by the specific absorption rate (SAR), an extrinsic parameter related to physio-chemical properties of particles such as composition, shape, size or magnetic shell thickness [2].

**Experimental Methods**

Shaped-anisotropic bi-magnetic nanoparticles have been synthesized by solvothermal route and their structural and magnetic properties have been studied. In the first step, we synthesized several types of nanoparticles as seed component. Secondly, magnetically core-shell nanoparticles were obtained by the seed-mediated route where a shell was over-grown by thermal decomposition onto the surface of the core nanoparticle. The nanoparticle shape, size and shell thickness has been controlled through their compositions, surfactant molar ratio of precursors, heating rate and reaction time. The structure and morphology of the core-shell nanostructures have been demonstrated by Raman spectroscopy, X-ray photoelectron spectroscopy (XPS) selected area electron diffraction (SAED), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Magnetic inductive measurements were performed to assay the heating capacity (i.e. SAR) of colloidal nanoparticles under an alternating magnetic field (AMF) (f=100 kHz, B=20 mT).

**Results and Discussion**

In this study various magnetic core-shell nanoparticles with shape anisotropy have been studied: Co$_{0.6}$Zn$_{0.4}$Fe$_2$O$_4$@CoFe$_2$O$_4$ (CZF), MnFe$_2$O$_4$@CoFe$_2$O$_4$ (MF), Fe$_3$O$_4$@CoFe$_2$O$_4$ (IO) and Fe$_3$O$_4$@Mn$_{0.5}$Zn$_{0.5}$Fe$_2$O$_4$ (MZF). The Raman peaks related to A$_{1g}$ and T$_{2g}$ vibrational modes matched the spinel structure of all studied systems. TEM images showed hexagonal (CZF), octahedron/polyhedral (IO), truncated-octahedron (MF) or cubic-type (MZF) morphology with mean size of particles between 8-15 nm. Dark field-TEM (DF-TEM) and X-ray photoelectron spectroscopy (XPS) results confirmed the formation of bi-magnetic core-shell nanoparticles. The shell thickness has been evaluated between 1-5 nm. For inductive heating measurements, the surface of magnetic nanoparticle was modified to provide water-based colloidal stability with a concentration of 5 mg/ml for all aliquots. The SAR results of core-shell nanoparticles demonstrated the superior heating properties compared to the core ones. This effect is attributed to the exchange-coupled interactions at the core-shell magnetic interface [3]. The SAR values of bi-magnetic samples (63 W/g to 176 W/g) with hexagonal and polyhedral morphologies did not show a strong variation compared with their seeds. Instead, the cubic-shaped system revealed approximately twofold value than the core counterpart. The thickness of the core-shell magnetic nanoparticles on SAR has been controlled by varying the precursor concentration. We observed a non-linear dependence of SAR on the shell thickness increase with the highest heating capacity corresponding at around 2 nm of the layer.

**Conclusion**

We synthesized four type of bi-magnetic nanoparticles with different morphology. The structural analysis demonstrated a spinel-type crystalline structure of all the samples. In the same experimental conditions, the inductive measurements of water-based colloids have shown greater heating rate of magnetically core-shell nanoparticles compared with the magnetic cores due to exchange-coupled spin mechanism occurred at physical interface between...
layers. With respect of nanoparticle mean size, the cubic-shaped nanoparticles (MZF) have proven the highest heating capability ($\text{SAR}=176 \text{ W/g, } f=100 \text{ kHz, } B=20 \text{ mT}$). Thus, the control of SAR can be performed by tailoring the material characteristics in terms of shape, size, composition and core-shell design with different chemical and physical structures. The designed anisotropic bi-magnetic nanoparticles could be used in a variety of systems as heat induction applications like magnetic hyperthermia, drug delivery vehicles or self-healing materials.

**Acknowledgement**

This research has received funding from AIRC and the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 800924.

**References**


Large-scale Superparamagnetic Iron Oxide Nanoparticles Coated with Dimercaptosuccinic Acid for Biomedical Applications

Manuel Estévez¹, Alvaro Gallo-Cordova², Julián Crespo³, Sara García⁴, Juana Serrano⁴, M. Puerto Morales², Montserrat Colilla¹, Montserrat Colilla¹, Blanca González¹, Isabel Izquierdo-Barba¹, María Vallet-Regí¹

¹ Universidad Complutense de Madrid, Departamento de Química en Ciencias Farmacéuticas, Instituto de Investigación Sanitaria, Hospital 12 de Octubre i+12, Plaza Ramón y Cajal s/n, 28040, Madrid, ES; ² Instituto de Ciencia de Materiales de Madrid, ICMM/CSIC, Sor Juana Inés de la Cruz 3, 28049, Madrid, ES; ³ Tecnología Navarra de Nanoproductos S.L. (TECNAN), Área industrial PERGUITA, C/A, Nº1, 31210, Los Arcos (Navarra), ES; ⁴ Fundación Jiménez Díaz, UAM, Experimental Hematology Lab, IIS, 28040, Madrid, ES; ⁵ Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, ES

Introduction
In recent years, Superparamagnetic Iron Oxide Nanoparticles (SPIONs) coated with dimercaptosuccinic acid (DMSA) have been widely described as good candidates for biomedical applications such as targeted drug delivery, magnetic separation, biomedical imaging or hyperthermia due to their low toxicity and excellent magnetic properties [1]. Among the different existing methods to synthesize SPIONs, Flame Spray Pyrolysis (FSP) technology allows large-scale production and control of the size and properties of the nanoparticles [2]. Since most nanoparticles designed for biomedical applications will encounter blood while being transported to the target tissue, it is important to assess their hemocompatibility to ensure their safety for clinical applications [3].

In this study, SPIONs were produced by a FSP process and surface-activated to allow their coating with DMSA. The resulting nanoparticles were characterized in terms of physico-chemical and magnetic properties and a bioanalytical evaluation, including a hemocompatibility study, was performed to explore their potential in biomedical applications.

Experimental Methods
SPIONs were synthesized and optimized employing FSP technology. Briefly, SPIONs were obtained passing an optimized iron-based organometallic precursor mixture in organic solvent through a flame constituted by oxygen and methane. The oxidative conditions of the flame, concentration of the organometallic precursor, ratio precursor/oxygen 0.5 approx., feeding flow and/or gas career were the controlled parameters of synthesis. The particles were further treated with 2 M HNO₃ under agitation for 15 minutes. The supernatant was then removed by magnetic separation and 1 M Fe(NO₃)₃ was added to the particles. The mixture was refluxed under stirring for 30 min and subsequently treated with 2 M HNO₃ and washed with water (3x). To perform the coating, a solution of 15 mg of DMSA in 10 mL of distilled water was added to a suspension of the nanoparticles in 20 mL of distilled water at pH 3 achieving a concentration in Fe of 3 mg/mL. The mixture was gently stirred manually, the pH adjusted to 11 and after 20 min of sonication, it was dialyzed against 5 L of water for 2 days. Finally, the pH was adjusted to 7 and the colloidal suspension filtered through a 0.22 mm filter. The nanoparticles were characterized by Transmission Electron Microscopy (TEM), X-ray diffraction (XRD), Dynamic Light Scattering (DLS) and Fourier Transform Infrared spectroscopy (FT-IR). Concentration in Fe₂O₃ was determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). Magnetic characterization was carried out in a vibrating sample magnetometer (VSM). To determine platelets activation, nanoparticles were incubated with platelet-rich plasma for 20 min at 37 °C in a platelet aggregometer TA-8V (Stago, France). Circulating monocytes activation was analyzed by fluorescence-activated cell sorter (FACS) with human anti-CD14, anti-CD16 and anti-CD11B after 2 h of exposure to the SPIONs. Stained cells were acquired in flow cytometry Canto-II equipped with FACSDIVA™ software (BD, Biosciences).

Results and Discussion
SPIONs between 10 and 18 nm in diameter have been successfully synthesized by FSP and subsequently coated with DMSA obtaining a time-stable colloidal water dispersion. The DLS result showed a great improvement in nanoparticle dispersion after acid treatment and DMSA coating. The infrared spectrum confirmed the DMSA coating. Finally, a biological assessment was performed confirming that our IONPs coated with DMSA failed to induce significant platelets clotting, reaching 0.52% and 4.33% of aggregation in IONPs and IONPs-DMSA respectively (control with platelets receptors agonists was 77.3%). Moreover, increasing in the expression of activating molecules on circulating monocytes such as CD11B was absent in presence of IONPs-DMSA after flow cytometry analysis. Thus, fold increase of CD11B in scaled dose of IONPs vs IONPs-DMSA was: 1.59±0.00014 vs 1.095±0.021 (50 μg/mL), P<0.001; 1.48±0.049 vs 0.88±0.042 (100 μg/mL), P<0.01 and 1.36±0.16 vs 0.99±0.16 (200 μg/mL).

**Conclusion**

Large-scale SPIONs produced by a FSP process have been coated with DMSA for the first time and their characterization and hemocompatibility results demonstrate their potential for biomedical applications.

**Acknowledgement**

This project has received funding from the European Union’s Horizon 2020 research and innovation program under grant agreement No. 814410.

References


Manganese Dioxide-based Nanoreactors for Reduction of Oxidative Stress and MRI Imaging

Soraia V. Lopes1,2, Piotr Walczak3, Miroslaw Janowski4, Rui L. Reis1,2, Joana Silva-Correia1,2, Joaquim M. Oliveira1,2

1 University of Minho, 3Bs Research Group, Guimarães, PT; 2 ICVS/3B’s - PT Government Associate Laboratory, Braga/Guimarães, PT; 3 University of Maryland, 3Department of Diagnostic Radiology and Nuclear Medicine, Baltimore, US; 4 Mossakowski Medical Research Centre, NeuroRepair Department, Warsaw, PL

Introduction
The use of nanoparticles in imaging techniques and therapies targeting inflammation has been increasing due to their small size and easy manipulation [1]. In particular, manganese (Mn2+) based nanoparticles have been greatly explored. This is due to the fact that manganese ions are naturally present in the brain, accumulate in the mitochondria of brain cells and can act as a contrast agent, therefore increasing signal intensity and specificity in magnetic resonance imaging (MRI) [2]. Additionally, manganese ions are able to decompose reactive oxygen species, commonly present in the inflammatory process of neurodegenerative diseases [3]. In this study, we present the development of nanoreactors with applications in cell delivery, Manganese-enhanced MRI (MEMRI), and reducing oxidation in degenerated tissues.

Experimental Methods
Different types of manganese dioxide nanoparticles and blends of methacrylated gellan gum and hyaluronic acid (HA) were investigated. Chemical and morphological analysis were performed by dynamic light scattering, scanning electron microscopy, in vitro degradation, and swelling ability. Round-shaped particles with overall average size of 70 nm were achieved. The addition of different concentrations of particles and different HA ratios do not seem to affect the hydrogels' stability. The in vitro cytotoxicity assessment of the materials was evaluated using immortalized rat lung fibroblast cell line (L929 cells) after 24 and 72 hours of culture.

Results and Discussion
Overall, differences could be seen with the addition of the different nanoparticle types and hyaluronic acid. The presence of the MnO2_S (synthesized MnO2) nanoparticles led to a lower cytocompatibility whereas, the MnO2_C1 (Manganese (IV) oxide ReagentPlus®, ≥99%) type presented the lowest toxicity to cells. Additionally, a lower percentage of hyaluronic acid in the formulations seems to be more favorable for cell proliferation and viability. Looking at the different nanoparticle concentration, for the MnO2_S and MnO2_C1, higher concentrations (75 and 100 ng/mL) do not seem to negatively affect cell viability. The opposite can be seen for the MnO2_C2 (Manganese(IV) oxide activated, ~85%, <10 μm) nanoparticle type.

Conclusion
This work evaluated different types of manganese nanoparticles and identified potential nanoreactor formulations suitable for combined use in MEMRI techniques and for reduction of oxidative stress in tissues.

Acknowledgement
The authors would like to acknowledge the financial support provided through the EC Funded project NanoTech4ALS (ENMed/0008/2015) and the funding provided under the project FROnTHERA (No. NORTE-01-0145-FEDER-000023), supported by Norte Portugal Regional Operational Programme (No. NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). S.V. Lopes thanks to the Portuguese Foundation for Science and Technology (FCT) for the PhD grant (SFRH/BD/143954/2019). The FCT distinction to J. Silva-Correia (IF/00115/2015) under the Investigator FCT program is also greatly acknowledged.
References
PS2-12-384

The Guiding Effect of Self-assembling Hydroxyapatite Nanorods on Bone Regeneration

Jishizhan Chen, Wenhui Song

University College London, Centre for Biomaterials in Surgical Reconstruction and Regeneration, Division of Surgery and Interventional Sciences, London, GB

Introduction

Liquid crystal-like, low-dimensional and long-range ordered structures (LC) are found in human tissues including bones. The excellent mechanical and biological functions of natural bones entail the well-orchestrated nano-scale hydroxyapatite (HAp) crystals within collagen fibres matrix. However, the understanding of the self-assembling of HAp nanocrystals and formation of hierarchically ordered bone structure are still elusive. Our study aims to develop an in situ traceable anisotropic HAp nanorods doped with citrate and terbium (Cit/Tb-HAp NRs), investigate their self-assembling behaviour and illuminate their potential and pathway of guiding ordered osteogenesis at multi-scale in vitro.

Experimental Methods

The Cit/Tb-HAp NRs were synthesised utilising a hydrothermal method. The lyotropic LC behaviours and fluorescence was observed. The toxicity, influence on ALP activity, and traceability of free NRs were initially investigated on NIH 3T3 cells. The large-scale alignment of NRs was achieved by spin coating technic and characterised by optical and electron microscopy. The study on proliferation and osteogenesis differentiation of human bone marrow-derived stem cells (hBMSCs) in response to aligned Cit/Tb-HAp NRs are performed.

Results and Discussion

The Cit/Tb-HAp NRs can self-assemble into a liquid crystalline phase and are then trackable under both polarised light and fluorescence microscopy. It is found that the NR suspension with a concentration of 100 μg/mL or lower is a relatively safe dose for 3T3 cells compared to controls and may be beneficial to osteogenic differentiation. The entry of the NRs into 3T3 cells are visualised directly utilising a confocal microscope. The spin coating affiliates the alignment of NRs at a macroscale, which induces elongation of cells into spindle-like shape and large-scale orientation along the NRs.

Conclusion

This study demonstrates the self-assembly, biosafety, traceability, and large-scale guiding effect of Cit/Tb-HAp NRs. Further study of the large-scale ordered osteogenesis of hBMSCs will be conducted in the future.

References

Fluorination of G4 PAMAM dendrimers for gene delivery

Lydia N. Dos Orfaos¹, Helena M. Tomás¹, João M. Rodrigues¹,²

¹ CQM - Centro de Química da Madeira - Universidade da Madeira, Ciências Exatas e da Engenharia, Funchal, PT; ² School of Materials Science and Engineering/Center for Nano Energy Materials, Northwestern Polytechnical University, Xi’an, CN

Introduction
Dendrimers are polymers that are widely used as drug/gene delivery vehicles since they present interesting properties, such as a well defined 3D structure, internal void spaces, and the ability of functionalization of their terminal groups. Fluorine has been incorporated in several drugs since it improves their bioavailability, protein-ligand interaction, and metabolic stability. Moreover, the presence of this element in polymers has also been shown to increase their transfection efficiency both in vitro and in vivo.

This work aimed at the fluorination of generation 4 poly(amidoamine) dendrimers (PAMAMG₄-NH₂) with 2,3,5,6-tetrafluoro-4-hydroxybenzoic acid (TFHBA) to obtain a new gene delivery vector with high cytocompatibility, increased transfection efficiency, and the possibility of traceability by magnetic resonance imaging (MRI). For comparison purposes, a non-fluorinated compound, the 4-hydroxybenzoic acid (HBA), was also used due to its resemblance with TFHBA.

Experimental Methods
The functionalization of the dendrimer with the TFHBA or HBA compounds involved their dropwise addition, at different molar equivalents (compound/dendrimer ratios), under stirring, to commercial PAMAMG₄-NH₂ solutions. The reactions were left to occur for two days. Conjugates were prepared at 8.5, 16.5, 32.5, 48.5, and 64.5 compound/dendrimer ratios. After a purification step by dialysis, dendrimers with different functionalization degrees were obtained and characterized by NMR (e.g., ¹⁹F, ¹H, ¹³C), FTIR, UV/visible, photoluminescence, and elemental analysis. The ability of the dendrimers to condense pDNA was assessed by the PicoGreen® assay. Cytotoxicity of the dendrimers and dendriplexes was evaluated using HEK 293T cells and the resazurin metabolic activity assay. Transfection efficiency was studied using the green fluorescence protein (GFP) and luciferase reporter genes by fluorescence microscopy and enzyme activity determination, respectively.

Results and Discussion
The conjugation of TFHBA or HBA with PAMAMG₄-NH₂ dendrimers was confirmed based on the analysis of the obtained structural data. Reaction yields were good, ranging from 88% to 97%.

The biological studies showed that the cytotoxic behaviour of the conjugates varied with the degree of functionalization. In general, the dendrimers prepared at high compound/dendrimer ratios were noncytotoxic at concentrations up to 90μg/mL, being the TFHBA conjugates less cytotoxic than their HBA counterparts and the pristine dendrimers PAMAMG₄-NH₂. Furthermore, the fluorinated dendrimers were able to condense pDNA and transfect HEK 293T cells more than the PAMAMG₄-NH₂ dendrimer by itself.

Conclusion
The functionalization of PAMAMG₄-NH₂ with the selected compounds HBA and TFHBA, at different ratios, was successfully achieved. The biological assays showed that these fluorinated dendrimers present low cytotoxicity and good transfection efficiency, clearly pointing out their potential application as new gene delivery vectors. Work is underway to explore the antibacterial activity of the compounds and their possible use against HIV and/or cancer.

Acknowledgement
This work was supported by FCT - Fundação para a Ciência e a Tecnologia through the CQM Base Fund - UIDB/00674/2020, and Programmatic Fund - UIDP/00674/2020, and by ARDITI-Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação through the project M1420-01-0145-FEDER-000005 - Centro de Química da Madeira - CQM* (Madeira 14-20).

Scheme
Poly(amidoamine) dendrimer functionalized with 2,3,5,6-tetrafluoro-4-hydroxybenzoic acid or 4-hydroxybenzoic acid.

References
Ascorbic acid and hydrazine as reducing agents for the synthesis of Cu DENPs

Duarte Fernandes¹, Carla S. Alves¹, João Rodrigues¹², Pedro Pires¹

¹ Universidade da Madeira, CQM - Centro de Química da Madeira, Funchal, PT; ² Northwestern Polytechnical University, School of Materials Science and Engineering/Center for Nano Energy Materials, Xi’an, CN

Introduction
As opposed to more expensive and less earth-abundant metals like silver and gold, copper-based nanoparticles (Cu NPs) are expected to be a more sustainable material. Also, the good electronic, antimicrobial, optical, and other physicochemical properties displayed by copper materials make them attractive for industrial applications. However, the use of Cu NPs is limited due to their intrinsic instability under atmospheric conditions, making them prone to oxidation. To increase the stability of Cu NPs, various efforts have been made involving, for instance, the association of Cu NPs and organic compounds such as polymers.

Experimental Methods
The purpose of this work was the development of a synthesis procedure of long-term stable Cu-based dendrimer entrapped NPs (Cu DENPs) and their characterization. Fourth-generation hydroxyl-terminated polyamidoamine (G4-OH PAMAM) dendrimer was used as a template for the controlled growth of the Cu NPs. Three temperature conditions were tested, 0º, 25º and 50 ºC. The environmentally friendly ascorbic acid was used as a reducing agent and an antioxidant. Hydrazine was also tried as a reducing agent. Experiments were performed using various ratios of only one of the reducing agents or both at the same time. The final product was purified by dialysis.

Results and Discussion
In contrast to ascorbic acid, hydrazine by itself was not suitable for this synthesis method, since it led to the formation of particles of sizes in the order of hundred nanometers as observed by Scanning Electron Microscopy (SEM). Over time ultraviolet-visible (UV-Vis) measurements of the prepared Cu DENPs, at optimal conditions, suggested a considerable stability of the NPs. Analysis by nuclear magnetic resonance (NMR) Spectroscopy indicated that the signals from the dendrimer were highly dependent on the nature of the Cu DENPs, which is most likely related to the Cu oxidation state. The NPs presented some fluorescence under UV light, and thus were also characterized by Photoluminescence (PL) Spectroscopy.

Conclusion
Although the results indicate the successful realization of our goal, complementary characterization, namely Transmission Electron Microscopy (TEM), are underway to better elucidate the size, structure, and chemical nature of the final product. Thereafter, biological studies will be performed to evaluate the cytotoxicity and antimicrobial activity of the final product.

Acknowledgement
This work was supported by the FCT - Fundação para a Ciência e a Tecnologia through the CQM Strategic Project PEst-OE/QUI/UI0674/2019, CQM Base Fund - UIDB/00674/2020, and Programmatic Fund - UIDP/00674/2020, and by the ARDITI - Agência Regional para o Desenvolvimento da Investigação, Tecnologia e Inovação through the project M1420-01-0145-FEDER-000005 - Centro de Química da Madeira - CQM+ (Madeira 14-20 Program). DF also acknowledge the ARDITI for a research grant (ARDITI-CQM-2019-007-MDG) through the project M1420-01-0145-FEDER-000005.

References

Page 1856 of 2028

PS2-12-390

Functionalized lipid nanocapsules for brain delivery of retinoic acid towards a dual assault on multiple sclerosis

Rui P. Moura1,2,3, Ana P. Pêgo1,2,4, Anne des Rieux5, Bruno Sarmento1,2,3

1 i3s - Instituto de Investigação e Inovação em Saúde, Nanomedicines & Translational Drug Delivery Research Group, Porto, PT; 2 ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Porto, PT; 3 CESPU - iiNFACTS Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde ), Gandra, PT; 4 FEUP - Faculdade de Engenharia da Universidade do Porto, Porto, PT; 5 UCL-LDRI - Université Catholique de Louvain, Louvain Drug Research Institute, Advanced Drug Delivery and Biomaterials, Brussels, BE

Introduction

Acquired demyelinating diseases, namely multiple sclerosis, have a plethora of detrimental consequences. Currently, treatments which involve substances that promote remyelination are hindered by failure of active molecules reaching therapeutic concentrations in the brain. Through the use of a safe, solvent free, and rapid to scale up process of development of lipid nanocapsules we propose to tackle this limitation [1]. This work aims to establish functionalized lipid nanocapsules containing retinoic acid, a drug with dual demonstrated effects in oligodendrocyte progenitor differentiation into mature oligodendrocytes [2, 3], responsible for production of myelin, and inflammation and microglia activation control. The rationale behind the encapsulation and targeting is ensuring retinoic acid reaches effective, active concentrations at the central nervous system (CNS), where it would otherwise be impaired by the blood-brain barrier.

Experimental Methods

Lipid nanocapsules (LNC) were developed using a standard phase-inversion protocol [4] using our adaptations to tailor the nanocapsules for our suiting. Retinoic acid was previously dissolved in dimethyl sulfoxide and added as such to the nanocapsules. Super paramagnetic iron oxide nanoparticles (SPIONs), stabilized by oleic acid, were encapsulated within LNC using the same protocol. Size, PdI and zeta-potential measurements were performed using a Malvern Zetasizer ZS and the encapsulation efficiency of retinoic acid was measured by HPLC [5]. Stability assays were performed at pre-established ICH conditions. Transmission electron microscopy imaging of retinoic acid LNC and retinoic acid-SPION-LNC was performed using a Joel JEM 1400 microscope. A release assay under simulated biological media (PBS, pH 7.4, 37°C) was performed, assuring sink conditions.

The LNC were surface-functionalized with a transferrin-binding peptide through a post-manufacture procedure wherein NH2-Peg-Maleimide was incorporated into the shell, and linked by thiol conjugation via a terminal cysteine. Cell viability and uptake assays were performed in hCMEC/d3 cell line (passages between 51-59 were used). The viability assays were performed using the MTT reduction assay at 4- and 24-hours incubation time. The uptake assay was performed using Imaging Flow Cytometry (ImageStream). Cell viability is also being assessed in microglia cell lines. Furthermore, activation of this cell line by LPS and the impact of RA-LNC in inflammation resolution is currently underway.

Results and Discussion

RA-LNC were developed with 70 nm, a small, nominal size ideal for central nervous system, with a monodisperse cell population with zeta-potential of -5 mV. The encapsulation efficiency of retinoic acid was approximately 88%, with an effective drug loading of 0.20%. The SPION encapsulation was confirmed by TEM. LNC demonstrated stability at 4°C for upwards of 9 months, maintaining their physico-chemical properties and preserving RA, an otherwise unstable molecule, within its shell. The LNC release in a 196-hour window less than 0.5% of its payload,
demonstrating the ability to preserve the payload adequately and only release it when in contact with a cell through membrane fusion.

The functionalization process was assessed by size alterations in the LNC and by elemental analysis for the presence of Sulphur.

Cell viability showed a concentration related toxicity, namely due to the high amount of lipids in the LNC but was safe and tolerable to hCMEC/d3 at 1-10µg/mL of retinoic acid, well above its active concentration.

The uptake assay demonstrated high uptake of the RA-LNC by the endothelial cells, with preserved cell morphology. It is foreseen that the results from microglia will show strong uptake, as well as the resolution of inflammation in an LPS-activation assay (ongoing).

Conclusion

To sum, size-tailored, monodisperse LNC encapsulation both RA and SPIONs were developed and optimized, and assessed for their in vitro potential, showing good potential for therapy in demyelinating disorders. As of yet, only a safety profile and interaction with endothelial cells was assessed for the LNC, but results in microglia are soon to follow and an effect on the control of the activation and the inflammation that is typical of neurodegenerations is an expected result of the LNC.

Acknowledgement

This project was financed by the project NORTE-01-0145-FEDER-000012 by Norte Portugal Regional Operational Programme (NORTE 2020), and COMPETE 2020 - Operacional Programme for Competitiveness and Internationalization (POCI), under the PORTUGAL 2020 Partnership Agreement, through the FEDER - Fundo Europeu de Desenvolvimento Regional, and by Portuguese funds through FCT - Fundação para a Ciência e a Tecnologia/ Ministério da Ciência, Tecnologia e Ensino Superior in the framework of the project "Institute for Research and Innovation in Health Sciences". UID/BIM/04293/2019. Rui Pedro Moura thanks Fundação para a Ciência e a Tecnologia (FCT), Portugal for financial support (Grant SFRH/BD/143196/2019). Anne des Rieux is a FNRS senior research associate.

References


Norbornene-functionalized chitosan nanogels as a preventive strategy to fight infections in orthopaedic implants

Bruna Costa1,2,3, Pedro Alves1,2,3, Guillermo Martínez-de-Tejada4, M. Cristina L. Martins1,2,5, Fabiola Costa1,2

1 I3s - Instituto de Investigação e Inovação em Saúde, Porto, PT; 2 INEB- Instituto de Engenharia Biomédica, Porto, PT; 3 FEUP- Faculdade de Engenharia, Universidade do Porto, Porto, PT; 4 Department of Microbiology and Parasitology. University of Navarra, Irunlarrea, 1; and Navarra Institute for Health Research (IdiSNA), Pamplona, ES; 5 ICBAS- Instituto de Ciências Biomédicas Abel Salazar, Porto, PT

Introduction
Orthopaedic implants, in consequence of the aging population, are frequently needed medical devices. Periprosthetic joint implant-associated infections (PJIs) are currently one of the leading causes of failure in primary and revision total knee and total hip arthroplasty. In USA and UK only, these infections are forecasted to have an annual rate of 38K-270K by 2030 [1]. PJIs are very difficult to treat particularly in patients with immune depressed systems, or relevant co-morbidities (e.g. diabetes), as well as in cases of trauma (infection rates around 30%) or after second surgery (revision surgery, up to 40%) [2]. Current treatment relies on systemically-administered antibiotics. However, the poor peri-implant vascularization may contribute for a low local antibiotic concentration insufficient to control infection. Moreover, antibiotic use may promote further antibiotic-resistance bacteria, as already seen by the significant prevalence of resistant strains isolated from PJIs [3]. Consequently, there is a demand for antibiotic-free alternatives, such as the exploration of antimicrobial peptides (AMPs)-based solutions.

Experimental Methods
Chitosan (acetylation degree of 6% and Mw= 342-393 kDa) was used for nanogel production due to its biocompatibility, ease of functionalization, degradation potential, and also due to its antimicrobial and osteogenic activity [4]. Chitosan was purified and functionalized with norbornenes (Norchip), the “enes” with highest reactivity, through the reaction with carbic anhydride [5].

Norchip nanogels were produced by water-in-oil nanoemulsion through thiol-ene photo-crosslinking using 1,4-Dithiothreitol (DTT), a di-sulphydryl linker, and non-toxic photoinitiator (VA-086) under UV photoactivation. After production, crosslinked nanogels were recovered by centrifugation, and subsequently washed with ethanol and type II water gradients. The hydrodynamic diameter (dh) and ζ potential of the nanogels were both measured with a Malvern NanoZetasizer. Later, to optimize the nanogels conjugation with AMP, a surrogate was used (the aminoacid N-acetyl-cysteine (NAC)). Then, the same conjugation protocol was applied with an AMP and immobilization was confirmed by Fourier Transform Infrared Spectroscopy analysis (FTIR).

For the stability assays, nanogels were immersed in phosphate buffer solution (pH 5.8) at room temperature during 7, 14 and 1 month, and then characterized by Zetasizer.

Results and Discussion
The obtained nanoemulsions, revealed particles with homogenous size distribution (~120 nm, PDI 0.2) and with a neutral charge. The UV-crosslinking was successfully achieved allowing the formation of particles that were stable during 14 days at pH 5.8. In addition, the conjugation of nanogels with NAC proved feasibility of the conjugation protocol, allowing the effective immobilization of AMP as demonstrated by FTIR.

Conclusion
Norbornene functionalized chitosan is a dual benefit biomaterial as under UV photoactivation allowed the production of nanogels and biological molecule interest immobilization. The obtained homogeneous conjugated-nanogels are
currently under *in vitro* bacterial tests. Altogether, this strategy has the potential to create a new biomaterial with efficient antimicrobial activity, to mitigate PJI’s hurdle without promoting further antibiotic resistance.

**Acknowledgement**

The authors would like to thank the Portuguese Foundation for Science and Technology (FCT) (Ref. SFRH/BD/147027/2019) and FCT/MCTES (Ref. CEECIND/01921/2017).

**References**


2:00 p.m. – 3:30 p.m.

Poster floor

**PS2-13 | Cell and Protein-Biomaterial Interactions**
Factors affecting bio-artificial liver prototype performance

Neda Heidari, Susan Sandeman, Richard Faragher
university of brighton, school of pharmacy and biomolecular sciences, Brighton, GB

Introduction

Acute liver failure (ALF) and acute chronic liver failure (ACLF) are life-threatening conditions with mortality rates of up to 85%. The only reliable treatment is orthotopic liver transplantation but there is a shortage of organ donors. In the UK ~29% of patients wait for a transplant for more than 6 months and 8% die before receiving one. This shortage of organ donors and the high mortality rate for patients with liver failure has led to the development of alternative liver replacement strategies to either bridge patients to transplantation or temporarily support native liver function for recovery to occur. Bioartificial livers (BALs) have been developed to overcome the limitations associated with non-biological devices and also to replace the metabolic activities of the liver (1). There are different designs incorporating hepatocytes within a biocompatible matrix. However, none have shown a significant survival benefit for patients in clinical trials to date. One potential limitation for these devices is the rapid reduction in hepatocyte function after exposure to patient plasma over time. Evidence from studies exposing hepatocytes to toxin containing ALF plasma suggests that there is a detrimental effect on hepatocyte function leading to the hypothesis that factors in liver toxin loaded plasma plays a role in altered hepatocyte physiology and reduced BAL function (2). The aim of this study was therefore to investigate the role and significance of liver toxin exposure on hepatocyte cell cycling and other phenotypic characteristics linked to function in a macroporous cryogel bioartificial liver prototype material.

Experimental Methods

In this study, HHL7 and HepG2 cells characterisation was established by immunocytochemistry assay for the presence of non-parenchymal and parenchymal phenotype marker in liver cells lines. Their characteristics were also compared with non-liver cell lines 3T3.

In order to establish a hepatocyte extracellular matrix model in the BAL prototype, HEMA-MBA cryogels were synthesised and modified with alginate to provide a 3-D environment for HepG2 and HHL-7 cell lines. HepG2 and HHL-7 cells viability was measured by MTT and live and dead assays on HEMA-MBA-Alginate cryogels for 7 days. Key metabolic functions such as albumin synthesis and urea production were measured in cryogels (3-D surface)seeded with hepatocytes and their functions were compared with hepatocytes on 2-dimensional surfaces. Also, an effective method was improved to measure hepatocytes cells proliferation and viability after exposure to different water and lipid-soluble liver toxins. Albumin and urea production was measured using standard assay kits in 3D cultures of HepG2 cells exposed to medium loaded toxins over clinically relevant periods of time (6 to 8 h).

Results and Discussion

The results of the ICC assay shows that parenchymal marker CK8 was present in all cell lines (HHL-7 and HepG2) with the exception of the non-liver cell (3T3 cell line). The non-parenchymal phenotype marker CK7 was found in HHL-7 but was absent from the HepG2 and 3T3. Therefore, HHL cell lines retained the expression of CK7 and CK8 and maintained primary hepatocyte phenotype.

The 3D porous scaffold from hydroxyethyl methacrylate -based cryogels provided a conducive microenvironment for HHL-7 and HepG2 cells to proliferate and improved their function compared to two-dimensional surfaces. The cell viability for both HepG2 and HHL-7 cell lines increased after 7 days of incubation. However, the difference is not statistically significant. The data obtained shows that this polymer could maintain cell viability over 7 days of incubation. Also, the function of liver cells including albumin production and urea synthesis grown on three-dimensional scaffolds was enhanced compared to the activity of cells grown on two-dimensional surfaces. The results

Page 1863 of 2028
suggest that HepG2 and HHL-7 liver cell function in three-dimensional environments more closely mimic physiological responses than existing two-dimensional culture systems. Further, the data in this study shows that exposing HepG2 cells to liver toxins treatment caused a significant alteration in cells phenotype and a disturbance in the cell cycle. Also, a subsequent decline in synthetic functions of HepG2 cells was observed after liver toxins treatment.

Conclusion
The 3-D culture system in this study was biocompatible and retrained the hallmarks of hepatocytes-specific functions by albumin and urea production. Also, liver toxins treatment had negatively affect hepatocytes proliferation and functions on both 2-D and 3-D surfaces. In future work, a vitro model of liver failure will be set up to assess the impact of liver toxins treatment on bioartificial liver prototype performance and develop methods to suppress these effects in the BAL prototype.

Acknowledgement
The authors would like to thank the University of Brighton for support and providing the facilities to accomplished this research.
Urea synthesis by HepG2 and HHL-7 cells in 2-D and 3-D surfaces

Figure 5. Synthesis of urea by HepG2 and HHL-7 cells cultured on HEMA-MBA-Alginate (3-D surfaces) and 6-well plates (2-D surfaces) after incubation for 7 days. The concentration of the urea in the sample was determined by interpolating the blank control subtracted absorbance values against the standard curve. Data represent the mean SD from three independent cultures and determinations.

References
Investigating Cell ‘Visco-Transduction’ in Engineered Viscoelastic Hydrogels

Ludovica Cacopardo, Nicole Guzzelli, Simona Piaggi, Alessandro Corti, Arti Ahluwalia

University of Pisa, Pisa, IT

Introduction
Despite tissue intrinsic viscoelasticity, only recently mechanobiology studies started investigating cell response in the light of time dependent substrate properties. However, these studies are usually based on hydrogels where both viscous and elastic properties are altered at the same time [1-2]. For this reason, we engineered hydrogels with a constant equilibrium elastic modulus and different characteristic relaxation times that can be modulated varying the liquid phase viscosity without modifying the crosslinking of the solid network [2].

Here, we optimised agarose gel properties for the culture of adipose-derived mesenchymal stem cells (ADSCs) in order to understand if they are able to sense and respond to substrates with different viscous properties.

Experimental Methods
0.5% w/v agarose gel were fabricated using aqueous solutions with increasing dextran concentrations (0, 3, 4 % w/v), and hence viscosities. Mechanical properties were investigated using the epsilon-dot method [3]. ADSCs (50.000 cells/cm²) were cultured for 21 days on the gels with 0 (high τ) and 4% (low τ) w/v dextran coated with 5% w/v gelatin and in 96-well multiwell plates (τ → ∞) as controls. The experimental setup is schematized in Figure 1D. The Alamar blue assay was performed at different time points and cell viability was normalised for the cell number obtained via image analysis. In order to optimise culture times for cell differentiation, at day 7 and 21, controls cells were fixed with 4% paraformaldehyde and permeabilized with 0.01% w/v Triton-X. Samples were thus incubated overnight with Yes-associated protein (YAP) and CD45 (negative marker for cell stemness) primary antibodies and, then, for 2h with the respective secondary antibody. Finally, nuclei were stained with DAPI and actin with rhodamine-conjugated phalloidin (ThermoFisher, USA). Images were acquired with a confocal microscope (Nikon A1, Japan).

All reagents were purchased from Sigma-Aldrich (Milano, Italy), unless differently specified.

Results and Discussion
Results showed a significant decrease of the instantaneous elastic moduli (Einst) and of the relaxation time (τ), while the equilibrium elastic modulus (Eeq) did not vary significantly (Figure 1A-B). Moreover, Eeq resulted in the optimal range to mimic the mechanical properties of the stem cell niche (≅ 3 kPa [2]). In all culture conditions, cells showed an increasing viability with increasing culture time (Figure 1C). Finally, in Figure 1E-G, it is possible to observe that, in the controls, cells started to differentiate at 21 days as shown by YAP migration in the nucleus and CD45 expression in the nuclear membrane.

Further experiments are ongoing to better analyse cell differentiation in all the substrates by means of tissue specific stainings, morphological and gene expression analysis. After the investigation of cell ‘visco-transduction’, further studies will be performed to understand if cell behaviour is influenced from past viscous stimuli.

Conclusion
Hydrogels with tunable viscoelastic properties were adapted for the culture of ADSCs, thus allowing to study cell response to substrate with different τ. In particular, culture times were optimised observing cell differentiation in the controls after 21 days. The final goal is the definition of optimal substrates to preserve their undifferentiated status in
culture, in order to develop more physiologically relevant in-vitro models to study cell responses to mechanical alterations of their environment.

![Figure 1](image)

**Figure 1:**
A) Einst, Eeq and B) τ of agarose samples as a function of different dextran concentrations. (*p* < 0.05, 1-way ANOVA); C) Viability normalised for cell number with respect to day 0 control (different letters indicate significantly different values); D) schematic illustration of the experimental setup; control samples imaging at day 7 (B) and 21 (C).

**References**


Tissue-mimicking Gelatin Microgels Implement 3D In Vitro Nanotoxicity Screening

Eunheui Gwag\textsuperscript{1,2}, Leon Abelmann\textsuperscript{1,3}, Andreas Manz\textsuperscript{1,4}, Baeckkyoung Sung\textsuperscript{1,2}

\textsuperscript{1} KIST-Europe, The Environmental Safety Group, Saarbrucken, DE; \textsuperscript{2} University of Science and Technology, The department of Energy & Environmental Engineering, Daejeon, KR; \textsuperscript{3} University of Twente, MESA+ Institute for Nanotechnology, Enschede, NL; \textsuperscript{4} Saarland University, Department of Systems Engineering, Saarbruecken, DE

Introduction

Although metallic nanoparticles (NPs) have been extensively studied in pharmaceutics applications, the potential adverse effects of the NPs on the human health and related biological mechanisms are not clear\textsuperscript{1,2}. Conventional \textit{in vitro} nanotoxicity assessments based on 2D cell culture methods are limited in their capability to provide mechanistic information on the NP-cell interactions that occur in the 3D microenvironments of biological tissues\textsuperscript{3}. The nanotoxicity can be demonstrated using human liver cell (HepG2 cell line) models as the nanoparticles can accumulated in the liver regardless of the administration method\textsuperscript{4}.

In this presentation, we show a 3D nanotoxicity screening platform in which HepG2 cells are encapsulated in covalently cross-linked gelatin microgels. Using this colloidal platform, we investigate the toxicity of silver NPs on 3D cultures of HepG2 cells, and compare the (sub-)cellular responses and effects of toxicity with the conventional 2D cell cultures.

Experimental Methods

HepG2 cell-laden gelatin droplets were generated using water-in-oil mini-emulsification. After physical gelation by cooling, a plant-derived cross-linker genipin was added for \textit{in situ} covalent cross-linking of gelatin chains to form stable microgel bead\textsuperscript{5}. The suspension of cell-embedded microgels was then exposed to Ag-NPs for 24-hour with varying dosage at 37 °C with 5% CO\textsubscript{2}. Liver cytotoxicity was quantified by measurement of the cell viability and lipid droplet formation using fluorescence microscopy. The extent of oxidative stress was measured by microplate spectrophotometer. For this, we used the assays of Live/Dead\textsuperscript{®} fluorescence, 2',7'-dichlorofluorescein diacetate (DCFDA), and boron-dipyrromethene (BODIPY 505/515) staining. The cells were indicated using 4',6-diamidino-2-phenylindole (DAPI) staining. The amount of Ag\textsuperscript{+} from dissolved Ag-NPs was quantified using inductively coupled plasma mass spectrometry (ICP-MS), and the cytotoxicity of Ag\textsuperscript{+} was investigated using Ag\textsubscript{2}NO\textsubscript{3}. The results of the microgel-based 3D cell cultures were compared to standard 2D culture. The Ag-NP suspensions in cell culture media were characterized using dynamic light scattering (DLS) and zeta potential measurements.

Results and Discussion

Spherical gelatin microgels had an average diameter of 343±7 μm and encapsulated 4-120 cells depending on the size. Cytotoxicity comparisons were made between the dose-response curves of 2D and 3D cultures of HepG2 cells. The cell viability results showed that the cells encapsulated in a gelatin microgel were less affected by Ag-NP. Furthermore, Ag\textsuperscript{+} had no significant differences in 2D and 3D cultures in terms of cytotoxicity. The dependence of cell viability on the strength of oxidative stress was quantified as a function of Ag-NP concentration. We observed an increase in oxidative stress that induced mitochondrial dysfunction and elevated lipid droplet accumulation. The differences in lipid metabolism and cell viability for 2D and 3D cultures indicated that the nanotoxicity mechanisms in different tissue microenvironments on a subcellular level.

Conclusion

We conclude that the HepG2 cells in 3D gelatin microgel are less sensitive to the adverse effects of Ag-NPs in the level of cell membrane integrity and mitochondrial activities.
Optical microscopic image (DIC/fluorescence) of HepG2 cells encapsulated in gelatin microgels. Blue fluorescence indicates the DAPI-stained cells.

Cell viabilities (%) in the standard 2D cell culture and the 3D cell-encapsulated microgel. Cell viabilities plotted against the logarithm of the Ag-NPs concentration (μg/mL). Error bars indicate the standard deviation. The examination was in triplicate.

References


Control of Nuclear Deformation of Polarized Macrophages on Micropatterned PMMA Surfaces

Ezgi Antmen1, Eric Mathieu4, Nihal E. Vrana3,4, Vasif Hasirci1,2

1 Middle East Technical University, Center of Excellence in Biomaterials and Tissue Engineering, Ankara, TR; 2 Acibadem University, Biomaterials R&A Center and Department of Medical Engineering, Istanbul, TR; 3 SPARTHA Medical, 14B Rue de la Canardiere, Strasbourg, FR; 4 INSERM Unité 1121, Biomaterials and Bioengineering, CRBS, 1 Rue Eugène Boeckel, Strasbourg, FR

Introduction
Chronic inflammation and foreign body response are crucial in the long term in vivo performance of implants. Macrophages are an important part of this response and their phenotype is a defining determinant of how an implant will integrate with the host. For proper implant integration, polarization of macrophage subpopulations should shift from an M1 phenotype (pro-inflammatory) to an M2 phenotype (anti-inflammatory). M1 macrophages are known as classically activated macrophages and show a pro-inflammatory phenotype during the early stages of the tissue repair process. On the other hand, M2 macrophages are known as alternatively activated macrophages and show an anti-inflammatory expression profile.1 Thus, to prevent excessive inflammation, macrophage polarization should be towards M2. We hypothesize that as highly plastic cells biomaterial surfaces can modulate the nuclear deformation of macrophages and hence their polarization since shape, migration and adhesion of cells are influenced by the surface topography of a substrate. Several studies, including ours, have shown that many cell types react strongly to microtopography.2 Control of the material surface topography gives the opportunity to change macrophage function, phenotype and polarization.3 Previously, the effect of nanogratings and microscale protein patch patterns on macrophage polarization have been shown.4,5 However, the effect of micropatterned substrates on polarization of the macrophage nuclei has never been tested. We hypothesize that this line of study would give important information about the mechanisms of polarization of the macrophages on biomaterials. Thus, in the proposed project, nuclear distortions of the macrophages on micropatterned surfaces were analyzed by using an image analysis software and a correlation of the nuclear deformation with the polarization of macrophage cells were correlated.

Experimental Methods
Micropatterned PMMA surfaces designed with square prism shaped pillars with 4x4 µm² area with 4 µm gap were used. Surfaces were produced by soft lithography and solvent casting method. RAW 246.7 monocytic cell lines were studied on these micropatterned surfaces. Cells were seeded on micropatterned surfaces first and then they were differentiated into M1 and M2 phenotypes with LPS and IL-4, respectively. After 24 h culture duration, nuclear morphology and cytokine secretions of the cells were analyzed with the help of confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM).

Results and Discussion
RAW 246.7 macrophage cells were seeded on micropatterned surfaces and they were polarized into M1 (with LPS) and M2 (with IL-4) phenotypes. With CLSM and SEM, nuclear deformability and cell cluster formation were analyzed. The results show that there is a distinct difference between M1 and M2 macrophages in terms of their nuclear deformation, adhesion and cluster formation which has not been reported before in the literature. CSLM showed that nuclei of M1 cells deformed completely whereas M2 did not show any nuclear deformation (Figure 1). SEM showed that M2 macrophages formed more distinct clusters whereas M1 macrophages spread more on the surfaces (Figure 2). Both cells resulted in pillar bending but with M2 it was more distinct probably due to the crowded clusters of cells which applied much force on the pillars.
Conclusion
In this study, the effect of micropatterns on the polarization of the same starting cells is presented (same passage, same conditions, only stimulation is different. With the use of surface topography, immunomodulation of the incoming immune cells was achieved.

Acknowledgement
EA acknowledges French Government and French Embassy in Turkey for the French Research Fellowship for Postdoctoral Researchers. EA and VH acknowledge METU BIOMATEN. NEV acknowledges the European Union's Horizon 2020 research and innovation program under grant agreements no. 760921 (PANBioRA).

References

Nanophase surface roughness enhances neural cell functions

Didem Mimiroglu¹,², Tulin Yanik³,¹, Batur Ercan⁴,⁵,⁶

¹ Middle East Technical University, Biochemistry, Ankara, TR; ² Sivas Cumhuriyet University, Biochemistry, Sivas, TR; ³ Middle East Technical University, Department of Biological Sciences, Ankara, TR; ⁴ Middle East Technical University, Department of Metallurgical and Materials Engineering, Ankara, TR; ⁵ Middle East Technical University, BIOMATEN, Ankara, TR; ⁶ Middle East Technical University, Department of Biomedical Engineering, Ankara, TR

Introduction
There are various factors important in the design of nerve guidance conduits (NGCs) used in peripheral nerve injuries. These factors include biodegradability, conductivity, permeability and supporting neural cell adhesion, proliferation and migration. Since the structure of molecules and cell-ECM interactions occur within the nanoscale, the surface of NGCs implanted should mimic the nanophase topography of ECM. While there are many studies investigating the effects of nanophase topography on various cells types, i.e. endothelial cells, mesenchymal stem cells, fibroblasts, etc., studies focusing on the effects of nanophase (<100nm) topography on neural cell functions are limited. As a matter of fact, most studies in neural tissue engineering focus on submicron level (>100nm) surface topography. Therefore, the aim of the study was to investigate the effects of <100nm topographical structures on poly(lactic-co-glycolic) acid (PLGA) on neural cell functions.

Experimental Methods
Nanopit features possessing 30 and 80 nm diameters were formed on stainless steel by anodization, and these structures were transferred onto PLGA surfaces via replica molding method. To confirm whether nanophase topographies were correctly obtained, scanning electron microscopy (SEM) and atomic force microscopy (AFM) characterizations were conducted. Additionally, hydrophobicity of the surfaces were determined using sessile drop water contact angle measurements. For the neural cell viability (mouse neuroblastoma N2a cell line), MTT analysis was conducted. To understand neural cell spreading, cells were stained with DAPI, vinculin, and f-actin. Finally, neural cell activity was assessed with c-fos protein expression levels by Western-blotting.

Results and Discussion
It was confirmed that nanophase topographies were successfully transferred onto PLGA surfaces. Sessile drop water contact angle measurements showed that hydrophilicity increased on surfaces having nanophase topographical features. For the proliferation studies, cellular density was found to increase up to 2-folds for nanophase surfaces compared to control ones on the 3rd and 5th days of culture. As a result of the neural cell spreading, it was found that more neurite extensions were formed on nanophase topographical surfaces (A and B) than the control (C, Fig. 1). Additionally, c-fos protein expression levels increased up to 2-fold for nanophase topographical surfaces than the control.

Conclusion
As a conclusion, the nanophase topographies successfully transferred onto PLGA surfaces, and these topographies were correlated with the increase in hydrophilicity. Additionally, it was observed that nanophase surface topography had favorable effects for cellular proliferation, cell spreading and cellular activity on neural cells. All of these results showed that fabrication of <100nm sized topographical features is a promising approach for NGC applications.

Acknowledgement
This work was financially supported by The Scientific and Technological Research Council of Turkey (Grant no: 217M952). The authors would like to thank Corbion Purac (Amsterdam, The Netherlands) for PURASORB® poly

Page 1874 of 2028
(lactic-co-glycolic acid) (w/w, 50:50), BIOMATEN-METU Center of Excellence in Biomaterials and Tissue Engineering (BIOMATEN), Middle East Technical University (METU) Central Laboratory.

**Immunofluorescence staining results of N2a cells**
The N2a cells stained with phalloidin for F-actin (red fluorescence), DAPI for nucleus (blue fluorescence) and Alexa-Fluor 488 for vinculin (green fluorescence) on control and nanophase topographical surfaces after 72 h incubation. The merged images scale bars are 20 mm.

**References**
Assessment of cell-biomaterial interactions in three dimensional hybrid spheroidal assemblies

Maria Gabriella Fois, Niloofar Z. Tahmasebi Birgani, Alexander P.M. Guttenplan, Clemens van Blitterswijk, Stefan Giselbrecht, Pamela Habibović, Roman Truckenmüller

Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, MAASTRICHT, NL

Introduction

Conventional 'two-dimensional' (2D) cell culture on (bio)material surfaces is still commonly employed for the early-stage assessment of interactions of cells or tissues with materials considered for biomedical applications. However, simplified 2D culture models often fail to mimic key aspects of the 'three-dimensional' (3D) microenvironment of cells in human tissues [1], and therefore, are increasingly complemented or replaced by 3D models. The spheroid model [2] presents a more close-to-native spatial configuration of cell-cell and cell-matrix contacts and more realistically recapitulates gradients of soluble factors such as signaling molecules existing in human tissues. Spheroids are more and more the culture model of choice for toxicity and efficacy testing of pharmaceutical or other soluble compounds [3]. Here, we present a novel in vitro platform based on spheroids from cells and microsized biomaterials, as a 3D model of the interaction between cells and biomaterials in human tissues. The presented cell-microbiomaterial spheroid model can be a valuable tool for assessing interactions of cells and either materials for classical biomedical devices or dedicated/designed microbiomaterials. The latter in turn can be applied for building hybrid microtissues for academic fundamental R&D, for industrial toxicity and efficacy testing, or for clinical (regenerative medicine) applications, for example, via bioprinting or bottom-up tissue engineering.

Experimental Methods

We showcase our approach by the example of MG63 osteoblast-like cells and human mesenchymal stromal cells (hMSCs), and a small library of shape- and size-harmonized model microbiomaterials. Practically, the microbiomaterials are three-dimensionally dispersed in the spheroids by co-aggregation. The cell behavior in response to the interaction with the micromaterials is then evaluated according to the considered target application.

Results and Discussion

The self-assembly of the microbiomaterials and the cells is supported by low-attachment geometrical confinements in the form of functionalized round-bottom microwells densely packed in arrays. Fabricated from thin and highly transparent polycarbonate films, these advanced microwell arrays are suitable for both high-throughput and -content imaging. We validated our approach by addressing cell-biomaterial interactions such as cell adhesion to the material, the hybrid spheroids' morphology, material-related cell death/cytotoxicity of the material, cell metabolic activity, cell proliferation and (osteogenic) differentiation. The cells in the hybrid spheroids were viable and metabolically active after 7 days of culture, presenting acceptable levels of cell death compliant to the selected (micro)biomaterials. To demonstrate the use of the hybrid spheroidal model for the evaluation microbiomaterials for bottom-up tissue engineering of bone, we investigated osteogenic differentiation in spheroids aggregated from cells and microparticles of bionert, e.g. smooth titanium, and bioactive, e.g. tricalcium phosphate (TCP), biomaterials. High levels of ALP activity, a well-known early osteogenic marker, were measured for hybrid spheroids containing TCP biomaterials, in the absence of stimulation through soluble osteogenic factors. The obtained results were benchmarked against a conventional 2D culture counterpart, by the evaluation of the aforementioned cell behaviors on flat surfaces presenting chemistries similar to that of the microparticles.

Conclusion
Overall, using bioassays with different readouts, we showed that the cell response to differences in the properties of the microbiomaterials, their amounts or dosage, and the duration of interaction with or times of exposure to them can be accurately measured in the hybrid spheroid model. Outlooks for this technology include normalization and standardization efforts, for example, in conjunction with developing consistent methods for microbiomaterial processing, increasing throughput via automated workflows, and further advancing miniaturization, for example, by integrating microfluidic-based technologies. Biologically, the aim is to branch out to the generation of more complex in vitro assemblies, thereby recapitulating native multicellularities and structural organization in the hybrid spheroids.

Acknowledgement

This research was funded by the European Union/Interreg Flanders-The Netherlands (project ‘Biomat on microfluidic chip’). We acknowledge financial support by the Dutch province of Limburg (program ‘Limburg INvesteert in haar Kenniseconomie/LINK’), the Gravitation Program of the Netherlands Organisation for Scientific Research (NWO) (project ‘Materials-Driven Regeneration’) and the NWO Incentive Grants for Women in STEM.

Figure 1

A: Fluorescence microscopy images of hMSC-microbiomaterial spheroids (7 days of culture) showing cells stained with LIVE/DEAD Fixable Far Red Dead Cell Stain (red) and HUECHST (blue), and Phalloidin (green) to label the dead cells, cell nuclei and cytoskeletons, respectively. Scale bar = 100 µm. B: Quantification of cell mortality in hMSC-microbiomaterial spheroids after 7 days of culture (n = 3). C: Normalized enzymatic ALP activity of hMSCs after 7 and 14 days of culture (n = 3). ** and **** indicates p values < 0.01 and 0.0001 respectively.

References

[1] Duval, Physiology, 2017. 32(4)
Directing Mesenchymal Stem Cells osteogenic differentiation by varying hydrogel stress relaxation

Emilie Prouvé1,2, Murielle Rémy2, Pascale Chevallier1, Drouin Bernard1, Marie-Christine Durrieu2, Gaétan Laroche1

1 Laval University, Laboratoire d’Ingénierie de Surface, Centre de Recherche sur les Matériaux Avancés, Département de Génie des Mines, de la Métallurgie et des Matériaux, Université Laval, Axe Médecine Régénératrice, Centre de Recherche du Centre Hospitalier Universitaire, Quebec, CA; 2 Bordeaux University, Univ. Bordeaux, CNRS, Bordeaux INP, Chimie et Biologie des Membranes et Nano-Objets UMR5248 CBMN, Pessac, FR

Introduction
Mesenchymal Stem Cells (MSCs) are adult stem cells which have gained high interest in regenerative medicine considering their self-renewal ability, multipotency, ease of access, and high proliferative rate. However, the use of MSCs in clinical applications still requires a better understanding of their biological behavior to provide the ability to control their in-vitro differentiation into a specific lineage needed for therapy1. While many studies have evaluated the impact of hydrogel stiffness on stem cells behavior, it is now acknowledged that hydrogels, as well as biological tissues, are intrinsically viscoelastic. In addition, some recent studies demonstrated that hydrogel stress relaxation can influence cell differentiation2. However, these studies sometimes led to contradictory results, and their number is still limited. Consequently, it is of particular interest to supply hydrogels with different controllable stress relaxation properties, to further assess their impact on MSCs differentiation.

Experimental Methods
Poly(acrylamide-co-acrylic acid) hydrogels were synthesized by varying the amount of crosslinker (bis-acrylamide) and the ratio between the two monomers to obtain hydrogels with controlled stiffness and relaxation profiles. The mechanical properties of the gels were measured by compressing the gel disks to 5 consecutive steps of 3% strain with a deformation rate of 1 mm.min⁻¹ and a relaxation time of 2 hours, using a Mach-1 V500CS (Biomomentum, Canada) apparatus. For cell culture experiments, the hydrogels were functionalized with a BMP-2 mimetic peptide via sulfo SANPAH under UV irradiation. After ethanol sterilization, human bone marrow MSCs were seeded on the gels at a density of 3 000 cells per cm² and cultured in Osteogenic Differentiation Medium (Promocell, Germany) at 37°C and 5% CO₂ for two weeks. The expression of Runx-2, osteopontin and E11 were assessed by incubating cells for 1 h at 37 °C with rabbit monoclonal anti-Runx2 (1:1600 dilution), or mouse monoclonal anti-osteopontin (1:200 dilution), or anti-podoplanin primary antibody (2 µg/mL), then with the secondary antibodies Alexa Fluor™ 647 goat anti-mouse IgG and Alexa Fluor™ 488 goat anti-rabbit IgG (1:400 dilution) for 1 h at 37 °C.

Results and Discussion
In this study, poly(acrylamide-co-acrylic acid) hydrogels were synthesized with an almost identical stiffness of around 55 kPa, as this value lies within the range of stiffnesses that have been found to promote MSCs osteogenic commitment. Then, it has been found that the stress relaxation increases with the amount of acrylic acid in the gel, with hydrogels containing 0%, 10%, or 18% of acrylic acid exhibiting an average relaxation of 15%, 30%, and 70%, respectively. Subsequently, the hydrogels were functionalized with a mimetic peptide of the BMP-2 protein, known to favor MSCs osteogenic differentiation3,4. Thereafter, human bone marrow MSCs were cultivated on the hydrogels with different stress relaxation for two weeks in osteogenic culture medium. The morphology of the cells was first considered by visualizing cell cytoskeleton. As shown in Figure 1, MSCs on glass in osteogenic culture medium (Fig. 1a) are large, highly spread, and exhibit a cuboidal morphology typical of osteoblasts, while the cells on the different...
hydrogels (Fig. 1b-c-d) present a smaller cell body and a dendritic morphology characteristic of osteocytes. Quantification of Runx-2 (Fig. 1e) expressed in osteoblasts, osteopontin (Fig. 1f), expressed in osteoblasts and osteocytes, and E11 (Fig. g), strongly expressed in early osteocytes, revealed a higher expression of the three markers on hydrogels with 30% and 70% relaxation, as compared to the hydrogel with 15% relaxation and to MSCs on glass.

**Conclusion**

This study shows the importance of considering the impact of hydrogels viscoelastic properties on MSCs differentiation, as hydrogels with higher stress relaxation (30% and 70%) promoted higher expression of Runx-2, osteopontin, and E11, which indicates a more advanced differentiation, as compared to pure polyacrylamide hydrogels presenting a low relaxation (15%). Furthermore, this study highlighted that the combination of the BMP-2 mimetic peptide with these hydrogels seems to promote a differentiation into osteocytes, which is unprecedented in vitro, and therefore constitutes a major step towards the study of hMSCs osteogenic differentiation. Future work will aim at evaluating the expression of other osteocyte markers, such as DMP-1 and sclerostin, in order to identify the best hydrogel mechanical properties for MSCs osteogenic differentiation, as well as assessing the impact of different biomolecules on MSCs differentiation.

**Acknowledgement**

This work was supported by the University of Bordeaux, the National Sciences and Engineering Research Council of Canada, the Centre Québécois sur les Matériaux Fonctionnels, and the ANR EchoCell. The authors thank Dr. Delphine Maurel (BioTis, Bordeaux University, France) for sharing her knowledge about osteocytes and osteocytes markers.

**References**


An improved 2-aminoimidazole based anti-biofilm coating for orthopedic implants: activity, stability, and in vivo biocompatibility

Guglielmo A. Coppola1,2, Jolien Onsea3,4, Ti F. Moriarty5, Dirk Nehrbass5, Caroline Constant5, Stephan Zeiter5, Merve K. Aktan6, Annabel Braem6, Erik V. Van der Eycken1,7, Hans P. Steenackers2, Willem-Jan Metsemakers3,4

1 KU Leuven, Department of Chemistry, Laboratory for Organic & Microwave-Assisted Chemistry (LOMAC), Leuven, BE; 2 KU Leuven, Department of Microbial and Molecular Systems / Centre of Microbial and Plant Genetics (CMPG), Leuven, BE; 3 University Hospitals Leuven, Department of Trauma Surgery, Leuven, BE; 4 KU Leuven, Department of Development and Regeneration, Leuven, BE; 5 AO Research Institute, Davos, CH; 6 KU Leuven, Department of Materials Engineering (MTM) / Biomaterials and Tissue Engineering Research Group, Leuven, BE; 7 Peoples’ Friendship University of Russia, Moscow, RU

Introduction
Orthopedic device-related infection (ODRI) represents a major threat to the success of orthopedic and trauma surgeries. With an incidence ranging from 1 or 2% in case of elective joint replacement up to 30% in complex open fractures, ODRI significantly affects patients’ recovery and compliance. Due to the inaccessibility of the infection site, current therapies rely on high dosage prolonged systemic antibiotic treatments, which can increase the risk of resistance development. Moreover, when antibiotics are not resolutive, surgical revision is required causing not neglectable burden on the healthcare system. ODRI is mainly associated with contamination happening during the surgery which cannot be avoided in toto. Nonetheless, hematogenous infections can jeopardize even first successful operations. Upon contamination, bacteria adhere to the surface of the implant and form a biofilm through the production of extracellular polymeric substances (EPS). These form a matrix that acts as a barrier hampering the action of immune response and antibiotic treatments.

Our group has recently reported the application of an in-house discovered antibiofilm compound, based on the 2-aminoimidazole core, for the prevention of Staphylococcus aureus biofilm formation on titanium surfaces1. The active compound LC0024-NH2 was covalently bonded ensuring activity retention in the long term.

In this work we present an optimized coating procedure that resulted in higher loading and enhanced activity in vitro. The coating was challenged with common sterilization procedures and co-administration with antibiotic was also performed. Finally, an in vivo biocompatibility experiment was carried out with coated implants on a rabbit osteotomy model.

Experimental Methods
LC0024-NH2 was covalently bonded onto the surface of titanium disks and orthopedic implants (i.e. locking compression plates (LCPs) and screws) by formation of ureidic bonds. To this mean the titanium surface was first functionalized by treatment with 3-aminopropyltriethoxy silane. Hexamethylene diisocyanate was employed as a linker between the aminated surface and LC0024-NH2. Loading of the active compound was assessed by fluorescence spectroscopy upon hydrolytic cleavage. The antibiofilm activity of the coating was tested against a clinical isolate of Staphylococcus aureus (JAR060131) by growing the biofilm on the surface of the titanium disks in static conditions for 24 h. The number of planktonic and biofilm cells was determined by plate counting. The effect on biofilm formation was also visualized by scanning electron microscopy (SEM) upon fixation with glutaraldehyde. The effect of antibiotic co-administration was assessed by treatment of 24h biofilms with cefuroxime. Stability of the coating against sterilization procedure (steam sterilization and exposure to ethylene oxide) was evaluated by means of both chemical analysis and in vitro assays. In vivo biocompatibility of the coated implants was assessed in a rabbit model.
osteotomy model using female New Zealand white rabbits. The effect on fracture healing was assessed 8 weeks after the surgery.

Results and Discussion
The optimized coating allowed for a 5-fold increase in loading while shortening the procedure with one step as compared to our previous report. The coating proved to be resilient to common sterilization procedures. The increased loading also resulted in a higher activity against biofilm formation, causing a 1 log reduction in biofilm cells without affecting planktonic cell viability. This biofilm-specific activity is a key feature of our approach since, by specifically interfering with the production of biofilm public goods, the chance of resistance development is reduced. As reported in our recent study on *Salmonella*, strains sensitive to the inhibitor are able to exploit the public goods produced by the resistant strains. This lets the sensitive population overcome the resistant one which is in turn counter-selected. When treated with antibiotic the biofilm grown on the coated surface experienced an additional reduction in biofilm cells as compared to the biofilm grown on the control uncoated titanium disks. Finally, the coating was successfully applied on locking compression plates (LCPs) and screws used in the *in vivo* study. The coating did not interfere with fracture healing nor enhanced the inflammatory response.

Conclusion
In this study we reported an optimized coating procedure to covalently bind the antibiofilm compound LC0024-NH₂ on titanium surfaces. This resulted in higher loading and enhanced activity *in vitro*. When applied to fracture fixation devices the coating did not negatively affect fracture healing in a rabbit osteotomy model. These positive results pave the way for future translational studies where we aim to confirm the effectiveness of the optimized coating against infection in vivo. Furthermore, they put our technology one step closer to clinical trials, confirming its potential in fighting ODRI without inducing local toxicity.

Figure 1. Coating structure and antibiofilm activity
Chemical structure of the antibiofilm compound LC0024-NH₂ and schematic representation of the coating sub-units are shown (top).
*In vitro* characterization of planktonic growth and biofilm formation of *S. aureus* JAR060131 on LC0024-Ti disks. The coating showed no effect on planktonic cells whilst the number of biofilm cells was significantly reduced compared to the control grown on uncoated disks (bottom).
Figure 2. SEM pictures and rabbit osteotomy model
SEM-BSE images of fixed biofilms grown on control-Ti (uncoated) and LC0024-Ti (coated) disks are shown (top). The surface of the coated disks showed mainly isolated cells whereas aggregates of biofilm cells are visible on the surface of the control uncoated disks. Intra-operative pictures of the surgical site after placement of uncoated and coated plates are shown. The radiographic pictures show complete bridging of the osteotomy gap and formation of periosteal callus (bottom).

References
How graphene surface features impact plasma protein adsorption: the power of proteomics

Patrícia C. Henriques\textsuperscript{1,2,3}, Hugo Osório\textsuperscript{1,4}, Fernão D. Magalhães\textsuperscript{3}, Inês C. Gonçalves\textsuperscript{1,2}

\textsuperscript{1} i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, PT; \textsuperscript{2} INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Porto, PT; \textsuperscript{3} LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Porto, PT; \textsuperscript{4} IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, PT

Introduction
Proteins are the first biological component to arrive to the surface of any medical device inserted in the body, thus determining subsequent interactions with other biological systems (mammalian cells or bacteria), and ultimately the device’s biological fate. Graphene-based materials (GBMs), promising candidates for biomedical applications [1,2], have been shown to interact strongly with proteins [3]. The physicochemical features of GBMs-integrated surfaces, as surface roughness, chemistry and wettability, drive the mechanisms of interaction and the type of proteins that adsorb [3, 4], and so it is of paramount relevance to better understand this interaction. Studies focused on the potential and impact of this GBMs-protein interaction are however scarce, with proteins being often neglected in biological studies. Here, films produced with GBMs with different features were incubated with human plasma proteins and evaluated by proteomic analysis, in order to clarify the impact of surface features, as oxidation degree, roughness, or wettability, on protein adsorption.

Experimental Methods
GBMs with different layer thickness and oxidation degree, namely graphene oxide (GO) and reduced GO (rGO) (0.34 nm thick) and graphene nanoplatelets (FLG) and their oxidized form (FLGO) (6-8 nm thick), were used to produce films by vacuum filtration. GBMs films were characterized regarding elemental composition and functional groups (XPS), crystallinity and structural properties (XRD), surface topography (SEM, AFM), and wettability (contact angle measurements). Protein adsorption to films was conducted using a pool of four donors of human plasma, to reduce the impact of plasma donor variability. Plasma was added on top of GBMs films and incubated for 3 h at 37 °C under static conditions. Surfaces were then gently rinsed with dH\textsubscript{2}O to remove unbound proteins. Adsorbed proteins were solubilized, and each sample was processed for proteomics analysis, following the solid-phase-enhanced sample-preparation protocol [5]. Enzymatic digestion was performed, and peptide concentration determined using the quantitative fluorometric peptide assay. Peptides from each condition were the injected in the nanoscale liquid chromatography coupled with mass spectrometry (nanoLC-MS/MS) for protein identification and quantification. Raw data was processed using Proteome Discoverer 2.4.0.305 software and searched against the UniProt database for Homo sapiens Proteome 2019_09. At least two unique peptides were considered for protein analysis.

Results and Discussion
XPS shows high content of oxygen-containing groups in oxidized films (GO and FLGO, \sim32\%), with intermediate levels in rGO (13.5\%) and negligible amounts in FLG (3.5\%) films. XRD confirms that intrinsic crystallinity and structural properties of raw GBMs is kept in films. SEM shows sharp edges exposed in FLG films, while a wrinkled surface with fused platelets is observed on oxidized and rGO films. AFM, however, highlights some differences: GO and FLGO films have a smoother surface, nanotopography, and low height variation, while rGO and FLG present microtopography, high peak to valley height heterogeneity, and more flat areas. Oxidized surfaces are hydrophilic, while rGO and FLG get closer to hydrophobicity.
Plasma proteins incubation with films clearly shows that proteins adsorb in higher amounts to reduced or non-oxidized films (26.5% on rGO, 49.3% on FLG), contrary to oxidized surfaces (8.3% on GO, 7.6% on FLGO), highlighting the impact of GBMs oxidation degree on protein adsorption. The most abundant proteins in human plasma and adsorbed to GBMs films are similar: albumin (∼75%), immunoglobulins (∼8%), serotransferrin (∼4%), and fibrinogen (∼1−2%), with these percentages being proportional to the total amount of adsorbed proteins. Focusing on the most abundant proteins (higher than 1%), differences appear between GBMs films. Proteins such as complement C3 and α-2-macroglobulin adsorb more to oxidized surfaces, while proteins as α-1-antitrypsin and haptoglobin prefer reduced and nonoxidized films. Apolipoprotein A-I (major component of HDL) is identified only in oxidized surfaces without being within the 1% most abundant proteins in the plasma. In some cases, despite belonging to the 1% most abundant proteins in oxidized films (e.g. α-2-macroglobulin and complement C3), higher amounts can be found on rGO and FLG films.

Conclusion

Overall, human plasma proteins adsorb less to oxidized films than to rGO and FLG, highlighting the importance of fine-tuning surface features for a better control of the biological outcome. Proteins adsorbed in higher amounts are mainly related with complement activation (complement factor H, complement C3), coagulation (fibrinogen, antithrombin-III), lipid metabolism (apo A-I, apo B-100), and protease inhibitors (α-1-antitrypsin, α-2-macroglobulin). Such insights on the type and amount of adsorbed proteins to each surface are valuable to better tune and design surfaces according to an envisaged biomedical application.

Acknowledgement


References

PS2-13-414

Dependence of surface topography parameters on the strength of cell adhesion

Przemyslaw Kurtyka1,2,3, Roman Major3, Maciej Gawlikowski2, Marcin Kaczmarek2, Roman Kustosz1

1 Foundation of Cardiac Surgery Development, Artificial Heart Laboratory, Zabrze, PL; 2 Silesian University of Technology, Faculty of Biomedical Engineering, Zabrze, PL; 3 Polish Academy of Sciences, Institute of Metallurgy and Materials Science, Krakow, PL

Introduction
Modifications of the surface of biomaterials currently offer enormous opportunities to obtain a stable connection of the biomaterial-tissue system. The phenomenon of bone tissue accretion has already been well understood and described in the literature and has been used in clinical practice. There are no data on the phenomenon of connective tissue adhesion to the surface of the biomaterial and research describing the influence of the biomaterial surface topography features on this phenomenon. The project includes material tests to parameterize the obtained surfaces and biological tests determining the effect of shear stress on the number of residual cells as a result of rotational motion.

Experimental Methods
The paper presents vacuum sintering modification performed on titanium alloy Ti6Al7Nb in form of cylinders Ø14mm x H 3mm. The compliance of the base material with the standard was confirmed on the basis of the microstructure study, the chemical composition analysis and the study of mechanical properties. Before sintering, samples were subjected to tumbling to obtain a homogeneous surface topography. The roughness of the base material was measured with the use of contact profilometry and resulted Ra=1.5µm and Rz=12.5µm. The sintering was performed using CpTi powder differed in size and morphology. The modified surfaces were characterized by material examinations including scanning electron microscope [SEM], contact angle measurement, contact profilometry and digital microscope [DM]. Samples colonized with fibroblasts were incubated until full confluence. The strength of adhesion of the obtained cell layer to the material substrate was examined using the developed and validated device. Therefore it was possible to assess the impact of the analysed biomaterial surface on the flowing medium under conditions of high shear stress. The system forced the medium flow between the rotating disc made of titanium alloy with modified surface and the immobile polished surface made of surgical steel. The gap size was selected experimentally within the scope of 0.05-0.3mm, and the rotational speed in range od 500-3000RPM. The cell adhesion force was determined comparatively for all samples as the decrease in number of cells per area unit. The number of cells was estimated using the Confocal Scanning Microscope LSM EXCITER 5.

Results and Discussion
The results have shown that surface after PS is characterized by high roughness of about ≈ Ra=35µm, high porosity ≈64% and wettability ≈100°. The DM cross section images revealed its complex 3D morphology. On the basis of SEM photos, the presence of interconnected pores was observed with various degrees of tortuosity, which may positively influence cell adhesion and tissue formation. On the basis of the conducted research, it was possible to determine the correlation of the shape and size of the powders used for modification on topographic parameters and the strength of cell adhesion.

Conclusion
On the basis of the obtained results, the dependence of surface topographic parameters on cell adhesion was observed. The research confirmed the effectiveness of the presented rotational method in order to estimate the...
adhesive strength comparing different modifications. The method, on the other hand, is not free from errors, however the modernization of the construction may result in improved accuracy.

Acknowledgement
National Science Centre, Poland: 2018/31/N/ST8/01085
Characterization of adsorbed HSA protein layers on UV-C modified Ti6Al4V surfaces

Margarita Hierro-Oliva\textsuperscript{1,2,3}, Amparo M. Gallardo-Moreno\textsuperscript{1,2,3}, M. Luisa Gonzalez-Martin\textsuperscript{1,2,3}

\textsuperscript{1} University of Extremadura, Badajoz, ES; \textsuperscript{2} CIBER-BBN, Badajoz, ES; \textsuperscript{3} INUBE, Badajoz, ES

Introduction
Human serum albumin (HSA) is a very abundant protein in the blood plasma. Coatings in biomaterials with this protein are commonly used to decrease platelet adhesion and to reduce bacterial adherence [1]. The surface properties of biomaterials, such as chemical composition and hydrophobicity, are decisive for the adsorption of proteins to their surface [2]. It is possible to modify the hydrophobicity of titanium alloys by UV-C radiation without modifying its chemical composition [3]. In this context, the purpose of this work is to study how the change in Ti6Al4V hydrophobicity affects the properties of adsorbed HSA layers.

Experimental Methods
Experiments were carried out with commercially available Ti6Al4V disks (DKSH, Switzerland). Some of them were treated with an ultraviolet lamp during 15 hours. Protein adsorption experiments were performed at 37 °C for 2 hours of incubation, in PBS buffer, using 1 ml solution of 30 µg/ml of HSA (Sigma-Aldrich, Germany). After adsorption, the samples were rinsed three times in deionized water and dried in air. Contact angles on discs were measured using the sessile drop technique with a G20 goniometer (Krüss, Germany). Deionized water (DW), formamide (F, Fluka, Germany) and diiodomethane (D, Sigma-Aldrich, Germany) liquids were used to measure static contact angles at room temperature. X-ray photoelectron spectroscopy (XPS) measurements were performed in a K-Alpha (Thermo Scientific) with monochromatic AlKα (1486.68 eV) X-ray source with a spot size of 400 μm.

Results and Discussion
The results obtained by XPS confirm the existence of the adsorbed protein layer on the surface of the titanium alloy by increasing the intensity of the N1s peak. It is also possible to detect the amide group, existing in proteins, by deconvolution of the C1s peak. The modification of hydrophobicity of the titanium alloy surfaces does not present significant changes in the layer of adsorbed proteins that can be detected by XPS. Measurements made on the Ti6Al4V surfaces of contact angles show that surfaces that have not undergone UV-C treatment have a hydrophobic character and seem to have a tendency to decrease their average water contact angle with the presence of the protein surface layer. On the other hand, samples that have been subjected to UV-C radiation show a superhydrophilic character and the adsorption of the protein layer on these samples makes them hydrophobic.

Conclusion
After adsorption of HSA on Ti6Al4V surfaces, differences in the hydrophobicity of the substratum becomes shadowed by the protein layer, as the similar water contact angle value obtained after protein adsorption.

Acknowledgement
This work was supported by Junta de Extremadura and FEDER (grant number GR18153) and proyecto RTI2018-096862-B-I00, supported by FEDER/ Ministerio de Ciencia e Innovación - Agencia Española de Investigacion respectively. XPS measurements was performed by the ICTS “NANBOSIS”, by the Surface Characterization and Calorimetry Unit of the CIBER in Bioengineering, Biomaterials & Nanomedicine (CIBER-BBN) and the SACSS-SAIUEx of the University of Extremadura (UEX).

References
Exploring the antiamyloidogenic properties of dendrimers for an Alzheimer’s disease nanotherapy

Débora A. Moreira\textsuperscript{1,2}, Sofia D. Santos\textsuperscript{1}, Victoria Leiro\textsuperscript{1}, Ana P. Pêgo\textsuperscript{1,2,3}

\textsuperscript{1} University of Porto, i3S/INEB - Instituto de Inovação e Investigação em Saúde/Instituto de Engenharia Biomédica, Porto, PT; \textsuperscript{2} University of Porto, FEUP - Faculdade de Engenharia da Universidade do Porto, Porto, PT; \textsuperscript{3} University of Porto, ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Porto, PT

Introduction

There are more than 50 million people worldwide living with dementia, and this number is expected to triple by 2050 \cite{1}. Alzheimer’s disease (AD) is the most prevalent form, corresponding to 60-70\% of all cases \cite{2}. The disease is characterized by extracellular aggregation of amyloid ß (Aß) peptide and intracellular aggregates of hyperphosphorylated tau protein, both leading to toxicity and neuronal death. The available therapies for AD act only on symptoms attenuation, hence cognitive impairment is not only irreversible but also inevitable \cite{3}. Therefore, there is an urgent need to develop disease-modifying therapies for AD.

Nanotechnology shows high potential for AD therapies, as the nanoscale size and controllable surface properties of nanoparticles make them able to cross the blood-brain barrier, reach the AD brain, deliver therapeutics in a specific and controlled way, and improve their pharmacokinetic and pharmacodynamics. Dendrimers are specially interesting as nanotherapeutics for AD treatment due to their unique structural characteristics. They are globular, have a well-defined and very branched structure, controllable nanosize (<15 nm) and multivalency. These intrinsic features allow them to act as efficient and multivalent carriers of different therapeutic molecules. In addition, these nanosystems have also been used as a drug \textit{per se} \cite{4}. With that in mind, a proprietary family of dendrimers was explored to hamper with Aß (1-42) peptide aggregation.

Experimental Methods

The structure of the dendrimers was characterized by nuclear magnetic resonance and Fourier-transform infrared spectroscopy, while its size and zeta potential by dynamic light scattering. Aß aggregation kinetics was studied by the fluorescence probe thioflavin T (ThT) when in the presence of different peptide/dendrimer ratios.

Results and Discussion

Results showed that the dendrimers were able to reduce Aß fibrillation when peptide/dendrimer ratios were equal or higher than 1. For ratios below 1, the dendrimers increased and altered Aß aggregation kinetics to different extents, as shown by a ThT destabilized signal. These preliminary results seem to indicate a dual effect of these dendrimers and their impact and potential in AD therapeutics: (i) at high peptide/dendrimer ratio they can inhibit Aß fibrillation, thus inhibiting the formation of toxic Aß species; (ii) at low peptide/dendrimer ratio they seem to promote the aggregation/fibrillation, altering the kinetics of aggregation.

Conclusion

Therefore, these dendrimers show relevant \textit{in vitro} antiamyloidogenic properties. Future studies will dissect the mechanism of interaction of the dendrimers and Aß and explore their impact in relevant cell lines.

Acknowledgement

This study has been funded by the projects NORTE-01-0145-FEDER-000008, i3S (POCI-01-0145-FEDER-007274 and by FCT (UI/BD/150831/2021) and Norma Transitória – DL 57/2016/CP1360/CT0013. The authors would like to acknowledge the support of the i3S Scientific Platform Biointerfaces and Nanotechnology.

References


Generation of a 3D artificial liver model using electrospun PCL scaffolds and footprint-free hiPSC-derived hepatocytes

Josefin Weber¹, Christiane Lörch¹, Carsten Linti², Michael Doser², Meltem Avci-Adali¹

¹ University Hospital Tuebingen, Department of Thoracic and Cardiovascular Surgery, Tübingen, DE; ² German Institutes of Textile and Fiber Research Denkendorf DITF, Biomedical Engineering, Denkendorf, DE

Introduction
Liver tissue engineering aims to provide close-to-nature 3D models to get further insights into the various functions, complex structures, and disease mechanisms of the liver and to establish future therapeutic approaches. The generation of human-induced pluripotent stem cells (hiPSCs) from patients' somatic cells and the subsequent differentiation into desired cell types, like hepatocytes, opens up numerous possibilities in regenerative medicine and tissue engineering. The use of hiPSCs represents an unlimited stem cell source for the generation of hepatocytes. In this study, using self-replicating mRNA footprint-free hiPSCs were generated from urine-derived epithelial cells. After a 2D pre-differentiation of these hiPSCs into hepatoblasts, the cells were seeded on poly-ε-caprolactone (PCL) scaffolds produced by an innovative combination of fused filament fabrication (FFF) 3D printing with melt electrospinning and differentiated into hepatocytes.

Experimental Methods
3D PCL scaffolds were produced by DITF Denkendorf. The scaffolds were coated with vitronectin to enable cell adhesion and proliferation. Afterwards, the scaffolds were populated with 5x10⁵ hiPSC-derived hepatoblasts and differentiated into mature hepatocytes using a cocktail of growth factors and small molecules. After two weeks of differentiation and maturation, the attachment, spreading, and proliferation of the cells, as well as the expression of hepatocyte-specific markers and the functionality, such as albumin secretion, were evaluated by performing fluorescence microscopy, qRT-PCR, flow cytometry, and ELISA. The 3D differentiation efficiency was compared with the hepatic differentiation under 2D conditions.

Results and Discussion
The seeding of hiPSC-derived hepatoblasts on vitronectin-coated scaffolds resulted in uniform attachment of the cells to the PCL fibers. In the course of the following two weeks of differentiation and maturation, the cells started to proliferate leading to build a 3D network across the fibers. The successful differentiation of the hepatoblasts into mature hepatocytes was confirmed by the expression of the hepatocyte-specific markers albumin, apolipoprotein A-II (APOA2), alpha-1-antitrypsin (A1AT), cytochrome P450 3A4 (CYP3A4), and alpha-1-fetoprotein (AFP). The differentiation efficiency of the 3D generated hepatocytes was compared to hepatocytes generated under 2D conditions, using fluorescence microscopy, qRT-PCR, flow cytometry, and ELISA.

Conclusion
The footprint-free hiPSCs generated by the application of self-replicating mRNA from urine-derived cells were successfully differentiated in combination with 3D PCL scaffolds into hepatocytes and created a simplified 3D liver-like model with specific hepatic functions. This model can be applied to study specific genetic diseases and novel treatment methods. Furthermore, the influence of drugs on patient-specific hepatocytes can be evaluated.
Miniaturized microenvironment as a versatile platform to study mechanosensing at single-cell resolution

Castro Johnbosco, Malin Becker, Kannan Govindaraj, Tom Kamperman, Jeroen Leijten

University Twente, Department of Bioengineering, Enschede, NL

Introduction
Cell-matrix interfaces govern cellular behavior and dictate virtually all levels of cellular function ranging from protein expression to cellular phenotype. The interplay between microenvironmental mechanics and cellular biology effectively guides cell function and fate. Although cellular heterogeneity in primary cell populations is widely accepted, single-cell resolution data on the heterogeneity in mechanobiology and mechanotransduction have remained scarce as current state of art systems are mostly geared towards multi-cellular cultures and population-level analyses.

Experimental Methods
Thereby, we propose a miniaturized microsystem to scrutinize the mechanistic activities of a single cell with its immediate pericellular environment. Droplet-based microfluidic emulsification was used to position individual single mesenchymal stem cells inside the center of a picoliter droplet of designer polymer solutions, in a high throughput manner. To this end, we used polysaccharides such as dextran that were functionalized with tyramine moieties to produce single-cell microdroplets which then were enzymatically crosslinked into single-cell microgels facilitating temporal tuning of biochemical, mechanical, and biophysical entities of these single-cell microgels. The microrheological properties of various single cell-laden micromaterials were determined using nanoindentation techniques.

Results and Discussion
We demonstrated that the encapsulated cells switched their phenotype depending on the microenvironmental stiffness and mapped this behavior at single-cell resolution. Immunostainings revealed that matrix protein production was strongly correlated with alterations in mechanical properties. Gene expression analysis corroborated that the dependency in stemness maintenance was affected by the mechanics of the cell’s mechanical surrounding.

Conclusion
Thus, we endorse a platform that bestows a highly tunable microenvironment to individual cells that reports the mechanobiological crosstalk at single-cell resolution.
The production of microgels via droplet microfluidics. The nanoindentation analysis of cell-laden microgels using a nanoindenter. Confocal microscopy of single cell-laden microgel stained for actin and nucleus.

References
2:00 p.m. – 3:30 p.m.

Poster floor

**PS2-14 | Biomaterials for Tissue and Organ Models**
PS2-14-424

Engineering the colorectal tumor on-a-chip

Mariana Carvalho\textsuperscript{1,2}, David Barata\textsuperscript{3}, Liliana Teixeira\textsuperscript{4}, Stefan Giselbrecht\textsuperscript{4}, Rui L. Reis\textsuperscript{1,2}, Joaquim Oliveira\textsuperscript{1,2}, Pamela Habibović\textsuperscript{4}, Roman Truckenmüller\textsuperscript{3}, J. Miguel Oliveira\textsuperscript{5}

\textsuperscript{1} 13B’s Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, GMR, PT; \textsuperscript{2} ICVS/3B’s - PT Government Associate Laboratory, Braga/Guimarães, PT; \textsuperscript{3} MERLN, Maastricht University, Department of Instructive Biomaterials Engineering, Maastricht, NL; \textsuperscript{4} MERLN, Maastricht University, Department of Complex Tissue Regeneration, Maastricht, NL; \textsuperscript{5} University of Minho, 3Bs Research Group, Institute 3Bs, Guimarães, PT

Introduction

Colorectal cancer is the third most common cancer world wide and its incidence increases with ageing. Understanding the mechanisms of tumor growth rely in further advances to unveil cancer-causing agents, drug screening and in the development of personalized therapies. Standard 2D in vitro models and in vivo animal models have undoubtedly contributed to the development of anti-cancer drug candidates. Yet their translation into successful clinical trials is critically low, which reinforces the need of a deeper understanding of tumorigenesis [1]. Therefore, 3D models integrating tissue engineering (TE) strategies with microfluidic technology have sparked the expectation on physiologically relevant microfluidic in vitro models [2].

Experimental Methods

Human colon cancer HCT-116 cells (ATCC) and intestinal microvascular endothelial cells (Innoprot) were cultured with specific media according to supplier’s specifications, at 37°C in a humidified atmosphere containing 5% CO\textsubscript{2}. The model includes a core region defined by human colorectal cancer cells (HCoMECs) embedded in a soft 3D matrix (Matrigel\textsuperscript{®}) being laterally surrounded by perfused engineered endothelial microvessels (Figure 1d).

Results and Discussion

Herein, we describe an “on chip in vitro tumor disease model” (Figure 1 a-b) that works as a screening tool for the generation and maintenance of stable gradients of Gemcitabine-loaded dendrimer nanoparticles (Figure 1c). The model includes a core region defined by human colorectal cancer cells (HCoMECs) embedded in a soft 3D matrix (Matrigel\textsuperscript{®}) being laterally surrounded by perfused engineered endothelial microvessels (Figure 1d). We achieved an “on chip in vitro tumor disease model” (Figure 1a and b)) that works as a screening tool for the generation and maintenance of stable gradients of Gemcitabine-loaded dendrimer nanoparticles. Subsequent Viability studies based on automated field of view imaging of the microfluidic 3D model corroborate the fact that cells are exposed to a gradient of Gemcitabine.

Conclusion

The developed on-chip model allows a co-culture of colorectal cancer cells and colonic endothelial cells, comprising both cancer and vascular parts of tumor microenvironment. The design of the model allows for the obtention of molecule gradients, which is foreseen to play an important role in assessing the influence of chemoattractants and drugs. Moreover, the model offers the potential to include complex microenvironments by means of including other cell types, such as immune cells or patient-derived cells, which can be key to bringing us closer to an effective treatment for colon cancer.

Acknowledgement

Mariana Carvalho acknowledges her PhD scholarship NORTE-08-5369-FSE-000044, funded by Programa Operacional Regional do Norte, Fundo Social Europeu, Norte2020 TERM&SC and EMBO Short-Term Fellowship 7232. J.M.O. thanks FCT for his distinction attributed under the FCT Investigator Program (IF/00423/2012 and IF/01285/2015).

Page 1896 of 2028
**Colorectal cancer-on-a-chip**

Figure 1: a) Image of microfluidic chip; b) Schematic of chip design: central chamber filled with Matrigel and two independent lateral channels for media perfusion; c) FITC-labeled nanoparticles’ gradient at 1min and 12 hours (in green); d) Microvascular 3D microenvironment at day 5: DAPI/Phalloidin staining of HCT-116 cells in Matrigel (central chamber) and HCoMECs (lateral microchannels).

**References**


Developing a coronary artery-on-chip model to study arterial calcification

Danielle Baptista¹², Armand Jaminon², Nilooftar Tahmasebi¹, Barend Mees², Leon Schurgers², Roman Truckenmüller¹

¹ University of Maastricht, MERLN, MAASTRICHT, NL; ² University of Maastricht, CARIM, MAASTRICHT, NL

Introduction
Vascular calcification is a well-known marker of subclinical atherosclerosis and, when present in coronary arteries, an independent predictor of coronary heart disease [1]. Although numerous studies have explored the link between vascular calcification and various biological and mechanical factors, a unifying mechanism that explains the initiation and development of this orchestrated condition has not yet been unraveled. Conventional research models are often based on in vivo animal models, ex vivo settings and in vitro static two-dimensional (2D) models [2]. Ex vivo models do not represent the complexity under which vascular calcification is initiated and progresses in blood vessels, and consequently fail to reproduce extensive calcification that is usually seen in pathological conditions in vivo. On the other hand, animal-based in vivo models fall short in providing clinically translatable outcome. Hence, there is still a large unmet demand for reliable and predictive human in vitro models to mimic pathological calcification of blood vessels. Recent advances in microfluidic and organ-on-chip technologies have resulted in the development of more complex human-based in vitro models to recapitulate the unique anatomical and biomechanical aspects of vessels combined with the biochemical and biological microenvironment that the corresponding cells experience [3]. However, to the best of our knowledge, current organ-on-chip models have not yet been applied for vascular calcification. Here, we present the development of a coronary artery-on-chip model that enables investigating the role of dynamic culture conditions on the onset of in vitro calcification. The model consists of a microfluidic chip with a channel representing the coronary artery. In the microchannel, vascular smooth muscle (VSMCs) and endothelial cells (ECs) will be co-cultured and emulates the arterial wall, which subsequently will be calcified. This co-culture will be subjected to stimuli, such as different shear stress regimes, mimicking both physiological and pathological conditions, with the aim to study effects on vascular calcification.

Experimental Methods
We have developed a microfluidic chip comprised of a straight channel that represents human adult coronaries. The first-generation chip was fabricated from polydimethylsiloxane (PDMS) via soft lithography and bonded to a polycarbonate (PC) film with PDMS mortar as described elsewhere [4]. As a first step of developing a co-culture on chip, we cultured human primary VSMCs, known to be key players in arterial calcification in vitro [5], inside the channels to obtain a VSMC monolayer. For this, VSMCs were seeded inside the channels that were pre-coated with either laminin or fibronectin. In addition, varying seeding densities were tested in order to maximize cell coverage and to ensure the formation of a confluent monolayer. Current experiments include the characterization of VSMCs with respect to their phenotypes. Within these conditions, we analyzed expression of common biomarkers including alpha smooth muscle actin (αSMA), calponin (CNN1), phosphorylated myosin light chain (pMLC) and F-actin.

Results and Discussion
We successfully fabricated a microfluidic chip consisting of a PDMS layer, featuring three identical straight channels, bonded to a 50 µm transparent PC film that is comparable to commonly used cell culture plastics, is optically transparent and renders a device easy to handle. Additionally, since the PC film can be peeled off the PDMS housing...
and easily cut, one device enables multiple analysis. The bonding between both parts resulted in stable constructs with no leakage (Fig. 1 a).

To efficiently obtain VSMC monolayers prior to perfusion on-chip, we optimized seeding density and surface coating (Fig. 1 b). A density of 10000 cells/µl in conjunction with fibronectin-coated channels showed superior cell adhesion and the resulting VSMC monolayer exhibited a contractile-like phenotype within 24 hours after seeding. To further characterize VSMCs, expression of contractile markers was assessed through immunocytochemical analysis. The cells cultured with fibronectin and laminin (Fig. 2 a-b) displayed similar expression of F-actin. CNN1 expression appeared stronger for cells on fibronectin while αSMA expression appeared stronger for cells cultured with laminin.

pMLC, despite being a very specific marker for contractile VSMCs, was not detected for any condition, possibly due to the late passage of VSMCs that were used. Next steps will include perfusion and calcifying conditions on-chip, as well as incorporation of ECs into the model.

**Conclusion**

In this study, we successfully took the first steps towards developing a physiological and clinically relevant coronary artery model that combines organ-on-chip technology with primary human cells. The results, despite preliminary, highlight the potential of the model that will be used to study vascular calcification under dynamic conditions.

**Acknowledgement**

Work supported by the partners of Regenerative Medicine Crossing Borders (RegMed XB), a public-private partnership that uses regenerative medicine strategies to cure common chronic diseases. This collaboration project is financed by the Dutch Ministry of Economic Affairs by means of the PPP Allowance made available by the Top Sector Life Sciences & Health to stimulate public-private partnerships.

We acknowledge financial support by the Dutch province of Limburg (program ‘Limburg INvesteert in haar Kenniseconomie/LINK’), the European Union/Interreg Flanders-The Netherlands (project ‘Biomat on microfluidic chip’) and the NWO Incentive Grants for Women in STEM.
Fig. 2
Confocal top view image of VSMCs cultured inside channels coated with fibronectin (a) and laminin (b), showing expression of CNN1 (green), F-actin (red) and αSMA (yellow). Scale bars: 100 µm.

References


Quantifying the minimal metabolic requirements to support stem cell survival using a gradient generating dynamic microfluidic cell culture platform

Melvin Gurian, Yvonne W. Schreurs, Liliana S. Moreira Teixeira, Iris E. Allijn, Jeroen Leijten

University of Twente, Developmental BioEngineering, Enschede, NL

Introduction
Metabolites are essential for cell survival, function, and fate of cells [1, 2]. Indeed, nutrient deprivation contributes to the rapid loss of implanted cells and engineered tissues. Knowledge on the minimal levels of nutrients that are required to maintain cell viability could aid predictable survival of living implants. Current knowledge is typically based on static culture systems in which cells are exposed to a culture medium that is initially chemically defined. However, the nutrient content of media in static systems decreases rapidly over time [3]. As starvation-induced cell death occurs in a delayed manner, it has remained unknown what the critical level of metabolite concentrations is to ensure the survival and function of implanted cells and tissues. Here, we report on the development and use of a dynamic microfluidic-based cell culture platform for the determination of minimal metabolic requirements for mesenchymal stem cell (MSC) survival and function at physiologically relevant oxygen concentrations.

Experimental Methods
Soft lithography and replica molding was used to create microfluidic devices that were composed of a gradient generator that was followed by an array of parallelly placed cell culture chambers. Approximately 3500 MSCs were seeded per culture chamber and exposed to a range of metabolite concentrations. All platforms were used inside a XVivo hypoxia chamber to effectively control the oxygen concentration. Fluorescent microscopy was used to visualize cell morphology and survival over time.

Results and Discussion
Perfusing the microfluidic device with fluorescein dyes validated that the gradient generator offered long-term stable and linear control over the media in each individual cell culture chamber. Subsequently, chemically defined media with quantified nutrient content was flown through the microfluidic device at 8ul/min, which offered neglectable amount of nutrient loss e.g. due to absorption to the microfluidic channels. Furthermore, we confirmed with cell laden chambers the dynamic flow to be sufficiently fast to maintain constant metabolite concentrations, while keeping the hydrodynamic shear sufficiently low (<0.3dyn/cm2 [4]) to avoid causing adverse effects on cell behavior. Cells were then cultured over a maximal period of seven days to quantitatively determine the minimal concentrations to sustain cell survival, as well as function for a panel of relevant metabolites in a time resolved manner.

Conclusion
This approach allowed the determination of the relative importance of individual metabolites on the survival of MSCs, which offers novel fundamental insights into what is required to avoid starvation-induced cell loss of implanted cells and prevascular tissues.

Acknowledgement
Financial support was received from the European Research Council (ERC, Starting Grant, #759425).

References

Impact of three-dimensional cell culture with electrospun nanofibers on estrogen receptor positive breast cancer cells

Marc Rabionet1,2, Jennifer Sims-Mourtada3, Lynn M. Opdenaker3, April M. Kloxin4,5, Samantha E. Cassel4, Joaquim Ciurana2, Teresa Puig1

1 University of Girona, New Therapeutic Targets Laboratory (TargetsLab) - Oncology Unit, Department of Medical Sciences, Faculty of Medicine, Girona, ES; 2 University of Girona, Product, Process and Production Engineering Research Group (GREP), Department of Mechanical Engineering and Industrial Construction, Girona, ES; 3 Christiana Care Health Services, Inc, Center for Translational Cancer Research, Helen F Graham Cancer Center and Research Institute, Newark, US; 4 University of Delaware, Chemical and Biomolecular Engineering, Newark, US; 5 University of Delaware, Materials Science and Engineering, Newark, US

Introduction
Plastic surfaces as in vitro cell culture supports have greatly facilitated the study of cell behavior. However, flat surfaces only allow the development of two-dimensional (2D) cell culture, where cells grow by forming a monolayer. Cells adopt a flattened morphology that affects gene and protein expression [1]. Therefore, cell behavior on monolayer culture differs from the in vivo pattern, where cells are embedded in the extracellular matrix (ECM), a three-dimensional (3D) network composed by fibrous proteins and array of molecules. Some studies have demonstrated that this key difference in cellular surroundings may influence cell metabolism and the differentiation state of the cells [2]. In fact, there is small subpopulation within the tumor that exhibits an undifferentiated state and other characteristics similar to the mammary stem cells. They are so-called cancer stem cells (CSCs) and have the ability to initiate tumors, are resistance to many current anticancer therapies, and are thought to be partially responsible for tumor recurrence. Targeting CSC population could represent an effective approach for oncologic treatments. CSCs can be identified by increased activity of the aldehyde dehydrogenase (ALDH) enzyme and, in breast cancer, they can be isolated based on their expression pattern of CD44+/CD24−/low and high expression of CD49f and ESA surface proteins [3]. Additionally, the induction of epithelial-to-mesenchymal transition (EMT), through decreased E-cadherin expression and upregulation of vimentin, results in stem properties.

All things considered, it is important to maintain the differentiation state of the cells to properly study CSC niche. For this reason, 3D structures have emerged as innovative cell culture supports that aim to emulate the in vivo cell environment. Scaffolds, which are composed by a network of filaments mostly made by synthetic materials, mimic aspects of the physiological surrounding of cells [4]. Therefore, estrogen receptor positive (ER+) breast cancer cells are proposed in the present work in order to study the impact of 3D scaffolds culture on the stem-like population. Knowing how cell surroundings modulate stemness state could favor CSC experimentation and, consequently, the development of targeted therapies.

Experimental Methods
Polycaprolactone (PCL) and electrospinning were chosen as biopolymer and technology to manufacture 15% w/v PCL scaffolds. Two ER+ breast cancer cell models were used, luminal A HER2- T47D and luminal B HER2+ BT474 cells. Both cell lines were cultured on scaffolds, and cell proliferation was analyzed by MTT assay. Cell cycle was also evaluated on cells cultured on 2D and 3D scaffolds. For T47D cell model, the T47D Fluorescent Ubiquitination-based Cell Cycle Indicator (FUCCI) cells were used, where cell cycle activity can be visualized by monitoring the oscillation dynamics of fluorescently tagged cell cycle fusion proteins. For BT474 cells, an EdU (5-ethyl-2'-deoxyuridine) assay was performed to analyze cell cycle status. In order to discern a possible impact of 3D culture on the cancer stem-like cell population, several stemness features were analyzed. ALDH activity was quantified with an ALDEFLUORä kit, as well as the protein expression pattern of CD44, CD24, CD49f, and ESA by flow cytometry.
The expression of EMT-related genes was evaluated such as SNAIL, SLUG, TWIST, ZEB1, ZEB2, ECADH and VIM, along with the expression of OCT4 and NANOG stemness-related genes. Finally, cells derived from ER+HER2- and ER+HER2+ primary tissue samples were cultured on the PCL meshes and a MTT assay was performed.

Results and Discussion
Impact of 3D culture with PCL scaffolds are summarized in Table 1. T47D cells acquired a more quiescent and mesenchymal phenotype during scaffolds culture, based on analyses of cell proliferation kinetics and ECADH/VIM expression, which led to an overactivation of the ALDH enzyme, a cancer stem cell marker. However, a -lower percentage of CD44+/CD24-low and CD49f cells and lower EMT transcription factors expression was also found in T47D cells cultured in scaffolds relative to monolayer culture. On the other hand, BT474 cells showed a similar cell proliferation rate in scaffolds relative to monolayer culture, as well as ALDH activity. 3D-cultured BT474 cells displayed less CD24 and CD49f levels, accompanied by an epithelial phenotype with lower EMT transcription factors expression. Importantly, ES 15% PCL scaffolds proved to be suitable for the culture of primary ER+ breast cancer cells, which adopted a similar cell growth behavior to that of tested model cell lines.

Conclusion
In summary, presented results provide evidence of the suitability of electrospun PCL nanofibers to accommodate 3D ER+ breast cancer cells culture, either established cell lines or primary samples. A modulation of the differentiation and stemness state of the cells was observed to occur within 3D surroundings. However, more efforts are needed to fully understand the mechanisms underlying these alterations.

Acknowledgement
The authors are grateful to the pre-doctoral and mobility grant from the University of Girona (IFUdG2017/62 and UdGMOB19, respectively). The authors are also grateful to the flow cytometry core at the Cawley Center for Translational Research, Helen F Graham Cancer Center, which is supported by the Delaware INBRE program, with a grant from the National Institute of General Medical Sciences – NIGMS (P20 GM103446) from the National Institutes of Health and the State of Delaware.
Table 1
Summary of the impact of 3D cell culture with ES 15% PCL scaffolds on the ER+ breast cancer cell models T47D and BT474

References
https://doi.org/10.1073/pnas.032668799.
https://doi.org/10.1158/0008-5472.CAN-11-3567.
https://doi.org/10.3390/cancers12123765.
A Fast Alternative to Soft-Lithography for the Fabrication of Organ-On-a-Chip Elastomeric-Based Devices and Microactuators

Daniel A. Ferreira1,2,3, Mario Rothbauer4,5, João P. Conde6,7, Peter Ertl8, Carla Oliveira1,9,10, Pedro L. Granja1,2,3

1 Universidade do Porto, i3S, Porto, PT; 2 Universidade do Porto, INEB, Porto, PT; 3 Universidade do Porto, ICBAS, Porto, PT; 4 Medical University of Vienna, Department of Orthopedics and Trauma Surgery, Karl Chiari Lab for Orthopaedic Biology, Vienna, AT; 5 Vienna University of Technology (TUW), Institute of Applied Synthetic Chemistry, Vienna, AT; 6 Universidade de Lisboa, Department of Bioengineering, Instituto Superior Técnico, Lisboa, PT; 7 INESC-MN, Instituto de Engenharia de Sistemas e Computadores – Microsistemas e Nanotecnologia (INESC MN), Lisboa, PT; 8 Vienna University of Technology (TUW), Faculty of Technical Chemistry, Vienna, AT; 9 University of Porto, Ipatimup – Institute of Molecular Pathology and Immunology of the University of Porto, Porto, PT; 10 University of Porto, Department of Pathology, Faculty of Medicine, Porto, PT

Introduction
Organ-on-a-chip technology promises to revolutionize how pre-clinical human trials are conducted. Engineering an in-vitro environment that mimics the functionality and architecture of human physiology is essential towards building better platforms for drug development and personalized medicine. However, the complex nature of these devices requires specialized, time-consuming and expensive fabrication methodologies. Alternatives that reduce design-to-prototype time are needed, in order to fulfill the potential of these devices. Here, a streamlined approach is proposed for the fabrication of organ-on-a-chip devices with incorporated microactuators, by using an adaptation of xurography. This method can generate multi-layered, membrane-integrated biochips in a matter of hours, using low-cost benchtop equipment.

Results and Discussion
Devices produced with the proposed technology are capable of withstanding considerable pressure without delamination. Furthermore, this method is suitable for the integration of flexible membranes, required for organ-on-a-chip applications, such as mechanical actuation or the establishment of biological barrier function. The devices are compatible with cell culture applications and present no cytotoxic effects or observable alterations on cellular homeostasis. A proof of principle stomach-on-a-chip device was designed to emulate the outer strata of the gastric epithelium. The engineered stomach-on-a-chip was shown to display enhanced phenotypical and functional traits characteristic of the normal gastric mucosa.

Conclusion
The proposed fabrication method can rapidly generate organ-on-a-chip prototypes for a fraction of cost and time, in comparison to conventional soft lithography, constituting an interesting alternative to the current fabrication methods.
Graphical Abstract

CAD design

CAD to
organ-on-a-chip
in
3 steps

Assembly

Plotter cutting
Tunable magnetic hydrogel for cardiomyocytes training

Baptiste Le Roi\textsuperscript{1,3}, Ben M. Maoz\textsuperscript{1,2,3}

\textsuperscript{1} Tel Aviv University, Biomedical Engineering, Tel Aviv, IL; \textsuperscript{2} Tel Aviv University, Sagol School of Neuroscience, Tel Aviv, IL; \textsuperscript{3} Tel Aviv University, Center for Nanoscience and Nanotechnology, Tel Aviv, IL

Introduction
Heart failure is a major cause of death in Europe as 2\% of the European population die from it every year. Heart failure naturally comes with the age but also with the regular consumption of toxics such as tobacco or alcohol. The main cause of heart failure is called cardiomyopathy and corresponds to a permanent loss of contraction strength of cardiac cells. Current treatment are palliative and do not target cardiac cells but arteries which become more compliant.

The contractile force generated by cardiomyocytes (CMs), a key indication of heart functionality, is dependent in part on the levels of external pressure to which the CMs are exposed before and during contraction (preload and afterload, respectively). Yet, current in vitro methods are unable to mimic the conditions that create such pressure. Consequently, these methods cannot provide sufficient information regarding CM functionality and drug response.

The proposed work aims to create an in vitro platform that overcomes this limitation, thereby revolutionizing current cardiac in vitro paradigms. The presented platform, involves the use of magnetic fields to apply dynamic forces to CM tissue, thereby mimicking in vivo conditions.

Experimental Methods
In the presented work, we aim to overcome limitations of current in vitro cardiac models by creating a human-relevant in vitro model of the cardiovascular system that mimics the dynamic environmental pressures to which CMs are subjected in vivo. In this work, we were able to:

• Develop a magnetically controllable soft substrate that uses magnetic fields to mimic preload and afterload pressures.
• Integrate CM into the magnetic substrate and identify their response to the substrate.
• Assess CM functionality under various preload/afterload conditions, and compare it to known PV loops

Results and Discussion
We have achieved a proof of concept for some of the key elements of the proposed platform (Fig. 2). Specifically, we have successfully synthesized MNPs and embedded them in biocompatible gels (gelatin) \cite{1}. In addition, a permanent magnet was used to demonstrate the gels’ response to a magnetic field \cite{2}. Then we applied an Ogden model of second order on uniaxial compression test led on gels, we determined that gels present a stiffness of 3.7 kPa which is coherent with values found in literature \cite{3} and that MNP do not act as stiffener. Finally we demonstrated that Mgels are not toxic to CM and are optimizing the growth of CMs (up to 14 days currently). Building on these promising preliminary results, we proceed to carry out the remaining steps of our research plan, including: characterising CM functionality in the Mgels and identifying how MNPs affect CM properties, developing magnetic and electric controls, and most importantly, developing an in vitro–in vivo extrapolation methodology.

Conclusion
So far, synthesis of the material is fully mastered and biocompatibility is now demonstrated. Our end goal is to determine the effect of electro mechanical stimulation, by evaluating the variation of the pace and of the number of sarcomeric unit of cardiomyocytes over different conditions.
Scientific Gap and concept
Currently, there is not in vitro system that can emulate the forces applied on the heart.

Preliminary results
(Top) The experimental design that will culminate in the establishment of a new in vitro platform for cardiac research. (Bottom) Preliminary results which demonstrate a proof of concept.

References
A biomimetic lung on chip device

Michela Licciardello1,2, Chiara Tonda-Turo1,2, Eleonora Palumbo1, Simone L. Marasso3,4, Matteo Cocuzza3,4, Giorgio Scordo3, Alberto Ballesio3, Gianluca Ciardelli1,2,5

1 Politecnico di Torino, Department of Mechanical and Aerospace Engineering, Torino, IT; 2 Politecnico di Torino, POLITO BIOMedLAB, Torino, IT; 3 Politecnico di Torino, Chilab - Materials and Microsystems Laboratory, Department of Applied Science and Technology, Torino, IT; 4 CNR-IMEM, Parma, IT; 5 Institute for the Chemical and Physical Processes (CNR-IPCF UOS), Department for Materials and Devices of the National Research Council, Pisa, IT; 6 Politecnico di Torino, Department of Mechanical and Aerospace Engineering, Torino, IT

Introduction

Lung cancer is the leading cause of cancer death around the world and non–small cell lung cancer (NSCLC) is the most frequent histologic type. Among the different type of NSCLC, adenocarcinoma is the most common one (about 40% of lung cancer) and arises from the epithelial alveolar cells [1]. The development of safe and effective therapies is currently constrained by the lack of a robust preclinical and experimental models that can reproduce the behaviour of the lung tissue. For these reasons, there is a crucial need for in vitro models that can quickly and reliably predict drug safety and efficacy in humans. In literature, several examples of microfluidic lung-on-a-chip devices were reported reproducing the physiological multicellular composition, 3D architecture and vascular perfusion of the human lung [2,3]. However, reported devices did not mimic the extracellular environment due to the use of non-biomimetic materials (mainly polydimethylsiloxane microporous membranes)[4,5]. Importantly, new biomaterial-based structures with ECM biomimetic features should be designed to have a physiological, district specific and bioartificial composition. Here, we propose the implementation of a novel technological biomimetic device to mimic the physiological condition by reproducing the basement membrane of alveolar wall and the physical stimuli characteristic of the blood-air barrier.

Experimental Methods

The biomimetic lung-on-a-chip device consists of an upper and a bottom layer made of polydimethylsiloxane (PDMS) moulded from two poly (methyl methacrylate) (PMMA) masters obtained through laser ablation method. These two layers are separated by an ECM-like electrospon membrane of Polycaprolactone/Gelatin (PCL/GL) that resembles the structural properties of the alveolar basement membrane serving as a substrate for the co-culture of epithelial and endothelial alveolar cells (Fig 1. a). The upper layer consists of a 3 mm central well completely open to atmosphere for recreating the air-liquid interface (ALI) culture condition of alveolar epithelial cells. The bottom layer has a central channel (1.5 x 16 mm) which allows the seeding of the endothelial cells in the bottom side of the membrane and guarantees cell survival by fresh medium flow. In addition, two lateral channels have been created in both layers for mechanical stimulation of the PCL/GL membrane to mimic breathing. HULEC-5a cells (human lung microvascular endothelium cells) were seeded on the bottom side of membrane and the chip was flipped over to promote cell adhesion. After 2 h, the chip was flipped over and A549 cells (human alveolar basal epithelial cells) were cultured on the upper holed chamber. To recreate the ALI condition, the culture medium on the upper chamber was removed when confluence of A549 cells was reached. Immunostaining and Live/Dead assays were performed to assess cell adhesion, proliferation and viability. Then, the formation of epithelial tight junction was investigated.

Results and Discussion

Leakage tests were performed to evaluate the successful sealing of the device channels. The bottom channel was filled with water containing blue dye and observed under optical microscope. Fig 1. b shows that the liquid flowed and remained in the channel, which demonstrated good sealing. Live/dead assay demonstrated the viability of cells after 72 h. The nuclei of A549 and HULEC-5a cells were stained with DAPI (Fig 1. c, d) confirming the adhesion of Page 1910 of 2028
cells on the two sides of the electrospun membrane. Furthermore, after 72 h in ALI condition, A549 cells demonstrated to express cell type-specific markers of both type I and II epithelial alveolar cells and the formation of epithelial tight junctions.

**Conclusion**

A biomimetic lung-on-a-chip device was obtained to better mimic the architecture of the basement membrane of the alveolar wall. The obtained results suggest that the proposed microfluidic system could be used to reproduce the microenvironment of both healthy and pathological tissues with higher fidelity thanks to the implementation of dynamic and three-dimensional cell culture conditions.

**Acknowledgement**

This project has been partially financed from Regione Piemonte under the Digital iEchnology For Lung Cancer Treatment (DEFLeCT) project.

---

Fig 1.

a) Schematic representation of a biomimetic alveolar unit composed by a PCL/GL membrane seeded with A549 and HULEC-5a in ALI and medium culture conditions, respectively; b) Representative image of a leakage test of device channels. Fluorescent microscopy images of c) A549 cells and d) HULEC-5a cells stained with DAPI (blue) (bar scale = 100 µm).

---

**References**


Recreating lung microbiota in vitro to determine antimicrobial susceptibility

Daniela P. Pacheco¹, Anna Ziccarelli¹, Federico Bertoglio², Natalia Suarez Vargas¹, Giuseppe Guagliano¹, Francesco Briatico Vangosa¹, Cosmin S. Butnarasu³, Elena Crotti⁴, Sebastião van Uden¹,⁶, Sonja Visentin³, Livia Visai²,⁵, Paola Petrini¹

¹ Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering, Milano, IT; ² University of Pavia, Center for Health Technologies (CHT), UdR INSTM, Pavia, IT; ³ University of Torino, Molecular Biotechnology and Health Sciences Department, Torino, IT; ⁴ Université degli studi di Milano, Department of Food, Environmental and Nutritional Sciences (DeFENS), Milano, IT; ⁵ Istituti Clinici Scientifici (ICS) Maugeri, IRCCS, Medicina Clinica-Specialistica, UORS Laboratorio di Nanotecnologie, Pavia, IT; ⁶ Bac3Gel, Lda, TagusPark - Edifício Inovação II, Lisbon, PT

Introduction

Bacterial aggregates are one of the most relevant causes of persistent infections. At least 65% of all bacterial infections are associated with it. Mucus is a human-designed barrier that in the lungs protect us against the constant exposure to inhaled pathogens, particles and toxic molecules, as well as physical insults. Yet, in some airway disorders, like cystic fibrosis (CF), mucus hypersecretion and accumulation provide a suitable site for bacterial infections to thrive resulting in chronic infections that lead to morbidity and mortality of patients. Infections of CF patients are characterised by the presence of different bacteria, which microbial complexity and microenvironment are difficult to recreate in current bacterial culture substrates and animal models. Therefore, novel models are needed to either screen already available antimicrobial agents in a patient-specific manner or design new molecules to tackle airway mucus infections [1]. Antimicrobial treatment of airway CF infections has been of main importance to increase the survival rates in patients with CF. However, bacteria in CF airways evolve in the form of aggregates, which makes them difficult to eradicate.

Experimental Methods

Three-dimensional mucus models were adopted, CF-Muc³Gel, as models of CF mucus to recreate lung microbiota in vitro. CF-Muc³Gel is mostly composed of 2.5 % (w/v) commercial mucins (porcine stomach type III that contains Muc5AC - one of the most important mucins found in airway mucus) and 0.71 % (w/v) NaCl that fall within the ranges determined on CF sputum. Extensive rheological analyses were carried out to characterize the viscoelastic properties of CF-Muc³Gel. In vitro infections were induced within CF-Muc³Gel by culturing Pseudomonas aeruginosa and/or Staphylococcus aureus, the prevalent bacteria colonizing the airway CF mucus. The bacterial organization was characterized through confocal microscopy. Vertical microprofiles of oxygen tension throughout the CF-Muc³Gel structure were determined through O₂ microsensor measurements of chemical gradients with or without cultured bacteria. The ability of CF-Muc³Gel to act as a platform to determine antimicrobial susceptibility was further assessed by treating the different generated in vitro infections with three antimicrobial agents routinely employed in the clinics to manage chronic infections by these bacteria. Their effectiveness was further compared to standard bacteria cultures.

Results and Discussion

CF-Muc³Gel exhibits similar viscoelastic properties alterations to those reported for CF mucus with the presence of structural gradients. CF-Muc³Gel successfully sustained the growth of P. aeruginosa and S. aureus either in monoculture or co-culture with a bacterial number representative of CF patients after 24 h of infection. Bacteria cultured within CF-Muc³Gel exhibited many pathophysiological features, as these not only were able to colonise all its structure but also generate microcolony aggregates, which size and shape resembled those observed in the...
mucus of CF patients. CF-Muc\textsuperscript{3}Gel was also able to sustain the growth of \textit{P. aeruginosa} and \textit{S. aureus} derived from clinical isolates. Measurements of O\textsubscript{2} microprofiles in CF-Muc\textsuperscript{3}Gel without bacteria, revealed a gradual decrease of O\textsubscript{2} tension. Similar to what was previously reported in CF sputum with chronic \textit{P. aeruginosa} airway infections, CF-Muc\textsuperscript{3}Gel infected with either \textit{P. aeruginosa} or \textit{S. aureus} exhibited dual O\textsubscript{2} distribution with an upper aerobic region and a lower region that was completely anoxic after 48 hours of infection. The interplay of all these features resulted in a similar barrier to antimicrobial treatment. Bacteria resulted more susceptible to antimicrobial treatment under planktonic conditions than when cultured within CF-Muc\textsuperscript{3}Gel, where these instead displayed increased antibiotic tolerances even at high concentrations of antimicrobial drugs (10 MIC). The sensibility differences between CF-Muc\textsuperscript{3}Gel and planktonic cultures confirmed the well-reported mismatch between planktonically cultured bacteria and clinical outcomes.

**Conclusion**

CF-Muc\textsuperscript{3}Gel is a very versatile bacteria culture substrate that can be further complicated with other components, such as proteins, which can integrate different culture media and be exploited for different culture applications to model the mucus of different body districts. In addition, from the antimicrobial studies, rheological data and microstructural inspections, it is possible to propose CF-Muc\textsuperscript{3}Gel as a new, valid tool for the screening of antimicrobial agents for the research and development of new antimicrobial agents. CF-Muc\textsuperscript{3}Gel is ready-to-use without requiring any technical skills and offers superior reproducibility.

**Acknowledgement**

The authors would like to thank the Switch2Product grant (UA.A.RRR.ARICID.SVRA.AUTO.AZ18VAR110), Politecnico di Milano, and EIT Health Headstart programme for partially funding the validation of Bac3Gel technology.

**References**

A new printable alginate / hyaluronic acid / gelatin hydrogel suitable for biofabrication of in vitro and in vivo metastatic melanoma models

Rafael Schmid¹, Sonja K. Schmidt², Rainer Detsch³, Hannes Horder⁴, Torsten Blunk⁴, Stefan Schrüfer⁵, Dirk W. Schubert⁶, Lena Fischer⁶, Ingo Thievesen⁶, Stefanie heltmann-Meyer¹, Dominik Steiner¹, Dominik Schneidereit⁷, Oliver Friedrich⁷, Anika Grüneboom⁸, Hanna Amouei⁹, Harald Wajant⁹, Raymund E. Horch¹, Anja K. Bosserhoff⁵, Andreas Arkudas¹, Annika Kengelbach-Weigand¹

¹ University Hospital Erlangen, Department of Plastic and Hand Surgery, Erlangen, DE; ² Friedrich-Alexander University of Erlangen-Nürnberg, Institute of Biochemistry, Erlangen, DE; ³ Friedrich-Alexander University of Erlangen-Nürnberg, Institute of Biomaterials, Erlangen, DE; ⁴ University of Würzburg, Department of Trauma, Hand, Plastic and Reconstructive Surgery, Würzburg, DE; ⁵ Friedrich-Alexander University of Erlangen-Nürnberg, Institute of Polymer Materials, Erlangen, DE; ⁶ Friedrich-Alexander University of Erlangen-Nürnberg, Biophysics Group, Department of Physics, Erlangen, DE; ⁷ Friedrich-Alexander University of Erlangen-Nürnberg, Institute of Medical Biotechnology, Erlangen, DE; ⁸ ISAS e.V., Bioimaging, Dortmund, DE; ⁹ University Hospital Würzburg, Division Molecular Internal Medicine, Department of Internal Medicine II, Würzburg, DE

Introduction
Scientists have made several advances over the years in order to study tumor pathophysiology. Formerly, usually artificial 2D cultures have been used but their results are often not transferable to the in vivo situation. There has been a trend to more natural 3D models that have a more promising outcome. Additionally, the field of biofabrication has provided the possibility to enhance these models even more, for in vitro as well as in vivo applications.

Experimental Methods
With this study, we created a printable hydrogel consisting of alginate, hyaluronic acid, and gelatin (Alg/HA/Gel). We characterized its printability, mechanical properties, and suitability as a basis for in vitro melanoma models mimicking the tumor microenvironment and, in vivo, its effect on tumor progression, vascularization, and metastasis in a defined and isolated arteriovenous (AV) loop model in the rat.

Results and Discussion
The hydrogel had mainly elastic properties with a storage modulus of 10.5 kPa at 1 rad s⁻¹ using dynamic mechanical analysis. In vitro, the bioink showed good printability and shape-fidelity with high survival rates of the human melanoma cell line Mel Im. Cell cycle analysis visualized with a FUCCI cell cycle reporter of these cells revealed no impact on cell cycle populations over one week. Adipose-derived stem cells were able to differentiate into the adipogenic and the osteogenic lineage over 21 days, demonstrated via Oil Red O and Alizarin red stainings, and qPCR. Using a GpL fusion protein producing cell line, diffusion of proteins with a size of 150 kDa through the hydrogel was confirmed. In vivo, the hydrogel facilitated good tumor progression, vascularization and reliable metastases, closely resembling the human morphology and course of disease, over four weeks. Histological sections (HMB-45) and whole-mount light sheet fluorescence microscopy (anti-CD31) of the explants supported these findings.

Conclusion
In summary, this makes the bioink a versatile tool for basic and applied cancer research. In combination with the AV loop model, it is a unique in vivo model to study melanoma and possible therapis.

Acknowledgement
The work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation), Project number 326998133, TRR 225 (subprojects C03 and A01, A07, B06, B08, C02, C04).
A dynamic microscale mid-throughput fibrosis model to investigate the effects of different ratios of cardiomyocytes and fibroblasts

Andrea Mainardi\textsuperscript{1,2}, Francesca Carminati\textsuperscript{1,2}, Giovanni S. Ugolini\textsuperscript{2}, Paola Occhetta\textsuperscript{1,2}, Giuseppe Isu\textsuperscript{1}, Diana Robles Diaz\textsuperscript{1}, Gregory Reid\textsuperscript{1}, Roberta Visone\textsuperscript{2}, Marco Rasponi\textsuperscript{2}, Anna Marsano\textsuperscript{1}

\textsuperscript{1} University of Basel, Department of Biomedical Engineering, Basel, CH; \textsuperscript{2} Politecnico di Milano, Biomechanics Group, Milano, IT

Introduction
Cardiac fibrosis is a maladaptive remodeling of the myocardium hallmarked by a contraction impairing, adverse, extracellular matrix deposition (ECM). The disease's progression, nevertheless, remains poorly understood and present treatments are not capable of stopping the scarring process. This lack correlates with the absence of physiologically relevant, easily operable, and low cost \textit{in-vitro} models, which are of the utmost importance to uncover pathological mechanisms and highlight possible targets for anti-fibrotic therapies. In classic models, fibrosis' features are obtained using substrates with scar mimicking stiffnesses and/or supplementation of morphogens such as Transforming growth factor b1 (TGF-b1). Qualities such as the interplay between activated fibroblasts (FBs) and cardiomyocytes (CMs) and the mechanically active, three-dimensional (3D) environment, are, however, neglected or obtained at the expense of the experimental yield.

Experimental Methods
We engineered a micro-physiological system (MPS) where multiple 3D microconstructs can be subjected to cyclical stretching. Up to six different biologically independent samples are obtained in a single device, increasing the experimental throughput, paving the way for higher yielding drug screening campaigns. We studied the effect of different ratios of neonatal rat FBs and CMs in the assumption and modulation of fibrosis traits, without the addition of morphogens, and in soft substrates.

Results and Discussion
The expression of contractile stress fibers and of degradative enzymes, as well as the deposition of fibronectin and type I collagen were superior in microtissues with a low amount of CMs. Moreover, high CMs-based microconstructs, simulating a ratio similar to that of healthy tissues, subjected to both cyclic stretch and TGF-b1 did not show any of the investigated fibrotic signs, indicating a CMs fibrosis modulating effect.

Conclusion
Overall, this \textit{in vitro} wound healing model could help to uncover new disease aspects, studying with higher throughput and in a mechanically active, physiologically relevant environment the crosstalk between the most abundant cell types involved in fibrosis.
2:00 p.m. – 3:30 p.m.

Poster floor

**PS2-15 | Surface-Modified Biomaterials**
Surface modification of neural electrodes via Pulsed-DC Electrophoretic Deposition and their *in-vivo* application

Vaijayanthi Ramesh¹, Christoph Rehbock¹, Svilen D. Angelov², Kerstin Schwabe², Joachim K. Krauss², Stephan Barcikowski¹

¹ University of Duisburg-Essen, Institute of Technical Chemistry I, Essen, DE; ² Hannover Medical School, Department of Neurosurgery, Hannover, DE

**Introduction**

Motor neuron diseases like Parkinson’s can induce severe tremors in later stages, which seriously impair the daily life of elderly patients, particularly when drug-based treatments have ceased to work. A possible way to improve the patients’ quality of life is deep brain stimulation (DBS) treatments. Here, platinum neural electrodes are implanted into the brain, providing electric pulses to stimulate the subthalamic nucleus (STN) and in turn tune neuronal activity[1]. Even though this treatment is routinely applied in clinical practice, difficulties such as low stimulation efficiency and reduced battery capacity of the pulse generator due to the increase of impedance over time between electrode tip and brain tissue occur[2].

**Experimental Methods**

Here we show that the impedance of neural electrodes can be significantly reduced in case their surface area is increased by sub-monolayer deposition of ligand-free laser-fabricated platinum nanoparticles (PtNPs)[3]. The coating of 3D electrode surfaces is done using electrophoretic deposition (EPD) in a custom-designed chamber as depicted in Figure 1 with a field strength of 5 V/cm and a platinum mass concentration of 100 µg/ml. We compared the impact of DC (5 min) and pulsed-DC EPD (period of 1 µs and a duty cycle of 50%, 10 min) on stimulation conditions.

**Results and Discussion**

Our findings reveal that the pulsed-DC coating procedure yields a more homogeneous surface coating and also the impedance is significantly reduced in contrast to the uncoated controls (Figure 2), an effect which could not be observed for DC-EPD, as previously described in literature[2].

**Conclusion**

In consecutive studies, the coated electrodes will undergo long term *in vitro* stimulations in saline solutions and *in vivo* stimulations in rat models and the influence of DC and pulsed DC electric fields on their functionality will be evaluated.
Electrode EPD chamber

In vitro impedance of electrodes (N = 15, p = 0.05)

References
Antioxidant polyphenolic implant coatings for improved wound healing

Florian Weber, Quang Huy Quach, Per H Nilsson, Hanna Tiainen

1 University of Oslo, Department of Biomaterials, Institute of Clinical Dentistry, Oslo, NO; 2 University of Oslo, Department of Immunology, Institute of Clinical Medicine, Oslo, NO; 3 Linnaeus University, Department of Chemistry and Biomedical Sciences, Kalmar, SE

Introduction
Biomaterial associated infections and lack of tissue integration are still a widespread problem preventing the long-term success of implants.[1] To overcome the risk of implant failure and revision surgeries, various surface modifications are being studied to alter the foreign body response and prevent microbial colonization. Thereby, the aim is to create a close contact of the surrounding tissue to the implant surface forming a natural barrier, which prevents infections.

Improved tissue integration is closely connected to the inflammatory response governed by the foreign body reaction and the surgical trauma. To relieve this inflammatory response and support wound healing processes, polyphenolic molecules have emerged as potential candidates.[2] Their anti-inflammatory properties are attributed to their capability to reduce oxidative stress and inhibit pro-inflammatory cytokine expression. Further, polyphenolic surface modifications may cope with adverse foreign body reactions.[3] Therefore, we evaluated innate immune response towards tannic acid and pyrogallol coatings. In particular, the effect of these polyphenolic coatings on blood coagulation and complement activation was assessed.

Experimental Methods
Tannic acid (TA) and pyrogallol (PG) coatings were prepared by immersing Ti substrates in 100 mM HEPES buffered solutions containing 1 mg/ml TA or PG. TA solutions contained 600 mM NaCl and 100 mM Siaq at either pH = 6.8 or pH = 7.8. PG solutions contained 100 mM MgCl₂ at pH = 7.0.

Human whole blood was obtained from three donors. Informed written consent was obtained from each donor and experiments with human blood were performed according to the ethical guidelines from the declaration of Helsinki with the approval of local ethical committee. Blood was collected in polypropylene tubes containing 0.5 ml of 500 mg/ml lepirudin. 400 ml aliquots were incubated with one coin each of the different surfaces in a rolling incubator at 37°C. Coagulation markers (TAT and F1+2) and complement markers (C3bBbP, C4d, TCC) were determined by ELISA assays after 30 min. Further, platelet, monocyte and granulocyte activation was determined after 30 min using flow cytometry. Expression of inflammatory cytokines was evaluated after 4 h using MultiPlex system. Experiments with blood from each donor were performed in triplicates (nₑ = 9).

Results and Discussion
The activation product of the classical and the lectin complement pathway, C4d, was detected at elevated levels on all surfaces (Figure 1A). Similarly, the marker of the alternative pathway, C3bBbP, was found in all samples (Figure 1B). This is in agreement with known activation of the alternative pathway on surfaces bearing nucleophiles, such as polyphenolic hydroxyl groups, and on protein layers formed on surfaces.[4] Regardless of the activation, all pathways of the complement system converge to form terminal complement complex (TCC). Although elevated levels of C4d and C3bBbP were detected for all surfaces, polyphenolic coatings did not induce increased levels of TCC (Figure 1C). These results suggest that the modified surfaces may inhibit the further amplification process of the complement cascade.

Ti surfaces and polyphenolic coatings induced high levels of both thrombin activation markers TAT and F1+2 (Figure 1D, E). High activation of the coagulation system by Ti is in accordance to literature and often correlated to the...
remarkable tissue integration of Ti surfaces. Our results showed that the coated surfaces maintain the thrombogenic activity of bare Ti, despite their altered surface chemistry. Activation of the coagulation system was also corroborated by platelet activation (Figure 2A).

The activation of monocytes and granulocytes was assessed as a hallmark for acute inflammation. Both activation markers CD11b and CD35 remained unchanged at the background level observed in the negative control (Figure 2B, C). These results imply that although different surface coatings activated the complement system, the stimulation was not sufficient to elicit a cellular response. This finding was in agreement with the expression profile of inflammatory cytokines (Figure 2 D-F). While TA-coated surfaces did not induce a significant change in expression of pro-inflammatory cytokines, PG coatings upregulated leukocyte chemotactic cytokines.

**Conclusion**

TA and PG coatings activated the complement system similar to Ti surfaces. However, the formation of TCC was reduced on the TA and PG coated surfaces. The thrombogenic properties of Ti were retained by TA and PG coated surfaces, which led to platelet activation. In contrast, monocytes and granulocytes were not activated by Ti or polyphenolic coatings, which was represented in the cytokine expression. Therefore, we conclude that polyphenolic coatings did not elicit inflammation or an adverse foreign body response, and are promising candidates for the further development of antioxidant implant coatings to support wound healing and tissue integration.

**Acknowledgement**

This work was financially supported by the Research Council of Norway, grant number 302590 and 274332.
Figure 2: Platelet, monocyte, granulocyte activation, and cytokine expression. (A) P-selectin (CD62P) and CD63 expression indicating activation and aggregation of platelets. (B, C) Expression of macrophage-1 antigen (CD11b) and complement receptor 1 (CD35) on monocytes and granulocytes as marker for inflammatory activation. (E - F) IL-2, IL-6, and IL-8 concentration in blood after a 4 h incubation period with polyphenol coated substrates.

References
**PS2-15-450**

**A bio-inspired antimicrobial peptide coating for dental magnesium implants**

**Dennis A. Böhner, Kathrin Bellmann-Sickert, Annette G. Beck-Sickinger**

*Leipzig University, Institute of Biochemistry / Faculty of Life Sciences, Leipzig, DE*

**Introduction**

The progressive aging of the population, which implies an increase in degenerative bone diseases, has significantly increased the market for dental implants. Traditionally, dental implants are made of stainless steel, titanium or cobalt-chromium alloys, which generally have a high overall survival rate. Nevertheless, material-related inflammation and post-operative infections are a high-risk factor for implant failure due to periprosthetic bone loss and subsequent loss of osseointegration [1]. Bacterial infections are associated with biofilm formation on the implant surface, which exhibits a higher resistance to antimicrobial agents. Thus, prevention of bacterial colonization at the implant surface is of high interest but remains challenging [2]. Recently, we developed a bio-inspired surface-binding peptide containing L-3,4-dihydroxyphenylalanine (DOPA) for the coating of titanium, polystyrene and polycaprolactone-collactide [3-5]. By immobilizing cell-binding peptides on titanium we significantly enhanced cell-surface interaction and cell viability of osteoblast-like cells [3]. Furthermore, functionalization of the carrier-peptide with chemokines such as the CXC chemokine ligand 12 improved wound healing [4]. Transferring this approach to dental implants has the potential to decrease to the risk of post-operative infections and implant failure. Here, we report on the synthesis of a surface-binding peptide for magnesium alloys.

**Experimental Methods**

For the synthesis of a DOPA-containing surface-binding peptide, we applied solid phase peptide synthesis using standard Fmoc/tBu strategy. For detection on magnesium surfaces, a biotin moiety was incorporated. Using biotinylated peptides, the immobilization was investigated by an on-surface ELISA-like assay with streptavidin coupled to an enzymatic reporter. To assess the antimicrobial power of the target antibiotics, broth dilution assays were used to determine the minimal inhibitory concentration of amoxicillin and ciprofloxacin against *Escherichia coli*.

**Results and Discussion**

Using on-surface ELISA the efficient surface binding of the carrier peptide on magnesium alloys was demonstrated. Further, we demonstrate the antimicrobial activity of two commonly applied antibiotics in a cell-based setup.

**Conclusion**

Our surface-binding peptide represents a promising approach to immobilize different antibiotics like amoxicillin and ciprofloxacin commonly used in dental surgeries on magnesium alloy implants.

**Acknowledgement**

The authors are thankful for the financial support from the M-era.Net, the EU as well as of the Graduate School BuildMoNa.

**References**


---

Page 1922 of 208
Development and morphological tuning of Plasma Electrolytic Oxidation titania coatings in a novel sodium borate electrolyte in galvanostatic and pulsed conditions

Matteo Pavarini, Monica Moscatelli, Luigi De Nardo, Roberto Chiesa

Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering, Milano, IT

Introduction
Plasma Electrolytic Oxidation (PEO) is a simple and effective electrochemical surface modification technique that can be applied to the so called “valve metals”, such as titanium and its alloys, to produce thick oxide coatings. This process has raised great interest in the biomedical field for its ability to produce porous biocompatible surfaces [1], especially by fine tuning of the electrochemical process parameters. However, conventional PEO electrolytes, mainly based on Ca-P containing compounds, typically need strict thermal control to produce optimal coatings or conversely require very high applied current densities and voltages, thus reducing efficiency. Thereby, alternative electrolytes, such as borates and silicates, can be useful to overcome those limitations: the former can produce very hard and compact oxide film [2], while the latter lead to surfaces with high porosity [3].

In our work, we focused on the development of an efficient PEO treatment, in both galvanostatic and pulsed conditions, capable to produce coatings with a surface morphology tuned for bone-contact applications.

Experimental Methods
Grade 2 titanium specimens were treated in a novel alkaline electrolyte made of 0.1M Na₂B₄O₇, 0.1M NaOH and 0.02M Na₂SiO₃. The PEO treatment in galvanostatic conditions was conducted by applying different current densities (25 ÷ 200 mA/cm²) and voltage limits (180 ÷ 400V) for variable times (30 s ÷ 15 min), at room temperature. In pulsed DC conditions, three different duty cycles (10%, 50% and 90%) and two treatment frequencies (100 and 800Hz) were explored. The treated samples were then observed via SEM and characterized in terms of pore diameters and average porosity through the ImageJ software, to identify possible relations between the applied treatment parameters and the coatings’ properties. The cross-sectional features of the coatings were then assessed by direct observation of their metallographic sections.

Results and Discussion
The borate-based electrolyte showed high thermal stability in all the treatment conditions. In galvanostatic regime, the pore size and porosity of the coatings showed a similar trend as a function of the applied current densities and final voltages, with a constant increase up to a certain threshold (50mA/cm², 300V), after which wider deviations, ascribable to an increase in surface inhomogeneity, could be observed. As for the process duration, samples treated for more than 7 min didn’t show any further difference in the coatings’ morphology, because of the discharge process’ interruption at constant voltage.

The coatings produced with optimized parameters (J=50mA/cm², 300V, 7 min) showed a microporous surface morphology (Fig. 1a), featuring “volcano-like” pores, with an average pore diameter of 900 nm and an 8% porosity. Moreover, the coatings’ cross-section shows an interesting bilayer structure (Fig. 1b), with a compact inner layer and a more porous surface one, probably due to the predominant presence of borate and silicate, respectively.

In pulsed conditions, the surface features of the produced coatings highlight clear trends with varying process parameters (Fig. 2): as the duty cycle is raised, the average pore size and surface homogeneity increase, with a steeper variation for duty cycles up to 50%, and only slight variations above this threshold, while, at high treatment frequency, the pores are smaller and less homogeneously distributed. Similar trends can be observed for the coating
thickness, with thicker oxide layers produced at low frequencies and high duties. In all the explored conditions, except for 10% duty, the obtained pore size is potentially optimal for perspective orthopedic applications, since pores with characteristic dimensions similar to the typical bacteria involved in periprosthetic joint infections (0.5÷2.5 µm) can inhibit their adhesion and proliferation [4] while promoting osteogenic cells’ growth.

Conclusion
The newly developed sodium borate electrolyte allowed the production of homogeneous and porous PEO coatings, with features optimized by the tuning of the process parameters in both galvanostatic and pulsed conditions, showing promising topographical features for potential use in bone-contact applications.

Fig. 1
SEM micrographs of PEO samples produced in optimized galvanostatic conditions. a Treated surface. b Coating’s cross-section.
Fig. 2
Surfaces of the PEO samples produced in different pulsed DC conditions. a 10%, 100Hz b 10%, 800Hz c 50%, 100Hz d 50%, 800Hz e 90%, 100Hz f 90%, 800Hz.

References
Immobilization of gelatin on electrospun fibers: a comparative analysis of aminolysis-based procedure and physisorption for three aliphatic polyesters

Oliwia Jeznach, Dorota Kołbuk, Paweł Sajkiewicz

Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, PL

Introduction
Immobilization of adhesive proteins, such as gelatin, collagen, fibronectin on the scaffold surface has become a widely reported method that can improve the interaction between scaffold and cells. It is achieved through various methods originated from chemical binding or physical interaction between biomolecule and polymer [1,2]. In this study, we modified the surface of three types of electrospun fibers using chemical immobilization of gelatin basing on aminolysis and glutaraldehyde cross-linking or physisorption of gelatin and evaluated the change of materials properties as well as L929 cells response.

Experimental Methods
Poly(caprolactone) (PCL), poly(L-lactide-co-caprolactone) 70:30 (PLCL) and poly(L-lactide) (PLLA) fibers were obtained by electrospinning. The aminolysis reaction was carried out by immersing nonwovens in ethylenediamine solution in isopropanol under various treatment conditions to test the susceptibility of fibers to the reaction. Two sets of conditions were chosen to evaluate immobilization for samples with lower and higher concentrations of free NH₂ groups. Then, gelatin was chemically immobilized on the surface of given samples using glutaraldehyde as a cross-linking agent. Samples with physisorbed gelatin were prepared by simple immersion in gelatin solution. Ninhydrin test and BCA test were used to measure the density of amine groups and gelatin on the surface, respectively. Change of morphology, average molecular weight and crystallinity were determined using scanning electron microscopy, gel permeation chromatography and wide-angle X-ray scattering technique, respectively. The wettability of modified samples was measured by a goniometer. Mechanical properties were determined using uniaxial tension testing. The stability of the coating originated from chemical binding or physical interaction was verified via incubation in phosphate-buffered saline (PBS) from 1 to 90 days. L929 cells were cultured on modified samples to investigate the biological response to modified samples after 3 and 5 days.

Results and Discussion
It was shown that aminolysis-based immobilization could provide a higher concentration of gelatin on the fiber surface in the case of all investigated polyesters (Fig.1.). However, gelatin concentration was relatively high in the case of physisorption, especially when comparing to samples aminolyzed under mild reaction conditions. However, the incubation test showed that the chemically immobilized gelatin layer is more stable than physisorbed one. After 90 days of immersion, more than 60% of the initial concentration of protein was detected for a physically modified PCL sample while the values were higher than 90% and 80% for aminolyzed samples with lower and higher concentration of free NH₂ groups, respectively (Fig.2.). Despite a similar concentration of free NH₂ groups among all polyesters, the concentration of gelatin was significantly higher in the case of PCL fibers. The cause of this phenomenon could be the difference in the accessibility of groups on the surface, the difference in fiber diameter or roughness, which could influence the amount of immobilized gelatin for each polymer. For chosen conditions, there was no difference in fibers morphology between chemically and physically modified samples, however, it is known that aminolysis could cause fracturing of fibers [3]. Gelatin immobilization contributed to the change of the fibers properties from hydrophobic to completely hydrophilic after each studied modification. Additionally, the time of water drop absorption...
was correlated with the density of gelatin on the surface of the fibers. Young's modulus, stress and strain at break results were very similar for samples subjected to physisorption and mild chemical immobilization. In the case of PLCL and PLLA samples, aminolysis conditions that were applied to provide a higher concentration of free NH2 groups result in a significant decrease of stress and strain at break. PCL sample with a similar concentration of free NH2 groups was much more resistant to loss of mechanical strength. Improvement of L929 cells morphology and metabolic activity was observed for all modified samples, wherein the nonsignificant differences between samples subjected to physisorption and mild chemical immobilization were observed.

Conclusion

Our study showed that aminolysis-based chemical immobilization of gelatin could provide a higher concentration of protein on the surface than physisorption. Chemically attached gelatin was additionally more stable than a physisorbed one. However, it is important to optimize reaction conditions depending on the scaffold application because aminolysis can significantly reduce the average molecular weight of the polymer and the resulting mechanical properties of the scaffold. Morphology and metabolic activity of L929 cells were improved in the case of each modification, but the most significant differences were observed for PCL samples and PLLA sample with a higher concentration of free amine groups.

Acknowledgement

This research was funded by the Polish National Science Center (NCN), grant number 2016/23/B/ST8/03409 and supported by ESF, POWR.03.02.00-00-1028/17-00.
Fig. 2. Concentrations of gelatin on PCL fibers after incubation in PBS for various time points.

References


Engineering micro/nanoscale surface topographies for corneal tissue regeneration

Gozde Sahin, Nello Formisano, Rhiannon Grant, Roman Truckenmüller, Stefan Giselbrecht

Maastricht University, Department of Instructive Biomaterials Engineering / MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, NL

Introduction
The cornea is a transparent tissue located at the outer part of the eye, whose function is to protect the eye from external damage and to provide most of its refractive power. Trauma or disease affecting the cornea can cause vision loss and subsequent blindness. This can be overcome via corneal transplantation, but suitable donor tissue is only available to a fraction of patients worldwide [1]. One of the most explored alternative approaches to address this shortage is to expand primary corneal cells in vitro. However, current cell culture protocols result in limited expansion of corneal cells and loss of their phenotypes. To overcome this limitation, instructive culture substrates comprising topographical features and materials properties that mimic essential cues present in their native corneal microenvironment may be a key tool. Fabricating multiscale structures with precise control over micro/nano-features, as those present in in vivo corneas, is challenging to achieve by a single technique and would likely require a combination of multiple nanofabrication techniques, some of which have not been entirely employed in this field [2]. To address this challenge, we created a library of tools for fabricating substrate topographies from micro- to nanoscale on various biocompatible polymer films to systematically screen for desired cell responses.

Experimental Methods
First, to test and optimize the fabrication of surface topographies by conventional photolithography, we designed and produced polygonal features with lateral dimensions between 10 μm and 100 μm (Figure 1a). Then, we established soft-lithography and nanoimprint lithography protocols to replicate these topographies onto thin thermoplastic biocompatible polymer films (Figure 1b-e), such as polycarbonate (PC), polystyrene (PS), polylactic acid (PLA), poly(methyl methacrylate) (PMMA) and cyclic olefin copolymers (COC).

Results and Discussion
The imprinting quality (incomplete filling, surface roughness, demoulding damages, etc.) is characterized for each engineered substrate by optical profilometry and scanning electron microscopy (SEM). Preliminary results indicate that cell morphology, alignment and proliferation can be tuned by these cell substrates. Further analysis of these parameters, together with analysis of phenotypical markers of corneal cells will be performed for the engineered substrate.

Conclusion
These engineered surfaces serve as a novel, high-throughput and robust method of screening instructive substrates for corneal cells, increasing cell proliferation and maintaining their corneal phenotype. Subsequently, this platform can be expanded to provide an infinite cell source for cell-based therapies and tissue-engineered equivalents to overcome the shortage of cornea donors, and other tissue types.

Acknowledgement
This work was performed under the framework of Chemelot InSciTe.
Figure 1
Optical profilometer images of microfeatures on silicon wafer after photolithography (a) and polycarbonate film after nanoimprint lithography (b-e). a) Polygon test patterns with dimensions between 10 to 100 μm, b) polycarbonate film that include microchannels with an approximate width and height of 30 μm, c) concentric circles with a width of 30 μm, d) micropillars with a diameter of 35 μm and e) rectangles with dimensions of 30 μm x 60 μm.

References
Development of advanced bioactive glass-based coatings by electrostatic spray deposition

Verónica Müller¹, Matías Jobbagy², Elisabeth Djurado¹

¹ Univ. Grenoble Alpes, Univ. Savoie Mont Blanc, Grenoble INP, LEPMI, Grenoble, FR; ² Univ. Buenos Aires, FCEyN, INQUIMAE-DQIAQF, Buenos Aires, AR

Introduction

Bioactive glasses (BG) are the subject of intensive investigation as a bone replacement material since the discovery of a 4-component glass in 1969 by Hench, nowadays known as 45S5 (46.1 SiO₂ – 24.4 Na₂O – 26.9 CaO – 2.6 P₂O₅ % mol) [1]. However, due to brittleness and poor mechanical properties, direct BG clinical applications are limited to non-load-bearing implants. Nevertheless, BG can be employed as coatings on a metallic substrate to combine their biological performance with mechanical strength. Many deposition processes are currently investigated to produce BG coatings [2]. However, the fabrication of BG coatings by sol-gel method coupled with the electrostatic spray deposition (ESD) has never been attempted to the far of our knowledge.

Based on electrohydrodynamics laws, ESD is a simple and low-cost technique that allows the deposition of films with precise control of thickness, microstructure, and chemical composition. In our previous work, by coupling the sol-gel method with the ESD technique, we have obtained a robust, one-step method for the fabrication of single-phase nanocrystalline hydroxyapatite coatings [3]. This study focuses on manufacturing BG coatings belonging to the SiO₂-CaO-P₂O₅ system, starting from homogeneous liquid precursor solutions deposited on Ti6Al4V substrates. Here, through this technique, we have successfully obtained one-step coatings with optimized S85 (85 SiO₂ – 10 CaO – 5 P₂O₅ mol. %), S75 (75 SiO₂ – 20 CaO – 5 P₂O₅ mol. %) and S58 (58 SiO₂ – 37 CaO – 5 P₂O₅ mol. %) compositions [4].

Experimental Methods

Bioactive glasses (BG) coatings were prepared using a vertical ESD set-up on polished commercial Ti6Al4V ELI plates. Precursor solutions were prepared by using either triethyl phosphate, referred to as TEP (Aldrich, 99.9%) or H₃PO₄ (Aldrich, 85 %) as P(V) source, tetraethyl orthosilicate, TEOS (Aldrich, 99.999%) as Si(IV) source and Ca(NO₃)₂·4H₂O (Merck, 99.95%) as Ca(II) source. Methanol (CH₃-OH, MetOH), Ethanol (CH₃-CH₂OH, EtOH), and diethylene glycol monobutyl ether, referred to as butyl carbitol (C₄H₉(OCH₂CH₂)₂OH, BC), were chosen as solvents for the preparation of the precursor solution. To investigate the influence of processing conditions on the characteristics of the coatings, such as composition, structural and microstructural properties, several ESD parameters were considered -i.e. nature of P(V) precursor, absolute precursor solution concentrations, solvent composition, substrate temperature, and deposition time.

The microstructure and composition of the obtained coatings were characterized by scanning electron microscopy (SEM) coupled with an EDS probe. Their structural properties were determined using X-ray diffraction (XRD) and Raman spectroscopy.

Assessment of in vitro bioactivity was carried out in simulated body fluid solution (SBF) according to the ISO standard norm (22317: 2014) on optimized S85, S75, and S58 coatings.

Results and Discussion

All BG-coatings deposited by ESD displayed an amorphous character as detected by XRD. Microstructures from denser to coral-like and highly porous morphologies were successfully obtained regardless of the coating composition (Fig. 1). EDX analyses show the presence of silicon, calcium, and phosphorus homogeneously distributed in the volume of films, according to the above formulations. Besides, their bioactivity behavior has been studied by Page 1932 of 2028
immersion in SBF solution for 24 h. The biological performance of BG was found to be strongly dependent on their texture. Highly porous coatings led to remarkable bioactivity of the films with S75 and S58 compositions, compared with more compacted ones. The films with S85 compositions, whatever is the morphology, were found highly reactive. Indeed, almost complete dissolution was carried out within 24 h of immersion.

**Conclusion**

The present work introduces an innovative preparation of glass-based coatings, prepared for the first time by coupling the sol-gel method with ESD. Coatings with S85, S75, S58 compositions were successfully obtained after optimization of i) the precursor solution and ii) the substrate temperature. All the obtained deposited coatings were crack-free and presented an amorphous environment with original coating microstructures. Morphologies ranging from highly porous coral-like toward more compact cauliflower-type were obtained showing the potential of ESD for easily tune the coating texture. Furthermore, the ESD method has proven to be very successful for developing highly reactive bioactive glass coatings that offer complete transformation into an apatite layer after 24 h of soaking in SBF for S75 and S58 BG coatings.

![Fig. 1](image)

**Fig. 1**

a) SEM surface micrographs of as-deposited BG coatings for S85, S75, and S58 formulations, obtained with different mixtures of solvents, M (MeOH: EtOH (8:2)), E (pure EtOH), and B (BC: EtOH (8:2)); b) SEM cross-sections of S58 formulation on top of Si-wafer using M, E and B solvent mixtures.

**References**

PS2-15-460

The Role of Surface Properties of Inorganic Materials for Improved Biological and Antimicrobial Response: Wettability, Zeta Potential and Biological Functionalization

Mari Lallukka, Francesca Gamna, Marta Miola, Sara Ferraris, Silvia Spriano, Enrica Verné

Politecnico di Torino, Turin, IT

Introduction
Surface properties of a biomaterial are one of the most influencing parameters in the context of a successful implant. Especially the complex phenomena occurring at the material - implant interface upon contact with physiological fluids and biological environment are yet to be completely understood. Here suggested innovative surface engineering methods could have potential to improve implant longevity through enhanced and selective protein adsorption and cell response, while simultaneously preventing biofilm formation.

Experimental Methods
In this work, a range of biomaterial surfaces are characterized and compared in terms of their zeta potential, wettability, and surface chemistry. The focus is especially on silica-based bioactive glasses, either doped with antibacterial silver (Ag) or copper (Cu) ions via ion exchange, or with other ions with therapeutic effects directly incorporated in the composition of the glass (zinc (Zn) ans strontium (Sr)). A separate focus is on the titanium alloy Ti64ELI surface treated to be suitable for functionalization.

The pristine and modified surfaces are characterized by means of SEM-EDS, XRD, zeta potential titration curves, and contact angle measurements. Furthermore, the release of therapeutic ions from the doped bioactive glasses either in Simulated Body Fluid (SBF) or in acidic (pH 5) sodium acetate buffer up to four weeks is evaluated by means of ICP-OES analysis. Finally, biological functionalization by grafting antimicrobial peptide nisin is studied and evaluated on both titanium and glass surfaces.

Results and Discussion
Silver and copper ions were successfully introduced on the bioactive glass surfaces without unwanted crystallization phenomenon, and without negatively affecting hydroxyapatite formation, and hence, bioactivity. The introduced ions also affect the surface zeta potential and wettability of the doped glasses. The release test of the antibacterial and osseoinductive ions, and the evaluation of the antibacterial efficacy of grafted nisin are in progress.

Acknowledgement
The authors received funding from European Union’s Horizon 2020 research and innovation program under grant agreement No. 860462 project PREMUROSA.
The Role of Surface Properties of the Implant Material
Artificial extracellular matrices containing bioactive glass nanoparticles promote osteogenic differentiation of human mesenchymal stem cells

Felix Allerdt¹, Lysann Kroschwald², Anne Bernhardt², Matthias Schnabelrauch³, Michael Hacker⁴, Michaela Schulz-Siegmund⁵, Kai Zheng⁶, Aldo R. Boccaccini⁶, Stefan Rammelt⁷, Vera Hintze¹

¹ Technische Universität Dresden, Max Bergmann Center of Biomaterials, Institute of Materials Science, Dresden, DE; ² Technische Universität Dresden and University Hospital Carl Gustav Carus, Germany, Centre for Translational Bone, Joint and Soft Tissue Research, Dresden, DE; ³ INNOVENT e.V. Jena, Biomaterials Department, Jena, DE; ⁴ University of Düsseldorf, Institute of Pharmaceutical Technology, Düsseldorf, DE; ⁵ University of Leipzig, Pharmaceutical Technology, Medical Faculty, Leipzig, DE; ⁶ University of Erlangen, Department of Materials Science and Engineering, Institute of Biomaterials, Erlangen, DE; ⁷ University Hospital Carl Gustav Carus of Technische Universität Dresden, University Centre for Orthopaedics and Trauma Surgery, Dresden, DE

Introduction

Due to the demographic development with an increasing number of patients with reduced healing capacities, there is a high need for treatment options improving the regeneration of damaged tissue, e.g. bone. Mimicking the cellular microenvironment by including components of the extracellular matrix (ECM) is one promising option to derive novel functional biomaterials for promoting tissue regeneration. In bone, glycosaminoglycans (GAG) like chondroitin sulfate (CS) or chemically sulfated hyaluronan (sHA3) have been implicated in supporting regenerative processes in vitro and in vivo [1]. As multifunctional components of artificial ECM (aECM), CS and sHA significantly influence bone healing by enhancing osteogenic differentiation of mesenchymal stem cells (hMSC) and premature osteoblast [1, 2]. Specifically, for bone-related applications, silicate-based bioactive glass nanoparticles (BGN) show unique properties for tissue regeneration and repair due to their small size and high specific surface area [3]. Based on their bioactivity and their capability of releasing ions like silicon and calcium, BGN can induce favorable cellular responses and promote rapid bone formation [4]. The aim of this study was to evaluate the potential synergistic effects of CS or sHA3 in combination with BGN on the osteogenic differentiation of hMSC as an important aspect of bone healing.

Experimental Methods

Tissue culture polystyrene samples were coated with collagen type I (col), col/CS or col/sHA3 and compared to similar coatings containing SiO₂-CaO BGN with diameters in the range of 300 - 400 nm. The stability of aECM coatings was qualitatively and quantitatively assessed by colorimetric assays directly after preparation and after incubation in phosphate buffered saline (PBS) for up to 31 days in the absence of cells. The morphology of coatings was assessed by scanning electron microscopy (SEM), while the viscoelastic properties were determined by oscillatory rheology. These aECM were further investigated regarding their effects on hMSC on osteogenic differentiation, comparing medium with and without dexamethasone (Dex) supplementation. Cellular proliferation and osteogenic differentiation were quantified via DNA content, alkaline phosphatase activity and calcium phosphate deposition (CPD) using hMSC isolated from the bone marrow of four different donors. The release of calcium and silicon ions from aECM coatings during cell culture but also in the absence of cells was quantified via inductively coupled plasma- optical emission spectrometry (ICP-OES).

Results and Discussion

Sirius red and Toluidine blue staining of aECM revealed the presence of collagen and GAG in the respective coatings as well as their homogeneous distribution irrespective of the presence of BGN. Directly after preparation, the collagen content was between 410–500 µg/cm² for GAG-containing aECM and up to 875 µg/cm² for collagen only. The initial
GAG amounts were between 50–85 µg/cm². Within the first day, we observed an initial release of collagen and GAG in PBS at 37°C that gradually ceased at later time points. After 13 days of incubation, more than half of the initial collagen content was still present. Neither the initial amounts nor the release was markedly influenced by the presence of BGN. The fibrillar structure of the collagen-based coatings was confirmed via SEM, while BGN formed equally distributed agglomerates. All samples exhibited viscoelastic behavior with predominantly elastic deformation. The complex viscosity of the coatings increased in the presence of BGN up to one order of magnitude. Cell proliferation was significantly increased on aECM without BGN compared to those with BGN, which was further enhanced by Dex. GAG and BGN promoted both, ALP activity and CPD. ALP activity was in particular increased on BGN-containing aECM in the presence of Dex. The CPD was increased on BGN-containing aECM irrespective of the presence of Dex, while, in case of coll/shA w/o BGN, Dex was necessary to induce CPD. For selected but not all donors a slightly synergistic effect of shA3 and BGN could be shown. Silicon ions were released into the cell culture supernatant from all BGN containing aECM and its concentration was significantly higher in the presence of cells.

**Conclusion**

aECM composed of col, GAG and BGN are promising bioinspired functional biomaterials for rapid mineralization in bone healing applications, which might be attributed to silicon release but also to GAG content and aECM stiffness. Further studies will investigate the mechanisms behind this effect as well as exploring the combination with physical stimulation, e. g. pulsed electric fields.

**Acknowledgement**

We acknowledge financial support by DFG, project number 59307082 - TRR67, subprojects A3, B5, Z3.

**References**

Hydrolysis, Plasma and Aminolysis: how a surface activation method affects gelatin attachment to PLCL nanofibres

Judyta Dulnik, Oliwia Jeznach, Paweł Sajkiewicz

Institute of Fundamental Technological Research, Polish Academy of Sciences, Laboratory of Polymers and Biomaterials, Warsaw, PL

Introduction
Aliphatic polyesters are materials widely used in tissue engineering because of a number of reasons – they can be formed with many different methods, resulting in a broad range of mechanical properties, they are biodegradable and biocompatible. To overcome their hydrophobicity and the lack of bioactivity, among other approaches, surface modification has been an extensively researched technique. Introducing proteins that are naturally present in the extracellular matrix to the surface of a scaffold material is a great way of solving both of these issues. Collagen, and its derivative – gelatin, has been shown to have a beneficial effect on mammalian cells’ proliferation and spreading [1]. Immobilization of gelatin through crosslinking to the surface of electrospun nanofibres made of aliphatic polyesters has been studied in our laboratory in the past years with the use of an aminolysis as a surface activation technique [2]. In this work a series of experiments were performed on poly(L-lactide-co-caprolactone) 70:30 (PLCL) nanofibres in order to compare three methods of surface activation - hydrolysis, cold oxygen plasma treatment and aminolysis, which were used as a first step of the process of gelatin immobilization to the material’s surface.

Experimental Methods
Nonwoven material was obtained through solution electrospinning of PLCL dissolved in hexafluoroisopropanol (HFIP) on a rotating drum collector. Alkaline hydrolysis (with NaOH solution) and plasma treatment were first optimized through a series of tests with varying process conditions in order to select a limited number of them for the final step of comparison. Aminolysis reaction conditions were chosen based on our previous results [2]. PLCL materials after hydrolysis and plasma treatment underwent gelatin crosslinking with 1-(3-dimethylaminopropyl)-N’-ethylcarbodimide hydrochloride/N-hydroxysuccinimide (EDC/NHS). For samples after aminolysis glutaraldehyde was additionally used.

Gel permeation chromatography (GPC), electron microscopy (SEM) and water contact angle measurement were used to assess how surface activation affected the samples. Toluidine blue O staining was used to assess the amount of COOH- groups on the surface of the fibres after hydrolysis and plasma treatment. The efficiency of gelatin immobilization, as well as how strong it was attached was measured with bicinchoninic acid assay (BCA) before and after biodegradation test.

Results and Discussion
GPC showed that each activation method affected the molecular weight of the polymer differently. Small change was observed for hydrolyzed samples, but a new peak for a very low molecular weight has appeared. Among the remaining two methods aminolysis proved to cause a greater drop in the molecular weight. Samples after both hydrolysis and plasma treatment showed elevated levels of COOH- groups on the surface of the fibres compared to control, but between the two methods hydrolysis resulted in up to 3 more times higher value. Apart from samples treated with aminolysis the rest gained hydrophilicity after surface activation, but later after biodegradation test all achieved 0°, which means that the water drop was immediately soaked into the material.

All tested methods were successful in gelatin immobilization as well maintaining its attachment after biodegradation test compared to control. Samples treated with aminolysis were leading in the strength of gelatin attachment.
treated materials were able to retain the highest amount of protein. Hydrolyzed samples in comparison, except for the one treated with the most concentrated NaOH solution, achieved average results in both regards. SEM imaging showed no significant change in fibres morphology, except for the ones treated with higher NaOH molarities. With increasing NaOH concentration a progressing in the damage of the very surface of the fibres would be observed. We believe these samples had achieved surprisingly high BCA result, as a consequence of their increased surface area caused by the frayed outer layer of the fibres.

Conclusion
All three tested surface activation methods were successful being a first step of the surface gelatin immobilization. Depending on the method, some properties of the electrospun PCLC material were affected more. The decision of which method is the most suitable should be based on a specific function that the final scaffold material is expected to fulfill and the properties it should have.

Acknowledgement
This research was funded by the Polish National Science Center (NCN), grant number 2016/23/B/ST8/03409

References
Hybrid HAp/tantalum-based bioactive coatings on titanium substrate

Amanda Bartkowiak¹, Yevhen Zabila¹, Elzbieta Menaszek², Arkadiusz Zarzycki¹, Marcin Perzanowski¹, Marta Marszalek¹

¹ Institute of Nuclear Physics Polish Academy of Sciences, Department of Magnetic Materials and Nanostructures, Krakow, PL; ² Jagiellonian University Medical College, Faculty of Pharmacy, Krakow, PL

Introduction

Tantalum (Ta) is recently attracting growing attention thanks to superior over other metals physicochemical stability, biocompatibility and osteoconductivity [1]. However, high density and stiffness unmatched with bone tissue, along with expensive costs of manufacturing, make bulk Ta material unsuitable for medical implants. One way to gain remarkable biological properties with currently used metallic implants is to modify their surface with Ta films. Another material used for successful surface modification to promote osseointegration at the implant-tissue interface is hydroxyapatite (HAp) [2]. Among different deposition techniques, hydrothermal method was found to produce highly crystalline and uniform HAp coatings on titanium (Ti), dealing with complex shapes typical for orthopedic implants [3]. To take full advantage of the beneficial properties of tantalum and hydroxyapatite, we modified the surface of commercially used titanium foil with hybrid HAp/tantalum-based bioactive coatings. Fabrication of the hybrid coatings was achieved by two methods approach: magnetron sputtering and hydrothermal synthesis. Samples were investigated for their structure, in vitro bioactivity and biocompatibility using osteoblast-like cell culture. In this study, we show that the synergetic properties of tantalum-based films and HAp coating have positive and superior effect on the osteoblast-like cells compared to titanium substrates only with HAp coatings.

Experimental Methods

Development of HAp/tantalum-based bioactive coatings was divided into two steps: (1) magnetron sputtering of tantalum on titanium foil and (2) hydrothermal synthesis of HAp coatings. Sample with intermediate tantalum oxide film was produced by thermal oxidation of the sputtered Ta film. The hydrothermal synthesis was carried out in an autoclave at 200°C from solution containing: calcium salt hydrate, diammonium phosphate and calcium chelating agent. The morphology and microstructure of prepared specimens were characterized using XRD, SEM/EDS and fluorescent microscopy. Cell viability was investigated using human osteoblast-like cell culture (MG-63) after 3 and 7 days of incubation. Bioactivity was assessed in vitro by immersion in simulated body fluid (SBF).

Results and Discussion

High crystallinity of HAp coating for implant applications is desirable since low crystallinity accelerates the speed of dissolution of HAp in the living body, causing the disappearance of coatings that bond with bone tissue at an early stage after implantation. The structural and morphological analysis confirmed that both tantalum and tantalum oxide films induce nucleation and growth of highly crystalline HAp coating under hydrothermal conditions. Cells cultured on these hybrid coatings were numerous, well spread and connected with filopodia extensions to form a cell network without any cytotoxic effect.

Conclusion

In this study, we present highly biocompatible and bioactive HAp/tantalum-based coatings on titanium substrates that were shown to enhance cell growth in vitro. The two method approach used to produce these hybrid coatings may be easily applicable on any other metallic substrate used in orthopedic field.
Bioactivity and biocompatibility analysis

References


Chair Index

A
Abrantes, João
Biomechanics 04..............................................973
Aguilar, Maria Rosa
K01 .................................................................74
N06 ...............................................................306
Ahluwalia, Arti
K09 .................................................................839
Ainsworth, Madison J.
S11 .................................................................694
Alaoui Selsouli, Yousra
N11 .................................................................562
Alini, Mauro
S15 .................................................................886
Alves Claro, Ana Paula R.
ESB-SLABO S ..................................................280
Ambrosio, Luigi
KdG AW01 ..........................................................374
Amedee, Joëlle
ESB-BIMAT S ......................................................431
Anand, Shivesh
K07 .................................................................751
Anselme, Karine
N16 .................................................................931
Aparicio, Conrado
S03 .................................................................112
Athamneh, Tamara K.
AERO InvF .........................................................617
Azevedo, Helena S.
N16 .................................................................931

B
Baker, Matthew
N05 .................................................................289
Bakirci, Ezgi
N03 .................................................................207
Baldini, Nicola
S07 .................................................................409
Barata, David
YSF WS .............................................................65
Barberis, Fabrizio
TR-S01 ..............................................................72
TR-S02 ..............................................................171
TR-S03 ..............................................................249
Barbosa, Mário A.
K05 .................................................................490
KdG AW02 ..........................................................649
Barrias, Cristina C.
K03 .................................................................251
K08 .................................................................768
Barrios, Joana
AERO InvF .........................................................617
YSF-AEROgELS WS ..........................................615
Bayon, Yves
TR-S01 ..............................................................72
TR-S02 ..............................................................171
Bhusari, Shardul
ESB-ISBF S ..........................................................532
Bidarra, Silvia J.
AERO S02 ..........................................................606
Bilardo, Roberta
NANOSTEM 02 ....................................................351
Boccaccini, Aldo R.
ESB-CSBM S ......................................................198
Bohner, Marc
S12 .................................................................784

C
Castilho, Miguel
N05 .................................................................289
Cerqueni, Giorgia
K01 .................................................................74
Chaparro, Catarina P.
S04 .................................................................188
Chatzinikolaidou, Maria
N11 .................................................................562
Chen, Hongyi
S13 .................................................................798
Chiono, Valeria
S09 .................................................................663
Ciardelli, Gianluca
S05 .................................................................265
Cirò, Ewelina
ESB-CRS S ..........................................................420
Ciofani, Gianni
N07 .................................................................327
S04 .................................................................188
Collins, Maurice
S07 .................................................................409
Contessi Negrini, Nicola
S10 .................................................................678
YSF-AERogELS WS ...........................................615
Corrales-Orovi, Rocio
ESB-SFB S ..........................................................807
Cortez, João
TR-S03 ..............................................................249

D
da Rocha Freire, Carmen Sofia
ESB-CRS S ..........................................................420
Daelman, Jeff
LS .................................................................487
<table>
<thead>
<tr>
<th>Name</th>
<th>Indexes</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dalton, Paul D.</td>
<td>N07</td>
<td>327</td>
</tr>
<tr>
<td>Dang, Phuong Anh</td>
<td>N04</td>
<td>229</td>
</tr>
<tr>
<td>Delgado Caceres, Manuel</td>
<td>N13</td>
<td>817</td>
</tr>
<tr>
<td></td>
<td>S05</td>
<td>265</td>
</tr>
<tr>
<td>D’Este, Matteo</td>
<td>N09</td>
<td>468</td>
</tr>
<tr>
<td>Döbelin, Nicola</td>
<td>S12</td>
<td>784</td>
</tr>
<tr>
<td>Doser, Michael</td>
<td>N04</td>
<td>229</td>
</tr>
<tr>
<td>Dubruel, Peter</td>
<td>JL AW01</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>TRS-S02</td>
<td>171</td>
</tr>
<tr>
<td></td>
<td>TRS-S03</td>
<td>249</td>
</tr>
<tr>
<td>Dunne, Nicholas</td>
<td>K06</td>
<td>650</td>
</tr>
<tr>
<td>Eeglin, David</td>
<td>N02</td>
<td>147</td>
</tr>
<tr>
<td>Eischen-Loges, Maria José R.</td>
<td>N16</td>
<td>931</td>
</tr>
<tr>
<td>Engel, Elisabeth</td>
<td>N02</td>
<td>147</td>
</tr>
<tr>
<td>Epitropaki, Eirini</td>
<td>NANOSTEM 01</td>
<td>346</td>
</tr>
<tr>
<td>Ercan, Batur</td>
<td>N12</td>
<td>731</td>
</tr>
<tr>
<td>Faré, Silvia</td>
<td>S10</td>
<td>678</td>
</tr>
<tr>
<td>Ferreira Duarte, Ana Marina</td>
<td>S11</td>
<td>694</td>
</tr>
<tr>
<td>Ferreira, Lino S.</td>
<td>S11</td>
<td>694</td>
</tr>
<tr>
<td>Fois, Maria Gabriella G.</td>
<td>K08</td>
<td>768</td>
</tr>
<tr>
<td>Fritschen, Anna</td>
<td>K04</td>
<td>378</td>
</tr>
<tr>
<td>Garcia, Andrés</td>
<td>ESB-SFB S</td>
<td>807</td>
</tr>
<tr>
<td>García-González, Carlos A.</td>
<td>AERO Closing</td>
<td>646</td>
</tr>
<tr>
<td></td>
<td>YSF-AERogELS WS</td>
<td>615</td>
</tr>
<tr>
<td>Gautrot, Julien</td>
<td>N06</td>
<td>306</td>
</tr>
<tr>
<td>Gelinsky, Michael</td>
<td>ESB-ISBF S</td>
<td>532</td>
</tr>
<tr>
<td>Gervaso, Francesca</td>
<td>S01</td>
<td>87</td>
</tr>
<tr>
<td>Ginebra, Maria-Pau</td>
<td>S13</td>
<td>798</td>
</tr>
<tr>
<td>Gomes, Manuela</td>
<td>N09</td>
<td>468</td>
</tr>
<tr>
<td>Gonçalves, Inês C.</td>
<td>ESB-SIBB S01</td>
<td>515</td>
</tr>
<tr>
<td></td>
<td>K04</td>
<td>378</td>
</tr>
<tr>
<td>Goncalves, Raquel M.</td>
<td>S15</td>
<td>886</td>
</tr>
<tr>
<td></td>
<td>SA1</td>
<td>584, 834</td>
</tr>
<tr>
<td>Granja, Pedro L.</td>
<td>CC</td>
<td>951</td>
</tr>
<tr>
<td></td>
<td>ESB-BIOMAT S</td>
<td>431</td>
</tr>
<tr>
<td></td>
<td>OC</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>PL2</td>
<td>583</td>
</tr>
<tr>
<td>Graziani, Gabriela</td>
<td>S07</td>
<td>409</td>
</tr>
<tr>
<td>Guagliano, Giuseppe</td>
<td>N05</td>
<td>289</td>
</tr>
<tr>
<td>Guarch Perez, Clara M.</td>
<td>S09</td>
<td>663</td>
</tr>
<tr>
<td>Guimarães, Sofia</td>
<td>K09</td>
<td>839</td>
</tr>
<tr>
<td>Habibovic, Pamela</td>
<td>ESB-BMSJ S</td>
<td>721</td>
</tr>
<tr>
<td></td>
<td>GW AW01</td>
<td>70</td>
</tr>
<tr>
<td>Hakimi, Osnat</td>
<td>S13</td>
<td>798</td>
</tr>
<tr>
<td>Heid, Susanne</td>
<td>S10</td>
<td>678</td>
</tr>
<tr>
<td>Hu, Jinlian</td>
<td>S04</td>
<td>188</td>
</tr>
<tr>
<td>Ji, Jian</td>
<td>ESB-CSBM S</td>
<td>198</td>
</tr>
<tr>
<td>Joyce, Michael</td>
<td>K02</td>
<td>173</td>
</tr>
<tr>
<td>Lamghari, Meriem</td>
<td>SA1</td>
<td>584, 834</td>
</tr>
<tr>
<td></td>
<td>K02</td>
<td>173</td>
</tr>
<tr>
<td>Larrañaga, Aitor</td>
<td>K04</td>
<td>378</td>
</tr>
<tr>
<td>Leijten, Jeroen</td>
<td>K07</td>
<td>751</td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td>487</td>
</tr>
<tr>
<td>Leiro, Victoria</td>
<td>ESB-SIBB S02</td>
<td>704</td>
</tr>
<tr>
<td>Leong, Kam W.</td>
<td>ESB-BMJ S</td>
<td>541</td>
</tr>
<tr>
<td>Letouerneur, Didier</td>
<td>N12</td>
<td>731</td>
</tr>
</tbody>
</table>
Chair Index

Levato, Riccardo
N14 ........................................ 895
L’Heureux, Nicolas
N08 ........................................ 449
Litowcenko-Cybul ska, Jagoda
K03 ........................................ 251
Liverani, Liliana
N07 ........................................ 327
YSF WS .................................. 65
Locs, Janis
S08 ........................................ 504
Lopez Serrano, Cristina
S15 ........................................ 886
López, Maria V.
S02 ........................................ 103
Lucchetti, Agnese
ESB-CSBM S .................................. 198

M

Maeztu Redin, Deyo
ESB-BIOMAT S .................................. 431
Mano, João
ESB-SIBB S02 .................................. 704
Mao, Hai-Quan
ESB-BMJ S .................................. 541
Marques, Paula A.
N14 ........................................ 895
Martins, M. Cristina L.
PL3 ........................................ 836
CC ........................................ 951
OC ........................................ 69
Mas-Moruno, Carles
S03 ........................................ 112
Massera, Jonathan
N03 ........................................ 207
Mattu, Clara
N15 ........................................ 915
Mignon, Arn
N08 ........................................ 449
YSF WS .................................. 65
Miklosic, Gregor
N14 ........................................ 895
Minsart, Manon
N09 ........................................ 468
Monteiro, Claudia
N10 ........................................ 543
Monteiro, Fernando
AERO Closing .................................. 646
K09 ........................................ 839
Mooney, David
K08 ........................................ 768
Moreira Marques, Joana
S08 ........................................ 504
Moreira Teixeira, Liliana
YSF WS .................................. 65

Moroni, Lorenzo
ESB-ISBF S .................................. 532
Mouchtaridi, Christina
S05 ........................................ 265
Mulder, Willem J.M.
K05 ........................................ 490

N
Navarro, Enrique
Biomechanics S .................................. 953

O
Oliveira, Ana
AERO S02 .................................. 606
N10 ........................................ 543
Oliveira, Hugo
N03 ........................................ 207
Oliveira, Mariana B.
S02 ........................................ 103
Oliveira, Miguel J.
ESB-SLABO S .................................. 280

P
Padmanaban, Prasanna
N06 ........................................ 306
Pandit, Abhay
ESB-SFB S .................................. 807
Parreira, Paula
S03 ........................................ 112
Pégo, Ana Paula
CC ........................................ 951
OC ........................................ 69
PL1 ........................................ 343
ESB-BMJ S .................................. 541
Persson, Cecilia
N13 ........................................ 817
Platti, Elisa
K05 ........................................ 490
Pinto Reis, Catarina
AERO S03 .................................. 636
Polini, Alessandro
S01 ........................................ 87
Prazeres, Hugo
TRS-S03 .................................. 249
Puglisi, Matteo
NANOSTEM 03 .................................. 358

Q
Quinn, James
S12 ........................................ 784

R
Raucci, Maria Grazia
N08 ........................................ 449
Reid, Gregory
ESB-SLABO S .................................. 280
<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribeiro, Cristina C.</td>
<td>650</td>
</tr>
<tr>
<td>Richards, Geoff</td>
<td>856</td>
</tr>
<tr>
<td>Rinaldi, Chiara</td>
<td>731</td>
</tr>
<tr>
<td>Rodrigues, João</td>
<td>915</td>
</tr>
<tr>
<td>Rodriguez Rius, Daniel</td>
<td>856</td>
</tr>
<tr>
<td>Rodriguez-Cabello, José Carlos</td>
<td>251</td>
</tr>
<tr>
<td>Rojas, Francisco J.</td>
<td>959</td>
</tr>
<tr>
<td>Rosales, Víctor S.</td>
<td>87</td>
</tr>
<tr>
<td>Salmeron Sanchez, Manuel</td>
<td>751</td>
</tr>
<tr>
<td>Santin, Matteo</td>
<td>833</td>
</tr>
<tr>
<td>Santos, Hélder</td>
<td>420</td>
</tr>
<tr>
<td>Sarmento, Bruno</td>
<td>636</td>
</tr>
<tr>
<td>Serrano, María C.</td>
<td>127</td>
</tr>
<tr>
<td>Simón, Rosana</td>
<td>586</td>
</tr>
<tr>
<td>Singh, Ankur</td>
<td>541</td>
</tr>
<tr>
<td>Soria, Federico</td>
<td>515</td>
</tr>
<tr>
<td>Southall, Maria</td>
<td>721</td>
</tr>
<tr>
<td>Sprio, Simone</td>
<td>871</td>
</tr>
<tr>
<td>Tahmasebi Birgani, Zeinab Nilofar</td>
<td>392</td>
</tr>
<tr>
<td>Tampieri, Anna</td>
<td>871</td>
</tr>
<tr>
<td>Tanner, Elizabeth</td>
<td>648</td>
</tr>
<tr>
<td>Tanzi, Maria Cristina</td>
<td>543</td>
</tr>
<tr>
<td>Tayyem, May A.</td>
<td>721</td>
</tr>
<tr>
<td>Tomasina, Clarissa</td>
<td>871</td>
</tr>
<tr>
<td>Torres, Ana L.</td>
<td>586</td>
</tr>
<tr>
<td>Traldi, Federico</td>
<td>367</td>
</tr>
<tr>
<td>Truckenmüller, Roman</td>
<td>173</td>
</tr>
<tr>
<td>Tsarnides, Ioannis</td>
<td>392</td>
</tr>
<tr>
<td>V</td>
<td>650</td>
</tr>
<tr>
<td>Valenti, Joan</td>
<td>954</td>
</tr>
<tr>
<td>van der Heide, Daphne</td>
<td>915</td>
</tr>
<tr>
<td>Van Vlierbergh, Sandra</td>
<td>376</td>
</tr>
<tr>
<td>Varoni, Elena Maria</td>
<td>147</td>
</tr>
<tr>
<td>Verné, Enrica</td>
<td>65</td>
</tr>
<tr>
<td>Viladot Voegeli, Antonio</td>
<td>504</td>
</tr>
<tr>
<td>Vilas-Boas, J. Paulo</td>
<td>954</td>
</tr>
<tr>
<td>Vlasceanu, George M.</td>
<td>957</td>
</tr>
<tr>
<td>von Witzleben, Max</td>
<td>392</td>
</tr>
<tr>
<td>Vranckx, Cédric</td>
<td>127</td>
</tr>
<tr>
<td>W</td>
<td>229</td>
</tr>
<tr>
<td>Weiss, Pierre</td>
<td>127</td>
</tr>
<tr>
<td>Wieringa, Paul</td>
<td>103</td>
</tr>
<tr>
<td>Wychowanicz, Jacek K.</td>
<td>817</td>
</tr>
<tr>
<td>Z</td>
<td>663</td>
</tr>
<tr>
<td>Zenobi-Wong, Marcy</td>
<td>817</td>
</tr>
<tr>
<td>Zoso, Alice</td>
<td>663</td>
</tr>
</tbody>
</table>
Author Index

A
A. García-Gonzaléz, Carlos
AERO InvF-01 .............................................. 618
Aabloo, Alvo
AERO PS01-02 ............................................ 599
Abakeviciene, Brigita
PS-01-051 .................................................. 1041
Abasolo, Ibane
ESB-SIBB S02-01 ...................................... 705
Abbah, Sunny
N01-05 ..................................................... 137
Abdel-Fattah, Wafa I.
N02-08 ..................................................... 164
PS2-07-230 .............................................. 1698
Abdo, Amir
ESB-BIOMAT S-03 .................................... 436
Abdulaziz, Dina
PS1-04-175 ............................................... 1162
Abel, Bernd
PS2-08-272 ............................................... 1736
Abelmann, Leon
PS2-13-398 .............................................. 1868
Abendroth, Philipp
PS2-08-258 ............................................... 1727
Abokeviciene, Brigita
PS01-051 .................................................. 1041
Abagut, Onnik
N01-05 ..................................................... 137
Abbas, Carlos
AERO S01-01 ............................................. 588
Addario, Gabriele
K02-03 ..................................................... 177
Addo, Esther
PS2-01-012 .............................................. 1484
Afonso, Carlos
PS2-05-182 ............................................... 1647
Afonso, Sónia
NANOSTEM 03-05 ..................................... 365
Ageitos, Jose Manuel
PS1-05-181 ............................................... 1170
Page 1946 of 2028

Agten, Hannah
ESB-SLABO S-03 ...................................... 283
Aguilar, Maria R.
N10-08 ..................................................... 558
Aguilar, Maria Rosa
ESB-SIBB S02-01 ..................................... 705
PS1-07-217 .............................................. 1211
Aguilar-Rodriguez, Hilda
PS1-02-103 ............................................... 1092
PS2-02-104 .............................................. 1574
Agustin, Melissa
PS1-05-197 ............................................... 1187
Ahlfeld, Tilman
N13-07 ..................................................... 829
PS2-01-040 ............................................... 1512
S08-05 ..................................................... 512
Ahlwalia, Arti
K07-05 ..................................................... 761
PS2-02-074 ............................................... 1544
PS2-13-396 ............................................... 1866
Aibibu, Dilbar
N14-06 ..................................................... 906
Aid-Launais, Rachida
PS1-09-307 ............................................... 1298
Alii, Daniel
PS2-03-110 ............................................... 1580
Ainsworth, Madison J.
N08-02 ..................................................... 452
N14-05 ..................................................... 904
Aissani, Abderrahmane W.
PS1-09-307 ............................................... 1298
Akgün, İşik S.
AERO S03-01 ............................................. 639
Akhavan, Behnam
N08-02 ..................................................... 452
Akbilbekova, Dana
PS2-04-134 ............................................... 1603
Akkineni, Ashwini Rahul
PS1-11-351 ............................................... 1348
Aktan, Merve K.
N03-01 ..................................................... 208
PS2-13-410 ............................................... 1881
Al Zoghool, Shahed
PS2-01-006 ............................................... 1477
Alaoui Selousli, Yousra
N09-06 ..................................................... 478
PS1-13-393 ............................................... 1391
Abererganti, Sebastiano
N05-09 .................................................... 304
<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albertini, Beatrice</td>
<td>117</td>
</tr>
<tr>
<td>Albino, Inês</td>
<td>311</td>
</tr>
<tr>
<td>N16-03</td>
<td></td>
</tr>
<tr>
<td>N13-03</td>
<td>820</td>
</tr>
<tr>
<td>S06-06</td>
<td>404</td>
</tr>
<tr>
<td>Aldana, Agustina</td>
<td>1706</td>
</tr>
<tr>
<td>N11-02</td>
<td>194</td>
</tr>
<tr>
<td>Almeida, Andreia</td>
<td>565</td>
</tr>
<tr>
<td>ESB-Biomaterials Science-KL02</td>
<td>723</td>
</tr>
<tr>
<td>Alkharusi, Ghayadah</td>
<td>818</td>
</tr>
<tr>
<td>Alkorta, Janire</td>
<td>323</td>
</tr>
<tr>
<td>Allan, Iain U.</td>
<td>1741</td>
</tr>
<tr>
<td>Allderdt, Felix</td>
<td>1936</td>
</tr>
<tr>
<td>Aliffin, Iris E.</td>
<td>1901</td>
</tr>
<tr>
<td>Almeida, Ana C.</td>
<td>1449</td>
</tr>
<tr>
<td>Almeida, Andreia</td>
<td>429</td>
</tr>
<tr>
<td>ESB-CRS S-05</td>
<td></td>
</tr>
<tr>
<td>PS1-10-323</td>
<td>1319</td>
</tr>
<tr>
<td>Almeida, Filipe V.</td>
<td>1235</td>
</tr>
<tr>
<td>PS1-07-243</td>
<td>1743</td>
</tr>
<tr>
<td>Almeida, José Carlos</td>
<td>1669</td>
</tr>
<tr>
<td>PS2-05-202</td>
<td></td>
</tr>
<tr>
<td>Almeida, Maria I.</td>
<td>1406</td>
</tr>
<tr>
<td>PS1-13-407</td>
<td></td>
</tr>
<tr>
<td>Almeida-Porada, Graça</td>
<td>893</td>
</tr>
<tr>
<td>S15-05</td>
<td></td>
</tr>
<tr>
<td>Alogna, Andrea</td>
<td>117</td>
</tr>
<tr>
<td>S03-03</td>
<td></td>
</tr>
<tr>
<td>Alongi, Mariilza</td>
<td>641</td>
</tr>
<tr>
<td>Alogna, Andrea</td>
<td>503</td>
</tr>
<tr>
<td>Alonso, Matilde</td>
<td></td>
</tr>
<tr>
<td>K05-07</td>
<td></td>
</tr>
<tr>
<td>Almeida, Filipe V.</td>
<td>1235</td>
</tr>
<tr>
<td>Almeida, José Carlos</td>
<td>1743</td>
</tr>
<tr>
<td>Almeida, Maria I.</td>
<td>1406</td>
</tr>
<tr>
<td>Almeida-Porada, Graça</td>
<td>893</td>
</tr>
<tr>
<td>Alogna, Andrea</td>
<td>117</td>
</tr>
<tr>
<td>Alonso, Matilde</td>
<td>503</td>
</tr>
<tr>
<td>Almeida, Filipe V.</td>
<td>1235</td>
</tr>
<tr>
<td>Almeida, José Carlos</td>
<td>1743</td>
</tr>
<tr>
<td>Almeida, Maria I.</td>
<td>1406</td>
</tr>
<tr>
<td>Almeida-Porada, Graça</td>
<td>893</td>
</tr>
<tr>
<td>Alogna, Andrea</td>
<td>117</td>
</tr>
<tr>
<td>Alonso, Matilde</td>
<td>503</td>
</tr>
<tr>
<td>Almeida, Filipe V.</td>
<td>1235</td>
</tr>
<tr>
<td>Almeida, José Carlos</td>
<td>1743</td>
</tr>
<tr>
<td>Almeida, Maria I.</td>
<td>1406</td>
</tr>
<tr>
<td>Almeida-Porada, Graça</td>
<td>893</td>
</tr>
<tr>
<td>Alogna, Andrea</td>
<td>117</td>
</tr>
<tr>
<td>Alonso, Matilde</td>
<td>503</td>
</tr>
<tr>
<td>Almeida, Filipe V.</td>
<td>1235</td>
</tr>
<tr>
<td>Almeida, José Carlos</td>
<td>1743</td>
</tr>
<tr>
<td>Almeida, Maria I.</td>
<td>1406</td>
</tr>
<tr>
<td>Almeida-Porada, Graça</td>
<td>893</td>
</tr>
<tr>
<td>Alogna, Andrea</td>
<td>117</td>
</tr>
<tr>
<td>Alonso, Matilde</td>
<td>503</td>
</tr>
<tr>
<td>Almeida, Filipe V.</td>
<td>1235</td>
</tr>
<tr>
<td>Almeida, José Carlos</td>
<td>1743</td>
</tr>
<tr>
<td>Almeida, Maria I.</td>
<td>1406</td>
</tr>
<tr>
<td>Almeida-Porada, Graça</td>
<td>893</td>
</tr>
<tr>
<td>Alogna, Andrea</td>
<td>117</td>
</tr>
<tr>
<td>Alonso, Matilde</td>
<td>503</td>
</tr>
<tr>
<td>Almeida, Filipe V.</td>
<td>1235</td>
</tr>
<tr>
<td>Almeida, José Carlos</td>
<td>1743</td>
</tr>
<tr>
<td>Almeida, Maria I.</td>
<td>1406</td>
</tr>
<tr>
<td>Almeida-Porada, Graça</td>
<td>893</td>
</tr>
<tr>
<td>Alogna, Andrea</td>
<td>117</td>
</tr>
<tr>
<td>Alonso, Matilde</td>
<td>503</td>
</tr>
<tr>
<td>Almeida, Filipe V.</td>
<td>1235</td>
</tr>
<tr>
<td>Almeida, José Carlos</td>
<td>1743</td>
</tr>
<tr>
<td>Almeida, Maria I.</td>
<td>1406</td>
</tr>
<tr>
<td>Almeida-Porada, Graça</td>
<td>893</td>
</tr>
<tr>
<td>Alogna, Andrea</td>
<td>117</td>
</tr>
<tr>
<td>Alonso, Matilde</td>
<td>503</td>
</tr>
<tr>
<td>Almeida, Filipe V.</td>
<td>1235</td>
</tr>
<tr>
<td>Almeida, José Carlos</td>
<td>1743</td>
</tr>
<tr>
<td>Almeida, Maria I.</td>
<td>1406</td>
</tr>
<tr>
<td>Almeida-Porada, Graça</td>
<td>893</td>
</tr>
<tr>
<td>Alogna, Andrea</td>
<td>117</td>
</tr>
<tr>
<td>Alonso, Matilde</td>
<td>503</td>
</tr>
<tr>
<td>Almeida, Filipe V.</td>
<td>1235</td>
</tr>
<tr>
<td>Almeida, José Carlos</td>
<td>1743</td>
</tr>
<tr>
<td>Almeida, Maria I.</td>
<td>1406</td>
</tr>
<tr>
<td>Almeida-Porada, Graça</td>
<td>893</td>
</tr>
<tr>
<td>Alogna, Andrea</td>
<td>117</td>
</tr>
<tr>
<td>Alonso, Matilde</td>
<td>503</td>
</tr>
<tr>
<td>Author Name</td>
<td>Affiliation</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>Ambrosio, Luigi</td>
<td>ESB-BIOMAT S-KL01</td>
</tr>
<tr>
<td></td>
<td>ESB-CSBM S-04</td>
</tr>
<tr>
<td></td>
<td>K08-07</td>
</tr>
<tr>
<td>N01-08</td>
<td></td>
</tr>
<tr>
<td>N02-08</td>
<td></td>
</tr>
<tr>
<td>N10-09</td>
<td></td>
</tr>
<tr>
<td>N15-05</td>
<td></td>
</tr>
<tr>
<td>PS2-07-03-118</td>
<td></td>
</tr>
<tr>
<td>PS2-07-07-230</td>
<td></td>
</tr>
<tr>
<td>PS2-07-07-244</td>
<td></td>
</tr>
<tr>
<td>PS2-07-07-246</td>
<td></td>
</tr>
<tr>
<td>S06-03</td>
<td></td>
</tr>
<tr>
<td>Amédée, Joelle</td>
<td></td>
</tr>
<tr>
<td>Amédié, Joelle</td>
<td></td>
</tr>
<tr>
<td>Amorim, M. Teresa P.</td>
<td></td>
</tr>
<tr>
<td>Amorim, Maria Teresa S.P.</td>
<td></td>
</tr>
<tr>
<td>Amouei, Hanna</td>
<td></td>
</tr>
<tr>
<td>Anagnostou, Fani</td>
<td></td>
</tr>
<tr>
<td>Anand, Shivesh</td>
<td></td>
</tr>
<tr>
<td>ESB-CRS S-03</td>
<td></td>
</tr>
<tr>
<td>S07-05</td>
<td></td>
</tr>
<tr>
<td>Anastasiou, Antonios</td>
<td></td>
</tr>
<tr>
<td>Anderson, Daniel G.</td>
<td>S11-KL01</td>
</tr>
<tr>
<td>Andreana, Ilaria</td>
<td></td>
</tr>
<tr>
<td>Andrée, Lea</td>
<td></td>
</tr>
<tr>
<td>Andrensen, Thomas L.</td>
<td></td>
</tr>
<tr>
<td>Angelats Lobo, David</td>
<td></td>
</tr>
<tr>
<td>Angelova Volponi, Ana</td>
<td></td>
</tr>
<tr>
<td>Anjos, Inês</td>
<td></td>
</tr>
<tr>
<td>Annabi, Nasim</td>
<td>ESB-Biomaterials Science-KL01</td>
</tr>
<tr>
<td>Anouz, Reema</td>
<td>ESB-SLABO S-05</td>
</tr>
<tr>
<td>Ansari, Aysha</td>
<td></td>
</tr>
<tr>
<td>Anselme, Karine</td>
<td></td>
</tr>
<tr>
<td>Antmen, Ezgi</td>
<td></td>
</tr>
<tr>
<td>S12-07</td>
<td></td>
</tr>
<tr>
<td>Anton-Sales, Irene</td>
<td></td>
</tr>
<tr>
<td>Antunes, Joana C.</td>
<td></td>
</tr>
<tr>
<td>ESB-SIBB S01-05</td>
<td></td>
</tr>
<tr>
<td>ESB-SIBB S01-07</td>
<td></td>
</tr>
<tr>
<td>N10-06</td>
<td></td>
</tr>
<tr>
<td>PS2-11-03-353</td>
<td></td>
</tr>
<tr>
<td>PS2-07-07-226</td>
<td></td>
</tr>
<tr>
<td>PS2-11-07-350</td>
<td></td>
</tr>
<tr>
<td>S12-03</td>
<td></td>
</tr>
<tr>
<td>Antúnez-Rodriguez, Cristina</td>
<td></td>
</tr>
<tr>
<td>Aparicio, Conrado</td>
<td></td>
</tr>
<tr>
<td>ESB-SFB S-03</td>
<td></td>
</tr>
<tr>
<td>Apetrei, Mihaela</td>
<td>N07-07</td>
</tr>
<tr>
<td>Apsite, Indra</td>
<td></td>
</tr>
<tr>
<td>Aquino, Rita P.</td>
<td></td>
</tr>
<tr>
<td>Aparicio, Conrado</td>
<td></td>
</tr>
<tr>
<td>Araque Marín, Pedronel</td>
<td></td>
</tr>
<tr>
<td>Araujo, Diana</td>
<td></td>
</tr>
<tr>
<td>Araújo, Marco</td>
<td></td>
</tr>
<tr>
<td>Araújo-Gomes, Nuno</td>
<td></td>
</tr>
<tr>
<td>Araújo, Marco</td>
<td></td>
</tr>
<tr>
<td>Araujo, Diana</td>
<td></td>
</tr>
<tr>
<td>Araújo-Gomes, Nuno</td>
<td></td>
</tr>
<tr>
<td>Arché-Núñez, Ana</td>
<td></td>
</tr>
<tr>
<td>Archit, Navendar</td>
<td></td>
</tr>
<tr>
<td>Biomechanics 04-03</td>
<td></td>
</tr>
<tr>
<td>Ardelean, Alina I.</td>
<td></td>
</tr>
<tr>
<td>Arefa, Anjumanara B.</td>
<td></td>
</tr>
<tr>
<td>S11-05</td>
<td></td>
</tr>
<tr>
<td>Arens, Jutta</td>
<td></td>
</tr>
<tr>
<td>Arias-González, Felipe</td>
<td></td>
</tr>
<tr>
<td>ESB-SIBB S01-06</td>
<td></td>
</tr>
</tbody>
</table>

Page 1948 of 2028
<table>
<thead>
<tr>
<th>Author</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aron, Vladimir B.</td>
<td>1246</td>
</tr>
<tr>
<td>Arakadas, Andreas</td>
<td>1444</td>
</tr>
<tr>
<td>Armanetti, Paolo</td>
<td>1221</td>
</tr>
<tr>
<td>Armãez, Iñaki G.</td>
<td>460</td>
</tr>
<tr>
<td>Arnold, Carole</td>
<td>796</td>
</tr>
<tr>
<td>Arntz, Youri</td>
<td>869</td>
</tr>
<tr>
<td>Arpicco, Silvia</td>
<td>1840</td>
</tr>
<tr>
<td>Arrico, Lorenzo</td>
<td>274</td>
</tr>
<tr>
<td>Arslan, Aysu</td>
<td>1741</td>
</tr>
<tr>
<td>Ascensão, Lia</td>
<td>621</td>
</tr>
<tr>
<td>Aslam, Zabedaa</td>
<td>1832</td>
</tr>
<tr>
<td>Atala, Anthony</td>
<td>136</td>
</tr>
<tr>
<td>Atanase, Leonard I.</td>
<td>1326</td>
</tr>
<tr>
<td>Athamneh, Tamara</td>
<td>590</td>
</tr>
<tr>
<td>Athanassiou, Athanassia</td>
<td>1701</td>
</tr>
<tr>
<td>Atif, Abdul Raouf</td>
<td>1143</td>
</tr>
<tr>
<td>Atif, Abdul-Raouf</td>
<td>805</td>
</tr>
<tr>
<td>Attallah, Moataz M.</td>
<td>119</td>
</tr>
<tr>
<td>Atz Dick, Teo</td>
<td>1793</td>
</tr>
<tr>
<td>Auricchio, Ferdinando</td>
<td>1080</td>
</tr>
<tr>
<td>Auzély-Velty, Rachel</td>
<td>330</td>
</tr>
<tr>
<td>Av, Thanusha</td>
<td>1003</td>
</tr>
<tr>
<td>Avci-Adali, Meltem</td>
<td>1528</td>
</tr>
<tr>
<td>Aveic, Sanja</td>
<td>1434</td>
</tr>
<tr>
<td>Avendaño-Soto, Esteban</td>
<td>633</td>
</tr>
<tr>
<td>Avendaño-Soto, Esteban D.</td>
<td>625</td>
</tr>
<tr>
<td>Avossa, Joshua</td>
<td>923</td>
</tr>
<tr>
<td>Awada, Hussein</td>
<td>82</td>
</tr>
<tr>
<td>Aylott, Jonathan W.</td>
<td>1321</td>
</tr>
<tr>
<td>Azevedo, António</td>
<td>241</td>
</tr>
<tr>
<td>Azevedo, Helena</td>
<td>876</td>
</tr>
<tr>
<td>Azevedo, Helena S.</td>
<td>1107</td>
</tr>
<tr>
<td>Babo, Pedro S.</td>
<td>916</td>
</tr>
<tr>
<td>Baboi, Ruxandra</td>
<td>697</td>
</tr>
<tr>
<td>Babu, Firuz</td>
<td>263</td>
</tr>
<tr>
<td>Bacakova, Lucie</td>
<td>328</td>
</tr>
<tr>
<td>Bačáková, Lucie</td>
<td>546</td>
</tr>
<tr>
<td>Bader, Rainer</td>
<td>1087</td>
</tr>
<tr>
<td>Bagheri, Shervin</td>
<td>1612</td>
</tr>
<tr>
<td>Bagnol, Romain</td>
<td>1143</td>
</tr>
<tr>
<td>Bailly, Lucie</td>
<td>444</td>
</tr>
<tr>
<td>Baker, Matt</td>
<td>1601</td>
</tr>
<tr>
<td>Baker, Matthew</td>
<td>1732</td>
</tr>
</tbody>
</table>

Page 1949 of 2028
Author Index

Baker, Matthew
N13-03 ........................................ 820
N14-01 ........................................ 896
N16-08 ........................................ 946
PS1-08-259 ................................ 1251
PS2-08-260 ................................ 1728

Baker, Matthew B.
N16-04 ........................................ 938
PS1-09-315 ................................ 1310
S06-06 ........................................ 404

Bakht, Syeda M.
K02-07 ........................................ 186

Bakina, Olga
N11-09 ........................................ 581

Bakirci, Ezgi
K08-06 ........................................ 779
N14-04 ........................................ 902
PS1-07-233 ................................ 1226

Balanuca, Brindusa
N05-02 ........................................ 292

Baldi, Francesco
S04-05 ........................................ 196

Baldini, Nicola
PS1-05-189 ................................ 1178

Ball, Vincent
K10-07 ........................................ 869

Ballerini, Laura
PS2-07-254 ................................ 1722

Ballesio, Alberto
PS1-14-435 ................................ 1435
PS2-14-438 ................................ 1910

Ballesteros, Yolanda
PS1-05-201 ................................ 1191

Ballesteros-Almanza, Ma. Lourdes
PS1-02-103 ................................ 1092

Banche-Niclot, Federica
N09-03 ........................................ 473

Barca, David
PS2-14-424 ................................ 1896
YSF WS-05 .................................. 66

Barata, Tânia
N06-03 ........................................ 311

Barbalexis, Panagiotis
PS2-10-342 ................................ 1808

Barbani, Niccoletta
K07-03 ........................................ 756

Barbe, Laurent
S13-04 ........................................ 805

Barber, Bill
TRS-S02-02 ................................ 172

Barberi, Jacopo
PS1-13-399 ................................ 1398

Barberis, Fabrizio
PS1-02-083 ................................ 1072
PS1-03-123 ................................ 1109
PS1-06-213 ................................ 1206
PS2-04-152 ................................ 1616

Barbosa, Catarina
PS1-10-325 ................................ 1321

Barbosa, Judite N.
K05-03 ........................................ 495
K07-06 ........................................ 763

Barbosa, Mário
N11-07 ........................................ 577

Barbosa, Mário A.
ESB-SFB S-05 ................................ 815
K05-03 ........................................ 495
KdG AW01-01 ................................ 374
N02-09 ........................................ 167
PS1-08-299 ................................ 1289
PS1-13-407 ................................ 1406
S15-03 ........................................ 889
S15-05 ........................................ 893

Barbugian, Federica
N05-09 ........................................ 304

Barbut, Clara
PS1-04-133 ................................ 1122

Barcaro, Giovanni
K07-03 ........................................ 756

Barcelona-Estaje, Eva
N08-08 ........................................ 464

Barcikowski, Stephan
PS1-12-369 ................................ 1367
PS2-15-446 ................................ 1917

Bardi, Ifigeneia
K07-02 ........................................ 753

Barbonnet, Raphael
PS1-04-173 ................................ 1161

Barreto, Yazmin B.
ESB-SLABO S-05 ................................ 287

Barkow, Phillip M.
PS1-09-317 ................................ 1311

Page 1950 of 2028
Author Index

Barou, Carole
N02-04 ........................................... 156
Barr, Jordan J.
PS1-09-313 .................................... 1307
Barrantes, Alejandro
ESB-SiBB S01-04 ................................... 522
Barreia, Angela
N06-05 ................................................. 316
Barrera, Angela M.
NANOSTEM 04-03 ..................................... 371
Barrias, Cristina
PS1-10-323 ......................................... 1319
PS1-14-427 ......................................... 1425
Barrias, Cristina C.
ESB-SiBB S02-03 ..................................... 709
K07-06 ................................................. 763
N05-06 ................................................. 297
N06-07 ................................................. 320
N16-02 ................................................ 934
PS1-13-419 ......................................... 1416
PS2-05-192 ......................................... 1657
PS2-07-256 ......................................... 1724
S09-03 ................................................. 668
S09-07 ................................................. 676
Barro, Oscar
S14-07 ................................................ 884
Barroca, Nathalie B.
K04-06 ................................................. 388
Barros da Silva, Patrícia
N05-06 ................................................. 297
Barros, Alexandre
TRS-S03-04 ......................................... 250
Barros, Andreia S.
N06-07 ................................................. 320
Bartkowiak, Amanda
PS2-15-466 ......................................... 1940
Bartley, Moresche G.
PS2-04-166 ......................................... 1628
Bártolo, Paulo J.
PS2-01-046 ......................................... 1516
Bassi, Giada
N09-05 ................................................. 476
PS2-08-282 ......................................... 1745
Battaglini, Matteo
K01-06 ................................................ 85
N01-02 ................................................. 131
N15-02 ................................................. 918
N15-03 ................................................. 921
Baudis, Stefan
PS2-09-314 ......................................... 1779
Bauer-Kreisel, Petra
K08-02 ................................................. 770
Baumberger, Tristan
PS1-10-321 ......................................... 1316
Bayon, Yves
TRS-S01-04 ......................................... 73
Becerrá, José
PS2-07-236 ......................................... 1706
Bechelany, Mikhael
N02-04 ................................................. 156
PS1-01-049 ......................................... 1039
Becker, Kevin
PS1-05-183 ......................................... 1172
Becker, Malin
PS2-13-422 ......................................... 1893
Becker, Malin L.
PS1-01-021 ......................................... 1013
S01-06 ................................................. 98
Beck-Sickinger, Annette G.
PS2-15-450 ......................................... 1922
Beeren, Ivo
PS2-04-132 ......................................... 1601
Begin-Colin, Sylvie
PS1-12-389 ......................................... 1385
Behrens, Peter
PS1-07-251 ......................................... 1241
PS2-04-138 ......................................... 1606
PS2-08-258 ......................................... 1727
PS2-10-340 ......................................... 1806
Behrens, Stephan
K06-05 ................................................. 659
Beketova, Anastasia
PS1-06-215 ......................................... 1208
Belabbes, Karima
PS1-01-027 ......................................... 1020
Belaid, Habib
N02-04 ................................................. 156
PS1-01-049 ......................................... 1039
Belcarz, Anna
PS1-12-381 ......................................... 1378
Belenli, Melike
N15-03 ............................................... 921
Bell, Alena K.
K02-02 ................................................. 175
Bellmann-Sickert, Kathrin
PS2-15-450 ......................................... 1922
Belton, Niall
PS1-01-011 ......................................... 1004
Belune, Kristine
PS1-08-277 ......................................... 1269
Ben Mouka, Amine
K10-07 ............................................... 869
Benecke, Lukas
N14-06 ................................................. 906
Benedicenti, Stefano
PS1-02-083 ......................................... 1072
Benfenati, Valentina
S10-03 ............................................... 684

Page 1951 of 2028
Author Index

Benn, Felix
PS2-02-068.............................................1537

Bennet-Powell, Jane
YSF WS-06..............................................67

Beresmeier, Sonja
PS1-03-129..............................................1117

Berg, Albrecht
N10-08..................................................558

Bergmeister, Helga
PS2-09-314..............................................1779

Bernard, Drouin
PS2-13-408..............................................1878

Bernardes, Beatriz G.
AERO InvF-01.............................................618
N10-05..................................................552

Bernardes, Nuno
PS1-10-323..............................................1319

Bernardo da Silva, Ana Paula
PS1-01-013..............................................1007

Bernardo-Castro, Sara
NANOSTEM 03-05........................................365

Bernhardt, Anne
N13-07..................................................829
PS1-01-011..............................................1004
PS2-01-040..............................................1512
PS2-15-462..............................................1936

Berniak, Krzysztof
PS1-13-397..............................................1396
PS2-04-140..............................................1608

Bernstein, Anke
PS2-10-332..............................................1797

Bertoglio, Federico
PS2-14-440..............................................1912

Bertorelli, Rosalia
PS2-07-232..............................................1701

Besecke, Karen
PS2-04-138..............................................1606

Bessa, José
PS2-02-100..............................................1572

Bessa-Gonçalves, Mafalda
K05-03...................................................495
PS2-07-256..............................................1724

Best, Serena M.
K05-06...................................................501
PS2-06-206..............................................1672

Bethy, Audrey
N04-02...................................................232
PS1-01-027..............................................1020

Bettega, Georges
N02-01...................................................148
PS1-04-165..............................................1153

Bettencourt, Ana
PS2-10-326..............................................1791

Bezzerri, Valentino
PS2-10-336..............................................1801

Bhusari, Shardul
K10-05...................................................865

Bialik-Wąs, Katarzyna
PS2-08-300..............................................1762
PS2-10-344..............................................1810

Bidarra, Silvia J.
N16-02...................................................934

Bidarra, Silvia J.
K07-06...................................................763
N05-06...................................................297

Bider, Faina
PS1-02-087..............................................1076

Biggs, Manus
N01-05...................................................137
N01-07...................................................141

Bigham, Ashkan
PS2-07-244..............................................1712

Bikianis, Dimitrios
PS2-01-058..............................................1526

Bikianis, Nikos
PS2-10-342..............................................1808

Bilardo, Roberta
NANOSTEM 03-01........................................359

Bilek, Marcela M.M.
N08-02...................................................452

Bilewicz, Renata
PS2-07-224..............................................1691

Billing, Florian
PS1-13-409..............................................1408

Biondi, Marco
S06-03...................................................398

Birch-Machin, Mark
PS1-05-187..............................................1176

Birgani, Zeinab T.
N02-07...................................................162

Biru, Elena I.
PS2-05-184..............................................1649

Bischof, Raffaele
K03-03...................................................257

Bishiti, Shaza
PS1-02-075..............................................1063

Bjørge, Isabel M.
N03-04...................................................215

Black, Kate
K02-06...................................................184

Blasen, Andreas
K02-02...................................................175
PS2-01-006..............................................1477

Blaker, Jonny J.
PS1-07-231............................................1224

Blanchefain, Nicolas
PS1-02-085..............................................1074
PS1-07-223............................................1217
<table>
<thead>
<tr>
<th>Author Index</th>
<th>Page 1953 of 2028</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS2-01-002............................................</td>
<td>1472</td>
</tr>
<tr>
<td>Blanco-Fernandez, Barbara</td>
<td></td>
</tr>
<tr>
<td>PS2-07-236.............................................</td>
<td>1706</td>
</tr>
<tr>
<td>Blanquer, Andreu</td>
<td></td>
</tr>
<tr>
<td>N07-01................................................................</td>
<td>328</td>
</tr>
<tr>
<td>N10-02................................................................</td>
<td>546</td>
</tr>
<tr>
<td>Blasi, Federica S.</td>
<td></td>
</tr>
<tr>
<td>PS2-05-200.................................................</td>
<td>1666</td>
</tr>
<tr>
<td>Blasi-Romero, Anna</td>
<td></td>
</tr>
<tr>
<td>N10-04................................................................</td>
<td>550</td>
</tr>
<tr>
<td>Blersch, Josephine</td>
<td></td>
</tr>
<tr>
<td>S11-04................................................................</td>
<td>699</td>
</tr>
<tr>
<td>Blitterswijk, Clemens V.</td>
<td></td>
</tr>
<tr>
<td>S06-06................................................................</td>
<td>404</td>
</tr>
<tr>
<td>Bloemen, Veerle</td>
<td></td>
</tr>
<tr>
<td>ESB-SLABO S-03.............................................</td>
<td>283</td>
</tr>
<tr>
<td>Bloise, Nora</td>
<td></td>
</tr>
<tr>
<td>PS2-07-252................................................</td>
<td>1720</td>
</tr>
<tr>
<td>Blokpoel Ferreras, Lia</td>
<td></td>
</tr>
<tr>
<td>S07-03................................................................</td>
<td>412</td>
</tr>
<tr>
<td>Blondeel, Eva</td>
<td></td>
</tr>
<tr>
<td>K08-05................................................................</td>
<td>777</td>
</tr>
<tr>
<td>Blondeel, Phillip</td>
<td></td>
</tr>
<tr>
<td>S10-KL01...................................................</td>
<td>679</td>
</tr>
<tr>
<td>Blunk, Torsten</td>
<td></td>
</tr>
<tr>
<td>K08-02................................................................</td>
<td>770</td>
</tr>
<tr>
<td>PS1-07-233................................................</td>
<td>1226</td>
</tr>
<tr>
<td>PS2-01-042..................................................</td>
<td>1514</td>
</tr>
<tr>
<td>PS2-14-442..................................................</td>
<td>1914</td>
</tr>
<tr>
<td>Boccaccini, Aldo</td>
<td></td>
</tr>
<tr>
<td>PS2-06-216..................................................</td>
<td>1684</td>
</tr>
<tr>
<td>Boccaccini, Aldo R</td>
<td></td>
</tr>
<tr>
<td>PS1-14-443..................................................</td>
<td>1444</td>
</tr>
<tr>
<td>Boccaccini, Aldo R.</td>
<td></td>
</tr>
<tr>
<td>PS1-02-087..................................................</td>
<td>1076</td>
</tr>
<tr>
<td>PS1-05-183..................................................</td>
<td>1172</td>
</tr>
<tr>
<td>PS1-06-215..................................................</td>
<td>1208</td>
</tr>
<tr>
<td>PS1-09-317..................................................</td>
<td>1311</td>
</tr>
<tr>
<td>PS1-11-349..................................................</td>
<td>1347</td>
</tr>
<tr>
<td>PS2-01-054..................................................</td>
<td>1525</td>
</tr>
<tr>
<td>PS2-03-116..................................................</td>
<td>1584</td>
</tr>
<tr>
<td>PS2-03-130..................................................</td>
<td>1598</td>
</tr>
<tr>
<td>PS2-15-462..................................................</td>
<td>1936</td>
</tr>
<tr>
<td>S03-06................................................................</td>
<td>123</td>
</tr>
<tr>
<td>S08-KL02...................................................</td>
<td>507</td>
</tr>
<tr>
<td>S10-04................................................................</td>
<td>686</td>
</tr>
<tr>
<td>Boda, Sunil K.</td>
<td></td>
</tr>
<tr>
<td>N03-03................................................................</td>
<td>212</td>
</tr>
<tr>
<td>Bodet, Tristan</td>
<td></td>
</tr>
<tr>
<td>N05-09................................................................</td>
<td>304</td>
</tr>
<tr>
<td>Boffito, Monica</td>
<td></td>
</tr>
<tr>
<td>N06-09................................................................</td>
<td>325</td>
</tr>
<tr>
<td>PS1-07-227..................................................</td>
<td>1221</td>
</tr>
<tr>
<td>PS2-05-178..................................................</td>
<td>1643</td>
</tr>
<tr>
<td>Bogas, Diana</td>
<td></td>
</tr>
<tr>
<td>K04-03................................................................</td>
<td>383</td>
</tr>
<tr>
<td>Bogdan, Sidonia</td>
<td></td>
</tr>
<tr>
<td>PS2-02-086..................................................</td>
<td>1558</td>
</tr>
<tr>
<td>Boggio, Elena</td>
<td></td>
</tr>
<tr>
<td>S08-04................................................................</td>
<td>510</td>
</tr>
<tr>
<td>Böhner, Dennis A.</td>
<td></td>
</tr>
<tr>
<td>PS2-15-450..................................................</td>
<td>1922</td>
</tr>
<tr>
<td>Boi, Marco</td>
<td></td>
</tr>
<tr>
<td>PS1-05-189..................................................</td>
<td>1178</td>
</tr>
<tr>
<td>Boissière, Michel</td>
<td></td>
</tr>
<tr>
<td>PS1-04-145..................................................</td>
<td>1134</td>
</tr>
<tr>
<td>PS1-04-173..................................................</td>
<td>1161</td>
</tr>
<tr>
<td>Böke, Frederik</td>
<td></td>
</tr>
<tr>
<td>PS1-02-075..................................................</td>
<td>1063</td>
</tr>
<tr>
<td>Bonadies, Irene</td>
<td></td>
</tr>
<tr>
<td>N01-08................................................................</td>
<td>143</td>
</tr>
<tr>
<td>Bonatti, Amedeo F.</td>
<td></td>
</tr>
<tr>
<td>N11-05................................................................</td>
<td>573</td>
</tr>
<tr>
<td>Bonetti, Lorenzo</td>
<td></td>
</tr>
<tr>
<td>PS1-08-265..................................................</td>
<td>1257</td>
</tr>
<tr>
<td>PS2-10-338..................................................</td>
<td>1803</td>
</tr>
<tr>
<td>Bonifacio, Ada</td>
<td></td>
</tr>
<tr>
<td>S01-07................................................................</td>
<td>101</td>
</tr>
<tr>
<td>Bono, Maria R.</td>
<td></td>
</tr>
<tr>
<td>PS1-07-257..................................................</td>
<td>1248</td>
</tr>
<tr>
<td>Boor, Peter</td>
<td></td>
</tr>
<tr>
<td>K02-03................................................................</td>
<td>177</td>
</tr>
<tr>
<td>Borciani, Giorgia</td>
<td></td>
</tr>
<tr>
<td>PS1-05-189..................................................</td>
<td>1178</td>
</tr>
<tr>
<td>S10-06................................................................</td>
<td>690</td>
</tr>
<tr>
<td>Bordat, Pascal</td>
<td></td>
</tr>
<tr>
<td>PS2-08-292..................................................</td>
<td>1755</td>
</tr>
<tr>
<td>Borges, João</td>
<td></td>
</tr>
<tr>
<td>PS1-02-093..................................................</td>
<td>1082</td>
</tr>
<tr>
<td>PS2-03-108..................................................</td>
<td>1578</td>
</tr>
<tr>
<td>S02-03................................................................</td>
<td>106</td>
</tr>
<tr>
<td>Borges, João P.</td>
<td></td>
</tr>
<tr>
<td>N01-03................................................................</td>
<td>133</td>
</tr>
<tr>
<td>PS1-03-117..................................................</td>
<td>1102</td>
</tr>
<tr>
<td>PS1-07-243..................................................</td>
<td>1235</td>
</tr>
<tr>
<td>PS1-08-291..................................................</td>
<td>1282</td>
</tr>
<tr>
<td>PS2-08-280..................................................</td>
<td>1743</td>
</tr>
<tr>
<td>PS2-08-288..................................................</td>
<td>1752</td>
</tr>
<tr>
<td>Borges, Sandra</td>
<td></td>
</tr>
<tr>
<td>N10-05................................................................</td>
<td>552</td>
</tr>
<tr>
<td>Bornitz, Matthias</td>
<td></td>
</tr>
<tr>
<td>N14-06................................................................</td>
<td>906</td>
</tr>
<tr>
<td>Borrás, Alejandro</td>
<td></td>
</tr>
<tr>
<td>AERO InvF-03................................................</td>
<td>623</td>
</tr>
<tr>
<td>Borrós, Salvador</td>
<td></td>
</tr>
<tr>
<td>PS1-12-367..................................................</td>
<td>1365</td>
</tr>
<tr>
<td>Borzacchiello, Assunta</td>
<td></td>
</tr>
<tr>
<td>N02-08................................................................</td>
<td>164</td>
</tr>
<tr>
<td>N15-05................................................................</td>
<td>925</td>
</tr>
<tr>
<td>Author Index</td>
<td>ESB 2021</td>
</tr>
<tr>
<td>-------------</td>
<td>---------</td>
</tr>
<tr>
<td>PS2-07-230</td>
<td>1698</td>
</tr>
<tr>
<td>S06-03</td>
<td>398</td>
</tr>
<tr>
<td>Boschetti, Federica</td>
<td>670</td>
</tr>
<tr>
<td>S09-04</td>
<td>307</td>
</tr>
<tr>
<td>Bosnard, Cédric</td>
<td>1444</td>
</tr>
<tr>
<td>N06-01</td>
<td>1914</td>
</tr>
<tr>
<td>Bosserhoff, Anja K</td>
<td>1409</td>
</tr>
<tr>
<td>PS1-14-443</td>
<td></td>
</tr>
<tr>
<td>Bosserhoff, Anja K.</td>
<td>796</td>
</tr>
<tr>
<td>PS2-14-442</td>
<td></td>
</tr>
<tr>
<td>Bossi, Arianna</td>
<td>1659</td>
</tr>
<tr>
<td>PS2-05-194</td>
<td></td>
</tr>
<tr>
<td>Botelho, Gabriela</td>
<td>579</td>
</tr>
<tr>
<td>N11-08</td>
<td>180</td>
</tr>
<tr>
<td>Böttcher, Bastian</td>
<td>786</td>
</tr>
<tr>
<td>K02-04</td>
<td></td>
</tr>
<tr>
<td>Bourhoven, Margaux</td>
<td>234</td>
</tr>
<tr>
<td>PS1-03-125</td>
<td>1112</td>
</tr>
<tr>
<td>Boutinguiza, Mohamed</td>
<td>527</td>
</tr>
<tr>
<td>ESB-SIBB S01-06</td>
<td>1452</td>
</tr>
<tr>
<td>Boutonnat, Jean</td>
<td>148</td>
</tr>
<tr>
<td>N02-01</td>
<td></td>
</tr>
<tr>
<td>Bouyer, Michael</td>
<td>194</td>
</tr>
<tr>
<td>N02-01</td>
<td></td>
</tr>
<tr>
<td>Bozzato, Elia</td>
<td>707</td>
</tr>
<tr>
<td>ESB-SIBB S02-02</td>
<td></td>
</tr>
<tr>
<td>Brachi, Giulia</td>
<td>208</td>
</tr>
<tr>
<td>PS1-07-227</td>
<td>1221</td>
</tr>
<tr>
<td>S11-KL02</td>
<td>696</td>
</tr>
<tr>
<td>Braem, Annabel</td>
<td>1881</td>
</tr>
<tr>
<td>N03-01</td>
<td></td>
</tr>
<tr>
<td>PS2-13-410</td>
<td></td>
</tr>
<tr>
<td>Branco, Ana</td>
<td>311</td>
</tr>
<tr>
<td>N06-03</td>
<td></td>
</tr>
<tr>
<td>Branco, Ana C.</td>
<td>1180</td>
</tr>
<tr>
<td>PS1-05-191</td>
<td></td>
</tr>
<tr>
<td>PS1-08-287</td>
<td>1278</td>
</tr>
<tr>
<td>PS2-08-286</td>
<td>1750</td>
</tr>
<tr>
<td>Brandt, Nico</td>
<td>1612</td>
</tr>
<tr>
<td>PS2-04-148</td>
<td></td>
</tr>
<tr>
<td>Brauchle, Eva</td>
<td>1408</td>
</tr>
<tr>
<td>PS1-13-409</td>
<td></td>
</tr>
<tr>
<td>Brauchle, Eva M.</td>
<td>1508</td>
</tr>
<tr>
<td>PS2-01-036</td>
<td></td>
</tr>
<tr>
<td>Braun, Joy</td>
<td>1345</td>
</tr>
<tr>
<td>PS1-11-347</td>
<td></td>
</tr>
<tr>
<td>Brenet, Marianne</td>
<td>1248</td>
</tr>
<tr>
<td>PS1-07-257</td>
<td></td>
</tr>
<tr>
<td>Brennan, Caroline H.</td>
<td>756</td>
</tr>
<tr>
<td>NANOSTEM 03-01</td>
<td>359</td>
</tr>
<tr>
<td>BRENOSTEM</td>
<td></td>
</tr>
<tr>
<td>Bresin, Anthony</td>
<td>1755</td>
</tr>
<tr>
<td>PS2-08-292</td>
<td></td>
</tr>
<tr>
<td>Briatico Vangosa, Francesco</td>
<td>1912</td>
</tr>
<tr>
<td>PS2-14-440</td>
<td></td>
</tr>
</tbody>
</table>

Page 1954 of 2028
Author Index

Bustos, Desiré V.
PS2-04-174 ........................................ 1637

Butnarasu, Cosmin S.
PS2-14-440 ........................................ 1912

Byun, Jiwon
PS1-05-183 ........................................ 1172

C

C H van Der Wal, Bart
K03-06 .............................................. 263

C. de Sousa, Herminio
PS1-07-245 ........................................ 1237

Caballer-Calero, Olga
N07-06 .............................................. 339

Cabello, José Carlos R.
PS1-07-241 ........................................ 1234
PS2-04-174 ........................................ 1637

Cabral, Horacio
ESB-CRS S-KL02 .................................. 422

Cabral, Joaquim M.S.
N07-03 .............................................. 332

Cacopardo, Ludovica
K07-05 .............................................. 761
PS2-02-074 ........................................ 1544
PS2-13-396 ........................................ 1866

Cadavid-Vargas, Juan F.
S06-07 .............................................. 406

Cadinoiu, Anca N.
PS1-10-329 ........................................ 1326
PS1-12-371 ........................................ 1369
PS2-10-324 ........................................ 1789

Caetano, Liliana
PS2-10-326 ........................................ 1791

Cafarchia, Claudia
K10-03 .............................................. 860

Cafarelli, Andrea
PS1-08-297 ........................................ 1287

Calessu, Ivo
PS2-07-254 ........................................ 1722

Caldeira, Joana
N11-07 .............................................. 577
S15-03 .............................................. 889

Calderón, Wilfredo
PS1-07-257 ........................................ 1248

Calderon-Jacinto, Rosa
ESB-BIOMAT S-05 ................................ 442

Calejo, Isabel
S05-04 .............................................. 272

Calligaris, Sonia
AERO S03-02 ...................................... 641
AERO S03-03 ...................................... 643

Camacho, Juan P.
PS1-07-257 ........................................ 1248

Camara-Sanchez, Patricia
ESB-SIBB S02-01 .................................. 705

Camarero Espinosa, Sandra
K01-02 .............................................. 76

Camarero, Julio
PS2-07-254 ........................................ 1722

Camarero-Espinosa, Sandra
ESB-ISBF S-03 ..................................... 535
PS2-04-132 ........................................ 1601
S14-03 .............................................. 876

Camasão, Dimitria B.
N12-02 .............................................. 734

Cameron, Ruth E.
K05-06 .............................................. 501
PS2-06-206 ........................................ 1672

Camman, Marie
PS1-03-105 ........................................ 1094

Campiglio, Chiara Emma
S09-04 .............................................. 670

Campodonii, Elisabetta
N09-05 .............................................. 476
PS2-01-018 ........................................ 1491
S10-03 .............................................. 684

Campodónico, Paola R.
PS1-11-365 ........................................ 1362

Campos Coll, José M.
Biomechanics 04-06 ................................ 988

Campos Coll, José María
Biomechanics 04-05 ................................ 985

Campos Granell, José
Biomechanics 04-06 ................................ 988

Campos, José
Biomechanics 04-05 ................................ 985

Camposeo, Andrea
PS2-07-224 ........................................ 1691

Campoy, Mariano
AERO PS01-04 ..................................... 603

Canal, Cristina
ESB-SIBB S02-06 .................................. 715
K09-05 .............................................. 848
N04-01 .............................................. 230
N16-06 .............................................. 941
PS2-09-304 ........................................ 1767
S14-KL01 ........................................... 872

Candiani, Gabriele
PS1-15-455 ........................................ 1454

Cantini, Marco
N08-08 .............................................. 464

Capella-Gutierrez, Salvador
S13-02 .............................................. 800

Capitão, Ana
PS2-01-004 ........................................ 1474

Caporal, Maria
K08-07 .............................................. 781

Cappelletti, Martina
PS1-05-189 ........................................ 1178
<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caraseva, Iulia</td>
<td>927</td>
</tr>
<tr>
<td>Carbajal-de-La-Torre, Georgina</td>
<td>1092</td>
</tr>
<tr>
<td>PS1-02-103</td>
<td></td>
</tr>
<tr>
<td>PS2-02-104</td>
<td>1574</td>
</tr>
<tr>
<td>Carballo, Jesús</td>
<td>1764</td>
</tr>
<tr>
<td>PS2-08-302</td>
<td></td>
</tr>
<tr>
<td>Carballo-Pedrares, Natalia</td>
<td>1328</td>
</tr>
<tr>
<td>PS1-10-331</td>
<td></td>
</tr>
<tr>
<td>Carella, Francesca</td>
<td>1614</td>
</tr>
<tr>
<td>PS2-04-150</td>
<td></td>
</tr>
<tr>
<td>PS2-04-162</td>
<td>1624</td>
</tr>
<tr>
<td>Careta, Oriol</td>
<td>1130</td>
</tr>
<tr>
<td>PS1-04-139</td>
<td></td>
</tr>
<tr>
<td>Carmagnola, Irene</td>
<td>732</td>
</tr>
<tr>
<td>N12-01</td>
<td></td>
</tr>
<tr>
<td>Carmignani, Alessio</td>
<td>85</td>
</tr>
<tr>
<td>K01-06</td>
<td></td>
</tr>
<tr>
<td>N15-02</td>
<td>918</td>
</tr>
<tr>
<td>PS2-12-378</td>
<td>1847</td>
</tr>
<tr>
<td>Carminati, Francesca</td>
<td>1915</td>
</tr>
<tr>
<td>PS2-14-444</td>
<td></td>
</tr>
<tr>
<td>Caro-León, Javier</td>
<td>1211</td>
</tr>
<tr>
<td>PS1-07-217</td>
<td></td>
</tr>
<tr>
<td>Caronna, Flavia</td>
<td>568</td>
</tr>
<tr>
<td>N11-03</td>
<td></td>
</tr>
<tr>
<td>Carouix, Julien</td>
<td>270</td>
</tr>
<tr>
<td>S05-03</td>
<td></td>
</tr>
<tr>
<td>Carpentier, Nathan</td>
<td>447</td>
</tr>
<tr>
<td>ESB-BIOMAT S-07</td>
<td></td>
</tr>
<tr>
<td>K08-05</td>
<td>777</td>
</tr>
<tr>
<td>Carrazone, Ryan</td>
<td>896</td>
</tr>
<tr>
<td>N14-01</td>
<td></td>
</tr>
<tr>
<td>Carrêlo, Henrique</td>
<td>1752</td>
</tr>
<tr>
<td>PS2-08-288</td>
<td></td>
</tr>
<tr>
<td>Carson, Louise</td>
<td>1354</td>
</tr>
<tr>
<td>PS1-11-357</td>
<td></td>
</tr>
<tr>
<td>PS1-11-363</td>
<td>1360</td>
</tr>
<tr>
<td>PS1-15-459</td>
<td>1460</td>
</tr>
<tr>
<td>PS2-11-348</td>
<td>1815</td>
</tr>
<tr>
<td>PS2-11-358</td>
<td>1825</td>
</tr>
<tr>
<td>PS2-11-362</td>
<td>1829</td>
</tr>
<tr>
<td>Carter, Luke</td>
<td>1448</td>
</tr>
<tr>
<td>PS1-15-447</td>
<td></td>
</tr>
<tr>
<td>Carter, Luke N.</td>
<td>119</td>
</tr>
<tr>
<td>S03-04</td>
<td></td>
</tr>
<tr>
<td>Carter, Sarah-Sophia D.</td>
<td>805</td>
</tr>
<tr>
<td>S13-04</td>
<td></td>
</tr>
<tr>
<td>Carvajal, Felipe</td>
<td>1248</td>
</tr>
<tr>
<td>PS1-07-257</td>
<td></td>
</tr>
<tr>
<td>Carvajal-Berrio, Daniel</td>
<td>1408</td>
</tr>
<tr>
<td>PS1-13-409</td>
<td></td>
</tr>
<tr>
<td>Carvalho, Angela</td>
<td>655</td>
</tr>
<tr>
<td>K06-03</td>
<td></td>
</tr>
<tr>
<td>Carvalho, Eugénia</td>
<td>311</td>
</tr>
<tr>
<td>N06-03</td>
<td></td>
</tr>
<tr>
<td>Carvalho, Eva</td>
<td>143</td>
</tr>
<tr>
<td>N01-08</td>
<td></td>
</tr>
<tr>
<td>Carvalho, Eva D.</td>
<td>1416</td>
</tr>
<tr>
<td>PS1-13-419</td>
<td></td>
</tr>
<tr>
<td>S09-03</td>
<td>668</td>
</tr>
<tr>
<td>Carvalho, Mariana</td>
<td>1896</td>
</tr>
<tr>
<td>PS2-14-424</td>
<td></td>
</tr>
<tr>
<td>Carvalho, Oscar</td>
<td>1184</td>
</tr>
<tr>
<td>PS1-05-195</td>
<td></td>
</tr>
<tr>
<td>Carvalho, Tiago</td>
<td>1777</td>
</tr>
<tr>
<td>PS2-09-312</td>
<td></td>
</tr>
<tr>
<td>Casadidio, Cristina</td>
<td>318</td>
</tr>
<tr>
<td>N06-06</td>
<td></td>
</tr>
<tr>
<td>Casal, Susana</td>
<td>427</td>
</tr>
<tr>
<td>ESB-CRS S-04</td>
<td></td>
</tr>
<tr>
<td>Casanova-Battle, Enric</td>
<td>1486</td>
</tr>
<tr>
<td>PS2-01-014</td>
<td></td>
</tr>
<tr>
<td>Casas-Luna, Mariano</td>
<td>1550</td>
</tr>
<tr>
<td>PS2-02-080</td>
<td></td>
</tr>
<tr>
<td>PS2-05-188</td>
<td>1652</td>
</tr>
<tr>
<td>Cases, Nuria B.</td>
<td></td>
</tr>
<tr>
<td>AERO PS01-03</td>
<td>601</td>
</tr>
<tr>
<td>Cassel, Samantha E.</td>
<td></td>
</tr>
<tr>
<td>PS2-14-432</td>
<td>1903</td>
</tr>
<tr>
<td>Castanhó, Miguel A. R. B.</td>
<td>1708</td>
</tr>
<tr>
<td>PS2-07-238</td>
<td></td>
</tr>
<tr>
<td>Castano Linares, Oscar</td>
<td>1423</td>
</tr>
<tr>
<td>PS1-14-425</td>
<td></td>
</tr>
<tr>
<td>Castano, Oscar</td>
<td>154</td>
</tr>
<tr>
<td>N02-03</td>
<td></td>
</tr>
<tr>
<td>PS1-07-235</td>
<td>1227</td>
</tr>
<tr>
<td>Castel-Branco, Miguel</td>
<td>365</td>
</tr>
<tr>
<td>NANOSTEM 03-05</td>
<td></td>
</tr>
<tr>
<td>Castilho, Miguel</td>
<td>263</td>
</tr>
<tr>
<td>K03-06</td>
<td></td>
</tr>
<tr>
<td>N08-02</td>
<td>452</td>
</tr>
<tr>
<td>N14-02</td>
<td>898</td>
</tr>
<tr>
<td>N14-05</td>
<td>904</td>
</tr>
<tr>
<td>Castilho, Miguel D.</td>
<td>482</td>
</tr>
<tr>
<td>N09-08</td>
<td></td>
</tr>
<tr>
<td>Castrillo, Aurelio V.</td>
<td>1637</td>
</tr>
<tr>
<td>PS2-04-174</td>
<td></td>
</tr>
<tr>
<td>Castro, Ana L.</td>
<td>1289</td>
</tr>
<tr>
<td>PS1-08-299</td>
<td></td>
</tr>
<tr>
<td>Castro, Filipa</td>
<td>1539</td>
</tr>
<tr>
<td>PS2-02-070</td>
<td></td>
</tr>
<tr>
<td>Castro, Flávia</td>
<td>429</td>
</tr>
<tr>
<td>ESB-CRS S-05</td>
<td></td>
</tr>
<tr>
<td>ESB-SFB S-05</td>
<td>815</td>
</tr>
<tr>
<td>PS1-10-323</td>
<td>1319</td>
</tr>
<tr>
<td>Catalano, Enrico</td>
<td>274</td>
</tr>
<tr>
<td>S05-05</td>
<td></td>
</tr>
</tbody>
</table>
Catarino, André
PS1-11-353……………………………………1350
Cauldbeck, Helen
PS1-07-237……………………………………1229
Cavailles, Vincent
PS1-01-049……………………………………1039
Cavailles, Vincent
N02-04………………………………………156
Cazaux, Frédéric
PS1-07-223……………………………………1217
Cecere, Renzo
PS1-09-319……………………………………1313
Cedillo-Servin, Gerardo
N14-05………………………………………904
Ceelen, Wim
K08-05………………………………………777
Cegielska, Olga
PS1-10-341……………………………………1338
Celiz, Adam D.
S10-02………………………………………682
Čelko, Ladislav
PS2-02-080……………………………………1550
PS2-05-188……………………………………1652
Censi, Roberta
N06-06………………………………………318
Cepero, Mar
Biomechanics 03-03…………………………963
Ceretti, Elisabetta
PS1-01-045……………………………………1036
Cermolacce, Alexia
S05-03………………………………………270
Cernencu, Alexandra I.
PS1-01-035……………………………………1027
Cerqueira, Andreia
N08-06………………………………………460
PS1-13-401……………………………………1400
Cerqueira, Rui
K07-02………………………………………753
Cerqueni, Giorgia
N04-07………………………………………243
N04-08………………………………………245
S01-07………………………………………101
Chagot, Lise
PS1-01-061……………………………………1048
Chai, Feng
PS1-02-085……………………………………1074
PS1-07-223……………………………………1217
Chakraborty, Juhi
PS2-04-168……………………………………1630
Champion, Eric
PS1-02-101……………………………………1091
PS1-03-119……………………………………1105
Chan, Chi Wai
PS1-15-459……………………………………1460
Chandrakar, Amit
N05-09………………………………………304
N14-03………………………………………900
PS1-02-085……………………………………1074
Chang, Lan
N06-04………………………………………314
Chanseau, Christel
ESB-BIOMAT S-02…………………………434
Chaparro, Catarina I.P.
N01-03………………………………………133
Chattahy, Kaoutar
N02-09………………………………………167
Chatzinikolaidou, Maria
N04-06………………………………………241
N11-05………………………………………573
PS2-02-096……………………………………1568
PS2-05-196……………………………………1662
S10-05………………………………………688
Chaubet, Frédéric
PS1-03-125……………………………………1112
PS1-08-271……………………………………1263
Chaudhuri, Ovijit
K08-KL01……………………………………769
Chaussain, Catherine
PS1-04-173……………………………………1161
Chausse, Victor
N12-04………………………………………738
PS2-09-304……………………………………1767
Chauvierre, Cédric
PS1-09-307……………………………………1298
Chavan, Bhaven
PS1-05-187……………………………………1176
Chawla, Shikha
PS2-04-168……………………………………1630
Chekireb, Nassim
PS1-12-381……………………………………1378
Chen, Christopher S.
S09-02………………………………………665
Chen, Honglin
PS2-08-262……………………………………1730
Chen, Hongyi
PS1-01-005……………………………………998
Chen, Jishizhan
PS2-12-384……………………………………1853
Chen, Menglin
ESB-CSBM S-05……………………………205
PS1-01-025……………………………………1018
Chen, Si
N09-09………………………………………484
Chen, Xiangzhong
PS1-12-375……………………………………1372
Chen, Zhaoyu
N14-06………………………………………906
Chennell, Philip
PS2-10-320……………………………………1784
<table>
<thead>
<tr>
<th>Author</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cherif, Chokri</td>
<td>906</td>
</tr>
<tr>
<td>N14-06</td>
<td></td>
</tr>
<tr>
<td>Cherry, Christopher</td>
<td>491</td>
</tr>
<tr>
<td>K05-KL01</td>
<td></td>
</tr>
<tr>
<td>Chevalier, Jerome</td>
<td>1502</td>
</tr>
<tr>
<td>PS2-01-028</td>
<td></td>
</tr>
<tr>
<td>Chevalier, Pascale</td>
<td>1878</td>
</tr>
<tr>
<td>PS2-13-408</td>
<td></td>
</tr>
<tr>
<td>Chiesa, Roberto</td>
<td>1454</td>
</tr>
<tr>
<td>PS1-15-455</td>
<td></td>
</tr>
<tr>
<td>PS2-15-452</td>
<td>1924</td>
</tr>
<tr>
<td>Chijcheapaza-Flores, Henry</td>
<td>1217</td>
</tr>
<tr>
<td>PS1-07-223</td>
<td></td>
</tr>
<tr>
<td>Chiono, Valeria</td>
<td>325</td>
</tr>
<tr>
<td>N06-09</td>
<td></td>
</tr>
<tr>
<td>N12-01</td>
<td>732</td>
</tr>
<tr>
<td>PS2-12-372</td>
<td>1840</td>
</tr>
<tr>
<td>Chiriac, Aurica</td>
<td>1734</td>
</tr>
<tr>
<td>PS2-08-268</td>
<td></td>
</tr>
<tr>
<td>Chiriac, Aurica P.</td>
<td>1831</td>
</tr>
<tr>
<td>PS2-11-364</td>
<td></td>
</tr>
<tr>
<td>Chitas, Rute</td>
<td>929</td>
</tr>
<tr>
<td>N15-07</td>
<td></td>
</tr>
<tr>
<td>PS2-08-298</td>
<td>1760</td>
</tr>
<tr>
<td>Chiussi, Stefano</td>
<td>1593</td>
</tr>
<tr>
<td>PS2-03-124</td>
<td></td>
</tr>
<tr>
<td>Choi, In sung S.</td>
<td>395</td>
</tr>
<tr>
<td>S06-02</td>
<td></td>
</tr>
<tr>
<td>Choudhary, Rajan</td>
<td>1677</td>
</tr>
<tr>
<td>PS2-06-210</td>
<td></td>
</tr>
<tr>
<td>Christ, Bastian</td>
<td>1025</td>
</tr>
<tr>
<td>PS1-01-033</td>
<td></td>
</tr>
<tr>
<td>PS1-05-185</td>
<td>1174</td>
</tr>
<tr>
<td>Christel, Chanseau</td>
<td>1265</td>
</tr>
<tr>
<td>PS1-08-273</td>
<td></td>
</tr>
<tr>
<td>Christin, Emilie</td>
<td>277</td>
</tr>
<tr>
<td>S05-06</td>
<td></td>
</tr>
<tr>
<td>Christmann, Hannah</td>
<td>1727</td>
</tr>
<tr>
<td>PS2-08-258</td>
<td></td>
</tr>
<tr>
<td>Christodoulou, Evi</td>
<td>1808</td>
</tr>
<tr>
<td>PS2-10-342</td>
<td></td>
</tr>
<tr>
<td>Ciapetti, Gabriela</td>
<td>690</td>
</tr>
<tr>
<td>S10-06</td>
<td></td>
</tr>
<tr>
<td>Ciardelli, Gianluca</td>
<td>775</td>
</tr>
<tr>
<td>K08-04</td>
<td></td>
</tr>
<tr>
<td>N06-09</td>
<td>325</td>
</tr>
<tr>
<td>PS1-01-017</td>
<td>1011</td>
</tr>
<tr>
<td>PS1-07-227</td>
<td>1221</td>
</tr>
<tr>
<td>PS1-14-435</td>
<td>1435</td>
</tr>
<tr>
<td>PS2-01-030</td>
<td>1504</td>
</tr>
<tr>
<td>PS2-05-178</td>
<td>1643</td>
</tr>
<tr>
<td>PS2-14-438</td>
<td>1910</td>
</tr>
<tr>
<td>S11-KL02</td>
<td>696</td>
</tr>
<tr>
<td>Cicha, Iwona</td>
<td>1076</td>
</tr>
<tr>
<td>PS1-02-087</td>
<td></td>
</tr>
<tr>
<td>Cichoń, Ewelina</td>
<td>151</td>
</tr>
<tr>
<td>N02-02</td>
<td></td>
</tr>
<tr>
<td>PS1-03-111</td>
<td>1098</td>
</tr>
<tr>
<td>PS1-04-147</td>
<td>1136</td>
</tr>
<tr>
<td>PS1-08-303</td>
<td>1293</td>
</tr>
<tr>
<td>PS2-03-112</td>
<td>1582</td>
</tr>
<tr>
<td>PS2-04-146</td>
<td>1610</td>
</tr>
<tr>
<td>Cidade, Maria T.</td>
<td>1752</td>
</tr>
<tr>
<td>PS2-08-288</td>
<td></td>
</tr>
<tr>
<td>Cinar, Goksu</td>
<td>106</td>
</tr>
<tr>
<td>S02-03</td>
<td></td>
</tr>
<tr>
<td>Ciocfani, Gianni</td>
<td>727</td>
</tr>
<tr>
<td>ESB-BMSJ S-04</td>
<td></td>
</tr>
<tr>
<td>K01-06</td>
<td>85</td>
</tr>
<tr>
<td>K09-02</td>
<td>842</td>
</tr>
<tr>
<td>N01-02</td>
<td>131</td>
</tr>
<tr>
<td>N15-02</td>
<td>918</td>
</tr>
<tr>
<td>N15-03</td>
<td>921</td>
</tr>
<tr>
<td>PS2-12-376</td>
<td>1845</td>
</tr>
<tr>
<td>PS2-12-378</td>
<td>1847</td>
</tr>
<tr>
<td>S04-KL02</td>
<td>191</td>
</tr>
<tr>
<td>S12-06</td>
<td>794</td>
</tr>
<tr>
<td>Cipolla, Laura</td>
<td>106</td>
</tr>
<tr>
<td>S02-03</td>
<td></td>
</tr>
<tr>
<td>Cipolli, Marco</td>
<td>1801</td>
</tr>
<tr>
<td>PS2-10-336</td>
<td></td>
</tr>
<tr>
<td>Cirstea, Matei</td>
<td>1074</td>
</tr>
<tr>
<td>PS1-02-085</td>
<td></td>
</tr>
<tr>
<td>Ciurana, Joaquim</td>
<td>711</td>
</tr>
<tr>
<td>ESB-SIBB S02-04</td>
<td></td>
</tr>
<tr>
<td>PS1-01-045</td>
<td>1036</td>
</tr>
<tr>
<td>PS2-01-014</td>
<td>1486</td>
</tr>
<tr>
<td>PS2-03-128</td>
<td>1595</td>
</tr>
<tr>
<td>PS2-14-432</td>
<td>1903</td>
</tr>
<tr>
<td>Claeyssens, Frederik</td>
<td>1089</td>
</tr>
<tr>
<td>PS1-02-099</td>
<td></td>
</tr>
<tr>
<td>Clauser, Johanna C.</td>
<td>1060</td>
</tr>
<tr>
<td>PS1-02-073</td>
<td></td>
</tr>
<tr>
<td>Claverol, Stéphane</td>
<td>402</td>
</tr>
<tr>
<td>S06-05</td>
<td></td>
</tr>
<tr>
<td>Clerkin, Shane</td>
<td>194</td>
</tr>
<tr>
<td>S04-04</td>
<td></td>
</tr>
<tr>
<td>Cleymand, Franck</td>
<td>136</td>
</tr>
<tr>
<td>N01-04</td>
<td></td>
</tr>
<tr>
<td>PS1-01-029</td>
<td>1022</td>
</tr>
<tr>
<td>Cocchi, Claudia</td>
<td>1624</td>
</tr>
<tr>
<td>PS2-04-162</td>
<td></td>
</tr>
<tr>
<td>Cochis, Andrea</td>
<td>1451</td>
</tr>
<tr>
<td>PS1-15-451</td>
<td></td>
</tr>
<tr>
<td>PS1-15-455</td>
<td>1454</td>
</tr>
<tr>
<td>PS2-10-338</td>
<td>1803</td>
</tr>
<tr>
<td>Cocuzza, Matteo</td>
<td>1435</td>
</tr>
<tr>
<td>PS1-14-435</td>
<td></td>
</tr>
<tr>
<td>PS2-14-438</td>
<td>1910</td>
</tr>
</tbody>
</table>
Author Index

Coelho, Catarina
N04-06 ........................................ 241

Coelho, João M.
AERO InvF-02 .......................... 621

Coelho, Mariana
N05-06 ........................................ 297

Coelho, Soraia
PS2-09-318 .................................. 1782

Coenye, Tom
K10-06 ........................................ 867

Colaço, Rogério
N04-04 ........................................ 237
PS1-05-191 .................................. 1180
PS1-08-287 .................................. 1278
PS2-08-286 .................................. 1750

Cönciencias
PS1-04-167 .................................. 1156

Coleman, Jonathan
K04-02 ........................................ 380

Collilla, Montserrat
PS2-12-380 .................................. 1849

Collins, Cheryl
N08-05 ........................................ 458
S12-02 ........................................ 786

Collins, Maurice
N07-05 ........................................ 337

Collins, Maurice N
PS1-01-009 .................................. 1003

Colomina, Laura
PS2-11-356 .................................. 1823

Combes, Christèle
N04-02 ........................................ 232
PS1-06-205 .................................. 1198
S12-05 ........................................ 792

Comesaña, Rafael
S14-07 ........................................ 884

Cometa, Stefania
PS1-15-455 .................................. 1454
S01-07 ........................................ 101

Cometta, Silvia
N13-07 ........................................ 829

Comperat, Leo
PS1-01-061 .................................. 1048
S14-05 ........................................ 880

Comperat, Léonard
PS2-01-052 .................................. 1523

Completo, António
PS2-01-004 .................................. 1474

Concheiro, Angel
N16-01 ........................................ 932
PS1-01-031 .................................. 1023
PS1-08-285 .................................. 1277
PS1-10-331 .................................. 1328
PS2-01-024 .................................. 1498

Conde, João P.
PS2-14-434 .................................. 1906

Conicella, Fabrizio
TRS-S02-03 .................................. 172

Connon, Che
PS1-01-041 .................................. 1033

Constâncio, Vera
K06-03 ........................................ 655

Constant, Caroline
PS2-13-410 .................................. 1881

Contessi Negrini, Nicola
PS1-07-239 .................................. 1231
PS2-05-194 .................................. 1659
S10-02 ........................................ 682

Contessi, Nicola
YSF-AERogels WS-01 ...................... 616

Conti, Sara
S09-04 ........................................ 670

Cooksley, Grace
PS1-13-405 .................................. 1403

Coppola, Guglielmo A.
PS2-13-410 .................................. 1881

Coradin, Thibaud
PS1-10-321 .................................. 1316

Corallo, Angelo
PS2-05-200 .................................. 1666

Coru, David
PS1-01-049 .................................. 1039

Coru, Olivier
N03-07 ........................................ 222

Corrales-Orovio, Rocío
PS1-07-257 .................................. 1248

Correia, Alexandra M.
PS2-09-312 .................................. 1777

Correia, Beatriz
PS1-08-291 .................................. 1282

Correia, Clara
N11-04 ........................................ 571

Correia, Clara R.
K09-06 ........................................ 850
N03-04 ........................................ 215
N04-05 ........................................ 239
PS2-09-306 .................................. 1770

Correia, Miguel V.
Biomechanics 04-04 ....................... 982

Correia, Tiago R.
PS1-02-093 .................................. 1082
PS2-03-108 .................................. 1578
S06-02 ........................................ 395

Corté, Laurent
ESB-BMSJ S-05 ......................... 729
PS1-13-423 .................................. 1420
PS2-08-274 .................................. 1739
S05-03 ........................................ 270
Author Index

Cortes Vesga, Catherine
PS2-01-040............................................. 1512
Corti, Alessandro
PS2-13-396............................................. 1866
Cortina, Jose Luis
N12-04................................................. 738
Cortinhas, João
S11-03................................................... 697
Corvi, Javier
PS2-02-082............................................. 1552
S13-02................................................... 800
Costa Lima, Sofia A.
ESB-CRS S-04........................................... 427
Costa, Beatriz
S03-02................................................... 115
Costa, Bruna
PS2-12-392............................................. 1860
Costa, Carolina
PS2-08-286............................................. 1750
Costa, Carolina M.
PS1-04-163............................................. 1151
Costa, Eduardo
AERO InvF-02........................................... 621
Costa, Fabiola
PS2-12-392............................................. 1860
Costa, Fabiola
S03-02................................................... 115
Costa, Madalena
K05-03................................................... 495
Costa, Raquel
AERO InvF-01........................................... 618
N10-05................................................... 552
Costa, Susana P.G.
PS1-07-221............................................. 1215
Costache, Marieta
PS1-07-219............................................. 1214
Courtial, Edwin J.
PS1-01-001............................................. 994
Couto, Cristiana
N02-09................................................... 167
Couto, Marina
PS2-04-156............................................. 1621
Couto, Ricardo
AERO S03-KL03........................................... 637
Couture, Olivier
PS1-09-307............................................. 1298
Cox, Sophie
PS1-15-447............................................. 1448
Cox, Sophie C.
S03-04................................................... 119
Crean, John
S04-04................................................... 194
Crespo, Julián
PS2-12-380............................................. 1849
Crespo-Cuadrado, Maria
PS1-05-187............................................. 1176
Crispim, João F.
K08-03................................................... 772
Cristallini, Caterina
K07-03................................................... 756
Croitoriu, Alexandra
PS1-11-355............................................. 1352
Crott, Elena
PS2-14-440............................................. 1912
Cryan, Sally-Ann
PS2-07-234............................................. 1704
Csaba, Noemi
ESB-SIBB S02-02...................................... 707
PS1-05-181............................................. 1170
PS2-10-330............................................. 1795
Cubo Mateo, Nieves
K03-02................................................... 255
Cucca, Lucia
PS1-13-413............................................. 1410
Cui, Sheng
ESB-CSBM S-03....................................... 201
Culebras Rubio, Mario
N07-05................................................... 337
Cuna, Anna
S03-05................................................... 121
Currie, Susan
PS2-09-316............................................. 1780
Custódio, Beatriz
PS2-02-100............................................. 1572
Custódio, Catarina A.
K05-05................................................... 499
N05-03................................................... 294
PS1-14-429............................................. 1428
S02-04................................................... 108
Czechowska, Joanna
N02-02................................................... 151
PS1-03-111............................................. 1098
PS1-04-147............................................. 1136
PS2-03-112............................................. 1582
PS2-04-146............................................. 1610

D
D. Angelov, Svilin
PS2-15-446............................................. 1917
D’Este, Matteo
PS1-01-039............................................. 1031
da Conceição, Katia
PS1-01-013............................................. 1007
da Silva, Daniela M.
K04-06................................................... 388
Da Silva, Nicolas
N12-03................................................... 736

Page 1960 of 2028
Author Index

ESB 2021 | Abstract Book

da Silva, Ricardo
K09-03 .................................................. 844
das Neves, José
PS1-11-359 ........................................ 1356
Dau, Michael
PS2-04-148 ........................................ 1612

Daelman, Jeff
LS-02 ................................................. 489
David, Robert
PS1-09-317 ........................................ 1311

Dalby, Matthew
K03-01 .................................................. 253
Davies, Alys E.
PS1-07-237 ........................................ 1229
N08-03 .................................................. 454
De Acutis, Aurora
N11-05 ................................................. 573
N08-03 .................................................. 454
De Angelis, Nicola
PS1-06-209 ........................................ 1202
PS2-04-160 .......................................... 1622
PS1-06-213 ........................................ 1206
S10-07 .................................................. 692
PS2-04-152 ........................................ 1616
del Aranda, Gonzalo
N10-03 ................................................. 548
del Boer, Jan
S13-KL01 ........................................... 799
del Boer, Leonie
S03-07 ............................................... 125
del Cola, Luisa
K01-KL01 ............................................ 75
del Donno, Chiara
PS1-02-091 .......................................... 1080
de Haan, Bart
K08-03 ............................................... 772
S11-05 ................................................. 701
del Hilster, Roderick H.J.
PS1-02-063 .......................................... 1052
de Jong, Vincent
K07-07 .................................................. 765
de la Cruz, Juan Carlos
Biomechanics 03-03 ................................. 963
de la Cruz, Julia E.
K07-07 .................................................. 765
de la Fuente, Jesús M.
ESB-SIBB S01-KL01 ................................. 516
del Rueda, Carlos
PL3-01 .............................................. 836
de Maria, Carmelo
N11-05 ................................................. 573
del Rueda, Carlos
PL3-01 .............................................. 836

De Maria, Carmelo
N11-05 ................................................. 573

Dalgarno, Kenneth
N10-01 .................................................. 544
del Rueda, Carlos
PL3-01 .............................................. 836
N11-05 .................................................. 573
PS1-01-007 ........................................... 1001
del Rueda, Carlos
PL3-01 .............................................. 836

Dalgarno, Kenny
N04-07 .................................................. 243
del Rueda, Carlos
PL3-01 .............................................. 836
N04-08 .................................................. 245
del Rueda, Carlos
PL3-01 .............................................. 836
PS1-05-187 .......................................... 1176
del Rueda, Carlos
PL3-01 .............................................. 836
S01-07 ............................................... 101
del Rueda, Carlos
PL3-01 .............................................. 836

D'Amora, Ugo
N10-09 .................................................. 560
del Rueda, Carlos
PL3-01 .............................................. 836
PS2-03-118 ........................................... 1586
del Rueda, Carlos
PL3-01 .............................................. 836

Dang, Phuong Anh
PS1-13-423 .......................................... 1420
del Rueda, Carlos
PL3-01 .............................................. 836
PS2-08-274 .......................................... 1739
del Rueda, Carlos
PL3-01 .............................................. 836

Dani, Sophie
K03-05 .................................................. 261
del Rueda, Carlos
PL3-01 .............................................. 836
S03-07 .................................................. 125
del Rueda, Carlos
PL3-01 .............................................. 836

Dankers, Patricia Y.
S03-07 .................................................. 125
del Rueda, Carlos
PL3-01 .............................................. 836
S03-07 .................................................. 125
del Rueda, Carlos
PL3-01 .............................................. 836

Dankers, Patricia Y.W.
PS1-09-315 ........................................... 1310
del Rueda, Carlos
PL3-01 .............................................. 836

Danti, Serena
ESB-CRS S-03 ........................................ 424
del Rueda, Carlos
PL3-01 .............................................. 836
S07-05 .................................................. 417
del Rueda, Carlos
PL3-01 .............................................. 836

Dapporto, Massimiliano
N09-05 .................................................. 476
del Rueda, Carlos
PL3-01 .............................................. 836
PS2-04-150 .......................................... 1614
del Rueda, Carlos
PL3-01 .............................................. 836
PS2-04-162 .......................................... 1624
del Rueda, Carlos
PL3-01 .............................................. 836

Darab, Oana M.
PS2-10-324 .......................................... 1789
del Rueda, Carlos
PL3-01 .............................................. 836

Darcos, Vincent
N04-02 .................................................. 232
del Rueda, Carlos
PL3-01 .............................................. 836

Page 1961 of 2028
<table>
<thead>
<tr>
<th>Name</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Nardo, Luigi</td>
<td>PS1-08-265: 1257</td>
</tr>
<tr>
<td></td>
<td>PS1-15-455: 1454</td>
</tr>
<tr>
<td></td>
<td>PS2-15-452: 1924</td>
</tr>
<tr>
<td>De Oliveira, Hugo</td>
<td>PS1-08-279: 1271</td>
</tr>
<tr>
<td></td>
<td>S14-05: 880</td>
</tr>
<tr>
<td>De Pasquale, Daniele</td>
<td>ESB-BMSJ S-04: 727</td>
</tr>
<tr>
<td></td>
<td>K09-02: 842</td>
</tr>
<tr>
<td>de Ruijter, Mylène</td>
<td>N04-03: 234</td>
</tr>
<tr>
<td></td>
<td>N14-02: 898</td>
</tr>
<tr>
<td></td>
<td>S01-04: 94</td>
</tr>
<tr>
<td>De Soricellis, Chiara</td>
<td>AERO S01-01: 588</td>
</tr>
<tr>
<td>de Sousa Trichès, Eliandra</td>
<td>PS1-01-013: 1007</td>
</tr>
<tr>
<td></td>
<td>PS2-03-130: 1598</td>
</tr>
<tr>
<td>de Sousa, Bárbara M.</td>
<td>N03-04: 215</td>
</tr>
<tr>
<td>De Vita, Alessandro</td>
<td>PS2-04-162: 1624</td>
</tr>
<tr>
<td>De Vita, Lorenzo</td>
<td>PS2-07-252: 1720</td>
</tr>
<tr>
<td>De Vitis, Eleonora</td>
<td>PS2-10-336: 1801</td>
</tr>
<tr>
<td>De Vlieghere, Elly</td>
<td>K08-05: 777</td>
</tr>
<tr>
<td></td>
<td>de Vos, Paul</td>
</tr>
<tr>
<td></td>
<td>K08-03: 772</td>
</tr>
<tr>
<td></td>
<td>S11-05: 701</td>
</tr>
<tr>
<td>de Vries, Jean-Paul P.</td>
<td>PS2-09-308: 1773</td>
</tr>
<tr>
<td>de Vries, Margreet</td>
<td>N06-03: 311</td>
</tr>
<tr>
<td>de Wever, Olivier</td>
<td>ESB-SFB S-05: 815</td>
</tr>
<tr>
<td>De Wever, Olivier</td>
<td>K08-05: 777</td>
</tr>
<tr>
<td>De Wilde, Lieven</td>
<td>S05-02: 267</td>
</tr>
<tr>
<td>de Windt, Leon</td>
<td>K07-04: 759</td>
</tr>
<tr>
<td>Debena, Nataliya</td>
<td>N10-03: 548</td>
</tr>
<tr>
<td>Débena, Nataliya</td>
<td>N16-02: 934</td>
</tr>
<tr>
<td>Debret, Romain</td>
<td>S05-06: 277</td>
</tr>
<tr>
<td>Decambon, Adeline</td>
<td>PS1-04-165: 1153</td>
</tr>
<tr>
<td>Decencière, Etienne</td>
<td>PS2-08-274: 1739</td>
</tr>
<tr>
<td>Deck, Anna</td>
<td>PS2-01-010: 1481</td>
</tr>
<tr>
<td>Deconinck, Liesbet</td>
<td>S05-02: 267</td>
</tr>
<tr>
<td>Dehmel, Susann</td>
<td>PS1-05-185: 1174</td>
</tr>
<tr>
<td>Dehouck, Lucie</td>
<td>NANOSTEM 03-03: 362</td>
</tr>
<tr>
<td>Dehouck, Marie Pierre</td>
<td>NANOSTEM 03-03: 362</td>
</tr>
<tr>
<td></td>
<td>NANOSTEM 03-01: 359</td>
</tr>
<tr>
<td></td>
<td>NANOSTEM 03-02: 360</td>
</tr>
<tr>
<td>Deie, Masataka</td>
<td>PS2-01-038: 1509</td>
</tr>
<tr>
<td>De-Juan-Pardo, Elena</td>
<td>PS1-03-129: 1117</td>
</tr>
<tr>
<td>del Campo, Aranzazu</td>
<td>S07-04: 415</td>
</tr>
<tr>
<td>del Campo, Aránzazu</td>
<td>K10-05: 865</td>
</tr>
<tr>
<td></td>
<td>PS2-01-034: 1506</td>
</tr>
<tr>
<td>Del Gaudio, Pasquale</td>
<td>AERO S01-01: 588</td>
</tr>
<tr>
<td>del Real, Juan C.</td>
<td>PS2-05-176: 1640</td>
</tr>
<tr>
<td>del Real, Juan Carlos</td>
<td>PS1-05-201: 1191</td>
</tr>
<tr>
<td>del Val, Jesús</td>
<td>ESB-SIBB S01-06: 527</td>
</tr>
<tr>
<td></td>
<td>S14-07: 884</td>
</tr>
<tr>
<td>Delaey, Jasper</td>
<td>N03-09: 227</td>
</tr>
<tr>
<td>Delaitre, Christelle</td>
<td>PS2-10-324: 1789</td>
</tr>
<tr>
<td>Delaney, Colm</td>
<td>N03-06: 219</td>
</tr>
<tr>
<td>Della Bella, Elena</td>
<td>N13-06: 827</td>
</tr>
<tr>
<td>Della Sala, Francesca</td>
<td>N02-08: 164</td>
</tr>
<tr>
<td></td>
<td>N15-05: 925</td>
</tr>
<tr>
<td></td>
<td>PS2-07-230: 1698</td>
</tr>
<tr>
<td></td>
<td>S06-03: 398</td>
</tr>
<tr>
<td>Dellaquila, Alessandra</td>
<td>N12-08: 745</td>
</tr>
<tr>
<td>Delplace, Vianney</td>
<td>N16-05: 940</td>
</tr>
<tr>
<td>Delrot, Paul</td>
<td>S01-05: 96</td>
</tr>
<tr>
<td>Demidov, Yan</td>
<td>AERO S02-KL02: 607</td>
</tr>
<tr>
<td>Demir, Enis</td>
<td>AERO S03-01: 639</td>
</tr>
<tr>
<td>Name</td>
<td>Page</td>
</tr>
<tr>
<td>-----------------------</td>
<td>------</td>
</tr>
<tr>
<td>Demitri, Christian</td>
<td>860</td>
</tr>
<tr>
<td>PS1-04-159.</td>
<td>1149</td>
</tr>
<tr>
<td>PS2-03-122.</td>
<td>1591</td>
</tr>
<tr>
<td>Dennison, Nicholas</td>
<td>1285</td>
</tr>
<tr>
<td>Dequidt, Alain</td>
<td>1784</td>
</tr>
<tr>
<td>Di Napoli, Michela</td>
<td>1932</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>661</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>1204</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>1698</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>294</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>117</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>661</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>1204</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>1698</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>294</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>117</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>661</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>1204</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>1698</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>294</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>117</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>661</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>1204</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>1698</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>294</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>117</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>661</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>1204</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>1698</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>294</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>117</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>661</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>1204</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>1698</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>294</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>117</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>661</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>1204</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>1698</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>294</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>117</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>661</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>1204</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>1698</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>294</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>117</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>661</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>1204</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>1698</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>294</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>117</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>661</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>1204</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>1698</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>294</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>117</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>661</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>1204</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>1698</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>294</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>117</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>661</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>1204</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>1698</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>294</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>117</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>661</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>1204</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>1698</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>294</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>117</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>661</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>1204</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>1698</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>294</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>117</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>661</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>1204</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>1698</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>294</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>117</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>661</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>1204</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>1698</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>294</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>117</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>661</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>1204</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>1698</td>
</tr>
<tr>
<td>Di Cio, Stefania</td>
<td>294</td>
</tr>
<tr>
<td>Di Filippo, Maria Francesca</td>
<td>117</td>
</tr>
<tr>
<td>di Gennaro, Mario</td>
<td>164</td>
</tr>
<tr>
<td>Di Martino, Piera</td>
<td>318</td>
</tr>
<tr>
<td>Author</td>
<td>Page</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Do, Thuy</td>
<td>1832</td>
</tr>
<tr>
<td>PS2-11-366.</td>
<td></td>
</tr>
<tr>
<td>Döbelin, Nicola</td>
<td>785</td>
</tr>
<tr>
<td>S12-KL01</td>
<td></td>
</tr>
<tr>
<td>Dobos, Agnes</td>
<td>537</td>
</tr>
<tr>
<td>ESB-ISBF S-04</td>
<td></td>
</tr>
<tr>
<td>Dobranici, Alexandra E.</td>
<td>1214</td>
</tr>
<tr>
<td>PS1-07-219.</td>
<td></td>
</tr>
<tr>
<td>Dobre, Oana</td>
<td>1246</td>
</tr>
<tr>
<td>S10-07</td>
<td>692</td>
</tr>
<tr>
<td>S14-04</td>
<td>878</td>
</tr>
<tr>
<td>Dobrov, Anatolie</td>
<td></td>
</tr>
<tr>
<td>PS1-07-255.</td>
<td></td>
</tr>
<tr>
<td>Doccini, Stefano</td>
<td>918</td>
</tr>
<tr>
<td>N15-02</td>
<td></td>
</tr>
<tr>
<td>S12-06</td>
<td>794</td>
</tr>
<tr>
<td>Docheva, Denitsa</td>
<td>272</td>
</tr>
<tr>
<td>S05-04</td>
<td></td>
</tr>
<tr>
<td>Dolan, Eimear B.</td>
<td>568</td>
</tr>
<tr>
<td>N11-03</td>
<td></td>
</tr>
<tr>
<td>Dolatsahi-Pirouz, Alireza</td>
<td>1267</td>
</tr>
<tr>
<td>PS1-08-275.</td>
<td></td>
</tr>
<tr>
<td>Dolatshah-Pirouz, Alireza</td>
<td>263</td>
</tr>
<tr>
<td>K03-06</td>
<td></td>
</tr>
<tr>
<td>Dolci, Luisa Stella</td>
<td>117</td>
</tr>
<tr>
<td>S03-03</td>
<td></td>
</tr>
<tr>
<td>Doll, Katharina</td>
<td>1727</td>
</tr>
<tr>
<td>PS2-08-258.</td>
<td></td>
</tr>
<tr>
<td>Dolzani, Paolo</td>
<td>1287</td>
</tr>
<tr>
<td>PS1-08-297.</td>
<td></td>
</tr>
<tr>
<td>Domingo, Concepción</td>
<td>623</td>
</tr>
<tr>
<td>AERO InvF-03</td>
<td></td>
</tr>
<tr>
<td>Domingues Vera, Samara</td>
<td>1007</td>
</tr>
<tr>
<td>PS1-01-013.</td>
<td></td>
</tr>
<tr>
<td>Domingues, Joana</td>
<td>530</td>
</tr>
<tr>
<td>ESB-SIBB S01-07</td>
<td></td>
</tr>
<tr>
<td>PS2-11-350.</td>
<td>1817</td>
</tr>
<tr>
<td>Domingues, Marco M.</td>
<td>697</td>
</tr>
<tr>
<td>S11-03</td>
<td></td>
</tr>
<tr>
<td>Domingues, Rui M.</td>
<td>522</td>
</tr>
<tr>
<td>ESB-SIBB S01-04</td>
<td></td>
</tr>
<tr>
<td>Domingues, Rui M.A.</td>
<td>272</td>
</tr>
<tr>
<td>N15-01</td>
<td>916</td>
</tr>
<tr>
<td>S05-04</td>
<td></td>
</tr>
<tr>
<td>Dominguez-Bajo, Ana</td>
<td>1722</td>
</tr>
<tr>
<td>PS2-07-254.</td>
<td></td>
</tr>
<tr>
<td>Donahue, Seth</td>
<td>563</td>
</tr>
<tr>
<td>N11-01</td>
<td></td>
</tr>
<tr>
<td>Dondi, Daniele</td>
<td>1080</td>
</tr>
<tr>
<td>PS1-02-091.</td>
<td></td>
</tr>
<tr>
<td>Dörsam, Edgar</td>
<td>1477</td>
</tr>
<tr>
<td>PS2-01-006.</td>
<td></td>
</tr>
<tr>
<td>Dos Orfaos, Lydia N.</td>
<td>1854</td>
</tr>
<tr>
<td>PS2-12-386.</td>
<td></td>
</tr>
<tr>
<td>Dos santos, Thomas</td>
<td>1409</td>
</tr>
<tr>
<td>PS1-13-411.</td>
<td></td>
</tr>
<tr>
<td>Doser, Michael</td>
<td>1528</td>
</tr>
<tr>
<td>PS2-01-060.</td>
<td></td>
</tr>
<tr>
<td>PS2-13-420.</td>
<td>1892</td>
</tr>
<tr>
<td>Dotti, Silvia</td>
<td>466</td>
</tr>
<tr>
<td>N08-09</td>
<td></td>
</tr>
<tr>
<td>Douglas, Timothy E.</td>
<td>1662</td>
</tr>
<tr>
<td>PS2-05-196.</td>
<td></td>
</tr>
<tr>
<td>Dragoj, Miodrag</td>
<td>1438</td>
</tr>
<tr>
<td>PS1-14-437.</td>
<td></td>
</tr>
<tr>
<td>Drake, Richard</td>
<td>137</td>
</tr>
<tr>
<td>N01-05</td>
<td></td>
</tr>
<tr>
<td>Dranseikiene, Dalia</td>
<td>257</td>
</tr>
<tr>
<td>PS1-05-177.</td>
<td></td>
</tr>
<tr>
<td>Drouglazet, Clémence</td>
<td>277</td>
</tr>
<tr>
<td>S05-06</td>
<td></td>
</tr>
<tr>
<td>Drucbert, Anne sophie</td>
<td>1074</td>
</tr>
<tr>
<td>PS1-02-085.</td>
<td></td>
</tr>
<tr>
<td>Du, Xiaoyu</td>
<td>858</td>
</tr>
<tr>
<td>K10-02</td>
<td></td>
</tr>
<tr>
<td>Duarte Campos, Daniela F.</td>
<td>1289</td>
</tr>
<tr>
<td>PS1-08-299.</td>
<td></td>
</tr>
<tr>
<td>Duarte, Iola F.</td>
<td>1651</td>
</tr>
<tr>
<td>ESB-SIBB S02-05</td>
<td>713</td>
</tr>
<tr>
<td>Dubnika, Arita</td>
<td>1334</td>
</tr>
<tr>
<td>PS1-10-337.</td>
<td></td>
</tr>
<tr>
<td>PS2-05-186.</td>
<td>1651</td>
</tr>
<tr>
<td>Dubrue, Peter</td>
<td>447</td>
</tr>
<tr>
<td>ESB-BIOMAT S-07.</td>
<td></td>
</tr>
<tr>
<td>ESB-ISBF S-04</td>
<td>537</td>
</tr>
<tr>
<td>N03-09</td>
<td>227</td>
</tr>
<tr>
<td>PS1-03-123.</td>
<td>1109</td>
</tr>
<tr>
<td>PS1-04-135.</td>
<td>1124</td>
</tr>
<tr>
<td>PS2-08-276.</td>
<td>1741</td>
</tr>
<tr>
<td>S05-02</td>
<td>267</td>
</tr>
<tr>
<td>S10-KL01</td>
<td>679</td>
</tr>
<tr>
<td>Duch, Joanna</td>
<td>1412</td>
</tr>
<tr>
<td>PS1-13-415.</td>
<td></td>
</tr>
<tr>
<td>Dudley, David</td>
<td>1378</td>
</tr>
<tr>
<td>PS1-12-381.</td>
<td></td>
</tr>
<tr>
<td>Duffy, Georgia L.</td>
<td>184</td>
</tr>
<tr>
<td>K02-06</td>
<td></td>
</tr>
<tr>
<td>Duimel, Hans</td>
<td>404</td>
</tr>
<tr>
<td>S06-06</td>
<td></td>
</tr>
<tr>
<td>Duin, Sarah</td>
<td>1687</td>
</tr>
<tr>
<td>PS2-07-220.</td>
<td></td>
</tr>
<tr>
<td>Dulnik, Judyta</td>
<td>1938</td>
</tr>
<tr>
<td>PS2-15-464.</td>
<td></td>
</tr>
<tr>
<td>Dumur, Adeline</td>
<td>1105</td>
</tr>
<tr>
<td>PS1-03-119.</td>
<td></td>
</tr>
<tr>
<td>Author Name</td>
<td>Page Numbers</td>
</tr>
<tr>
<td>------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Dunne, Nicholas</td>
<td></td>
</tr>
<tr>
<td>N11-01</td>
<td></td>
</tr>
<tr>
<td>N11-05</td>
<td></td>
</tr>
<tr>
<td>N13-02</td>
<td></td>
</tr>
<tr>
<td>N14-07</td>
<td></td>
</tr>
<tr>
<td>PS2-04-154</td>
<td></td>
</tr>
<tr>
<td>PS2-05-176</td>
<td></td>
</tr>
<tr>
<td>Dupin, Damien</td>
<td></td>
</tr>
<tr>
<td>N03-07</td>
<td></td>
</tr>
<tr>
<td>Duport-Gillain, Christine</td>
<td></td>
</tr>
<tr>
<td>N03-07</td>
<td></td>
</tr>
<tr>
<td>Duque Caballero, Edison</td>
<td></td>
</tr>
<tr>
<td>N11-01</td>
<td></td>
</tr>
<tr>
<td>Duque Ramírez, Jaime</td>
<td></td>
</tr>
<tr>
<td>N11-01</td>
<td></td>
</tr>
<tr>
<td>Dura, Gema</td>
<td></td>
</tr>
<tr>
<td>N14-07</td>
<td></td>
</tr>
<tr>
<td>Duraccio, Donatella</td>
<td></td>
</tr>
<tr>
<td>PS2-07-246</td>
<td></td>
</tr>
<tr>
<td>Durrieu, Marie C.</td>
<td></td>
</tr>
<tr>
<td>PS2-13-408</td>
<td></td>
</tr>
<tr>
<td>Durrieu, Marie-Christine</td>
<td></td>
</tr>
<tr>
<td>PS2-06-208</td>
<td></td>
</tr>
<tr>
<td>Dusserre, Nathalie</td>
<td></td>
</tr>
<tr>
<td>PS1-02-052</td>
<td></td>
</tr>
<tr>
<td>PS2-01-052</td>
<td></td>
</tr>
<tr>
<td>S01-03</td>
<td></td>
</tr>
<tr>
<td>S06-05</td>
<td></td>
</tr>
<tr>
<td>S14-05</td>
<td></td>
</tr>
<tr>
<td>Dutta, Deepanjalee</td>
<td></td>
</tr>
<tr>
<td>PS2-02-080</td>
<td></td>
</tr>
<tr>
<td>PS2-02-188</td>
<td></td>
</tr>
<tr>
<td>Dymond, Marcus</td>
<td></td>
</tr>
<tr>
<td>PS1-07-237</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
</tr>
<tr>
<td>E. Gomes, Manuela</td>
<td></td>
</tr>
<tr>
<td>PS2-01-018</td>
<td></td>
</tr>
<tr>
<td>Eganha, José T.</td>
<td></td>
</tr>
<tr>
<td>PS1-10-337</td>
<td></td>
</tr>
<tr>
<td>PS2-05-186</td>
<td></td>
</tr>
<tr>
<td>Eglin, David</td>
<td></td>
</tr>
<tr>
<td>PS2-01-028</td>
<td></td>
</tr>
<tr>
<td>Eglin, David O.</td>
<td></td>
</tr>
<tr>
<td>PS1-13-393</td>
<td></td>
</tr>
<tr>
<td>Eiesen-Loges, Maria José R.</td>
<td></td>
</tr>
<tr>
<td>PS1-06-209</td>
<td></td>
</tr>
<tr>
<td>PS1-06-213</td>
<td></td>
</tr>
<tr>
<td>Ehler, Nina</td>
<td></td>
</tr>
<tr>
<td>PS2-02-064</td>
<td></td>
</tr>
<tr>
<td>Ehrmann, Katharina</td>
<td></td>
</tr>
<tr>
<td>PS2-09-314</td>
<td></td>
</tr>
<tr>
<td>El Hafci, Hanane</td>
<td></td>
</tr>
<tr>
<td>PS2-07-250</td>
<td></td>
</tr>
<tr>
<td>El Halawani, Mohamed T.</td>
<td></td>
</tr>
<tr>
<td>PS1-06-213</td>
<td></td>
</tr>
<tr>
<td>El Khassawna, Thaqif</td>
<td></td>
</tr>
<tr>
<td>PS1-06-213</td>
<td></td>
</tr>
<tr>
<td>Elks-Glatz, Yvonne</td>
<td></td>
</tr>
<tr>
<td>PS2-13-405</td>
<td></td>
</tr>
<tr>
<td>Ellermann, Else</td>
<td></td>
</tr>
<tr>
<td>PS2-06-206</td>
<td></td>
</tr>
<tr>
<td>Ellmann, Daniel</td>
<td></td>
</tr>
<tr>
<td>PS1-01-018</td>
<td></td>
</tr>
<tr>
<td>Elortza, Félix</td>
<td></td>
</tr>
<tr>
<td>PS1-13-401</td>
<td></td>
</tr>
<tr>
<td>Elowsson Rendin, Linda</td>
<td></td>
</tr>
<tr>
<td>PS2-02-064</td>
<td></td>
</tr>
<tr>
<td>Elowe, Ahmad</td>
<td></td>
</tr>
<tr>
<td>PS2-02-064</td>
<td></td>
</tr>
<tr>
<td>Emanet, Melis</td>
<td></td>
</tr>
<tr>
<td>PS1-09-305</td>
<td></td>
</tr>
<tr>
<td>Engel López, Elisabeth</td>
<td></td>
</tr>
<tr>
<td>PS2-02-064</td>
<td></td>
</tr>
<tr>
<td>Engel, Elisabeth</td>
<td></td>
</tr>
<tr>
<td>PS2-02-064</td>
<td></td>
</tr>
</tbody>
</table>

Page 1965 of 2028
## Author Index

<table>
<thead>
<tr>
<th>Author</th>
<th>Session</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>N08-01</td>
<td>PS2-08</td>
<td>1227</td>
</tr>
<tr>
<td>PS2-07-235</td>
<td></td>
<td>1706</td>
</tr>
<tr>
<td>PS2-07-236</td>
<td></td>
<td>1764</td>
</tr>
<tr>
<td>PS2-08-302</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Engelke, Martin</td>
<td>PS1-05</td>
<td>1174</td>
</tr>
<tr>
<td>PS2-07-235</td>
<td></td>
<td>1706</td>
</tr>
<tr>
<td>PS2-07-236</td>
<td></td>
<td>1764</td>
</tr>
<tr>
<td>PS2-08-302</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Engqvist, Håkan</td>
<td>ESB-CSBM</td>
<td>201</td>
</tr>
<tr>
<td>ESB-CSBM S-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enrique, Navarro</td>
<td>Biomechanics</td>
<td>979</td>
</tr>
<tr>
<td>Biomechanics 04-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epitropaki, Eirini</td>
<td>NANOSTEM</td>
<td>354</td>
</tr>
<tr>
<td>NANOSTEM 02-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES1-12-383</td>
<td></td>
<td>1380</td>
</tr>
<tr>
<td>Epple, Matthias</td>
<td>PS1-12-385</td>
<td>1382</td>
</tr>
<tr>
<td>Ercan, Batur</td>
<td>PS1-06-207</td>
<td>1200</td>
</tr>
<tr>
<td>PS1-09-309</td>
<td></td>
<td>1301</td>
</tr>
<tr>
<td>PS2-03-120</td>
<td></td>
<td>1588</td>
</tr>
<tr>
<td>PS2-13-402</td>
<td></td>
<td>1874</td>
</tr>
<tr>
<td>Ercan, Utku K</td>
<td>S14-02</td>
<td>874</td>
</tr>
<tr>
<td>Erdogan, Yasar K</td>
<td>PS1-09-309</td>
<td>1301</td>
</tr>
<tr>
<td>Ergene, Emre</td>
<td>PS1-07-253</td>
<td>1244</td>
</tr>
<tr>
<td>Ergün, Süleyman</td>
<td>K08-02</td>
<td>770</td>
</tr>
<tr>
<td>Eriksson, Olle</td>
<td>PS1-01-003</td>
<td>996</td>
</tr>
<tr>
<td>Ertl, Peter</td>
<td>K02-KL01</td>
<td>174</td>
</tr>
<tr>
<td>Ertl, Peter</td>
<td>PS2-14-434</td>
<td>1906</td>
</tr>
<tr>
<td>Escalera Rodríguez, Maria D.</td>
<td>PS1-02-089</td>
<td>1079</td>
</tr>
<tr>
<td>Espanol, Montserrat</td>
<td>S14-KL01</td>
<td>872</td>
</tr>
<tr>
<td>Espinosa-Cano, Eva</td>
<td>ESB-SIBB</td>
<td>705</td>
</tr>
<tr>
<td>ESB-SIBB S02-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N10-08</td>
<td></td>
<td>558</td>
</tr>
<tr>
<td>PS1-07-217</td>
<td></td>
<td>1211</td>
</tr>
<tr>
<td>Espinosa-Medina, Marco A.</td>
<td>PS1-02-103</td>
<td>1092</td>
</tr>
<tr>
<td>Espino-Noguera, Albert</td>
<td>N04-01</td>
<td>230</td>
</tr>
<tr>
<td>Esteban, Sheila B</td>
<td>PS2-10-330</td>
<td>1795</td>
</tr>
<tr>
<td>Esteve, Jaume</td>
<td>N10-02</td>
<td>546</td>
</tr>
<tr>
<td>Estevez Amado, Manuel</td>
<td>K01-02</td>
<td>76</td>
</tr>
<tr>
<td>Estévez, Manuel</td>
<td>PS2-12-380</td>
<td>1849</td>
</tr>
<tr>
<td>Estevinho, Berta</td>
<td>K10-04</td>
<td>863</td>
</tr>
<tr>
<td>Eyckmans, Jeroen</td>
<td>S09-02</td>
<td>665</td>
</tr>
<tr>
<td>Ezami, Nazanin</td>
<td>PS2-09-312</td>
<td>1777</td>
</tr>
<tr>
<td>Ezquer, Marcelo</td>
<td>PS1-11-365</td>
<td>1362</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fabozzi, Antonio</td>
<td>N15-05</td>
<td>925</td>
</tr>
<tr>
<td>Fabry, Ben</td>
<td>K08-02</td>
<td>770</td>
</tr>
<tr>
<td>Faisca, Pedro</td>
<td>AERO InvF</td>
<td>621</td>
</tr>
<tr>
<td>Falini, Giuseppe</td>
<td>PS1-01-003</td>
<td>996</td>
</tr>
<tr>
<td>Faragher, Richard</td>
<td>PS2-13-394</td>
<td>1863</td>
</tr>
<tr>
<td>Fare, Silvia</td>
<td>K02-03</td>
<td>177</td>
</tr>
<tr>
<td>Fare, Silvia</td>
<td>PS1-07-239</td>
<td>1231</td>
</tr>
<tr>
<td>Fasolino, Ines</td>
<td>PS1-08-265</td>
<td>1257</td>
</tr>
<tr>
<td>Farto, Nicolette</td>
<td>PS1-10-321</td>
<td>1316</td>
</tr>
<tr>
<td>Farràs, Pau</td>
<td>ESB-BIOMAT</td>
<td>436</td>
</tr>
<tr>
<td>Fabar-Vaamonde, Xián</td>
<td>PS2-01-024</td>
<td>1498</td>
</tr>
<tr>
<td>Fasolino, Ines</td>
<td>K08-07</td>
<td>781</td>
</tr>
<tr>
<td>Fardo-Vaamonde, Xián</td>
<td>N01-08</td>
<td>143</td>
</tr>
<tr>
<td>Fedele, Chiara</td>
<td>S09-06</td>
<td>674</td>
</tr>
<tr>
<td>Fedrizzi, Lorenzo</td>
<td>PS2-11-354</td>
<td>1821</td>
</tr>
<tr>
<td>Fegueiras, Helena P</td>
<td>ESB-SIBB</td>
<td>524</td>
</tr>
<tr>
<td>ESB-SIBB S01-05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESB-SIBB S01-07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N10-06</td>
<td></td>
<td>554</td>
</tr>
<tr>
<td>PS1-07-221</td>
<td></td>
<td>1215</td>
</tr>
<tr>
<td>PS2-07-226</td>
<td></td>
<td>1693</td>
</tr>
<tr>
<td>PS2-11-353</td>
<td></td>
<td>1817</td>
</tr>
<tr>
<td>S07-08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fabar-Vaamonde, Xián</td>
<td>PS2-01-024</td>
<td>1498</td>
</tr>
<tr>
<td>Fasolino, Ines</td>
<td>K08-07</td>
<td>781</td>
</tr>
<tr>
<td>Fardo-Vaamonde, Xián</td>
<td>N01-08</td>
<td>143</td>
</tr>
<tr>
<td>Fedele, Chiara</td>
<td>S09-06</td>
<td>674</td>
</tr>
<tr>
<td>Fedrizzi, Lorenzo</td>
<td>PS2-11-354</td>
<td>1821</td>
</tr>
<tr>
<td>Fegueiras, Helena P</td>
<td>ESB-SIBB</td>
<td>524</td>
</tr>
<tr>
<td>ESB-SIBB S01-05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESB-SIBB S01-07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N10-06</td>
<td></td>
<td>554</td>
</tr>
<tr>
<td>PS1-07-221</td>
<td></td>
<td>1215</td>
</tr>
<tr>
<td>PS2-07-226</td>
<td></td>
<td>1693</td>
</tr>
<tr>
<td>PS2-11-353</td>
<td></td>
<td>1817</td>
</tr>
<tr>
<td>S12-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Author Name</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>Felici, Antonio</td>
<td>867</td>
<td></td>
</tr>
<tr>
<td>K10-06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feliciano, Antonio</td>
<td>1251</td>
<td></td>
</tr>
<tr>
<td>PS1-08-259</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS2-08-266</td>
<td>1732</td>
<td></td>
</tr>
<tr>
<td>Feliu, Lidia</td>
<td>711</td>
<td></td>
</tr>
<tr>
<td>ESB-SIBB S02-04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Félix, Carina</td>
<td>1488</td>
<td></td>
</tr>
<tr>
<td>PS2-01-016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Félix, Gautier</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>K01-05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Félix, Rafael</td>
<td>1488</td>
<td></td>
</tr>
<tr>
<td>PS2-01-016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feller, Karl-Heinz</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>K02-04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facendéz Villamärín, Silvia</td>
<td>1640</td>
<td></td>
</tr>
<tr>
<td>PS2-05-176</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feraru, Alexandra</td>
<td>1058</td>
<td></td>
</tr>
<tr>
<td>PS1-02-071</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS2-02-072</td>
<td>1542</td>
<td></td>
</tr>
<tr>
<td>Ferguson, Stephen J.</td>
<td>858</td>
<td></td>
</tr>
<tr>
<td>K10-02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS2-01-020</td>
<td>1493</td>
<td></td>
</tr>
<tr>
<td>Fernandes Gomes, Francisca L.</td>
<td>1465</td>
<td></td>
</tr>
<tr>
<td>PS1-15-463</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fernandes Duarte</td>
<td>1856</td>
<td></td>
</tr>
<tr>
<td>PS2-12-388</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fernandes, Francisco M.</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>S02-05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fernandes, Hugo</td>
<td>759</td>
<td></td>
</tr>
<tr>
<td>K07-04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S11-04</td>
<td>699</td>
<td></td>
</tr>
<tr>
<td>Fernandes, Hugo M.</td>
<td>311</td>
<td></td>
</tr>
<tr>
<td>N06-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fernandes, Inês J.</td>
<td>850</td>
<td></td>
</tr>
<tr>
<td>K09-06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fernandes, Maria Helena</td>
<td>1555</td>
<td></td>
</tr>
<tr>
<td>PS2-02-084</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fernandes, Maria Helena V.</td>
<td>1782</td>
<td></td>
</tr>
<tr>
<td>PS2-09-318</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fernandes, Ricardo</td>
<td>958</td>
<td></td>
</tr>
<tr>
<td>Biomechanics 02-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fernandez, Jose M.</td>
<td>800</td>
<td></td>
</tr>
<tr>
<td>S13-02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fernandez, Julio</td>
<td>1764</td>
<td></td>
</tr>
<tr>
<td>PS2-08-302</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fernandez-Arias, Mónica</td>
<td>527</td>
<td></td>
</tr>
<tr>
<td>ESB-SIBB S01-06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fernandez-Fernández, Julio</td>
<td>299</td>
<td></td>
</tr>
<tr>
<td>N05-07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fernandez-Grajera, Maria</td>
<td>1414</td>
<td></td>
</tr>
<tr>
<td>PS1-13-417</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fernández-Pérez, Julia</td>
<td>1310</td>
<td></td>
</tr>
<tr>
<td>PS1-09-315</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fernández-Quintero, Monica L.</td>
<td>347</td>
<td></td>
</tr>
<tr>
<td>NANOSTEM 01-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fernandez-Yague, Marc A.</td>
<td>665</td>
<td></td>
</tr>
<tr>
<td>S09-02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferrão, Rafaela</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>K01-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferrari, Livia</td>
<td>867</td>
<td></td>
</tr>
<tr>
<td>K10-06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferraris, Sara</td>
<td>1451</td>
<td></td>
</tr>
<tr>
<td>PS1-15-451</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS2-15-460</td>
<td>1934</td>
<td></td>
</tr>
<tr>
<td>Ferraz, Natalia</td>
<td>550</td>
<td></td>
</tr>
<tr>
<td>N10-04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS2-02-076</td>
<td>1546</td>
<td></td>
</tr>
<tr>
<td>Ferreira Fernandes, Beatriz</td>
<td>1184</td>
<td></td>
</tr>
<tr>
<td>PS1-05-195</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferreira, Carolina A.M.</td>
<td>1488</td>
<td></td>
</tr>
<tr>
<td>PS2-01-016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferreira, Daniel A.</td>
<td>1906</td>
<td></td>
</tr>
<tr>
<td>PS2-14-434</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferreira, David</td>
<td>621</td>
<td></td>
</tr>
<tr>
<td>AERO InvF-02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferreira, Diana P.</td>
<td>554</td>
<td></td>
</tr>
<tr>
<td>N10-06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferreira, Domingos</td>
<td>1383</td>
<td></td>
</tr>
<tr>
<td>PS1-12-387</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferreira, Flávio A.</td>
<td>332</td>
<td></td>
</tr>
<tr>
<td>N07-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferreira, Frederico C.</td>
<td>332</td>
<td></td>
</tr>
<tr>
<td>N07-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferreira, Gabriela</td>
<td>655</td>
<td></td>
</tr>
<tr>
<td>K06-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferreira, Helena</td>
<td>1657</td>
<td></td>
</tr>
<tr>
<td>PS2-05-192</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S09-03</td>
<td>668</td>
<td></td>
</tr>
<tr>
<td>Ferreira, Helena P.</td>
<td>1280</td>
<td></td>
</tr>
<tr>
<td>PS1-08-289</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferreira, Hugo</td>
<td>621</td>
<td></td>
</tr>
<tr>
<td>AERO InvF-02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferreira, Inês</td>
<td>1647</td>
<td></td>
</tr>
<tr>
<td>PS2-05-182</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS2-08-286</td>
<td>1750</td>
<td></td>
</tr>
<tr>
<td>Ferreira, Joana R.</td>
<td>889</td>
<td></td>
</tr>
<tr>
<td>S15-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferreira, Lino</td>
<td>893</td>
<td></td>
</tr>
<tr>
<td>S15-05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferreira, Lino</td>
<td>390</td>
<td></td>
</tr>
<tr>
<td>K04-07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K07-04</td>
<td>759</td>
<td></td>
</tr>
<tr>
<td>N06-03</td>
<td>311</td>
<td></td>
</tr>
<tr>
<td>N06-05</td>
<td>316</td>
<td></td>
</tr>
<tr>
<td>NANOSTEM 02-04</td>
<td>356</td>
<td></td>
</tr>
<tr>
<td>NANOSTEM 03-03</td>
<td>362</td>
<td></td>
</tr>
<tr>
<td>NANOSTEM 03-05</td>
<td>365</td>
<td></td>
</tr>
<tr>
<td>NANOSTEM 04-01</td>
<td>368</td>
<td></td>
</tr>
<tr>
<td>NANOSTEM 04-03</td>
<td>371</td>
<td></td>
</tr>
</tbody>
</table>

Page 1967 of 2028
Author Index

Ferreira, Luis P.
ESB-SBB S02-08 .................................. 719
Ferreira, Sônia
TRS-S02-01 ........................................ 172
Ferreira-Duarte, Ana
N10-01 ............................................. 544
PS1-01-007 ....................................... 1001
PS1-05-187 ....................................... 1176
Ferreira-Duarte, Ana Marina
S01-07 ............................................. 101
N04-07 ............................................ 243
N04-08 ............................................ 245
Ferreira-Gonçalves, Tânia
AERO InvF-02 .................................... 621
Ferri, Daniel
ESB-BIOMAT S-06 ................................ 444
Feurer, Nora
PS1-13-409 ....................................... 1408
Fialho, Arsénio M.
PS1-10-323 ....................................... 1319
Figliuzzi, Marina
S09-04 ............................................. 670
Figueiredo, Bruno
K04-07 ............................................. 390
Figueiredo, Isabel V.
AERO InvF-02 .................................... 621
Figueiredo, Patrícia
PS1-05-197 ....................................... 1187
Figueiredo-Pina, Célio G.
PS1-04-163 ....................................... 1151
PS1-05-191 ....................................... 1180
PS1-08-287 ....................................... 1278
PS2-08-286 ....................................... 1750
Figuerras, Antonio
PS2-02-090 ....................................... 1561
Filova, Elena
N10-02 ............................................. 546
Fini, Milena
PS1-06-211 ....................................... 1204
Fiorati, Andrea
PS2-05-190 ....................................... 1654
Fiordalisi, Morena F.
S15-03 ............................................. 889
Fiorelli, Roberto
PS2-07-224 ....................................... 1691
Fiorentini, Fabrizio
PS2-07-232 ....................................... 1701
Fiorilli, Sonia
K01-02 ............................................. 76
N04-06 ............................................ 241
N09-03 ............................................ 473
PS2-02-096 ....................................... 1568
S10-06 ............................................ 690
S14-03 ............................................ 876
S08-04 ............................................ 510
Fischbach, Claudia
ESB-SBB S02-03 ................................ 709
Fischer, Horst
PS1-02-075 ....................................... 1063
PS1-14-433 ....................................... 1434
PS2-01-008 ....................................... 1479
Fischer, Lena
PS1-14-443 ....................................... 1444
PS2-01-050 ....................................... 1520
PS2-14-442 ....................................... 1914
Fischer, Nicholas G.
ESB-SFB S-03 .................................... 810
Fischer, Stefan
PS1-14-443 ....................................... 1444
Fitzpatrick, Vincent
N02-01 ............................................. 148
PS2-01-010 ....................................... 1481
Flegeau, Killian
PS2-08-292 ....................................... 1755
Fleishman, Lada
PS2-02-064 ....................................... 1532
Flis, Agata
PS1-02-097 ....................................... 1087
Flori, Alessandra
PS1-07-227 ....................................... 1221
Flóra, Pablo
Biomechanics 03-02 ................................ 960
Florindo, Helena
PS1-12-387 ....................................... 1383
Flynn, Shauna
N03-05 ............................................. 217
Fois, Maria Gabriella
PS2-13-406 ....................................... 1876
Foije, Jaroslav
K06-04 ............................................. 657
Fonseca, Diana R.
K10-04 ............................................. 863
Font, Jordi
Biomechanics 01-03 ................................ 955
Forestier, Christiane
N06-01 ............................................. 307
Forgacs, Gabor
K03-KL01 ......................................... 252
Formisano, Nello
PS2-15-456 ....................................... 1930
Fornaguera, Cristina
PS1-12-367 ....................................... 1365
Fornell, Jordina
PS1-04-139 ....................................... 1130
Fornili, Arianna
PS1-12-373 ....................................... 1371
Author Index

Forooghi, Pourya
ESB-CSBM S-05 ........................................... 205
Forson, Abigail M.
PS-15-453 .................................................. 1452
Forster, Leonard
PS-1-07-233 .................................................. 1226
PS-2-01-042 .................................................. 1514
Fürster, Stephan
PS-1-01-059 .................................................. 1046
Foulc, Marie-Pierre
ESB-SFB S-04 .............................................. 813
Fournier, Louise J.
PS-1-09-307 .................................................. 1298
Franch, Jordi
S14-KL01 ...................................................... 872
Francisco, Vitor
S11-04 .......................................................... 699
Franconi, Florence
K01-05 .......................................................... 82
Frank, Johannes
PS-2-01-006 .................................................. 1477
Franz, Sandra
K05-04 .......................................................... 497
Freeley, Mark
NANOSTEM 01-04 ......................................... 349
Freire, Carmen S.R.
PS-2-09-312 .................................................. 1777
Freitas Mendes, Luis
ESB-SLABO S-03 .......................................... 283
Freitas, Alexandra
PS-2-04-156 .................................................. 1621
Freitas, Filomena
PS-2-05-182 .................................................. 1647
Freitas, Jaime
S15-05 .......................................................... 893
Freudenberg, Uwe
PS-1-08-295 .................................................. 1285
Fricain, Jean-Christophe
PS-1-01-061 .................................................. 1048
PS-2-01-052 .................................................. 1523
S01-03 .......................................................... 92
S14-05 .......................................................... 880
Friedrich, Oliver
PS-1-09-317 .................................................. 1311
PS-2-14-442 .................................................. 1914
Friedrichs, Jens
PS-1-10-345 .................................................. 1342
Fritschen, Anna
K02-02 .......................................................... 175
Friuli, Marco
K10-03 .......................................................... 860
Frogne, Thomas
PS-1-08-275 .................................................. 1267
Frugoli, Lisa
K05-02 .......................................................... 492
Page 1969 of 2028

Frutos Díaz-Alejo, Jesús
K03-02 .......................................................... 255
Fu, Jianping
S09-02 .......................................................... 665
Fuchslegger, Thomas A.
N14-09 .......................................................... 913
Fuentes, Marcos G.
PS-2-10-330 .................................................. 1795
Fuentes Lopez, Carla
S13-02 .......................................................... 800
PS-2-02-082 .................................................. 1552
Fuhrmann, Bodo
PS-1-08-283 .................................................. 1275
Fujiiwara, Masashi
N03-02 .......................................................... 210
Funnell, Jessica
K01-05 .......................................................... 82
Furlani, Franco
PS-2-08-282 .................................................. 1745
S10-03 .......................................................... 684
Fusenig, Maximilian
PS-1-08-295 .................................................. 1285

G

Gaballo, Antonio
PS-2-05-200 .................................................. 1666
Gabusi, Elena
PS-1-08-297 .................................................. 1287
PS-2-04-168 .................................................. 1630
Gache, Vincent
S05-06 .......................................................... 277
Gaétan, Laroche
PS-1-08-273 .................................................. 1265
Gala, Joana
PS-07-242 ...................................................... 1710
Galante, Camilla
PS-05-190 ...................................................... 1654
Galiastro Pedraz, Alberto
Biomechanics 04-01 ........................................ 974
Gall, Maxence
PS-1-13-423 .................................................. 1420
Gallardo, Xavier
PS-02-13-128 ................................................ 1595
Gallardo-Moreno, Amparo M.
PS-1-13-417 .................................................. 1414
PS-1-15-465 .................................................. 1468
PS-2-13-416 .................................................. 1888
Gallici-Nottiani, Duccio
PS-1-13-413 .................................................. 1410
Gallo, Nunzia
PS-2-05-200 .................................................. 1666
Gallo-Cordova, Alvaro
PS-2-12-380 .................................................. 1849
Galusek, Dušan
N09-09 .......................................................... 484
Author Index

Garna, Francesca
PS1-15-451................................. 1451
PS2-15-460................................. 1934
Gand, Adeline
PS1-13-423................................. 1420
Gandia, Antoni
PS1-02-091................................. 1080
Garaghoolee, Abdulkhaleq M.
PS1-06-209................................. 1202
Garanger, Elisabeth
PS1-08-279................................. 1271
Garbay, Bertrand
PS1-01-039................................. 1031
PS1-08-279................................. 1271
Garcia Del Valle, Inés
PS1-08-285................................. 1277
Garcia Martinez, Jorge M.
N07-06................................. 339
PS2-02-098................................. 1570
Garcia Mintegui, Claudia
N12-04................................. 738
Garca Urkia, Nerea
PS2-07-228................................. 1695
Garcia, Andres J.
ESB-SFB S-KL01............................. 808
N05-01................................. 290
S09-02................................. 665
Garcia, James
K04-02................................. 380
Garcia, Leonor
PS2-07-248................................. 1716
García, Rosalba Inés F.
AERO PS01-03............................. 601
Garcia, Sara
PS2-12-380................................. 1849
Garcia-Arnáez, Iñaki
PS1-13-401................................. 1400
Garcia-Fernandez, Coral
PS1-12-367................................. 1365
Garcia-Fernández, Luis
PS1-04-131................................. 1119
Garcia-Fernández, Maria José
PS1-07-223................................. 1217
PS2-01-002................................. 1472
Garcia-Fuentes, Marcos
ESB-SIBB S02-02.......................... 707
Garcia-González, Carlos
AERO InvF-06.............................. 630
AERO Closing-01.......................... 646
AERO InvF-02.............................. 621
AERO InvF-04.............................. 625
AERO S01-01.............................. 588
AERO S01-03.............................. 592
AERO S02-01.............................. 609
N11-02................................. 565
PS1-05-179................................. 1168
YSF-AERoGELS WS-01.................... 616
Garcia-Lecina, Eva
PS1-04-139................................. 1130
Garcia-López, Juan
Biomechanics 03-02........................ 960
Garcia-Martín, Jose M.
N07-06................................. 339
PS2-02-092................................. 1563
PS2-02-098................................. 1570
Garcia-Mazás, Carla
ESB-SIBB S02-02......................... 707
Garcia-Ramos, Amador
Biomechanics 03-03........................ 963
Biomechanics 04-02........................ 976
Garcia-Robledo, Hector
PS1-04-131................................. 1119
Garcia-Torreiro, Maria
PS1-02-091................................. 1080
Garizo, Ana R.
PS1-10-323................................. 1319
Garot, Charlotte
N02-01................................. 148
PS1-04-165................................. 1153
Garric, Xavier
PS1-01-027................................. 1020
Garrido López, Gonzalo
Biomechanics 04-01........................ 974
Garrudo, Fábio F.F.
N07-03................................. 332
Gartner, Fátima
ESB-SFB S-05.............................. 815
Gasco, Paolo
PS1-12-377................................. 1374
Gasik, Michael
PS1-05-195................................. 1184
Gaspar, Maria Manuela
AERO InvF-02.............................. 621
Gaspar, Vitor M.
ESB-SIBB S02-08.......................... 719
Gaspar, Vitor M.
ESB-SIBB S02-05.......................... 713
N05-08................................. 301
PS1-01-047................................. 1037
S06-02................................. 395
Gasperini, Luca
K02-05................................. 182
Gaucher, Valérie
PS2-01-002................................. 1472
Gautier, Hélène
PS2-08-292................................. 1755
Gautrot, Julien
K06-06................................. 661
N06-04................................. 314
S09-05................................. 672
<table>
<thead>
<tr>
<th>Author Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gavilán, Francisco R.</td>
<td>460</td>
</tr>
<tr>
<td>Gawlikowski, Maciej</td>
<td>1775</td>
</tr>
<tr>
<td>PS2-09-310</td>
<td>1886</td>
</tr>
<tr>
<td>Gazvoda (Udovč), Lea</td>
<td>1819</td>
</tr>
<tr>
<td>Gazzaniga, Andrea</td>
<td>196</td>
</tr>
<tr>
<td>Gbereck, Uwe</td>
<td>1664</td>
</tr>
<tr>
<td>Geerlof, Arie</td>
<td>370</td>
</tr>
<tr>
<td>Gehweiler, Dominic</td>
<td>160</td>
</tr>
<tr>
<td>Geissler, Michael</td>
<td>1348</td>
</tr>
<tr>
<td>Gelinsky, Michael</td>
<td>261</td>
</tr>
<tr>
<td>K03-05</td>
<td>480</td>
</tr>
<tr>
<td>N09-07</td>
<td>829</td>
</tr>
<tr>
<td>N13-07</td>
<td>1004</td>
</tr>
<tr>
<td>PS1-01-011</td>
<td>1348</td>
</tr>
<tr>
<td>PS2-01-040</td>
<td>1512</td>
</tr>
<tr>
<td>PS2-02-220</td>
<td>1687</td>
</tr>
<tr>
<td>S08-05</td>
<td>512</td>
</tr>
<tr>
<td>Gelpi, Josep-Lluis</td>
<td>800</td>
</tr>
<tr>
<td>Genc, Hatice</td>
<td>1076</td>
</tr>
<tr>
<td>PS1-02-087</td>
<td></td>
</tr>
<tr>
<td>Genchi, Giada Graziana</td>
<td>921</td>
</tr>
<tr>
<td>N15-03</td>
<td></td>
</tr>
<tr>
<td>Geng, Tianxiang</td>
<td>1224</td>
</tr>
<tr>
<td>PS1-07-231</td>
<td></td>
</tr>
<tr>
<td>Genta, Martina</td>
<td>128</td>
</tr>
<tr>
<td>N01-01</td>
<td></td>
</tr>
<tr>
<td>Gentile, Piergiorgio</td>
<td>243</td>
</tr>
<tr>
<td>N04-07</td>
<td>245</td>
</tr>
<tr>
<td>N10-01</td>
<td>544</td>
</tr>
<tr>
<td>PS1-01-007</td>
<td>1001</td>
</tr>
<tr>
<td>S01-07</td>
<td>101</td>
</tr>
<tr>
<td>Gentili, Valentina</td>
<td>117</td>
</tr>
<tr>
<td>S03-03</td>
<td></td>
</tr>
<tr>
<td>Gentleman, Eileen</td>
<td>844</td>
</tr>
<tr>
<td>K09-03</td>
<td></td>
</tr>
<tr>
<td>Geoghegan, Niamh</td>
<td>219</td>
</tr>
<tr>
<td>N03-06</td>
<td></td>
</tr>
<tr>
<td>Gering, Christine</td>
<td>1534</td>
</tr>
<tr>
<td>PS2-02-066</td>
<td></td>
</tr>
<tr>
<td>Gervaso, Francesca</td>
<td>1801</td>
</tr>
<tr>
<td>PS2-10-336</td>
<td></td>
</tr>
<tr>
<td>Gheduzzi, Sabina</td>
<td>891</td>
</tr>
<tr>
<td>S15-04</td>
<td></td>
</tr>
<tr>
<td>Gheysens, Tom</td>
<td>537</td>
</tr>
<tr>
<td>ESB-ISBF S-04</td>
<td></td>
</tr>
<tr>
<td>Ghezzi, Daniele</td>
<td>1178</td>
</tr>
<tr>
<td>PS1-05-189</td>
<td></td>
</tr>
<tr>
<td>Ghitman, Jana</td>
<td>292</td>
</tr>
<tr>
<td>N05-02</td>
<td>1649</td>
</tr>
<tr>
<td>PS2-05-184</td>
<td></td>
</tr>
<tr>
<td>Ghosh, Devlina</td>
<td>1452</td>
</tr>
<tr>
<td>PS1-15-453</td>
<td></td>
</tr>
<tr>
<td>Ghosh, Sourabh</td>
<td>1630</td>
</tr>
<tr>
<td>PS2-04-168</td>
<td></td>
</tr>
<tr>
<td>Giachino, Claudia</td>
<td>756</td>
</tr>
<tr>
<td>K07-03</td>
<td></td>
</tr>
<tr>
<td>Giannini, Cinzia</td>
<td>1666</td>
</tr>
<tr>
<td>PS2-05-200</td>
<td></td>
</tr>
<tr>
<td>Giannitelli, Sara M.</td>
<td>948</td>
</tr>
<tr>
<td>N16-09</td>
<td></td>
</tr>
<tr>
<td>Giannoudis, Peter</td>
<td>1162</td>
</tr>
<tr>
<td>PS1-04-175</td>
<td>1832</td>
</tr>
<tr>
<td>PS2-11-366</td>
<td></td>
</tr>
<tr>
<td>Gigli, Giuseppe</td>
<td>948</td>
</tr>
<tr>
<td>N16-09</td>
<td></td>
</tr>
<tr>
<td>Gigliotti, Casimiro L.</td>
<td>510</td>
</tr>
<tr>
<td>S08-04</td>
<td></td>
</tr>
<tr>
<td>Gil, Francisco J.</td>
<td>1378</td>
</tr>
<tr>
<td>ESB-SIBB S01-06</td>
<td>527</td>
</tr>
<tr>
<td>Gil, Maria Helena</td>
<td>1237</td>
</tr>
<tr>
<td>PS1-07-245</td>
<td></td>
</tr>
<tr>
<td>Gilbert, Ryan</td>
<td>82</td>
</tr>
<tr>
<td>K01-05</td>
<td>452</td>
</tr>
<tr>
<td>Gilmour, Aaron</td>
<td></td>
</tr>
<tr>
<td>N08-02</td>
<td></td>
</tr>
<tr>
<td>Ginalsak, Grazyna</td>
<td>1378</td>
</tr>
<tr>
<td>PS1-12-381</td>
<td></td>
</tr>
<tr>
<td>Gindraux, Florelle</td>
<td>796</td>
</tr>
<tr>
<td>S12-07</td>
<td></td>
</tr>
<tr>
<td>Ginebra, Maria P.</td>
<td>715</td>
</tr>
<tr>
<td>ESB-SIBB S02-06</td>
<td>848</td>
</tr>
<tr>
<td>K09-05</td>
<td>230</td>
</tr>
<tr>
<td>N04-01</td>
<td>456</td>
</tr>
<tr>
<td>N08-04</td>
<td>941</td>
</tr>
<tr>
<td>N16-06</td>
<td>1552</td>
</tr>
<tr>
<td>PS2-02-082</td>
<td>452</td>
</tr>
<tr>
<td>S13-02</td>
<td>1000</td>
</tr>
<tr>
<td>S14-KL01</td>
<td>872</td>
</tr>
<tr>
<td>Ginestra, Paola</td>
<td>1036</td>
</tr>
<tr>
<td>PS1-01-045</td>
<td></td>
</tr>
<tr>
<td>Giovannozzi, Andrea M.</td>
<td>1398</td>
</tr>
<tr>
<td>PS1-13-399</td>
<td></td>
</tr>
<tr>
<td>Giselbrecht, Stefan</td>
<td>1251</td>
</tr>
<tr>
<td>PS1-08-259</td>
<td>1876</td>
</tr>
<tr>
<td>PS2-13-406</td>
<td>1896</td>
</tr>
<tr>
<td>PS2-14-424</td>
<td>1930</td>
</tr>
<tr>
<td>PS2-15-456</td>
<td></td>
</tr>
</tbody>
</table>
Author Index

Giuri, Demetra
PS1-06-211 .................................. 1204
Gkiliopoulos, Dimitrios
PS1-12-391 .................................. 1388
Gladysz, Magdalena
S07-04 ........................................ 415
Gläser, Alisa
PS1-14-441 .................................. 1442
PS2-08-296 .................................. 1758
Gleeson, John P.
PS1-04-151 .................................. 1140
Glimpel, Nikola
N11-03 ........................................ 568
Gluais, Maude
N12-03 ........................................ 736
S06-05 ........................................ 402
Glynn, Sharon
ESB-BIOMAT S-03 .................................. 436
Gniesmer, Sarah
PS2-04-138 .................................. 1606
Godineau, Thibault
PS2-07-250 .................................. 1718
Goding, Josef
N01-01 ........................................ 128
Goebel, Uta
YSF-AERoGELS WS-03 .................................. 616
Gogotsi, Yury
PS1-13-405 .................................. 1403
Goker, Meric
PS1-07-253 .................................. 1244
S14-06 ........................................ 882
Gokyer, Seyda
PS1-07-253 .................................. 1244
Golafshan, Nasim
K03-06 ........................................ 263
Golda-Cepa, Monika
PS1-13-415 .................................. 1412
Golse, Nicolas
ESB-BMSJ S-05 .................................. 729
Gomes, Diana
PS2-11-356 .................................. 1823
Gomes, Manuela
S05-KL01 ...................................... 266
ESB-SIBB S01-04 .................................. 522
N15-01 ........................................ 916
S05-04 ........................................ 272
Gomes, Maria C.
N09-04 ........................................ 475
N11-04 ........................................ 571
Gomes, Paula
K10-04 ........................................ 863
PS1-11-359 .................................. 1356
S03-02 ........................................ 115
Gomes, Rita
K07-02 ........................................ 753
Gomes, Sandra
K10-04 ........................................ 863
PS2-08-298 .................................. 1760
Gomez d’Ayala, Giovanna
PS2-07-246 .................................. 1714
Gómez-Baño, Félix
S14-07 ........................................ 884
Gomez-Florit, Manuel
ESB-SIBB S01-04 .................................. 522
K02-07 ........................................ 186
S05-04 ........................................ 272
Gonçalves, Adriana
PS1-07-243 .................................. 1235
PS1-08-291 .................................. 1282
PS2-08-280 .................................. 1743
Gonçalves, Inês
PS1-08-289 .................................. 1280
K04-03 ........................................ 383
PS2-05-192 .................................. 1657
PS2-13-412 .................................. 1884
S04-03 ........................................ 192
Gonçalves, Leandro
N05-08 ........................................ 301
Gonçalves, Lidia
PS2-10-326 .................................. 1791
Gonçalves, Raquel
PS2-04-156 .................................. 1621
ESB-SFB S-05 .................................. 815
N02-09 ........................................ 167
PS1-08-299 .................................. 1289
S15-03 ........................................ 889
S15-05 ........................................ 893
Gofii, Isabel
N08-06 ........................................ 460
PS1-13-401 .................................. 1400
Gonjo, Tomohiro
Biomechanics 02-02 .................................. 958
Gonzalez de Langarica, Sergio
K04-05 ........................................ 386
González Gómez, Iciar
K03-02 ........................................ 255
Gonzalez Prada, Iago
PS1-01-031 ..................................... 1023
Gonzalez Vazquez, Arlyng
S07-03 ........................................ 412
González, Blanca
K01-02 ........................................ 76
PS2-12-380 .................................. 1849
Gonzalez, Javier G.
K04-02 ........................................ 380
K05-02 ........................................ 492
González, María T.
PS2-07-254 .................................. 1722
González, Maria Ujue
PS2-02-092........................................ 1563
PS2-02-098........................................ 1570
N07-06 ........................................ 339
González, Pio
PS2-02-090........................................ 1561
PS2-03-124........................................ 1593
Gonzalez-Fernandez, Felipe M.
PS1-12-377........................................ 1374
González-Frutos, Pablo
Biomechanics 03-06 ................................ 971
Gonzalez-Martin, M. Luisa
PS1-13-417........................................ 1414
PS1-15-465........................................ 1468
PS2-13-416........................................ 1888
González-Mayorga, Ankor
PS2-07-254........................................ 1722
González-Pérez, Fernando
K05-07 ........................................ 503
González-Pérez, Miguel
N12-02 ........................................ 734
González-Rodríguez, Laura
PS2-02-090........................................ 1561
PS2-03-124........................................ 1593
González-Vázquez, Arlyng
N02-06 ........................................ 160
Goodall, Russell
PS1-02-099........................................ 1089
Gori, Manuele
N16-09 ........................................ 948
Gorin, Caroline
PS1-04-173........................................ 1161
Gosselet, Fabien
NANOSTEM 03-02 ................................ 360
NANOSTEM 03-03 ................................ 362
Gotz, Magdalena
N06-05 ........................................ 316
Götz, Magdalena
NANOSTEM 04-02 ................................ 370
Goujon, Florent
PS2-10-320........................................ 1784
Gounari, Eleni
PS2-10-342........................................ 1808
Gouveia, Pedro J.
K04-02 ........................................ 380
K07-02 ........................................ 753
Govindaraj, Kannan
PS2-13-422........................................ 1893
Grabow, Niels
PS1-15-461........................................ 1463
Gracia, Raquel
N06-08 ........................................ 323
Gradisteau Pircalabioru, Gratiela
PS2-05-184........................................ 1649
Grainha, Tânia
PS1-11-361........................................ 1358
Grande, Hans-Jürgen
N06-08 ........................................ 323
Grandfils, Christian
PS2-03-106........................................ 1576
PS2-04-136........................................ 1605
Grelal, Henri
N06-01 ........................................ 307
Granja, Pedro L.
ESB-SIBB S02-03 ................................ 709
N10-05 ........................................ 552
PS2-10-343........................................ 1340
PS2-01-046........................................ 1516
PS2-14-434........................................ 1906
Grant, Rhianne
PS1-08-259........................................ 1251
PS2-15-456........................................ 1930
Grasl, Christian
PS2-09-314........................................ 1779
Grava, Andra
PS2-05-186........................................ 1651
Graziani, Gabriela
PS1-05-189........................................ 1178
Greant, Coralie
PS2-07-222........................................ 1689
Green, Rylie
N01-01 ........................................ 128
Gremse, Felix
PS2-02-068........................................ 1537
Grenho, Liliana
PS2-02-084........................................ 1555
Gresser, Goetz T.
PS2-01-060........................................ 1528
Gries, Thomas
N11-03 ........................................ 568
PS1-09-305........................................ 1296
PS1-09-311........................................ 1304
Griffith, Linda
PL2-01 ........................................ 583
Grijpma, Dirk W.
PS2-08-262........................................ 1730
Grimaudo, Maria A.
PS2-08-282........................................ 1745
Grippi, Valentina
PS2-07-224........................................ 1691
Gritsch, Lukas
N06-01 ........................................ 307
Griveau, Louise
S05-06 ........................................ 277
Grivet Brancot, Arianna
PS2-05-178........................................ 1643
Gröber-Becker, Florian
PS1-01-033........................................ 1025

Page 1973 of 2028
<table>
<thead>
<tr>
<th>Author</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groll, Jürgen</td>
<td>259</td>
</tr>
<tr>
<td>K03-04</td>
<td></td>
</tr>
<tr>
<td>K08-02</td>
<td>770</td>
</tr>
<tr>
<td>PS1-01-059</td>
<td>1046</td>
</tr>
<tr>
<td>PS1-02-087</td>
<td>1076</td>
</tr>
<tr>
<td>PS2-01-042</td>
<td>1514</td>
</tr>
<tr>
<td>Grosjean, Mathilde</td>
<td>1331</td>
</tr>
<tr>
<td>PS1-10-335</td>
<td></td>
</tr>
<tr>
<td>Grossin, David</td>
<td>232</td>
</tr>
<tr>
<td>N04-02</td>
<td></td>
</tr>
<tr>
<td>Groth, Thomas</td>
<td>439</td>
</tr>
<tr>
<td>ESB-BIOMAT S-04</td>
<td></td>
</tr>
<tr>
<td>ESB-SLABO S-05</td>
<td>287</td>
</tr>
<tr>
<td>N04-09</td>
<td>247</td>
</tr>
<tr>
<td>PS1-08-283</td>
<td>1275</td>
</tr>
<tr>
<td>Grover, Liam M.</td>
<td>119</td>
</tr>
<tr>
<td>S03-04</td>
<td></td>
</tr>
<tr>
<td>Guibeboom, Anika</td>
<td>1914</td>
</tr>
<tr>
<td>PS2-14-442</td>
<td></td>
</tr>
<tr>
<td>Guirlande, Lars G.</td>
<td>1267</td>
</tr>
<tr>
<td>PS1-08-275</td>
<td></td>
</tr>
<tr>
<td>Gruszczyński, Adam</td>
<td>1608</td>
</tr>
<tr>
<td>PS2-04-140</td>
<td></td>
</tr>
<tr>
<td>Grzeszczałk, Ana</td>
<td>996</td>
</tr>
<tr>
<td>PS1-01-003</td>
<td></td>
</tr>
<tr>
<td>Guagliano, Giuseppe</td>
<td>466</td>
</tr>
<tr>
<td>N08-09</td>
<td></td>
</tr>
<tr>
<td>PS2-14-440</td>
<td>1912</td>
</tr>
<tr>
<td>Guarch Perez, Clara M.</td>
<td>867</td>
</tr>
<tr>
<td>K10-06</td>
<td></td>
</tr>
<tr>
<td>Guari, Yannick</td>
<td>82</td>
</tr>
<tr>
<td>K01-05</td>
<td></td>
</tr>
<tr>
<td>Guasch, Judith</td>
<td>450</td>
</tr>
<tr>
<td>N08-01</td>
<td></td>
</tr>
<tr>
<td>Guaza Lasheras, Mar</td>
<td>500</td>
</tr>
<tr>
<td>K08-02</td>
<td></td>
</tr>
<tr>
<td>Guazzelli, Nicole</td>
<td>761</td>
</tr>
<tr>
<td>K07-05</td>
<td></td>
</tr>
<tr>
<td>PS2-02-074</td>
<td>1544</td>
</tr>
<tr>
<td>Guerri, Vera</td>
<td>1004</td>
</tr>
<tr>
<td>PS1-01-011</td>
<td></td>
</tr>
<tr>
<td>Guerra, Antonio J.</td>
<td>1486</td>
</tr>
<tr>
<td>PS2-01-014</td>
<td></td>
</tr>
<tr>
<td>Guerra-Debello, Marta</td>
<td>1365</td>
</tr>
<tr>
<td>PS1-12-367</td>
<td></td>
</tr>
<tr>
<td>Guerreiro, Sara</td>
<td>1009</td>
</tr>
<tr>
<td>PS1-01-015</td>
<td></td>
</tr>
<tr>
<td>Guicheux, Jérôme</td>
<td>940</td>
</tr>
<tr>
<td>N16-05</td>
<td></td>
</tr>
<tr>
<td>Guimarães, Carlos F.</td>
<td>182</td>
</tr>
<tr>
<td>K02-05</td>
<td></td>
</tr>
<tr>
<td>Guimond, Stefanie</td>
<td>1532</td>
</tr>
<tr>
<td>PS2-02-064</td>
<td></td>
</tr>
<tr>
<td>Guler, Mustafa O.</td>
<td>106</td>
</tr>
<tr>
<td>S02-03</td>
<td></td>
</tr>
<tr>
<td>Gulino, Maurizio</td>
<td>1372</td>
</tr>
<tr>
<td>PS1-12-375</td>
<td></td>
</tr>
<tr>
<td>Gulyeva, Medina</td>
<td>1842</td>
</tr>
<tr>
<td>PS1-12-374</td>
<td></td>
</tr>
<tr>
<td>Gülker, Lisa</td>
<td>1606</td>
</tr>
<tr>
<td>PS2-04-138</td>
<td></td>
</tr>
<tr>
<td>Güven, Gülchin</td>
<td>1836</td>
</tr>
<tr>
<td>PS2-12-368</td>
<td></td>
</tr>
<tr>
<td>Günday, Cemre</td>
<td>1842</td>
</tr>
<tr>
<td>ESB-CRS S-03</td>
<td>424</td>
</tr>
<tr>
<td>Günday-Türel, Nazende</td>
<td>352</td>
</tr>
<tr>
<td>NANOSTEM 02-02</td>
<td>360</td>
</tr>
<tr>
<td>GUANOSTEM 03-02</td>
<td></td>
</tr>
<tr>
<td>Gupta, Amita</td>
<td>1901</td>
</tr>
<tr>
<td>PS2-12-374</td>
<td></td>
</tr>
<tr>
<td>Gurian, Melvin</td>
<td>1189</td>
</tr>
<tr>
<td>PS2-14-430</td>
<td></td>
</tr>
<tr>
<td>Gurikov, Pavel</td>
<td>590</td>
</tr>
<tr>
<td>AERO S01-02</td>
<td></td>
</tr>
<tr>
<td>PS1-05-199</td>
<td></td>
</tr>
<tr>
<td>Guruchaga, Mariló</td>
<td>460</td>
</tr>
<tr>
<td>N08-06</td>
<td></td>
</tr>
<tr>
<td>PS1-13-401</td>
<td>1400</td>
</tr>
<tr>
<td>Gusano, Diana J.</td>
<td>1234</td>
</tr>
<tr>
<td>PS1-07-241</td>
<td></td>
</tr>
<tr>
<td>Gutierrez Dávila, Marcos</td>
<td>988</td>
</tr>
<tr>
<td>Biomechanics 04-05</td>
<td>988</td>
</tr>
<tr>
<td>Biomechanics 04-06</td>
<td></td>
</tr>
<tr>
<td>Guzman, Elazar</td>
<td>581</td>
</tr>
<tr>
<td>N11-09</td>
<td></td>
</tr>
<tr>
<td>Guttenplan, Alexander P.M.</td>
<td>1876</td>
</tr>
<tr>
<td>PS2-13-406</td>
<td></td>
</tr>
<tr>
<td>Guzi de Moraes, Elisângela</td>
<td>1598</td>
</tr>
<tr>
<td>PS2-03-130</td>
<td></td>
</tr>
<tr>
<td>Guzik, Maciej</td>
<td>1293</td>
</tr>
<tr>
<td>PS1-08-303</td>
<td></td>
</tr>
<tr>
<td>Guzmán, Christian D.</td>
<td>1248</td>
</tr>
<tr>
<td>PS1-07-257</td>
<td></td>
</tr>
<tr>
<td>Guzzelli, Nicole</td>
<td>1866</td>
</tr>
<tr>
<td>PS2-13-396</td>
<td></td>
</tr>
<tr>
<td>Guzzi, Elia A.</td>
<td>257</td>
</tr>
<tr>
<td>K03-03</td>
<td></td>
</tr>
<tr>
<td>Gwag, Eunheui</td>
<td>1868</td>
</tr>
<tr>
<td>PS2-13-398</td>
<td></td>
</tr>
<tr>
<td>Gyulavari, Tamas</td>
<td>1166</td>
</tr>
<tr>
<td>PS1-05-177</td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Pages</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Habib, Mosaieb</td>
<td>1806</td>
</tr>
<tr>
<td>Habibović, Pamela</td>
<td></td>
</tr>
<tr>
<td>N02-07</td>
<td>162</td>
</tr>
<tr>
<td>N09-06</td>
<td>478</td>
</tr>
<tr>
<td>PS1-04-157</td>
<td>1148</td>
</tr>
<tr>
<td>PS2-10-322</td>
<td>1787</td>
</tr>
<tr>
<td>PS1-13-393</td>
<td>1391</td>
</tr>
<tr>
<td>PS2-13-406</td>
<td>1876</td>
</tr>
<tr>
<td>PS2-14-424</td>
<td>1896</td>
</tr>
<tr>
<td>Hacker, Michael</td>
<td></td>
</tr>
<tr>
<td>PS2-15-462</td>
<td>1936</td>
</tr>
<tr>
<td>K09-07</td>
<td>853</td>
</tr>
<tr>
<td>Hafeez, Shahzad</td>
<td></td>
</tr>
<tr>
<td>S06-06</td>
<td>404</td>
</tr>
<tr>
<td>Hager, Pia</td>
<td></td>
</tr>
<tr>
<td>PS2-09-314</td>
<td>1779</td>
</tr>
<tr>
<td>Hahn, Clemens</td>
<td></td>
</tr>
<tr>
<td>PS2-09-314</td>
<td>1779</td>
</tr>
<tr>
<td>Hahn, Olga</td>
<td></td>
</tr>
<tr>
<td>PS1-15-457</td>
<td>1457</td>
</tr>
<tr>
<td>Hajnal, Anja</td>
<td></td>
</tr>
<tr>
<td>PS1-05-199</td>
<td>1189</td>
</tr>
<tr>
<td>Hakimi, Osnat</td>
<td></td>
</tr>
<tr>
<td>PS2-02-082</td>
<td>1552</td>
</tr>
<tr>
<td>S13-02</td>
<td>800</td>
</tr>
<tr>
<td>Halfter, Norbert</td>
<td></td>
</tr>
<tr>
<td>K05-04</td>
<td>497</td>
</tr>
<tr>
<td>N10-08</td>
<td>558</td>
</tr>
<tr>
<td>Halfwerk, Frank R.</td>
<td></td>
</tr>
<tr>
<td>PS1-02-073</td>
<td>1060</td>
</tr>
<tr>
<td>Halgand, Boris</td>
<td></td>
</tr>
<tr>
<td>N16-05</td>
<td>940</td>
</tr>
<tr>
<td>Hallen, Sonia</td>
<td></td>
</tr>
<tr>
<td>YSF WS-07</td>
<td>67</td>
</tr>
<tr>
<td>Hamar, Peter</td>
<td></td>
</tr>
<tr>
<td>PS1-10-343</td>
<td>1340</td>
</tr>
<tr>
<td>Hamoh, Thamir</td>
<td></td>
</tr>
<tr>
<td>K06-02</td>
<td>653</td>
</tr>
<tr>
<td>Han, Xingting</td>
<td></td>
</tr>
<tr>
<td>PS1-04-155</td>
<td>1145</td>
</tr>
<tr>
<td>Handschin, Charles</td>
<td></td>
</tr>
<tr>
<td>S01-03</td>
<td>92</td>
</tr>
<tr>
<td>S14-05</td>
<td>880</td>
</tr>
<tr>
<td>Hansmann, Jan</td>
<td></td>
</tr>
<tr>
<td>PS1-01-033</td>
<td>1025</td>
</tr>
<tr>
<td>Hanson, Peter</td>
<td></td>
</tr>
<tr>
<td>PS1-05-187</td>
<td>1176</td>
</tr>
<tr>
<td>Harazna, Katarzyna</td>
<td></td>
</tr>
<tr>
<td>PS1-08-303</td>
<td>1293</td>
</tr>
<tr>
<td>Hardouin, Julie</td>
<td></td>
</tr>
<tr>
<td>K10-07</td>
<td>869</td>
</tr>
<tr>
<td>Hardt, Wolf-Dietrich</td>
<td></td>
</tr>
<tr>
<td>K10-02</td>
<td>858</td>
</tr>
<tr>
<td>Harmsen, Martin C.</td>
<td></td>
</tr>
<tr>
<td>PS1-02-063</td>
<td>1052</td>
</tr>
<tr>
<td>Harre, Jennifer</td>
<td></td>
</tr>
<tr>
<td>PS2-10-340</td>
<td>1806</td>
</tr>
<tr>
<td>Hartmann, Hanna</td>
<td></td>
</tr>
<tr>
<td>PS2-01-036</td>
<td>1508</td>
</tr>
<tr>
<td>Hartmann, Malte</td>
<td></td>
</tr>
<tr>
<td>PS2-03-130</td>
<td>1598</td>
</tr>
<tr>
<td>Hartwig, Henning</td>
<td></td>
</tr>
<tr>
<td>PS2-04-138</td>
<td>1606</td>
</tr>
<tr>
<td>PS2-08-258</td>
<td>1727</td>
</tr>
<tr>
<td>Hasirci, Vasif</td>
<td></td>
</tr>
<tr>
<td>PS2-13-400</td>
<td>1871</td>
</tr>
<tr>
<td>Hatayama, Kyosuke</td>
<td></td>
</tr>
<tr>
<td>N03-08</td>
<td>225</td>
</tr>
<tr>
<td>Hattori, Tomokazu</td>
<td></td>
</tr>
<tr>
<td>PS2-01-038</td>
<td>1509</td>
</tr>
<tr>
<td>Hauck, Sophia</td>
<td></td>
</tr>
<tr>
<td>K05-04</td>
<td>497</td>
</tr>
<tr>
<td>Haugen, Håvard J.</td>
<td></td>
</tr>
<tr>
<td>N03-04</td>
<td>215</td>
</tr>
<tr>
<td>PS1-02-067</td>
<td>1057</td>
</tr>
<tr>
<td>PS1-07-231</td>
<td>1224</td>
</tr>
<tr>
<td>Hauptstein, Julia</td>
<td></td>
</tr>
<tr>
<td>PS1-07-233</td>
<td>1226</td>
</tr>
<tr>
<td>PS2-01-042</td>
<td>1514</td>
</tr>
<tr>
<td>Hauschild, Stephan</td>
<td></td>
</tr>
<tr>
<td>PS1-01-059</td>
<td>1046</td>
</tr>
<tr>
<td>Hayward, Stephan</td>
<td></td>
</tr>
<tr>
<td>S15-04</td>
<td>891</td>
</tr>
<tr>
<td>Hazur, Jonas</td>
<td></td>
</tr>
<tr>
<td>PS1-02-087</td>
<td>1076</td>
</tr>
<tr>
<td>S10-04</td>
<td>686</td>
</tr>
<tr>
<td>Hecker, Christine</td>
<td></td>
</tr>
<tr>
<td>N09-07</td>
<td>480</td>
</tr>
<tr>
<td>Hedtke, Tobias</td>
<td></td>
</tr>
<tr>
<td>ESB-BIOMAT S-04.</td>
<td>439</td>
</tr>
<tr>
<td>Heid, Susanne</td>
<td></td>
</tr>
<tr>
<td>PS1-05-183</td>
<td>1172</td>
</tr>
<tr>
<td>Heidari, Neda</td>
<td></td>
</tr>
<tr>
<td>PS2-13-394</td>
<td>1863</td>
</tr>
<tr>
<td>Heitmeijer, Marije</td>
<td></td>
</tr>
<tr>
<td>S06-04</td>
<td>400</td>
</tr>
<tr>
<td>Helary, Christophe</td>
<td></td>
</tr>
<tr>
<td>PS1-10-321</td>
<td>1316</td>
</tr>
<tr>
<td>Hélary, Christophe</td>
<td></td>
</tr>
<tr>
<td>PS1-03-105</td>
<td>1094</td>
</tr>
<tr>
<td>PS2-01-020</td>
<td>1493</td>
</tr>
<tr>
<td>Heltmann-Meyer, Stefanie</td>
<td></td>
</tr>
<tr>
<td>PS2-14-442</td>
<td>1914</td>
</tr>
<tr>
<td>Henke, Heinz-Werner</td>
<td></td>
</tr>
<tr>
<td>PS1-09-305</td>
<td>1296</td>
</tr>
<tr>
<td>Author Index</td>
<td>ESB 2021</td>
</tr>
<tr>
<td>--------------</td>
<td>---------</td>
</tr>
<tr>
<td>Henke, Sieger</td>
<td>K08-03</td>
</tr>
<tr>
<td>Hennink, Wim E.</td>
<td>N06-06</td>
</tr>
<tr>
<td>Henrich, Dirk</td>
<td>PS2-01-006</td>
</tr>
<tr>
<td>Henriques, Margarida</td>
<td>PS2-10-326</td>
</tr>
<tr>
<td>Henriques, Patrícia C.</td>
<td>PS1-08-289</td>
</tr>
<tr>
<td></td>
<td>K04-03</td>
</tr>
<tr>
<td></td>
<td>PS2-13-412</td>
</tr>
<tr>
<td>Herbault-Barres, Béatrice</td>
<td>N04-02</td>
</tr>
<tr>
<td>Herbig, Johannes</td>
<td>K08-02</td>
</tr>
<tr>
<td>Herland, Anna</td>
<td>PS2-03-110</td>
</tr>
<tr>
<td>Hernadi, Klara</td>
<td>PS2-02-072</td>
</tr>
<tr>
<td>Herrero-Molleda, Alba</td>
<td>Biomechanics 03-02</td>
</tr>
<tr>
<td>Hess, Julian</td>
<td>PS2-10-332</td>
</tr>
<tr>
<td>Heying, Ruth</td>
<td>PS1-12-379</td>
</tr>
<tr>
<td>Heyward, Catherine A.</td>
<td>PS1-07-231</td>
</tr>
<tr>
<td>Hibbits, Alan</td>
<td>K05-02</td>
</tr>
<tr>
<td>Hierro-Oliva, Margarita</td>
<td>PS1-15-465</td>
</tr>
<tr>
<td></td>
<td>PS2-13-416</td>
</tr>
<tr>
<td>Hildebrand, Feng</td>
<td>PS2-01-002</td>
</tr>
<tr>
<td>Hillebrands, Jan L.</td>
<td>PS2-09-308</td>
</tr>
<tr>
<td>Himmler, Marcus</td>
<td>N14-09</td>
</tr>
<tr>
<td>Hintze, Vera</td>
<td>K05-04</td>
</tr>
<tr>
<td></td>
<td>N10-08</td>
</tr>
<tr>
<td></td>
<td>PS2-15-462</td>
</tr>
<tr>
<td>Hiramitsu, Akihiro</td>
<td>PS2-01-038</td>
</tr>
<tr>
<td>Hjörvarsson, Björgvin</td>
<td>PS2-02-076</td>
</tr>
<tr>
<td>Hodgkinson, Tom</td>
<td>N02-05</td>
</tr>
<tr>
<td></td>
<td>S07-03</td>
</tr>
<tr>
<td>Hoehenwarter, Wolfgang</td>
<td>ESB-BIOMAT S-04</td>
</tr>
<tr>
<td>Hoene, Andreas</td>
<td>PS1-15-457</td>
</tr>
<tr>
<td>Hoffmann, Andrea</td>
<td>PS2-04-138</td>
</tr>
<tr>
<td>Hofman, Anno</td>
<td>S07-04</td>
</tr>
<tr>
<td>Holland, Elijah N.</td>
<td>S09-02</td>
</tr>
<tr>
<td>Holtzhausen, Stefan</td>
<td>N09-01</td>
</tr>
<tr>
<td>Holweg, Patrick</td>
<td>N11-06</td>
</tr>
<tr>
<td>Homem, Natália C.</td>
<td>ESB-SIBB S01-05</td>
</tr>
<tr>
<td></td>
<td>PS1-07-221</td>
</tr>
<tr>
<td></td>
<td>PS2-07-226</td>
</tr>
<tr>
<td>Höning, Ellena</td>
<td>PS2-01-036</td>
</tr>
<tr>
<td>Hoogenboom, Richard</td>
<td>N05-09</td>
</tr>
<tr>
<td>Hoppe, Sandrine</td>
<td>PS1-01-029</td>
</tr>
<tr>
<td>Horch, Raymund E.</td>
<td>PS2-14-442</td>
</tr>
<tr>
<td>Horder, Hannes</td>
<td>K08-02</td>
</tr>
<tr>
<td></td>
<td>PS2-14-442</td>
</tr>
<tr>
<td>Horejs, Christine</td>
<td>YSF-AEROGELS WS-04</td>
</tr>
<tr>
<td>Horvat, Gabrijela</td>
<td>AERO S02-02</td>
</tr>
<tr>
<td>Hosseini, Shabnam</td>
<td>PS1-12-385</td>
</tr>
<tr>
<td>Houauoi, Amel</td>
<td>PS1-04-145</td>
</tr>
<tr>
<td>Houben, Sofie</td>
<td>N16-04</td>
</tr>
<tr>
<td>Hrynevich, Andrei</td>
<td>N14-04</td>
</tr>
<tr>
<td></td>
<td>N14-05</td>
</tr>
<tr>
<td>Hu, Jinlian</td>
<td>S04-K101</td>
</tr>
<tr>
<td>Huang, Jie</td>
<td>PS1-01-005</td>
</tr>
<tr>
<td>Hubbe, Hendrik</td>
<td>S09-03</td>
</tr>
<tr>
<td>Huerta-Madróñal, Miguel</td>
<td>ESB-SIBB S02-01</td>
</tr>
<tr>
<td></td>
<td>PS1-07-217</td>
</tr>
<tr>
<td>Hulsart Billström, Gry</td>
<td>PS1-13-03</td>
</tr>
<tr>
<td>Huri, Pinar Y.</td>
<td>S14-06</td>
</tr>
<tr>
<td>Hurlet, Jérôme</td>
<td>PS2-03-106</td>
</tr>
<tr>
<td>Hutsky, André</td>
<td>N09-01</td>
</tr>
</tbody>
</table>

Page 1976 of 2028
Author Index

Hybásekov, Vojtech
K06-04 .................................................. 657
Hylkema, Machtedl N.
PS1-02-063 ......................................... 1052

I
Iafisco, Michele
PS2-04-150 ........................................... 1614
PS2-04-162 ........................................... 1624
Ibáñez Fonseca, Arturo
PS1-14-431 ........................................... 1431
Ibáñez, Elena
PS1-04-139 ........................................... 1130
Ibáñez-Fonseca, Arturo
K05-07 ................................................... 503
Ibarretxe, Gaskon
K04-05 ................................................... 386
PS2-07-228 ........................................... 1695
Iberite, Federica
S05-05 .................................................... 274
Ibrahim, Toni
PS2-04-150 ........................................... 1614
Ichim, Daniela L.
PS2-10-324 ........................................... 1789
Iglesias, Cláudia
PS2-09-304 ........................................... 1767
Iglesias-Mejuto, Ana
AERO S02-01 ...................................... 609
Ignatius, Anita
PS2-05-198 ........................................... 1664
Ihalainen, Teemu O.
S09-06 ................................................... 674
Ikebata, Atsushi
PS2-01-038 ........................................... 1509
Illera, Pilar A.
AERO PS01-03 ...................................... 601
Illing-Günther, Heike
N09-07 ................................................... 480
Illoz, Ibon
PS1-13-401 ........................................... 1400
Insley, Gerard
PS2-04-154 ........................................... 1618
Intini, Claudio
N02-05 ................................................... 158
PS1-04-151 ........................................... 1140
S07-03 ................................................... 412
Inverardi, Nicoletta
S04-05 ................................................... 196
Ionta, Mariana
K04-04 ................................................... 385
Iono, Leonid
PS2-01-012 ........................................... 1484
PS2-01-022 ........................................... 1496
Iovu, Horia
N05-02 ................................................... 292

PS1-01-035 ........................................... 1027
PS2-05-184 ........................................... 1649
Iqbal, Neelam
PS1-04-175 ........................................... 1162
PS2-11-366 ........................................... 1832
Irastorza, Igor
PS2-07-228 ........................................... 1695
Irbe, Zilgma
PS1-04-143 ........................................... 1132
Irwin, Nicola
PS1-11-357 ........................................... 1354
PS1-11-363 ........................................... 1360
PS2-11-360 ........................................... 1827
PS2-11-362 ........................................... 1829
PS2-11-348 ........................................... 1815
Işık, Murat
AERO S03-01 ...................................... 639
Iskhahtova, Kamila
PS1-02-067 ........................................... 1057
Ismar, Ezgi
PS2-08-294 ........................................... 1757
Ismakki, Mari
S09-06 ................................................... 674
Isu, Giuseppe
PS2-14-444 .......................................... 1915
Itho, Manano
PS2-01-038 .......................................... 1509
Ito, Keita
N14-02 ................................................... 898
Iurciuc (Tincu), Camelia E.
PS1-08-269 .......................................... 1261
Izotte, Julien
ESB-SFB S-04 ..................................... 813
Izquierdo-Barba, Isabel
K01-02 ................................................... 76
PS2-12-380 .......................................... 1849

J
Jahns, Mandy
PS2-10-340 .......................................... 1806
Jaisser, Frédéric
PS1-10-321 .......................................... 1316
Jakobi, Meike
PS1-13-409 .......................................... 1408
Jallot, Edouard
N06-01 ................................................... 307
Jaminon, Armand
PS2-14-426 .......................................... 1898
Janicijevic, Danica
Biomechanics 03-03 ................................ 963
Biomechanics 04-02 ................................ 976
Janner, Davide
PS1-13-413 .......................................... 1410

Page 1977 of 2028
Author Index

Jasch, Katarzyna
PS1-10-339 ........................................ 1336
Jaszczyk, Marek
PS1-07-225 ................................. 1219
Javen, Rueda
Biomechanics 04-03 ....................... 979
Jayawardena, Vineetha
K03-01 ........................................... 253
PS2-04-160 .................................... 1622
Jehl, Jean-Philippe
PS1-01-029 .................................... 1022
Jeu, Gavin
N09-02 ......................................... 471
N10-07 ......................................... 556
PS1-14-445 .................................... 1445
PS2-06-216 .................................... 1684
PS2-12-374 .................................... 1842
S08-03 ......................................... 508
Jerome, Christine
PS1-08-269 .................................... 1261
Jerónimo, Carmen
K06-03 ......................................... 655
Jesus, Ana
K09-04 ......................................... 846
N01-06 ......................................... 139
Jezierkas-Woźniak, Katarzyna
PS2-07-224 .................................... 1691
Jeznach, Olwia
PS2-15-454 .................................... 1927
PS2-15-464 .................................... 1938
Jha, Animesh
PS1-04-175 .................................... 1162
PS2-04-172 .................................... 1635
PS2-11-366 .................................... 1832
Ji, Zili
PS1-11-357 .................................... 1354
Jiang, Xinquan
S03-06 ......................................... 123
Jin, Minye
PS1-14-441 .................................... 1442
PS2-08-296 .................................... 1758
Jiru, Jitrenka
K06-04 ......................................... 657
Jobbagy, Matias
PS2-15-458 .................................... 1932
Page 1978 of 2028
Jockenhoevel, Stefan
PS1-09-305 .................................... 1296
Johannson, Linh
ESB-SIBB S02-06 ......................... 715
Johnbocso, Castro
PS2-13-422 .................................... 1893
Jones, David
PS1-11-363 .................................... 1360
Jones, David S.
PS2-11-348 .................................... 1815
Jones, Julian
N10-07 ......................................... 556
Jonkheijm, Pascal
PS1-15-463 .................................... 1465
Jorcano, José Luis
N10-03 ......................................... 548
Jorda, Noemi
PS2-07-242 .................................... 1710
Jorge, Carole
ESB-SIBB S02-08 ......................... 719
Jørgensen, Mathias L.
ESB-CSBM S-05 ............................ 205
Joseph, Yvonne
N09-07 ......................................... 480
Joshi, Arundhati
S12-04 ......................................... 790
Joska, Ludek
K06-04 ......................................... 657
Josselin, Romane
PS1-01-039 .................................... 1031
Josserand, Véronique
N02-01 ......................................... 148
PS1-04-165 .................................... 1153
Jouenne, Thierry
K10-07 ......................................... 869
Jööl, Pia
AERO PS01-05 .............................. 605
Jowett, Geraldine
K09-03 ......................................... 844
Joyce, Michael
S07-03 ......................................... 412
Ju, Young Min
N01-04 ......................................... 136
Jugie, Clotilde
S14-05 ......................................... 880
Julius, Matthew
PS2-04-164 .................................... 1626
Juncos Bombin, Adrain D.
N14-07 ......................................... 909
Jurczak, Klaudia M.
PS2-09-308 .................................... 1773
Jury, Michael
PS2-03-110 .................................... 1580
<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td></td>
</tr>
<tr>
<td>K. Krauss, Joachim</td>
<td>1917</td>
</tr>
<tr>
<td>PS2-15-446</td>
<td></td>
</tr>
<tr>
<td>Kabirian, Fatemeh</td>
<td>1376</td>
</tr>
<tr>
<td>PS1-12-379</td>
<td></td>
</tr>
<tr>
<td>Kaczmarek, Marcin</td>
<td>1866</td>
</tr>
<tr>
<td>PS2-13-414</td>
<td></td>
</tr>
<tr>
<td>Kade, Juliane C.</td>
<td>335</td>
</tr>
<tr>
<td>N07-04</td>
<td></td>
</tr>
<tr>
<td>Kadefors, Måns</td>
<td>1431</td>
</tr>
<tr>
<td>PS1-14-431</td>
<td></td>
</tr>
<tr>
<td>Kadumudi, Firoz B.</td>
<td>1267</td>
</tr>
<tr>
<td>PS1-08-275</td>
<td></td>
</tr>
<tr>
<td>Kafali, Melisa</td>
<td>1588</td>
</tr>
<tr>
<td>PS2-03-120</td>
<td></td>
</tr>
<tr>
<td>Kaiser, Friederike</td>
<td>1664</td>
</tr>
<tr>
<td>PS2-05-198</td>
<td></td>
</tr>
<tr>
<td>Kaiser, Jozef</td>
<td>1652</td>
</tr>
<tr>
<td>PS2-05-188</td>
<td></td>
</tr>
<tr>
<td>Kakpenova, Aïnur</td>
<td>497</td>
</tr>
<tr>
<td>K05-04</td>
<td></td>
</tr>
<tr>
<td>Kalaskar, Deepak</td>
<td>1518</td>
</tr>
<tr>
<td>PS2-01-048</td>
<td></td>
</tr>
<tr>
<td>Kalmar, Jozef</td>
<td>613</td>
</tr>
<tr>
<td>AERO S02-03</td>
<td></td>
</tr>
<tr>
<td>Kamenik, Anna S.</td>
<td>347</td>
</tr>
<tr>
<td>NANOSTEM 01-03</td>
<td></td>
</tr>
<tr>
<td>Kamperman, Marleen</td>
<td>415</td>
</tr>
<tr>
<td>S07-04</td>
<td></td>
</tr>
<tr>
<td>Kamperman, Tom</td>
<td></td>
</tr>
<tr>
<td>K07-07</td>
<td>765</td>
</tr>
<tr>
<td>K08-03</td>
<td>772</td>
</tr>
<tr>
<td>PS2-13-422</td>
<td></td>
</tr>
<tr>
<td>Kampmann, Andreas</td>
<td>1893</td>
</tr>
<tr>
<td>PS2-04-138</td>
<td></td>
</tr>
<tr>
<td>Kaniuk, Łukasz</td>
<td>1606</td>
</tr>
<tr>
<td>PS2-04-140</td>
<td></td>
</tr>
<tr>
<td>Kantiranis, Nikolaos</td>
<td>1632</td>
</tr>
<tr>
<td>PS2-04-170</td>
<td></td>
</tr>
<tr>
<td>Kapeller, Barbara</td>
<td>1779</td>
</tr>
<tr>
<td>PS2-09-314</td>
<td></td>
</tr>
<tr>
<td>Kapis, Artur</td>
<td>1566</td>
</tr>
<tr>
<td>PS2-02-094</td>
<td></td>
</tr>
<tr>
<td>Kaplan, David</td>
<td>1481</td>
</tr>
<tr>
<td>PS2-01-010</td>
<td></td>
</tr>
<tr>
<td>Kappelmann-Fenzl, Melanie</td>
<td>1444</td>
</tr>
<tr>
<td>PS1-14-443</td>
<td></td>
</tr>
<tr>
<td>Karakaya, Emine</td>
<td>1076</td>
</tr>
<tr>
<td>PS1-02-087</td>
<td></td>
</tr>
<tr>
<td>Karaman, Ozan</td>
<td>874</td>
</tr>
<tr>
<td>S14-02</td>
<td></td>
</tr>
<tr>
<td>Karava, Vasiliki</td>
<td>1808</td>
</tr>
<tr>
<td>PS2-10-342</td>
<td></td>
</tr>
<tr>
<td>Karbowniczek, Joanna</td>
<td>1396</td>
</tr>
<tr>
<td>PS1-13-397</td>
<td></td>
</tr>
<tr>
<td>Karbowniczek, Joanna E.</td>
<td>1608</td>
</tr>
<tr>
<td>PS2-04-140</td>
<td></td>
</tr>
<tr>
<td>Karpenko, Andrey</td>
<td>1548</td>
</tr>
<tr>
<td>PS2-02-078</td>
<td></td>
</tr>
<tr>
<td>Karperien, Marcel</td>
<td>701</td>
</tr>
<tr>
<td>K07-07</td>
<td>765</td>
</tr>
<tr>
<td>K08-03</td>
<td>772</td>
</tr>
<tr>
<td>S11-05</td>
<td></td>
</tr>
<tr>
<td>Kashimbetova, Adelia</td>
<td>1550</td>
</tr>
<tr>
<td>PS2-02-080</td>
<td></td>
</tr>
<tr>
<td>PS2-05-188</td>
<td>1652</td>
</tr>
<tr>
<td>Kasprzyk, Wiktor</td>
<td>1087</td>
</tr>
<tr>
<td>PS1-02-097</td>
<td></td>
</tr>
<tr>
<td>Katewongsa, Kanlaya P.</td>
<td>1799</td>
</tr>
<tr>
<td>PS2-10-334</td>
<td></td>
</tr>
<tr>
<td>Kattar, Axel</td>
<td>1328</td>
</tr>
<tr>
<td>PS1-10-331</td>
<td></td>
</tr>
<tr>
<td>Kaur, Kulwinder</td>
<td>944</td>
</tr>
<tr>
<td>N16-07</td>
<td></td>
</tr>
<tr>
<td>Kawalko, Jakub</td>
<td>653</td>
</tr>
<tr>
<td>K06-02</td>
<td></td>
</tr>
<tr>
<td>Kawecki, Fabien</td>
<td>813</td>
</tr>
<tr>
<td>ESB-SFB S-04</td>
<td>402</td>
</tr>
<tr>
<td>Kc, Remant</td>
<td></td>
</tr>
<tr>
<td>PS2-10-346</td>
<td>1812</td>
</tr>
<tr>
<td>Kearns, Victoria</td>
<td>1229</td>
</tr>
<tr>
<td>PS1-07-237</td>
<td></td>
</tr>
<tr>
<td>Kebel, Kristina</td>
<td>1241</td>
</tr>
<tr>
<td>PS1-07-251</td>
<td></td>
</tr>
<tr>
<td>Kelleher, Susan</td>
<td>219</td>
</tr>
<tr>
<td>N03-05</td>
<td>217</td>
</tr>
<tr>
<td>N03-06</td>
<td></td>
</tr>
<tr>
<td>Kellomäki, Minna</td>
<td>1534</td>
</tr>
<tr>
<td>PS2-02-066</td>
<td></td>
</tr>
<tr>
<td>Kelly, Daniel</td>
<td>1029</td>
</tr>
<tr>
<td>PS1-01-037</td>
<td></td>
</tr>
<tr>
<td>Kelly, Daniel J.</td>
<td>412</td>
</tr>
<tr>
<td>S07-03</td>
<td></td>
</tr>
<tr>
<td>Kelly, David</td>
<td>1618</td>
</tr>
<tr>
<td>PS2-04-154</td>
<td></td>
</tr>
<tr>
<td>Kelly, Domhnall</td>
<td>158</td>
</tr>
<tr>
<td>N02-05</td>
<td></td>
</tr>
<tr>
<td>Kelly, Helena M.</td>
<td>1747</td>
</tr>
<tr>
<td>PS2-08-284</td>
<td></td>
</tr>
<tr>
<td>Kermter, Elisabeth</td>
<td>1687</td>
</tr>
<tr>
<td>PS2-07-220</td>
<td></td>
</tr>
<tr>
<td>Kengelbach-Weigand, Annika</td>
<td>1444</td>
</tr>
<tr>
<td>PS1-14-443</td>
<td></td>
</tr>
<tr>
<td>PS2-14-442</td>
<td>1914</td>
</tr>
<tr>
<td>Keogh, Patrick</td>
<td>891</td>
</tr>
<tr>
<td>S15-04</td>
<td></td>
</tr>
<tr>
<td>Author</td>
<td>Page</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Kerr, Séan</td>
<td>492</td>
</tr>
<tr>
<td>Keskin, Damla</td>
<td>1452</td>
</tr>
<tr>
<td>Kesse, Xavier</td>
<td>1385</td>
</tr>
<tr>
<td>Khalid, Tehreem</td>
<td>1704</td>
</tr>
<tr>
<td>Khan, Ilyas</td>
<td>234</td>
</tr>
<tr>
<td>Khan, Sophia</td>
<td>458</td>
</tr>
<tr>
<td>Khong, Jia</td>
<td>998</td>
</tr>
<tr>
<td>Kibl, Michelle F.</td>
<td>1345</td>
</tr>
<tr>
<td>Kilian, David</td>
<td>829</td>
</tr>
<tr>
<td>Kim, Donghoon</td>
<td>1372</td>
</tr>
<tr>
<td>Kim, Ji Hyun</td>
<td>136</td>
</tr>
<tr>
<td>Kim, Michael</td>
<td>136</td>
</tr>
<tr>
<td>Kindi, Husnia</td>
<td>287</td>
</tr>
<tr>
<td>Kiriako, Georges</td>
<td>364</td>
</tr>
<tr>
<td>Kirnbauer, Stefan</td>
<td>137</td>
</tr>
<tr>
<td>Kiss, János</td>
<td>1542</td>
</tr>
<tr>
<td>Kloxin, April M.</td>
<td>1903</td>
</tr>
<tr>
<td>Knap, Karolina</td>
<td>1336</td>
</tr>
<tr>
<td>Knez, Zeljko</td>
<td>612</td>
</tr>
<tr>
<td>Kocer, Gülşistan</td>
<td>1442</td>
</tr>
<tr>
<td>Koch, Marcus</td>
<td>415</td>
</tr>
<tr>
<td>Koel, Mihkel</td>
<td>605</td>
</tr>
<tr>
<td>Koga, Tomoyuki</td>
<td>210</td>
</tr>
<tr>
<td>Koldis, Petros</td>
<td>1632</td>
</tr>
<tr>
<td>Kolbuk, Dorota</td>
<td>1927</td>
</tr>
<tr>
<td>Kollamaram, Gayathri</td>
<td>412</td>
</tr>
<tr>
<td>Kong, Dexu</td>
<td>672</td>
</tr>
<tr>
<td>Konka, Joanna</td>
<td>872</td>
</tr>
<tr>
<td>Kontogianni, Georgia-Ioanna</td>
<td>241</td>
</tr>
<tr>
<td>Kontonasaki, Eleana</td>
<td>1159</td>
</tr>
<tr>
<td>Kop, David</td>
<td>1148</td>
</tr>
<tr>
<td>Koper, Filip</td>
<td>1087</td>
</tr>
<tr>
<td>Kopp, Alexander</td>
<td>1296</td>
</tr>
<tr>
<td>Koppe, Charlotte</td>
<td>1537</td>
</tr>
<tr>
<td>Koppen, Carina</td>
<td>1612</td>
</tr>
<tr>
<td>Kopershoek, Jasmijn</td>
<td>1402</td>
</tr>
<tr>
<td>Kostenko, Anastassia</td>
<td>1033</td>
</tr>
<tr>
<td>Kostka, Kathrin</td>
<td>1382</td>
</tr>
<tr>
<td>Kostoglou, Margaritis</td>
<td>1808</td>
</tr>
<tr>
<td>Kotagudda Ranganath, Sindhu</td>
<td>1074</td>
</tr>
<tr>
<td>Kotarba, Andrzei</td>
<td>1412</td>
</tr>
<tr>
<td>Kotlarz, Marcin</td>
<td>544</td>
</tr>
<tr>
<td>Kotov, Nicholas A.</td>
<td>1001</td>
</tr>
<tr>
<td>Koutrikova, Ioanna</td>
<td>237</td>
</tr>
<tr>
<td>Kowalewski, Tomasz Aleksander</td>
<td>1526</td>
</tr>
<tr>
<td>Krajewski, Stefanie</td>
<td>1691</td>
</tr>
<tr>
<td>Kreiskötter, Kim D.</td>
<td>1291</td>
</tr>
<tr>
<td>Kreuger, Johan</td>
<td>996</td>
</tr>
</tbody>
</table>
Author Index

Krömmelbein, Catharina
  PS2-08-272 .......................................... 1736
Kroschwald, Lysann
  PS2-15-462 .......................................... 1936
Kroschwald, Lysann M.
  N09-01 ................................................ 469
Krujatz, Felix
  K03-05 ................................................ 261
Kruppke, Benjamin
  PS1-02-077 ........................................... 1065
Krysiak, Zuzanna J.
  PS1-07-229 ........................................... 1223
Kuhnt, Tobias
  N13-03 ................................................ 820
Kukhtar, Dmytro
  AERO PS01-03 ........................................... 601
Kurytyka, Przemyslaw
  PS2-02-094 ........................................... 1566
  PS2-13-414 .......................................... 1886
Kussauer, Sophie
  PS1-09-317 ........................................... 1311
Kustosz, Roman
  PS2-02-094 ........................................... 1566
  PS2-09-310 .......................................... 1775
  PS2-13-414 .......................................... 1886
Kwiecień, Konrad
  PS1-10-339 ........................................... 1336

L

L. Reis, Rui
  K02-07 ................................................ 186
Labay, Cédric
  ESB-SIBB S02-06 .................................... 715
  N12-04 ................................................ 738
  N16-06 ................................................ 941
Labrador-Rached, Claudia J.
  S05-04 ................................................ 272
Labrunie, Gaëlle
  ESB-SFB S-04 ......................................... 813
Lacey, Joseph
  PS1-13-405 ........................................... 1403
Lacis, Ugis
  PS1-04-153 ........................................... 1143
Lackington, William A.
  N02-06 ................................................ 160
  PS2-02-064 .......................................... 1532
Lackner, Juergen M.
  PS2-09-310 .......................................... 1775
Lacza, Zsombor
  PS1-10-343 .......................................... 1340
Lafont, Marianne
  S05-06 ................................................ 277
Lafourcade, Michaël
  ESB-SFB S-04 ......................................... 813
Laganenka, Leanid
  K10-02 ................................................ 858
Lagarrigue, Prescillia
  N04-02 ................................................ 232
  PS1-06-205 .......................................... 1198
Lagazzo, Alberto
  PS1-02-083 .......................................... 1072
  PS1-06-213 .......................................... 1206
  PS2-04-152 .......................................... 1616
Lagneau, Nathan
  N16-05 ................................................ 940
Lahtinen, Maarit
  PS1-05-197 .......................................... 1187
Lai, Dehui
  N01-04 ................................................ 136
Lallukka, Mari
  PS2-15-460 ........................................... 1934
Lally, Christopher
  PS1-03-127 ........................................... 1114
Lama, Raquel
  PS2-02-090 .......................................... 1561
Lambert, Stéphanie
  PS2-04-136 .......................................... 1605
Lamers, Tara
  K02-07 ................................................ 186
Lamghari, Meriem
  N02-09 ................................................ 167
  PS2-04-156 .......................................... 1621
  S15-05 ................................................ 893
Lämmerhirt, Lisa
  PS1-14-443 .......................................... 1444
Landon, Rebecca
  S12-05 ................................................ 792
Lang, Gregor
  PS2-01-050 .......................................... 1520
Lanzi, Massimiliano
  PS2-07-224 .......................................... 1691
Lao, Jonathan
  N06-01 ................................................ 307
LaPointe, Vanessa
  PS1-13-393 .......................................... 1391
Laranjeira, Marta S.
  ESB-SIBB S02-07 .................................... 717
Larionova, Joulia
  K01-05 ................................................ 82
Laroche, Gaëtan
  PS2-13-408 .......................................... 1878
Laromaine, Anna
  AERO PS01-03 ........................................... 601
  AERO PS01-04 ........................................... 603
  COST AERoGELS KL-01 ................................ 587
  PS1-05-193 .......................................... 1182
Larrañaga, Aitor
  K04-05 ................................................ 386
  S06-07 ................................................ 406

Page 1981 of 2028
Author Index

Larrañaga, Xabier
PS1-02-079........................................ 1067
Larraneta, Eneko
PS1-11-357....................................... 1354
Larsson, Lisa
PS2-02-076....................................... 1546
Lauer, Guenter
N09-01............................................... 469
Laurano, Rossella
N06-09.............................................. 325
Laurencin, Danielle
K01-05............................................... 82
Lavigne, Rob
N03-01............................................... 208
Lavrador, Pedro
N05-08............................................... 301
Lawrence, Drew
AERO S01-02...................................... 590
Le Bao, Chau
PS1-03-125........................................ 1112
Le Goff, Héloïse
S05-06............................................... 277
Le Roi, Baptiste
PS2-14-436........................................ 1908
Le Visage, Catherine
N16-05............................................... 940
S05-06............................................... 277
Leahy, Liam
K04-02............................................... 380
Leal, Ermelindo
N06-03............................................... 311
Lecommandoux, Sébastien
PS1-08-279........................................ 1271
Ledeza-Espinosa, Aura
AERO InvF-07...................................... 633
Lee, Sang Jin
S07-KL01............................................ 410
Lee, Seunghun S.
K10-02............................................... 858
Lee, Wooje
K08-03............................................... 772
Leeuwenburgh, Sander C.G.
S06-04............................................... 400
Lefaix, Laura
N07-01............................................... 328
N10-02............................................... 546
Legnardi, Manuela
N08-09............................................... 466
Lehmann, Susann
PS2-07-220........................................ 1687
Leijten, Jeroen
JL AW01-01......................................... 71
K07-07............................................... 765
K08-03............................................... 772
LS-01............................................... 488
Leiro, Victoria
K09-04............................................... 846
Lekka, Maria
PS2-11-354........................................ 1821
Lemaire, Laurent
K01-05............................................... 82
Le-Meins, Jean-François
PS1-01-039.......................................... 1031
Lemes, Ana Paula
PS1-01-013.......................................... 1007
Lemoine, Mark
PS1-01-037.......................................... 1029
PS1-04-151.......................................... 1140
S07-03............................................... 412
Lemos, Marco F.L.
PS2-01-016.......................................... 1488
Lenarz, Thomas
PS2-10-340.......................................... 1806
Lennon, Alex
N12-05............................................... 740
N12-06............................................... 742
PS1-09-313.......................................... 1307
Leosson, Kristian
PS2-12-370.......................................... 1838
Lepine, Clarence
S13-02............................................... 800
Leprince, Maxime
N07-02............................................... 330
Leriche, Anne
PS2-11-356.......................................... 1823
Lerner, Marat
N11-09............................................... 581
Leroux, Alice
S12-05............................................... 792
Leroux, Amélie
PS2-07-250.......................................... 1718
Leszczyński, Bartosz
PS1-08-303.......................................... 1293
Author Index

Letourneur, Didier
N12-08 ................................................. 745
PS1-03-125 ........................................ 1112
PS1-08-271 ........................................ 1263
PS1-09-307 ........................................ 1298
Leuvenink, Henri G.
PS2-09-308 ........................................ 1773
Levato, Riccardo
JL AW02-01 .......................................... 376
N04-03 .............................................. 234
N14-02 .............................................. 898
PS1-13-403 .......................................... 1402
S01-04 .............................................. 94
S01-05 .............................................. 96
Levingstone, Tanya
N13-02 ................................................ 818
Levingstone, Tanya J.
N06-02 .............................................. 309
PS2-04-154 .......................................... 1618
Levkin, Pavel A.
PS1-02-093 .......................................... 1082
Lewin, Susanne
PS1-01-003 .......................................... 996
L’Heureux, Nicolas
ESB-SFB S-04 ...................................... 813
N12-03 .............................................. 736
S01-03 .............................................. 92
S06-05 .............................................. 402
Li, Amy
N09-02 .............................................. 471
Li, Chunmei
PS2-01-010 .......................................... 1481
Li, Danyang
N06-04 .............................................. 314
Li, Dichen
PS1-04-155 .......................................... 1145
Li, Jia
PS1-11-363 .......................................... 1360
Li, Jiaping
PS1-04-157 .......................................... 1148
Li, Jinyu
S01-04 .............................................. 94
Li, Runrun
K06-02 .............................................. 653
Li, Wei
ESB-CSBM S-03 .................................... 201
Li, Yang
S01-05 .............................................. 96
Li, Yaya
PS1-12-369 .......................................... 1367
Lia, Riccardo P.
K10-03 .............................................. 860
Liang, He
K02-06 .............................................. 184
Liang, Jia
PS2-08-262 .......................................... 1730
Licarete, Emilia
PS2-02-072 .......................................... 1542
Licciardello, Michela
K08-04 .............................................. 775
PS1-01-017 .......................................... 1011
PS2-14-438 .......................................... 1910
Licciulli, Antonio
PS1-04-159 .......................................... 1149
Licini, Caterina
N09-03 .............................................. 473
Lizcnar, Patricia
N04-02 .............................................. 232
Lieber, Roman
PS2-09-314 .......................................... 1779
Liebig, Nadine
N09-07 .............................................. 480
Liedl, Klaus R.
NANOSTEM 01-03 .................................. 347
Lietzow, Marvin
PS2-04-138 .......................................... 1606
Ligier, Olivier
PS2-01-028 .......................................... 1502
Likotrafiti, Eleni
PS1-12-391 .......................................... 1388
Limasale, Yanuar D.
PS1-10-345 .......................................... 1342
Limones-Ahijón, Blanca
PS2-02-092 .......................................... 1563
Lin, Quankui
PS1-07-247 .......................................... 1239
Linckian, Cristina
PS2-05-190 .......................................... 1654
Lindh, Jonas
N10-04 .............................................. 550
Linhardt, Robert J.
N07-03 .............................................. 332
Lino, Miguel
N06-05 .............................................. 316
NANOSTEM 02-04 .................................. 356
Linti, Carsten
PS2-01-060 .......................................... 1528
PS2-13-420 .......................................... 1892
Lipovka, Anna
PS2-02-078 .......................................... 1548
Lisignoli, Gina
PS1-08-297 .......................................... 1287
PS2-04-168 .......................................... 1630
Litowczenko, Jagoda
PS1-13-421 .......................................... 1418
Little, Suzanne
N13-02 .............................................. 818
Liu, Changsheng
ESB-CSBM S-KL01 .................................. 199

Page 1983 of 2028
Author Index

Liu, Hao
N13-04 ........................................... 822
Liverani, Liliana
PS1-06-215 .................................... 1208
PS2-01-054 .................................... 1525
PS2-03-116 .................................... 1584
YSF Opening .................................... 66
Llanes, Luis
N08-04 ........................................... 456
Llop, Jordi
N06-08 ........................................... 323
Loca, Dagmija
PS1-04-133 .................................... 1122
PS1-04-143 .................................... 1132
PS1-06-203 .................................... 1195
PS1-08-277 .................................... 1269
PS1-10-327 .................................... 1324
Locs, Janis
PS1-04-133 .................................... 1122
PS1-10-327 .................................... 1324
PS2-06-204 .................................... 1671
PS2-06-212 .................................... 1679
PS2-06-214 .................................... 1681
Lode, Anja
K03-05 ........................................... 261
N13-07 ........................................... 829
PS1-01-011 .................................... 1004
PS1-11-351 .................................... 1348
PS2-07-220 .................................... 1687
S08-05 ........................................... 512
Loffredo, Augusto V.
N01-07 ........................................... 141
Logeart-Avramoglou, Delphine
PS1-04-165 .................................... 1153
Loinaz, Iraida
N06-08 ........................................... 323
Loll, François
N16-05 ........................................... 940
Lombardo, Sonia
NANOSTEM 03-02 .......................... 360
NANOSTEM 02-02 .......................... 352
Londoño López, Martha E.
PS1-04-167 .................................... 1156
Longhorn, Daniel
PS2-09-316 .................................... 1780
Lopera Echavarria, Aura M.
PS1-04-167 .................................... 1156
Lopes Martins, Maria Cristina
S03-02 .......................................... 115
Lopes, Jorge
PS1-13-407 .................................... 1406
Lopes, Maria C.
S02-03 .......................................... 106
Lopes, Soraia V.
PS2-12-382 .................................... 1851

Lopes, Susana P.
ESB-SIBB S01-03 ........................... 520
Lopes, Tânia
NANOSTEM 03-05 ........................... 365
López Canosa, Adrián
PS1-14-425 .................................... 1423
Lopez de Armentia, Sara
PS2-05-176 .................................... 1640
López de Armentia, Sara
PS1-05-201 .................................... 1191
Lopez Serrano, Cristina
PS1-08-273 .................................... 1265
López-Dolado, Elisa
PS2-07-254 .................................... 1722
López-Gállego, Fernando
N06-08 ........................................... 323
López-Iglesias, Clara
AERO InvF-01 ................................. 618
AERO InvF-06 ................................. 630
PS1-05-179 .................................... 1168
Lopez-Martinez, Maria J.
PS1-03-109 .................................... 1097
Lopez-Noriega, Adolfo
PS1-02-065 .................................... 1054
López-Periago, Ana M.
AERO InvF-03 ................................. 623
Lörch, Christiane
PS2-13-420 .................................... 1892
Lorenz, Christian
K09-03 .......................................... 844
Loterie, Damien
S01-05 .......................................... 96
Lotz, Oliver
N08-02 .......................................... 452
Loukelis, Konstantinos
S10-05 .......................................... 688
Lourenço, Bianca N.
ESB-SIBB S02-03 ........................... 709
PS2-01-046 .................................... 1516
Louth, Sophie E.
S03-04 .......................................... 119
Lozhkomoev, Aleksandr
N11-09 .......................................... 581
Lu, Hsuan-Heng
PS1-05-183 .................................... 1172
Lu, Yi-Tung
PS1-08-283 .................................... 1275
Lucchetti, Agnese
PS1-09-311 .................................... 1304
Luciani, Giuseppina
N15-04 .......................................... 923
S03-05 .......................................... 121
Lúcio, Marlène
ESB-CRS S-05 ............................... 429
Author Index

Ludwig, Barbara  
PS2-07-220..................................................1687

Luginina, Jevgenija  
PS1-08-301..................................................1291

Luiz Souto Borges, Alexandre  
PS1-01-013..................................................1007

Lukic, Ivana Z.  
AERO InvF-05..................................................628  
AERO PS01-01..................................................597  
AERO S01-04..................................................594

Lukk, Tit  
AERO PS01-05..................................................605

Lungu, Adriana  
PS1-01-035..................................................1027

Lupeti de Cena, Gabrielle  
PS1-01-013..................................................1007

Luque-Agudo, Veronica  
PS1-15-465..................................................1468

Lusquiños, Fernando  
S14-07.......................................................884

Lust, Suzette  
K09-03.......................................................844

Luxenhofer, Robert  
N07-04.......................................................335

Luyten, Frank P.  
ESB-SLABO S-03.............................................283

Luzuriaga, Jon  
K04-05.......................................................386

Lymboris, Andreas  
TRS-S02-04..................................................172

Lymeraki, Evgenia  
PS1-12-391..................................................1388

Lyngstadaas, S. Petter  
PS1-07-231..................................................1224

Lyu, Yaqi  
PS1-08-267..................................................1259

M

M. A. Domingues, Rui  
K02-07.......................................................186

Maas, Judith  
PS1-02-073..................................................1060

Macagnano, Antonella  
N15-04.......................................................923

Macedo, Maria H.  
N06-07.......................................................320

Machillot, Paul  
N02-01.......................................................148  
PS1-04-165..................................................1153

Maciejewska, Barbara M.  
PS1-13-421..................................................1418

Maciel, Marta M.  
PS1-02-093..................................................1082  
S06-02.......................................................395

MacManus, David  
N11-05.......................................................573

Madaghiela, Marta  
PS2-03-122..................................................1591

Madarieta, Iratxe  
PS2-07-228..................................................1695

Madrigal, Julián C.  
AERO PS01-03...............................................601

Maeztu Redin, Deyo  
S05-03.......................................................270

Magalhães, Fernão  
PS1-08-289..................................................1280  
K04-03.......................................................383  
PS2-05-192..................................................1657  
PS2-13-412..................................................1884

Magalhães, João  
PS1-08-291..................................................1282

Magalhães, Mariana  
N16-02.......................................................934

Magalhães, Natália  
PS1-10-333..................................................1329

Magalhães, Rui  
PS2-02-070..................................................1539

Magariños, Beatriz  
N11-02.......................................................565  
PS1-05-179..................................................1168

Mager, Ilona  
PS1-02-073..................................................1060

Maglio, Melania  
PS1-06-211..................................................1204

Magnan, Laure  
ESB-SFB S-04...............................................813

Magnaudeix, Amandine  
PS1-02-101..................................................1091  
PS1-03-119..................................................1105

Maguire, Oran  
PS2-12-374..................................................1842

Magyari, Klara  
PS1-05-177..................................................1166  
PS2-02-086..................................................1558

Magyari, Klára  
PS1-02-071..................................................1058  
PS2-02-072..................................................1542

Mahmoudi, Nadia  
PS1-08-279..................................................1271

Mahou, Redouan  
PS2-01-028..................................................1502

Maia Ferreira, Igor  
PS2-03-130..................................................1598

Mainardi, Andrea  
PS2-14-444..................................................1915

Mair, Vincent T.  
K03-04.......................................................259

Maire, Murielle  
PS1-09-307..................................................1298

Page 1985 of 2028
<table>
<thead>
<tr>
<th>Author Name</th>
<th>Index Number</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maitz, Manfred</td>
<td>K06-05</td>
<td>659</td>
</tr>
<tr>
<td>Majidi, Sara S.</td>
<td>ESB-CSBM S-05</td>
<td>205</td>
</tr>
<tr>
<td>Major, Roman</td>
<td>PS2-09-310</td>
<td>1775</td>
</tr>
<tr>
<td></td>
<td>PS2-13-414</td>
<td>1886</td>
</tr>
<tr>
<td>Mąkiewicz, Mariusz</td>
<td>PS2-08-290</td>
<td>1753</td>
</tr>
<tr>
<td>Maksimovic, Svetolik N.</td>
<td>AERO InvF-05</td>
<td>628</td>
</tr>
<tr>
<td>Maksymowicz, Wojciech</td>
<td>PS2-07-224</td>
<td>1691</td>
</tr>
<tr>
<td>Makvandi, Pooyan</td>
<td>N02-08</td>
<td>164</td>
</tr>
<tr>
<td>Makwandi, Pooyan</td>
<td>PS2-07-230</td>
<td>1698</td>
</tr>
<tr>
<td>Malda, Jos</td>
<td>K03-06</td>
<td>263</td>
</tr>
<tr>
<td></td>
<td>N04-03</td>
<td>234</td>
</tr>
<tr>
<td></td>
<td>N08-02</td>
<td>452</td>
</tr>
<tr>
<td></td>
<td>N09-08</td>
<td>482</td>
</tr>
<tr>
<td></td>
<td>N14-02</td>
<td>898</td>
</tr>
<tr>
<td></td>
<td>N14-05</td>
<td>904</td>
</tr>
<tr>
<td></td>
<td>PS1-13-403</td>
<td>1402</td>
</tr>
<tr>
<td></td>
<td>S01-04</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>S01-05</td>
<td>96</td>
</tr>
<tr>
<td>Maleki, Hajar H.</td>
<td>AERO S02-KL02</td>
<td>607</td>
</tr>
<tr>
<td>Malfreyt, Patrice</td>
<td>PS2-10-320</td>
<td>1784</td>
</tr>
<tr>
<td>Malina, Dagmara</td>
<td>PS2-08-300</td>
<td>1762</td>
</tr>
<tr>
<td>Malkmus, Christoph</td>
<td>PS1-01-033</td>
<td>1025</td>
</tr>
<tr>
<td>Mallet, Jhony</td>
<td>TRS-S01-04</td>
<td>73</td>
</tr>
<tr>
<td>Mallo, Javier</td>
<td>Biomechanics 03-06</td>
<td>971</td>
</tr>
<tr>
<td>Man, Kenny</td>
<td>PS1-15-447</td>
<td>1448</td>
</tr>
<tr>
<td>Manassero, Mathieu</td>
<td>ESB-BMSJ S-05</td>
<td>729</td>
</tr>
<tr>
<td></td>
<td>PS1-04-165</td>
<td>1153</td>
</tr>
<tr>
<td></td>
<td>PS2-07-250</td>
<td>1718</td>
</tr>
<tr>
<td></td>
<td>S05-03</td>
<td>270</td>
</tr>
<tr>
<td>Mandrice, Luisa</td>
<td>PS1-13-399</td>
<td>1398</td>
</tr>
<tr>
<td>Manferdini, Cristina</td>
<td>PS1-08-297</td>
<td>1287</td>
</tr>
<tr>
<td></td>
<td>PS2-04-168</td>
<td>1630</td>
</tr>
<tr>
<td>Manfredi, Marcello</td>
<td>PS2-10-338</td>
<td>1803</td>
</tr>
<tr>
<td>Manini, Paola</td>
<td>PS2-03-118</td>
<td>1586</td>
</tr>
<tr>
<td>Maniura, Katharina</td>
<td>PS2-02-064</td>
<td>1532</td>
</tr>
<tr>
<td>Mano, Joao</td>
<td>N01-04</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td>PS1-01-029</td>
<td>1022</td>
</tr>
<tr>
<td>Mano, João F.</td>
<td>ESB-BMSJ S-03</td>
<td>724</td>
</tr>
<tr>
<td></td>
<td>ESB-SIBB S02-05</td>
<td>713</td>
</tr>
<tr>
<td></td>
<td>ESB-SIBB S02-08</td>
<td>719</td>
</tr>
<tr>
<td></td>
<td>GW AW01-01</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>K05-05</td>
<td>499</td>
</tr>
<tr>
<td></td>
<td>K09-06</td>
<td>850</td>
</tr>
<tr>
<td></td>
<td>N03-04</td>
<td>215</td>
</tr>
<tr>
<td></td>
<td>N04-05</td>
<td>239</td>
</tr>
<tr>
<td></td>
<td>N05-03</td>
<td>294</td>
</tr>
<tr>
<td></td>
<td>N05-08</td>
<td>301</td>
</tr>
<tr>
<td></td>
<td>N09-04</td>
<td>475</td>
</tr>
<tr>
<td></td>
<td>N11-04</td>
<td>571</td>
</tr>
<tr>
<td></td>
<td>N16-03</td>
<td>936</td>
</tr>
<tr>
<td></td>
<td>PS1-01-047</td>
<td>1037</td>
</tr>
<tr>
<td></td>
<td>PS1-02-093</td>
<td>1082</td>
</tr>
<tr>
<td></td>
<td>PS1-14-429</td>
<td>1428</td>
</tr>
<tr>
<td></td>
<td>PS2-03-108</td>
<td>1578</td>
</tr>
<tr>
<td></td>
<td>PS2-09-306</td>
<td>1770</td>
</tr>
<tr>
<td></td>
<td>S01-02</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>S02-03</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>S02-04</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>S06-02</td>
<td>395</td>
</tr>
<tr>
<td>Mantovani, Diego</td>
<td>N12-02</td>
<td>734</td>
</tr>
<tr>
<td>Mäntylä, Elina</td>
<td>S09-06</td>
<td>674</td>
</tr>
<tr>
<td>Manz, Andreas</td>
<td>PS2-13-398</td>
<td>1868</td>
</tr>
<tr>
<td>Manzanares, Maria-Cristina</td>
<td>S14-KL01</td>
<td>872</td>
</tr>
<tr>
<td>Manzocoo, Lara</td>
<td>AERO S03-02</td>
<td>641</td>
</tr>
<tr>
<td></td>
<td>AERO S03-03</td>
<td>643</td>
</tr>
<tr>
<td>Maoz, Ben M.</td>
<td>K09-KL01</td>
<td>840</td>
</tr>
<tr>
<td></td>
<td>PS2-14-436</td>
<td>1908</td>
</tr>
<tr>
<td>Marais, Sébastien</td>
<td>ESB-SFB S-04</td>
<td>813</td>
</tr>
<tr>
<td>Marasso, Simone L.</td>
<td>PS1-14-435</td>
<td>1435</td>
</tr>
<tr>
<td></td>
<td>PS2-14-438</td>
<td>1910</td>
</tr>
<tr>
<td>Marattukalam, Jithin James</td>
<td>PS2-02-076</td>
<td>1546</td>
</tr>
<tr>
<td>Marchal, Philippe</td>
<td>PS1-01-029</td>
<td>1022</td>
</tr>
<tr>
<td>Marchandise, Pierre</td>
<td>PS1-02-085</td>
<td>1074</td>
</tr>
<tr>
<td>Marchetti-Déschmann, Martina</td>
<td>N01-05</td>
<td>137</td>
</tr>
<tr>
<td>Name</td>
<td>Session Code</td>
<td>Page</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>--------------</td>
<td>------</td>
</tr>
<tr>
<td>Marchiori, Gregorio</td>
<td>PS1-06-211</td>
<td>1204</td>
</tr>
<tr>
<td>Marie-Christine, Durrie</td>
<td>PS1-08-273</td>
<td>1265</td>
</tr>
<tr>
<td>Marin, Edurne</td>
<td>K04-05</td>
<td>386</td>
</tr>
<tr>
<td></td>
<td>S06-07</td>
<td>406</td>
</tr>
<tr>
<td>Marinho, Daniel</td>
<td>Biomechanics 02-01</td>
<td>958</td>
</tr>
<tr>
<td>Marino, Attilio</td>
<td>N01-02</td>
<td>131</td>
</tr>
<tr>
<td>Markhoff, Jana</td>
<td>PS1-15-461</td>
<td>1463</td>
</tr>
<tr>
<td></td>
<td>PS2-06-208</td>
<td>1675</td>
</tr>
<tr>
<td>Markovic, Marica</td>
<td>ESB-ISBF S-04</td>
<td>537</td>
</tr>
<tr>
<td>Maroni, Alessandra</td>
<td>S04-05</td>
<td>196</td>
</tr>
<tr>
<td>Marquaille, Pierre</td>
<td>PS2-08-274</td>
<td>1739</td>
</tr>
<tr>
<td>Marques, Alexandra P.</td>
<td>K02-05</td>
<td>182</td>
</tr>
<tr>
<td>Marques, Joana F.</td>
<td>PS2-02-100</td>
<td>1572</td>
</tr>
<tr>
<td>Marques, Paula A.A.P.</td>
<td>PS2-01-004</td>
<td>1474</td>
</tr>
<tr>
<td></td>
<td>K04-06</td>
<td>388</td>
</tr>
<tr>
<td>Marquette, Christophe</td>
<td>PS1-01-001</td>
<td>994</td>
</tr>
<tr>
<td>Marradi, Marco</td>
<td>N06-08</td>
<td>323</td>
</tr>
<tr>
<td>Marroquin-Garcia, Ramiro</td>
<td>N13-03</td>
<td>820</td>
</tr>
<tr>
<td>Marsano, Anna</td>
<td>PS2-14-444</td>
<td>1915</td>
</tr>
<tr>
<td>Marszalek, Marta</td>
<td>PS2-15-466</td>
<td>1940</td>
</tr>
<tr>
<td>Martel, Bernard</td>
<td>PS1-07-223</td>
<td>1217</td>
</tr>
<tr>
<td></td>
<td>PS2-01-002</td>
<td>1472</td>
</tr>
<tr>
<td>Martens, Ann</td>
<td>S05-02</td>
<td>267</td>
</tr>
<tr>
<td>Martin Moldes, Zaira</td>
<td>PS2-01-010</td>
<td>1481</td>
</tr>
<tr>
<td>Martin, Sara</td>
<td>N10-03</td>
<td>548</td>
</tr>
<tr>
<td>Martinelli, Chiara</td>
<td>N15-02</td>
<td>918</td>
</tr>
<tr>
<td>Martinez Garcia, Francisco D.</td>
<td>PS1-02-063</td>
<td>1052</td>
</tr>
<tr>
<td>Martinez Perez, David</td>
<td>K10-06</td>
<td>867</td>
</tr>
<tr>
<td>Martinez, Elena</td>
<td>N06-07</td>
<td>320</td>
</tr>
<tr>
<td>Martínez, Fabián A.</td>
<td>PS1-11-365</td>
<td>1362</td>
</tr>
<tr>
<td>Martínez-de-Tejada, Guillermo</td>
<td>PS2-12-392</td>
<td>1860</td>
</tr>
<tr>
<td>Martínez-Tong, Daniel</td>
<td>K04-05</td>
<td>386</td>
</tr>
<tr>
<td>Martín-González, Marisol</td>
<td>N07-06</td>
<td>339</td>
</tr>
<tr>
<td>Martinier, Isabelle</td>
<td>S02-05</td>
<td>110</td>
</tr>
<tr>
<td>Martinotti, Simona</td>
<td>PS1-07-227</td>
<td>1221</td>
</tr>
<tr>
<td>Martins, Ana S.</td>
<td>S11-03</td>
<td>697</td>
</tr>
<tr>
<td>Martins, Cláudia</td>
<td>PS1-10-323</td>
<td>1319</td>
</tr>
<tr>
<td></td>
<td>PS1-10-325</td>
<td>1321</td>
</tr>
<tr>
<td>Martins, Emanuel</td>
<td>PS1-01-006</td>
<td>1477</td>
</tr>
<tr>
<td>Martins, Maria C.</td>
<td>N15-07</td>
<td>929</td>
</tr>
<tr>
<td>Martorell, Ximena</td>
<td>PS1-07-257</td>
<td>1248</td>
</tr>
<tr>
<td>Marzi, Ingo</td>
<td>PS2-01-006</td>
<td>1477</td>
</tr>
<tr>
<td>Marzi, Julia</td>
<td>PS1-13-409</td>
<td>1408</td>
</tr>
<tr>
<td></td>
<td>PS2-01-036</td>
<td>1508</td>
</tr>
<tr>
<td>Mas Moruno, Carles</td>
<td>N12-04</td>
<td>738</td>
</tr>
<tr>
<td>Mashanov, Vladimir</td>
<td>N01-04</td>
<td>136</td>
</tr>
<tr>
<td>Mas-Moruno, Carles</td>
<td>N08-04</td>
<td>456</td>
</tr>
<tr>
<td>Masnou, Agnès</td>
<td>N04-02</td>
<td>232</td>
</tr>
<tr>
<td>Massera, Jonathan</td>
<td>PS1-04-145</td>
<td>1134</td>
</tr>
<tr>
<td></td>
<td>S08-KL01</td>
<td>505</td>
</tr>
<tr>
<td>Masuda, Yuki</td>
<td>PS1-03-113</td>
<td>1100</td>
</tr>
<tr>
<td>Mata-Leija, Aida</td>
<td>PS2-05-188</td>
<td>1652</td>
</tr>
<tr>
<td>Mateos Timoneda, Miguel Angel</td>
<td>N08-01</td>
<td>450</td>
</tr>
<tr>
<td>Mateos-Timoneda, Miguel Angel</td>
<td>N02-03</td>
<td>154</td>
</tr>
</tbody>
</table>

Page 1987 of 2028
Author Index

Mateu-Sanz, Miguel
K09-05 ........................................ 848

Mathieu, Eric
PS2-13-400 .................................... 1871

Mathieu, Noëlle
PS1-10-335 .................................... 1331

Maton, Mickael
PS1-07-223 .................................... 1217

Maton, Mickaël
PS2-01-002 .................................... 1472

Matos, Ricardo J.R.
PS1-03-117 .................................... 1102

Matricardi, Pietro
ESB-BIOMAT S-05 ............................ 442

Matson, John
N14-01 .......................................... 896

Matsusaki, Michiya
S01-04 .......................................... 94

Matthies, Alexander
PS1-14-443 .................................... 1444

Matthiesen, Isabelle
PS2-03-110 .................................... 1580

Mattioli-Belmonte, Monica
N04-07 .......................................... 243
N04-08 .......................................... 245
N09-03 .......................................... 473
S01-07 .......................................... 101

Mau, Clara
PS1-07-227 .................................... 1221
PS2-12-372 .................................... 1840
S11-KL02 ...................................... 696

Maubon, Nathalie
NANOSTEM 03-02 .............................. 360

Maughan, Jack
K04-02 .......................................... 380

Mauri, Emanuele
N16-09 .......................................... 948

Maurice, Emeline
PS1-04-165 .................................... 1153
PS2-07-250 .................................... 1718

Mayol, Laura
S06-03 .......................................... 398

Mayr, Hermann O.
PS2-10-332 .................................... 1797

Mayr, Stefan
PS2-08-272 .................................... 1736

M’Bengue, Marie-Stella
PS2-01-002 .................................... 1472

Mbundi, Lubinda M.
PS1-07-241 .................................... 1234

McAllister, Todd
S06-05 .......................................... 402

McCarthy, Helen
N14-07 .......................................... 909

McCarthy, Helen O.
N11-01 .......................................... 563
PS2-04-154 .................................... 1618

McCarthy, Hellen O.
N06-02 .......................................... 309

McClelland, Nicola
PS2-11-358 .................................... 1825

McCormick, Christopher
PS2-09-316 .................................... 1780

McCoy, Colin
PS1-11-357 .................................... 1354
PS1-11-363 .................................... 1360
PS2-11-358 .................................... 1825
PS2-11-360 .................................... 1827
PS2-11-362 .................................... 1829

McCoy, Colin P.
PS2-11-348 .................................... 1815

McDonald, Tom
PS1-07-237 .................................... 1229

McFadden, Ryan
PS1-15-459 .................................... 1460

McGarry, Katie
S04-04 .......................................... 194

McHugh, Peter E.
PS2-02-068 .................................... 1537

McKenzie, David R.
N08-02 .......................................... 452

McKiernan, Eoin
S04-04 .......................................... 194

McKitrick, Austin
PS2-02-082 .................................... 1552
S13-02 .......................................... 800

McLaren, Jane
PS2-04-160 .................................... 1622

MCormack, James
N03-05 .......................................... 217

Meazzo, Martina
PS1-03-123 .................................... 1109

Medina, Chantal
PS1-01-061 .................................... 1048
S01-03 .......................................... 92

Medrano David, Daniela
PS1-04-167 .................................... 1156

Mees, Barent
PS2-14-426 .................................... 1898

Meireles, Catarina
S15-05 .......................................... 893

Meissner, Heike
N09-01 .......................................... 469

Mekseriwattana, Wid
PS2-10-334 .................................... 1799

Mela, Petra
PS1-03-129 .................................... 1117
PS1-12-379 .................................... 1376
Michopoulou, Anna
PS2-01-058 ........................................... 1526

Migny, Jean-Marc
PS1-18-076 ........................................... 987

Migny, Véronique
PS2-07-250 ........................................... 1718

Mikko, Anneloes
ESB-SIBB PS2-01-043 .................................. 482

Mikko, Kirsi
PS1-10-197 ........................................... 1187

Miklosic, Gregor
PS2-01-020 ........................................... 1493

Milanese, Daniel
PS1-13-413 ........................................... 1410

Miles, Anthony
S15-04 ........................................... 891

Milewska, Adrianna
PS2-12-370 ........................................... 1838

Milivojevic, Milena
PS1-14-439 ........................................... 1440

Millan, Rosendo
PS1-01-031 ........................................... 1023

Milovanovic, Stojka L.
AERO InvF ........................................... 628
AERO PS01-01 ........................................... 597
AERO S01-04 ........................................... 594

Mimiroglu, Didem
PS2-13-402 ........................................... 1874

Minervini, Beatrice
PS1-14-435 ........................................... 1435

Minguela, Joaquim
N08-04 ........................................... 456

Minsart, Manon
PS2-08-276 ........................................... 1741

Miola, Marta
PS1-13-399 ........................................... 1398
PS2-03-116 ........................................... 1584
PS2-15-460 ........................................... 1934

Mira, Cristina
COST AEROGELS KL-01 ................................ 587

Miranda, Catarina S.
PS1-07-221 ........................................... 1215
PS2-07-226 ........................................... 1693

Miranda, Margarida S.
N15-01 ........................................... 916

Miranda, Rodolfo
PS2-07-254 ........................................... 1722

Miras-Moreno, Sergio
Biomechanics 03-03 ................................ 963
Biomechanics 04-02 ................................ 976

Mirzaali, Mohammad J.
PS1-01-043 ........................................... 1035

Mobini, Sahba
N07-06 ........................................... 339
PS2-02-092 ........................................... 1563

Page 1989 of 2028
<table>
<thead>
<tr>
<th>Author</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monteiro, Maria V.</td>
<td>ESB-SIBB S02-05</td>
<td>713</td>
</tr>
<tr>
<td>Monteiro-Rodriguez, Juan Jose</td>
<td>AERO InvF-04</td>
<td>625</td>
</tr>
<tr>
<td>Montesi, Monica</td>
<td>N09-05</td>
<td>476</td>
</tr>
<tr>
<td>Montoya Goez, Yesid D.J.</td>
<td>PS1-04-167</td>
<td>1156</td>
</tr>
<tr>
<td>Montufar, Edgar B.</td>
<td>PS2-02-080</td>
<td>1550</td>
</tr>
<tr>
<td>Mooney, David</td>
<td>Int AW-01</td>
<td>833</td>
</tr>
<tr>
<td>Moor, Jessica</td>
<td>PS2-11-358</td>
<td>1825</td>
</tr>
<tr>
<td>Moquin, Paul</td>
<td>AERO S03-KL03</td>
<td>637</td>
</tr>
<tr>
<td>Mora-Boza, Ana</td>
<td>N05-01</td>
<td>290</td>
</tr>
<tr>
<td>Moraes Saldanha de Oliveira, Rodrigo L.</td>
<td>PS2-03-130</td>
<td>1598</td>
</tr>
<tr>
<td>Morais, Miguel R.G.</td>
<td>PS1-13-419</td>
<td>1416</td>
</tr>
<tr>
<td>Morales, Bernardo J.</td>
<td>PS1-11-365</td>
<td>1362</td>
</tr>
<tr>
<td>Morales, M. Puerto</td>
<td>PS2-12-380</td>
<td>1849</td>
</tr>
<tr>
<td>Morand, Sanela</td>
<td>N02-01</td>
<td>148</td>
</tr>
<tr>
<td>Morch, Yrr</td>
<td>S12-02</td>
<td>786</td>
</tr>
<tr>
<td>Moreira Marques, Joana</td>
<td>PS1-12-387</td>
<td>1383</td>
</tr>
<tr>
<td>Moreira Teixeira, Liliana S.</td>
<td>PS2-14-430</td>
<td>1901</td>
</tr>
<tr>
<td>Moreira, Débora A.</td>
<td>PS2-13-418</td>
<td>1890</td>
</tr>
<tr>
<td>Moreira, Joana</td>
<td>N11-08</td>
<td>579</td>
</tr>
<tr>
<td>Moreira, Luís</td>
<td>PS1-11-359</td>
<td>1356</td>
</tr>
<tr>
<td>Moreno, Pedro M.</td>
<td>S11-03</td>
<td>697</td>
</tr>
<tr>
<td>Morgado, Jorge</td>
<td>N07-03</td>
<td>332</td>
</tr>
<tr>
<td>Morgan, Francis</td>
<td>N16-08</td>
<td>946</td>
</tr>
<tr>
<td>Moriarty, Ti F.</td>
<td>PS2-13-410</td>
<td>1881</td>
</tr>
<tr>
<td>Author</td>
<td>Index</td>
<td>Page</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-------------</td>
<td>------</td>
</tr>
<tr>
<td>Morita, Yusuke</td>
<td>N03-02</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>N03-08</td>
<td>225</td>
</tr>
<tr>
<td></td>
<td>PS1-02-081</td>
<td>1070</td>
</tr>
<tr>
<td></td>
<td>PS1-03-113</td>
<td>1100</td>
</tr>
<tr>
<td>Moroni, Lorenzo</td>
<td>ESB-CRS S-03</td>
<td>424</td>
</tr>
<tr>
<td></td>
<td>ESB-JSBF S-03</td>
<td>535</td>
</tr>
<tr>
<td></td>
<td>K01-02</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>K02-03</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>N05-09</td>
<td>304</td>
</tr>
<tr>
<td></td>
<td>N13-03</td>
<td>820</td>
</tr>
<tr>
<td></td>
<td>N14-01</td>
<td>896</td>
</tr>
<tr>
<td></td>
<td>N14-03</td>
<td>900</td>
</tr>
<tr>
<td></td>
<td>N16-04</td>
<td>938</td>
</tr>
<tr>
<td></td>
<td>N16-08</td>
<td>946</td>
</tr>
<tr>
<td></td>
<td>PS1-04-157</td>
<td>1148</td>
</tr>
<tr>
<td></td>
<td>PS1-09-315</td>
<td>1310</td>
</tr>
<tr>
<td></td>
<td>PS2-04-132</td>
<td>1601</td>
</tr>
<tr>
<td></td>
<td>S01-KL01</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>S06-06</td>
<td>404</td>
</tr>
<tr>
<td></td>
<td>S07-05</td>
<td>417</td>
</tr>
<tr>
<td></td>
<td>S09-07</td>
<td>676</td>
</tr>
<tr>
<td></td>
<td>S14-03</td>
<td>876</td>
</tr>
<tr>
<td>Morra, Emily</td>
<td>N12-06</td>
<td>742</td>
</tr>
<tr>
<td>Moscatelli, Monica</td>
<td>PS1-15-455</td>
<td>1454</td>
</tr>
<tr>
<td></td>
<td>PS2-15-452</td>
<td>1924</td>
</tr>
<tr>
<td>Moser, Christophe</td>
<td>S01-05</td>
<td>96</td>
</tr>
<tr>
<td>Mosina, Marika</td>
<td>PS2-06-204</td>
<td>1671</td>
</tr>
<tr>
<td></td>
<td>PS2-06-214</td>
<td>1681</td>
</tr>
<tr>
<td>Mota, Carlos</td>
<td>ESB-CRS S-03</td>
<td>424</td>
</tr>
<tr>
<td></td>
<td>K02-03</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>PS1-09-315</td>
<td>1310</td>
</tr>
<tr>
<td></td>
<td>PS2-04-132</td>
<td>1601</td>
</tr>
<tr>
<td></td>
<td>S07-05</td>
<td>417</td>
</tr>
<tr>
<td>Mota, Pedro</td>
<td>K09-04</td>
<td>846</td>
</tr>
<tr>
<td></td>
<td>N01-06</td>
<td>139</td>
</tr>
<tr>
<td>Motevallian, Saeed S.</td>
<td>PS2-12-374</td>
<td>1842</td>
</tr>
<tr>
<td>Mouchtaridi, Christina</td>
<td>PS1-02-089</td>
<td>1079</td>
</tr>
<tr>
<td></td>
<td>PS2-02-088</td>
<td>1560</td>
</tr>
<tr>
<td>Moura, Ana</td>
<td>K10-04</td>
<td>863</td>
</tr>
<tr>
<td>Moura, Duarte</td>
<td>PS1-08-289</td>
<td>1280</td>
</tr>
<tr>
<td></td>
<td>PS2-05-192</td>
<td>1657</td>
</tr>
<tr>
<td>Moura, Rui P.</td>
<td>PS2-12-390</td>
<td>1858</td>
</tr>
<tr>
<td>Mourão, André</td>
<td>NANOSTEM 04-02</td>
<td>370</td>
</tr>
<tr>
<td>Moya, Elisa L.</td>
<td>NANOSTEM 03-02</td>
<td>360</td>
</tr>
<tr>
<td>Mozetic, Pamela</td>
<td>N16-09</td>
<td>948</td>
</tr>
<tr>
<td>Muallah, David</td>
<td>N09-01</td>
<td>469</td>
</tr>
<tr>
<td>Mueller, Kilian M.A.</td>
<td>PS1-03-129</td>
<td>1117</td>
</tr>
<tr>
<td>Mulder, Willem J.M.</td>
<td>ESB-SFB S-KL02</td>
<td>809</td>
</tr>
<tr>
<td>Müller, Christoph</td>
<td>ESB-CSBM S-05</td>
<td>205</td>
</tr>
<tr>
<td>Muller, Quentin</td>
<td>PS1-01-039</td>
<td>1031</td>
</tr>
<tr>
<td>Müller, Verónica</td>
<td>PS2-15-458</td>
<td>1932</td>
</tr>
<tr>
<td>Müller-Newen, Gerhard</td>
<td>PS1-02-075</td>
<td>1063</td>
</tr>
<tr>
<td>Mutiginer Domínguez, Marta</td>
<td>PS1-02-089</td>
<td>1079</td>
</tr>
<tr>
<td>Munafò, Sara</td>
<td>ESB-CRS S-03</td>
<td>424</td>
</tr>
<tr>
<td>Munawar, Muhammad A.</td>
<td>PS1-01-023</td>
<td>1015</td>
</tr>
<tr>
<td></td>
<td>PS1-01-053</td>
<td>1044</td>
</tr>
<tr>
<td>Muñoz Hernández, Marta</td>
<td>PS1-02-089</td>
<td>1079</td>
</tr>
<tr>
<td></td>
<td>PS2-02-088</td>
<td>1560</td>
</tr>
<tr>
<td>Muñoz, Jone</td>
<td>N15-06</td>
<td>927</td>
</tr>
<tr>
<td>Munir, A</td>
<td>PS2-01-024</td>
<td>1498</td>
</tr>
<tr>
<td>Murillo, Gonzalo</td>
<td>N07-01</td>
<td>328</td>
</tr>
<tr>
<td></td>
<td>N10-02</td>
<td>546</td>
</tr>
<tr>
<td>Murphy, Ciara</td>
<td>N16-07</td>
<td>944</td>
</tr>
<tr>
<td></td>
<td>PS1-04-137</td>
<td>1127</td>
</tr>
<tr>
<td></td>
<td>PS2-08-284</td>
<td>1747</td>
</tr>
<tr>
<td>Mustafa, Kamal</td>
<td>PS2-01-018</td>
<td>1491</td>
</tr>
<tr>
<td>Myslorek, Caroline</td>
<td>NANOSTEM 03-01</td>
<td>359</td>
</tr>
<tr>
<td></td>
<td>NANOSTEM 03-03</td>
<td>362</td>
</tr>
<tr>
<td>Mzyk, Aldona</td>
<td>K06-02</td>
<td>653</td>
</tr>
<tr>
<td>Nadal, Jurandir</td>
<td>Biomechanics 04-04</td>
<td>982</td>
</tr>
</tbody>
</table>

Page 1991 of 2028
<table>
<thead>
<tr>
<th>Author</th>
<th>Index</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nadernezhad, Ali</td>
<td>K08-02</td>
<td>770</td>
</tr>
<tr>
<td></td>
<td>PS2-01-042</td>
<td>1514</td>
</tr>
<tr>
<td>Nadine, Sara</td>
<td>K09-06</td>
<td>850</td>
</tr>
<tr>
<td></td>
<td>N04-05</td>
<td>239</td>
</tr>
<tr>
<td></td>
<td>N11-04</td>
<td>571</td>
</tr>
<tr>
<td></td>
<td>PS2-09-306</td>
<td>1770</td>
</tr>
<tr>
<td>Nai, Kenneth</td>
<td>S03-04</td>
<td>119</td>
</tr>
<tr>
<td>Najmi, Ziba</td>
<td>PS1-15-455</td>
<td>1454</td>
</tr>
<tr>
<td>Nakano, Yuna</td>
<td>PS1-02-081</td>
<td>1070</td>
</tr>
<tr>
<td>Nakarada, Dura</td>
<td>PS1-07-255</td>
<td>1246</td>
</tr>
<tr>
<td>Nakieliski, Pawel</td>
<td>PS2-07-224</td>
<td>1691</td>
</tr>
<tr>
<td>Napio, Lucia</td>
<td>PS1-13-399</td>
<td>1398</td>
</tr>
<tr>
<td>Naren, Aruzhan</td>
<td>PS2-04-134</td>
<td>1603</td>
</tr>
<tr>
<td>Nascimento, Diana S.</td>
<td>K07-02</td>
<td>753</td>
</tr>
<tr>
<td></td>
<td>K07-06</td>
<td>763</td>
</tr>
<tr>
<td>Nasehi, Ramin</td>
<td>PS1-14-433</td>
<td>1434</td>
</tr>
<tr>
<td>Natali, María Lucia</td>
<td>PS2-05-200</td>
<td>1666</td>
</tr>
<tr>
<td>Navarro Cabello, Enrique</td>
<td>Biomechanics 04-01</td>
<td>974</td>
</tr>
<tr>
<td>Navarro, Enrique</td>
<td>Biomechanics 03-05</td>
<td>969</td>
</tr>
<tr>
<td></td>
<td>Biomechanics 03-06</td>
<td>971</td>
</tr>
<tr>
<td>Navascuez, Marcos</td>
<td>N06-08</td>
<td>323</td>
</tr>
<tr>
<td>Nawrotek, Katarzyna</td>
<td>PS2-08-290</td>
<td>1753</td>
</tr>
<tr>
<td>Nedelec, Jean-Marie</td>
<td>PS1-12-389</td>
<td>1385</td>
</tr>
<tr>
<td>Nehrass, Dirk</td>
<td>N02-06</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>PS2-13-410</td>
<td>1881</td>
</tr>
<tr>
<td>Nelson, Bradley J.</td>
<td>PL1-01</td>
<td>343</td>
</tr>
<tr>
<td>Neubauer, Martin</td>
<td>K08-03</td>
<td>772</td>
</tr>
<tr>
<td>Neuber, Christin</td>
<td>PS1-04-149</td>
<td>1138</td>
</tr>
<tr>
<td>Neudert, Marcus</td>
<td>N14-06</td>
<td>906</td>
</tr>
<tr>
<td></td>
<td>S07-05</td>
<td>417</td>
</tr>
<tr>
<td>Neumann, Hans-Georg</td>
<td>PS1-15-457</td>
<td>1457</td>
</tr>
<tr>
<td>Neuner, Andrea M.</td>
<td>NANO STEM 04-02</td>
<td>370</td>
</tr>
<tr>
<td>Neves, Joana</td>
<td>K09-03</td>
<td>844</td>
</tr>
<tr>
<td>Neves, Luisa</td>
<td>PS2-05-182</td>
<td>1647</td>
</tr>
<tr>
<td></td>
<td>PS2-07-242</td>
<td>1710</td>
</tr>
<tr>
<td>Neves, Mariana I.</td>
<td>S09-07</td>
<td>676</td>
</tr>
<tr>
<td>Neves, Nuno</td>
<td>PS1-13-407</td>
<td>1406</td>
</tr>
<tr>
<td>Neves, Sara C.</td>
<td>N05-06</td>
<td>297</td>
</tr>
<tr>
<td>Neves, Vera L.S.</td>
<td>N01-03</td>
<td>133</td>
</tr>
<tr>
<td>Nica, Valentin</td>
<td>PS2-12-378</td>
<td>1847</td>
</tr>
<tr>
<td>Nickel, Daniela</td>
<td>PS1-11-347</td>
<td>1345</td>
</tr>
<tr>
<td>Nicolett, Letizia</td>
<td>PS2-12-372</td>
<td>1840</td>
</tr>
<tr>
<td>Nicoli, Sara</td>
<td>PS1-12-377</td>
<td>1374</td>
</tr>
<tr>
<td>Nie, Guangjun</td>
<td>ESB-CSBM S-05</td>
<td>205</td>
</tr>
<tr>
<td>Niewolik, Daria</td>
<td>PS1-10-339</td>
<td>1336</td>
</tr>
<tr>
<td>Nijhuis, Suzanne</td>
<td>S11-05</td>
<td>701</td>
</tr>
<tr>
<td>Nikody, Martyna</td>
<td>PS1-04-157</td>
<td>1148</td>
</tr>
<tr>
<td>Nikolic, Sofija</td>
<td>N07-07</td>
<td>341</td>
</tr>
<tr>
<td>Nilsson, Per H</td>
<td>PS2-15-448</td>
<td>1919</td>
</tr>
<tr>
<td>Nilsson-Åhman, Hanna</td>
<td>N13-08</td>
<td>831</td>
</tr>
<tr>
<td>Nita, Loredana E.</td>
<td>PS2-08-268</td>
<td>1734</td>
</tr>
<tr>
<td></td>
<td>PS2-11-364</td>
<td>1831</td>
</tr>
<tr>
<td></td>
<td>PS2-11-355</td>
<td>1352</td>
</tr>
<tr>
<td>Nitti, Paola</td>
<td>PS1-04-159</td>
<td>1149</td>
</tr>
<tr>
<td></td>
<td>PS2-03-122</td>
<td>1591</td>
</tr>
<tr>
<td>Nogueira Alves, Ana P.</td>
<td>PS2-03-130</td>
<td>1598</td>
</tr>
<tr>
<td>Nogueira, Diogo E.S.</td>
<td>N07-03</td>
<td>332</td>
</tr>
<tr>
<td>Nogueira, Eugénia</td>
<td>PS1-11-361</td>
<td>1358</td>
</tr>
<tr>
<td>Nogueira, Liebert P.</td>
<td>N03-04</td>
<td>215</td>
</tr>
<tr>
<td>Nogués, Carme</td>
<td>PS1-04-139</td>
<td>1130</td>
</tr>
<tr>
<td>Name</td>
<td>Pages</td>
<td></td>
</tr>
<tr>
<td>------------------------------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td>Nolasco, Pedro</td>
<td>PS1-08-287</td>
<td>1278</td>
</tr>
<tr>
<td></td>
<td>PS2-08-286</td>
<td>1750</td>
</tr>
<tr>
<td>Norkus, Skirmantas</td>
<td>PS1-01-051</td>
<td>1041</td>
</tr>
<tr>
<td>Norman, Michael</td>
<td>K09-03</td>
<td>844</td>
</tr>
<tr>
<td>Norvez, Sophie</td>
<td>ESB-BMSJ S-05</td>
<td>729</td>
</tr>
<tr>
<td></td>
<td>PS1-13-423</td>
<td>1420</td>
</tr>
<tr>
<td></td>
<td>PS2-08-274</td>
<td>1739</td>
</tr>
<tr>
<td>Nottelet, Benjamin</td>
<td>K01-05</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>PS1-10-335</td>
<td>1331</td>
</tr>
<tr>
<td>Novaes de Oliveira, Antonio P.</td>
<td>PS2-03-130</td>
<td>1598</td>
</tr>
<tr>
<td>Novais, Sara C.N.</td>
<td>PS2-01-016</td>
<td>1488</td>
</tr>
<tr>
<td>Novak, Zoran</td>
<td>AERO S02-02</td>
<td>612</td>
</tr>
<tr>
<td>Novoa, Beatrix</td>
<td>PS2-02-090</td>
<td>1561</td>
</tr>
<tr>
<td>Nunes, César</td>
<td>NANOSTEM 03-05</td>
<td>365</td>
</tr>
<tr>
<td>Nunes, Cláudia</td>
<td>ESB-CRS S-04</td>
<td>427</td>
</tr>
<tr>
<td></td>
<td>N15-07</td>
<td>929</td>
</tr>
<tr>
<td></td>
<td>PS2-08-298</td>
<td>1760</td>
</tr>
<tr>
<td>Nunes, Nádia Sofia H.</td>
<td>K01-04</td>
<td>80</td>
</tr>
<tr>
<td>Nunes, Rute</td>
<td>PS1-12-387</td>
<td>1383</td>
</tr>
<tr>
<td>Nunez Bernal, Paulina</td>
<td>N14-02</td>
<td>898</td>
</tr>
<tr>
<td></td>
<td>S01-05</td>
<td>96</td>
</tr>
<tr>
<td>Nymark, Soille</td>
<td>S09-06</td>
<td>674</td>
</tr>
<tr>
<td>O</td>
<td>O'Brien, Fergal J.</td>
<td>K04-02</td>
</tr>
<tr>
<td></td>
<td>O'Reilly, Rachel</td>
<td>NANOSTEM 01-04</td>
</tr>
<tr>
<td></td>
<td>Obaíd, Miguel</td>
<td>PS1-07-257</td>
</tr>
<tr>
<td></td>
<td>Ober, Ciprian</td>
<td>PS2-02-086</td>
</tr>
<tr>
<td></td>
<td>Obradovic, Bojana</td>
<td>PS1-14-437</td>
</tr>
<tr>
<td></td>
<td>PS1-14-439</td>
<td>1440</td>
</tr>
<tr>
<td></td>
<td>O'Brien, Fergal</td>
<td>K05-02</td>
</tr>
<tr>
<td></td>
<td>N02-06</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>PS1-01-037</td>
<td>1029</td>
</tr>
<tr>
<td></td>
<td>PS1-04-137</td>
<td>1127</td>
</tr>
<tr>
<td></td>
<td>PS2-07-234</td>
<td>1704</td>
</tr>
<tr>
<td></td>
<td>N02-05</td>
<td>158</td>
</tr>
<tr>
<td></td>
<td>N11-01</td>
<td>563</td>
</tr>
<tr>
<td></td>
<td>PS1-04-151</td>
<td>1140</td>
</tr>
<tr>
<td></td>
<td>S07-03</td>
<td>412</td>
</tr>
<tr>
<td></td>
<td>O'Byrne, John</td>
<td>PS1-01-037</td>
</tr>
<tr>
<td></td>
<td>Ochetta, Paola</td>
<td>PS2-14-444</td>
</tr>
<tr>
<td></td>
<td>O'Cearbhaill, Eoin</td>
<td>N06-02</td>
</tr>
<tr>
<td></td>
<td>Ochiu, Lacramioara</td>
<td>PS1-08-269</td>
</tr>
<tr>
<td></td>
<td>Ochonska, Dorota</td>
<td>PS1-13-415</td>
</tr>
<tr>
<td></td>
<td>O'Connor, Cian</td>
<td>K05-02</td>
</tr>
<tr>
<td></td>
<td>Odabas, Sedat</td>
<td>PS1-07-253</td>
</tr>
<tr>
<td></td>
<td>Offerhaus, Herman L.</td>
<td>K08-03</td>
</tr>
<tr>
<td></td>
<td>Offioach, Ruben</td>
<td>PS2-11-354</td>
</tr>
<tr>
<td></td>
<td>Okke, Mohammad</td>
<td>PS2-07-252</td>
</tr>
<tr>
<td></td>
<td>Olaret, Elena</td>
<td>N05-02</td>
</tr>
<tr>
<td></td>
<td>PS1-07-219</td>
<td>1214</td>
</tr>
<tr>
<td></td>
<td>O'Leary, Cian</td>
<td>PS2-07-234</td>
</tr>
<tr>
<td></td>
<td>Olhero, Susana Maria H.</td>
<td>PS2-05-202</td>
</tr>
<tr>
<td></td>
<td>Olivares, Belén</td>
<td>PS1-11-365</td>
</tr>
<tr>
<td></td>
<td>Oliveira, Ana L.</td>
<td>AERO InvF-01</td>
</tr>
<tr>
<td></td>
<td>Oliveira, Susana Maria H.</td>
<td>PS2-05-202</td>
</tr>
<tr>
<td></td>
<td>Oliveira, Andreia</td>
<td>N04-04</td>
</tr>
<tr>
<td></td>
<td>PS1-04-163</td>
<td>1151</td>
</tr>
<tr>
<td></td>
<td>PS2-07-248</td>
<td>1716</td>
</tr>
<tr>
<td></td>
<td>Oliveira, Carla</td>
<td>ESB-SIBB S02-03</td>
</tr>
<tr>
<td></td>
<td>Oliveira, Claudia S.</td>
<td>N11-04</td>
</tr>
<tr>
<td></td>
<td>Oliveira, Hugo</td>
<td>PS1-01-039</td>
</tr>
<tr>
<td></td>
<td>PS1-01-061</td>
<td>1048</td>
</tr>
<tr>
<td></td>
<td>PS2-01-052</td>
<td>1523</td>
</tr>
<tr>
<td></td>
<td>S01-03</td>
<td>92</td>
</tr>
</tbody>
</table>

Page 1993 of 2028
<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oliveira, J. Miguel</td>
<td>PS2-14-424</td>
<td>1896</td>
</tr>
<tr>
<td>Oliveira, Joaquim</td>
<td>PS2-14-424</td>
<td>1896</td>
</tr>
<tr>
<td>K10-04</td>
<td></td>
<td>863</td>
</tr>
<tr>
<td>PS2-08-298</td>
<td></td>
<td>1760</td>
</tr>
<tr>
<td>PS2-12-382</td>
<td></td>
<td>1851</td>
</tr>
<tr>
<td>Oliveira, Maria</td>
<td>PS1-10-325</td>
<td>1321</td>
</tr>
<tr>
<td>ESB-SFB S-05</td>
<td></td>
<td>815</td>
</tr>
<tr>
<td>Oliveira, Mariana B.</td>
<td>S06-KL01</td>
<td>393</td>
</tr>
<tr>
<td>Oliveira, Miguel</td>
<td>N07-05</td>
<td>337</td>
</tr>
<tr>
<td>Oliveira, Ricardo</td>
<td>N05-06</td>
<td>297</td>
</tr>
<tr>
<td>Oliver-Urrutia, Carolina</td>
<td>PS2-02-080</td>
<td>1550</td>
</tr>
<tr>
<td>PS2-05-188</td>
<td></td>
<td>1652</td>
</tr>
<tr>
<td>Ollivier, Veronique</td>
<td>PS1-09-307</td>
<td>1298</td>
</tr>
<tr>
<td>Olmo, Laura</td>
<td>PS1-12-367</td>
<td>1365</td>
</tr>
<tr>
<td>O’Loughlin, Mark T.</td>
<td>N03-06</td>
<td>219</td>
</tr>
<tr>
<td>Onak Pulat, Günnur</td>
<td>S14-02</td>
<td>874</td>
</tr>
<tr>
<td>Onas, Andrea M.</td>
<td>N05-02</td>
<td>292</td>
</tr>
<tr>
<td>Onsea, Jolien</td>
<td>PS2-13-410</td>
<td>1881</td>
</tr>
<tr>
<td>Opdenaker, Lynn M.</td>
<td>PS2-14-432</td>
<td>1903</td>
</tr>
<tr>
<td>Oppmann, Maximilian</td>
<td>PS1-05-185</td>
<td>1174</td>
</tr>
<tr>
<td>O’Reilly, Rachel</td>
<td>NANOSTEM 02-03</td>
<td>354</td>
</tr>
<tr>
<td>PS1-12-383</td>
<td></td>
<td>1380</td>
</tr>
<tr>
<td>Orgéas, Laurent</td>
<td>ESB-BIOMAT S-06</td>
<td>444</td>
</tr>
<tr>
<td>Ornig, Martin</td>
<td>N11-06</td>
<td>575</td>
</tr>
<tr>
<td>Ortiz-Ortiz, Javier</td>
<td>PS1-02-103</td>
<td>1092</td>
</tr>
<tr>
<td>PS2-02-104</td>
<td></td>
<td>1574</td>
</tr>
<tr>
<td>Oschatz, Stefan T.</td>
<td>PS1-15-461</td>
<td>1463</td>
</tr>
<tr>
<td>Osório, Hugo</td>
<td>PS2-13-412</td>
<td>1884</td>
</tr>
<tr>
<td>Otero, Ana</td>
<td>PS2-01-024</td>
<td>1498</td>
</tr>
<tr>
<td>Otto, Paul</td>
<td>N07-04</td>
<td>335</td>
</tr>
<tr>
<td>Oude Egberink, Rik</td>
<td>S06-04</td>
<td>400</td>
</tr>
<tr>
<td>Ouedraogo, Sidzigu</td>
<td>PS1-10-335</td>
<td>1331</td>
</tr>
<tr>
<td>Ouyang, Liliang</td>
<td>S14-04</td>
<td>878</td>
</tr>
<tr>
<td>Ovtsianikov, Aleksandr</td>
<td>TRS-S03-03</td>
<td>250</td>
</tr>
<tr>
<td>ESB-ISBF S-04</td>
<td></td>
<td>537</td>
</tr>
<tr>
<td>S10-KL01</td>
<td></td>
<td>679</td>
</tr>
<tr>
<td>Öztürk, Abdullah</td>
<td>PS1-06-207</td>
<td>1200</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paar, Georg-Philipp</td>
<td>N11-03</td>
<td>568</td>
</tr>
<tr>
<td>Pacha-Olivenza, Miguel A.</td>
<td>PS1-13-417</td>
<td>1414</td>
</tr>
<tr>
<td>Pacheco, Daniela P.</td>
<td>PS2-14-440</td>
<td>1912</td>
</tr>
<tr>
<td>Pacci, Claudia</td>
<td>S05-05</td>
<td>274</td>
</tr>
<tr>
<td>Padmanabhan, Prasanna</td>
<td>N12-07</td>
<td>744</td>
</tr>
<tr>
<td>Padmanabhan, Sanosh K.</td>
<td>PS1-04-159</td>
<td>1149</td>
</tr>
<tr>
<td>Paez, Julieta I.</td>
<td>PS1-14-441</td>
<td>1442</td>
</tr>
<tr>
<td>PS2-08-296</td>
<td></td>
<td>1758</td>
</tr>
<tr>
<td>Pagani, Mattia</td>
<td>PS2-02-096</td>
<td>1568</td>
</tr>
<tr>
<td>S08-04</td>
<td></td>
<td>510</td>
</tr>
<tr>
<td>Pagani, Stefania</td>
<td>PS1-06-211</td>
<td>1204</td>
</tr>
<tr>
<td>Pagès, Esther</td>
<td>PS1-02-101</td>
<td>1091</td>
</tr>
<tr>
<td>PS1-03-119</td>
<td></td>
<td>1105</td>
</tr>
<tr>
<td>Paggi, Carlo A.</td>
<td>S01-06</td>
<td>98</td>
</tr>
<tr>
<td>Pahlavani, Helda</td>
<td>PS1-01-043</td>
<td>1035</td>
</tr>
<tr>
<td>Paiva dos Santos, Bruno</td>
<td>PS1-08-279</td>
<td>1271</td>
</tr>
<tr>
<td>Paiva, Silvia S.</td>
<td>PS1-04-137</td>
<td>1127</td>
</tr>
<tr>
<td>Paiva, Silvia T.</td>
<td>PS2-08-284</td>
<td>1747</td>
</tr>
<tr>
<td>Pajerski, Wojciech</td>
<td>PS1-13-415</td>
<td>1412</td>
</tr>
<tr>
<td>Pajnik, Jelena M.</td>
<td>AERO InvF-05</td>
<td>628</td>
</tr>
<tr>
<td>AERO PS01-01</td>
<td></td>
<td>597</td>
</tr>
<tr>
<td>AERO S01-04</td>
<td></td>
<td>594</td>
</tr>
<tr>
<td>Palierse, Estelle</td>
<td>ESB-BMSJ S-05</td>
<td>729</td>
</tr>
<tr>
<td>Palkowitz, Alena L.</td>
<td>PS1-02-075</td>
<td>1063</td>
</tr>
<tr>
<td>Author Name</td>
<td>Abstract Code</td>
<td>Page Number</td>
</tr>
<tr>
<td>-----------------------------------------------------------------------------</td>
<td>-----------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Pallavicini, Piersandro</td>
<td>PS2-07-252</td>
<td>1720</td>
</tr>
<tr>
<td>Palomeras, Sónia</td>
<td>PS1-01-045</td>
<td>1036</td>
</tr>
<tr>
<td>Palomino-Durand, Carla</td>
<td>PS1-13-423</td>
<td>1420</td>
</tr>
<tr>
<td>PS2-08-274</td>
<td></td>
<td>1739</td>
</tr>
<tr>
<td>Palo-Nieto, Carlos</td>
<td>N10-04</td>
<td>550</td>
</tr>
<tr>
<td>Palumbo, Eleonora</td>
<td>PS2-14-438</td>
<td>1910</td>
</tr>
<tr>
<td>Panamula, Elżbieta</td>
<td>PS1-02-097</td>
<td>1087</td>
</tr>
<tr>
<td>PS1-10-339</td>
<td></td>
<td>1336</td>
</tr>
<tr>
<td>Pandini, Stefano</td>
<td>S04-05</td>
<td>196</td>
</tr>
<tr>
<td>Pandit, Abhay</td>
<td>ESB-BIOMAT S-03</td>
<td>436</td>
</tr>
<tr>
<td>ESB-BMJ S-01</td>
<td></td>
<td>542</td>
</tr>
<tr>
<td>ESB-BMJ S-02</td>
<td></td>
<td>542</td>
</tr>
<tr>
<td>N01-05</td>
<td></td>
<td>137</td>
</tr>
<tr>
<td>N01-07</td>
<td></td>
<td>141</td>
</tr>
<tr>
<td>PS1-03-127</td>
<td></td>
<td>1114</td>
</tr>
<tr>
<td>S15-KL01</td>
<td></td>
<td>887</td>
</tr>
<tr>
<td>Pandoleon, Panagiotis</td>
<td>PS1-04-169</td>
<td>1159</td>
</tr>
<tr>
<td>Pané, Salvador</td>
<td>PS1-12-375</td>
<td>1372</td>
</tr>
<tr>
<td>Panseri, Silvia</td>
<td>N09-05</td>
<td>476</td>
</tr>
<tr>
<td>PS2-08-282</td>
<td></td>
<td>1745</td>
</tr>
<tr>
<td>S10-03</td>
<td></td>
<td>684</td>
</tr>
<tr>
<td>Pariktak, Piotr</td>
<td>PS1-04-147</td>
<td>1136</td>
</tr>
<tr>
<td>PS2-04-146</td>
<td></td>
<td>1610</td>
</tr>
<tr>
<td>Panzavolta, Silvia</td>
<td>PS1-06-211</td>
<td>1204</td>
</tr>
<tr>
<td>S03-03</td>
<td></td>
<td>117</td>
</tr>
<tr>
<td>Paoletti, Camilla</td>
<td>PS2-12-372</td>
<td>1840</td>
</tr>
<tr>
<td>Papadimitriou, Lina</td>
<td>K04-06</td>
<td>388</td>
</tr>
<tr>
<td>Papadogianni, Danai</td>
<td>S10-05</td>
<td>688</td>
</tr>
<tr>
<td>Papadopoulos, Triantafyllos</td>
<td>PS1-02-089</td>
<td>1079</td>
</tr>
<tr>
<td>PS2-02-088</td>
<td></td>
<td>1560</td>
</tr>
<tr>
<td>Papadopoulosou, Lambrini</td>
<td>PS2-04-170</td>
<td>1632</td>
</tr>
<tr>
<td>Papi, Massimiliano</td>
<td>K04-KL01</td>
<td>379</td>
</tr>
<tr>
<td>Paraskeveloulos, Konstantinos M.</td>
<td>PS1-12-391</td>
<td>1388</td>
</tr>
<tr>
<td>Pardo-Rodriguez, Beatriz</td>
<td>K04-05</td>
<td>386</td>
</tr>
<tr>
<td>Pardo, François</td>
<td>PS2-01-052</td>
<td>1523</td>
</tr>
<tr>
<td>Parisi, Cleo</td>
<td>S02-05</td>
<td>110</td>
</tr>
<tr>
<td>Park, Jongee</td>
<td>PS1-06-207</td>
<td>1200</td>
</tr>
<tr>
<td>Parkinson, Sam</td>
<td>NANOSTEM 02-03</td>
<td>354</td>
</tr>
<tr>
<td>Parmentier, Laurens</td>
<td>PS1-04-135</td>
<td>1124</td>
</tr>
<tr>
<td>Parreira, Paula</td>
<td>PS2-02-066</td>
<td>1534</td>
</tr>
<tr>
<td>Pasberg, Patrick</td>
<td>KL01</td>
<td>765</td>
</tr>
<tr>
<td>Pasini, Chiara</td>
<td>PS2-02-078</td>
<td>1548</td>
</tr>
<tr>
<td>Pasquale, Claudio</td>
<td>KL01</td>
<td>1520</td>
</tr>
<tr>
<td>Pattricio, Sónia G.</td>
<td>K09-06</td>
<td>850</td>
</tr>
<tr>
<td>Pasini, Chiara</td>
<td>N03-04</td>
<td>215</td>
</tr>
<tr>
<td>PS1-02-093</td>
<td></td>
<td>1082</td>
</tr>
<tr>
<td>PS2-03-108</td>
<td></td>
<td>1578</td>
</tr>
<tr>
<td>S01-02</td>
<td></td>
<td>89</td>
</tr>
<tr>
<td>S02-03</td>
<td></td>
<td>106</td>
</tr>
<tr>
<td>Patrick, Erin E.</td>
<td>PS1-01-059</td>
<td>339</td>
</tr>
<tr>
<td>Paulu, Ilona</td>
<td>K03-04</td>
<td>259</td>
</tr>
<tr>
<td>PS1-01-059</td>
<td></td>
<td>1046</td>
</tr>
<tr>
<td>Pauhthe, Emmanuel</td>
<td>ESB-BIOMAT S-05</td>
<td>442</td>
</tr>
<tr>
<td>PS1-04-145</td>
<td></td>
<td>1134</td>
</tr>
<tr>
<td>PS1-04-173</td>
<td></td>
<td>1161</td>
</tr>
<tr>
<td>PS1-13-423</td>
<td></td>
<td>1420</td>
</tr>
<tr>
<td>PS2-08-274</td>
<td></td>
<td>1739</td>
</tr>
<tr>
<td>Pavlovski, Marek</td>
<td>TRS-S03-02</td>
<td>285</td>
</tr>
<tr>
<td>Payen, Julien</td>
<td>PS1-15-455</td>
<td>1454</td>
</tr>
<tr>
<td>Payen, Julien</td>
<td>PS2-15-452</td>
<td>1924</td>
</tr>
<tr>
<td>Pave, Eva</td>
<td>PS1-05-201</td>
<td>1191</td>
</tr>
</tbody>
</table>
Author Index

Pereira, Amanda
NANOSTEM 01-04 ................................... 349

Pearce, Amanda K.
PS1-12-383 ............................................. 1380

Peeters, Ian
S05-02 .................................................. 267

Pêgo, Ana P.
PS2-11-354 ............................................. 1821

Pêgo, Ana P.
K09-04 ................................................... 846
N01-06 ................................................... 139
N01-08 ................................................... 143
PS1-10-333 ............................................. 1329
PS1-12-375 ............................................. 1372
PS1-13-419 ............................................. 1416
PS2-02-100 ............................................. 1572
S02-03 ................................................... 106
S09-03 ................................................... 668
S11-03 ................................................... 697
PS2-12-390 ............................................. 1858
PS2-13-418 ............................................. 1890

Pequeroles, Marta
N12-04 ................................................... 738
PS2-09-304 ............................................. 1767

Peikolainen, Anna Liisa
AERO PS01-02 ........................................... 599

Pellicer, Eva
PS1-04-139 ............................................. 1130

Peloso, Charlotte
PS1-02-065 ............................................. 1054

Penchev, Pavel
PS1-15-447 ............................................. 1448

Peng, Lihui
S09-05 ................................................... 672

Peppas, Nicholas A.
N15-01 ................................................... 916

Perbellini, Filippo
K07-02 ................................................... 753

Pereira, André M.
S04-03 ................................................... 192

Pereira, Andreia T.
K04-03 ................................................... 383
PS1-08-289 ............................................. 1280
PS2-05-192 ............................................. 1657
S04-03 ................................................... 192

Pereira, Catarina L.
ESB-SFB S-05 .......................................... 815
N02-09 ................................................... 167
PS2-04-156 ............................................. 1621

Pereira, Inês S.
PS1-03-109 ............................................. 1097

Pereira, Maria O.
ESB-SIBB S01-03 ...................................... 520
PS1-11-361 ............................................. 1358

Page 1996 of 2028
<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pestean, Cosmin</td>
<td>1558</td>
</tr>
<tr>
<td>Petaroudi, Michaela</td>
<td>462</td>
</tr>
<tr>
<td>Peters, Kirsten</td>
<td>1457</td>
</tr>
<tr>
<td>Petithory, Tatiana</td>
<td>1409</td>
</tr>
<tr>
<td>Petretta, Mauro</td>
<td>1630</td>
</tr>
<tr>
<td>Petrin, Paola</td>
<td>466</td>
</tr>
<tr>
<td>Petrovic, Jelena</td>
<td>1438</td>
</tr>
<tr>
<td>Pette, Dagmar</td>
<td>1342</td>
</tr>
<tr>
<td>Pezzella, Alessandro</td>
<td>143</td>
</tr>
<tr>
<td>Pfannstiel, Jessica</td>
<td>1508</td>
</tr>
<tr>
<td>Piaggi, Simona</td>
<td>1866</td>
</tr>
<tr>
<td>Piatti, Elisa</td>
<td>1584</td>
</tr>
<tr>
<td>Picart, Catherine</td>
<td>148</td>
</tr>
<tr>
<td>Pien, Nele</td>
<td>1153</td>
</tr>
<tr>
<td>Pierini, Filippo</td>
<td>267</td>
</tr>
<tr>
<td>Pietryga, Krzysztof</td>
<td>1662</td>
</tr>
<tr>
<td>Pietzsch, Jens</td>
<td>1138</td>
</tr>
<tr>
<td>Pieuchot, Laurent</td>
<td>1331</td>
</tr>
<tr>
<td>Pillet, Hélène</td>
<td>270</td>
</tr>
<tr>
<td>Pliluso, Susanna</td>
<td>482</td>
</tr>
<tr>
<td>Pineda, Jose Ramon</td>
<td>386</td>
</tr>
<tr>
<td>Pine, Celine</td>
<td>1020</td>
</tr>
<tr>
<td>Pinho, Ana R.</td>
<td>475</td>
</tr>
<tr>
<td>Pintado, Manuela</td>
<td>552</td>
</tr>
<tr>
<td>Pinto, Artur M.</td>
<td>383</td>
</tr>
<tr>
<td>Pinto, Marta L.</td>
<td>815</td>
</tr>
<tr>
<td>Pinto, Rita M.</td>
<td>889</td>
</tr>
<tr>
<td>Pinto, Susana C.</td>
<td>1474</td>
</tr>
<tr>
<td>Pinto, Susana Cristina</td>
<td>388</td>
</tr>
<tr>
<td>Pinto-do-Ô, Perpêtua</td>
<td>753</td>
</tr>
<tr>
<td>Pires, Pedro</td>
<td>1856</td>
</tr>
<tr>
<td>Pires, Ricardo A.</td>
<td>579</td>
</tr>
<tr>
<td>Pisignano, Dario</td>
<td>1449</td>
</tr>
<tr>
<td>Pistillo, Michele</td>
<td>243</td>
</tr>
<tr>
<td>Pizarek, John A.</td>
<td>101</td>
</tr>
<tr>
<td>Pitet, Louis</td>
<td>938</td>
</tr>
<tr>
<td>Pitrez, Patricia</td>
<td>371</td>
</tr>
<tr>
<td>Pilton, Mattiño</td>
<td>1231</td>
</tr>
<tr>
<td>Pizzillo, Michele</td>
<td>1659</td>
</tr>
<tr>
<td>Planas, Marta</td>
<td>810</td>
</tr>
<tr>
<td>Platania, Varvara</td>
<td>711</td>
</tr>
<tr>
<td>Plazzotto, Stella</td>
<td>641</td>
</tr>
<tr>
<td>Plomp, Saskia</td>
<td>234</td>
</tr>
<tr>
<td>Plougouven, Erwan</td>
<td>1576</td>
</tr>
<tr>
<td>Ploux, Lydie</td>
<td>869</td>
</tr>
<tr>
<td>Plumhoff, Berit Z.</td>
<td>1057</td>
</tr>
<tr>
<td>Pluuy, Isabelle</td>
<td>1762</td>
</tr>
<tr>
<td>Page 1997 of 2028</td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Pages</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Podesser, Bruno K.</td>
<td>1779</td>
</tr>
<tr>
<td>Podewitz, Mareen</td>
<td>347</td>
</tr>
<tr>
<td>Podlou, Lucia</td>
<td>217</td>
</tr>
<tr>
<td>Podstawczyk, Daria</td>
<td>539</td>
</tr>
<tr>
<td>Poirier, Aurelia</td>
<td>136, 1022</td>
</tr>
<tr>
<td>Pohl, Christopher</td>
<td>1457</td>
</tr>
<tr>
<td>Pohl, Christopher D.</td>
<td>1612</td>
</tr>
<tr>
<td>Polat, Oguzhan</td>
<td>1757</td>
</tr>
<tr>
<td>Polido, Mário</td>
<td>1180</td>
</tr>
<tr>
<td>Polini, Alessandro</td>
<td>1801</td>
</tr>
<tr>
<td>Polley, Christian</td>
<td>1311</td>
</tr>
<tr>
<td>Polo, Yurena</td>
<td>386</td>
</tr>
<tr>
<td>Polonio-Alcalá, Emma</td>
<td>711</td>
</tr>
<tr>
<td>Pombi, Marco</td>
<td>860</td>
</tr>
<tr>
<td>Ponche, Arnaud</td>
<td>1331</td>
</tr>
<tr>
<td>Pontremoli, Carlotta</td>
<td>510</td>
</tr>
<tr>
<td>Poosch, Friederike</td>
<td>1612</td>
</tr>
<tr>
<td>Poot, André A.</td>
<td>1730</td>
</tr>
<tr>
<td>Popa, Marcel</td>
<td>1261, 1326, 1369</td>
</tr>
<tr>
<td>Popescu, Andra</td>
<td>1558</td>
</tr>
<tr>
<td>Popović Bijelić, Ana</td>
<td>1246</td>
</tr>
<tr>
<td>Portillo Lara, Roberto</td>
<td>128</td>
</tr>
<tr>
<td>Pota, Giulio</td>
<td>923, 121</td>
</tr>
<tr>
<td>Potart, Diane</td>
<td>736</td>
</tr>
<tr>
<td>Pou, Juan</td>
<td>527, 884</td>
</tr>
<tr>
<td>Pout, André</td>
<td>1497</td>
</tr>
<tr>
<td>Poulo, Yurena</td>
<td>386</td>
</tr>
<tr>
<td>Polabil, Juan</td>
<td>386</td>
</tr>
<tr>
<td>Poulton, Pierre</td>
<td>1457</td>
</tr>
<tr>
<td>Pouret, Philippe</td>
<td>1180</td>
</tr>
<tr>
<td>Prach, Monika</td>
<td>1801</td>
</tr>
<tr>
<td>Prado, Monica</td>
<td>633</td>
</tr>
<tr>
<td>Prado-Porras, Monica</td>
<td>625</td>
</tr>
<tr>
<td>Prató, Mirko</td>
<td>921</td>
</tr>
<tr>
<td>Prate, Veronique</td>
<td>707</td>
</tr>
<tr>
<td>Priimagi, Arri</td>
<td>674</td>
</tr>
<tr>
<td>Prinzi, Cornelia</td>
<td>1457</td>
</tr>
<tr>
<td>Probst, Jörn</td>
<td>1025, 1174</td>
</tr>
<tr>
<td>Procter, Philip</td>
<td>1618</td>
</tr>
<tr>
<td>Prouve, Emilie</td>
<td>434</td>
</tr>
<tr>
<td>Prouvé, Emilie</td>
<td>1878</td>
</tr>
<tr>
<td>Puchova, Eva</td>
<td>657</td>
</tr>
<tr>
<td>Przekora-Kuśmierz, Agata</td>
<td>1378</td>
</tr>
<tr>
<td>Pucci, Carlotta</td>
<td>727</td>
</tr>
<tr>
<td>Puchwein, Paul</td>
<td>575</td>
</tr>
<tr>
<td>Puertas-Bartolomé, María</td>
<td>1506</td>
</tr>
<tr>
<td>Pugliese, Diego</td>
<td>1410</td>
</tr>
<tr>
<td>Puglisi, Matteo</td>
<td>370</td>
</tr>
<tr>
<td>Puig, Miquel, Teresa</td>
<td>1036</td>
</tr>
<tr>
<td>Puig, Teresa</td>
<td>1903</td>
</tr>
<tr>
<td>Pujari-Palmer, Michael</td>
<td>805</td>
</tr>
<tr>
<td>Pum, Jonathan</td>
<td>1506</td>
</tr>
<tr>
<td>Qu, Fengjing</td>
<td>314</td>
</tr>
<tr>
<td>Quach, Quang Huy</td>
<td>1919</td>
</tr>
<tr>
<td>Quadros, Paulo</td>
<td>241</td>
</tr>
</tbody>
</table>
# Author Index

| S14-03 | .................................................................................. | 876 |
| Quarta, Alessandra | PS2-14-432 | .......................................................... | 1903 |
| Quax, Paul | N06-03 | .......................................................... | 311 |
| Quillez, Cristina | N10-03 | .......................................................... | 548 |
| Quinn, James | PS1-15-459 | .......................................................... | 1460 |
| Quintanilla-Sierra, Luis | N05-07 | .......................................................... | 299 |
| Quoika, Patrick K. | NANOSTEM 01-03 | .......................................................... | 347 |
| Rabionet, Marc | PS2-14-432 | .......................................................... | 1903 |
| Radisic, Milica | K07-KL01 | .......................................................... | 752 |
| Radonjic, Mia | PS1-14-437 | .......................................................... | 1438 |
| Ramalho, Sidnei | PS1-14-439 | .......................................................... | 1440 |
| Rahman, Ruman | PS2-10-330 | .......................................................... | 1795 |
| Rahmati, Maryam | PS1-02-067 | .......................................................... | 1057 |
| Rai, Akhilesh | K01-03 | .......................................................... | 78 |
| Raif, Mostafa | PS2-11-366 | .......................................................... | 1832 |
| Rainer, Alberto | N16-09 | .......................................................... | 948 |
| Rampal, Sidnei | Biomechanics 03-04 | .......................................................... | 966 |
| Ramasesh, Vaijayanthi | PS2-15-446 | .......................................................... | 1917 |
| Ramirez-Sánchez, Karla | AERO InvF-04 | .......................................................... | 625 |
| Ramirez-Sánchez, Karla | AERO InvF-07 | .......................................................... | 633 |
| Ramittel, Stefan | PS1-04-149 | .......................................................... | 1138 |
| Ramôa, Antônio | PS1-25-426 | .......................................................... | 1936 |
| Rama, Juan | PS1-11-359 | .......................................................... | 1356 |
| Ramesh, Vaijayanthi | PS1-02-089 | .......................................................... | 1079 |
| Rana, Deepti | N12-09 | .......................................................... | 747 |
| Ranella, Anthi | K04-06 | .......................................................... | 388 |
| Rangel, André | PS2-07-250 | .......................................................... | 1718 |
| Rangel, Vincent | N12-09 | .......................................................... | 747 |

| Rannard, Steve | PS1-07-237 | .......................................................... | 1229 |
| Ranzato, Elia | PS1-07-227 | .......................................................... | 1221 |
| Rasponi, Marco | PS2-14-444 | .......................................................... | 1915 |
| Rata, Delia M. | PS1-10-329 | .......................................................... | 1326 |
| Rata, Delia M. | PS1-12-371 | .......................................................... | 1369 |
| Rata, Delia M. | PS2-10-324 | .......................................................... | 1789 |
| Ratera, Imma | N08-01 | .......................................................... | 450 |
| Rau, Julietta | ESB-SLABO S-KL01 | .......................................................... | 281 |
| Raucci, Maria Grazia | ESB-CSBM S-04 | .......................................................... | 203 |
| Rausch, Simona | K08-07 | .......................................................... | 781 |
| Rauzová, Simona | N01-08 | .......................................................... | 143 |
| RaverPan, Franco | PS2-03-118 | .......................................................... | 1586 |
| Raymold, Yago | PS2-07-244 | .......................................................... | 1712 |
| Raynold, Alex | PS2-07-246 | .......................................................... | 1714 |
| Razzaq, Hussman | S14-KL01 | .......................................................... | 872 |
| Rebe, Ana L. | N01-05 | .......................................................... | 137 |
| Rebe, Catarina | N11-04 | .......................................................... | 699 |
| Reches, Meital | K10-KL01 | .......................................................... | 857 |
| Reczyńska, Katarzyna | PS1-10-339 | .......................................................... | 1336 |
| Redmond, John | N11-01 | .......................................................... | 563 |
| Redondo, Marta | N16-04 | .......................................................... | 938 |
| Rege, Ameya | AERO S01-03 | .......................................................... | 592 |
| Rehbock, Christoph | PS1-12-369 | .......................................................... | 1367 |
| PS2-15-446 | .......................................................... | 1917 |
### Author Index

<table>
<thead>
<tr>
<th>Name</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reid, Andrew</td>
<td>PS2-04-164</td>
</tr>
<tr>
<td>Reid, Gregory</td>
<td>PS2-14-444</td>
</tr>
<tr>
<td>Reilly, Gwendolen C.</td>
<td>PS1-02-099</td>
</tr>
<tr>
<td>Reineke, Benjamin</td>
<td>PS1-01-059</td>
</tr>
<tr>
<td>Reinis, Aigars</td>
<td>PS1-08-281</td>
</tr>
<tr>
<td>Reis, Catarina P.</td>
<td>AERO InvF-02</td>
</tr>
<tr>
<td>Reis, Rui L.</td>
<td>K02-05</td>
</tr>
<tr>
<td>Reis, Salette</td>
<td>ESB-CRS S-04</td>
</tr>
<tr>
<td>Reis, Sara D.</td>
<td>S11-03</td>
</tr>
<tr>
<td>Reiter, Theresa</td>
<td>PS2-01-054</td>
</tr>
<tr>
<td>Remuzzi, Andrea</td>
<td>S09-04</td>
</tr>
<tr>
<td>Remy, Murielle</td>
<td>ESB-BIOMAT S-02</td>
</tr>
<tr>
<td>Rémy, Murielle</td>
<td>PS1-08-273</td>
</tr>
<tr>
<td>Renaudie, Emeline</td>
<td>PS1-03-119</td>
</tr>
<tr>
<td>Renkler, Nergis Z.</td>
<td>PS1-07-253</td>
</tr>
<tr>
<td>Renner, Lars D.</td>
<td>K06-05</td>
</tr>
<tr>
<td>Reseland, Janne E.</td>
<td>PS1-07-231</td>
</tr>
<tr>
<td>Resmini, Marina</td>
<td>NANOSTEM 01-04</td>
</tr>
<tr>
<td></td>
<td>NANOSTEM 01-05</td>
</tr>
<tr>
<td></td>
<td>NANOSTEM 01-Intro</td>
</tr>
<tr>
<td></td>
<td>NANOSTEM 02-KL01</td>
</tr>
<tr>
<td></td>
<td>NANOSTEM 03-01</td>
</tr>
<tr>
<td></td>
<td>NANOSTEM 03-03</td>
</tr>
<tr>
<td></td>
<td>PS1-12-373</td>
</tr>
<tr>
<td></td>
<td>PS2-08-270</td>
</tr>
<tr>
<td>Restivo, Elisa</td>
<td>PS1-13-413</td>
</tr>
<tr>
<td></td>
<td>PS2-04-150</td>
</tr>
<tr>
<td></td>
<td>PS2-07-252</td>
</tr>
<tr>
<td>Retheore, Gildas</td>
<td>PS2-08-292</td>
</tr>
<tr>
<td>Rey, Christian</td>
<td>PS1-06-205</td>
</tr>
<tr>
<td></td>
<td>S12-05</td>
</tr>
<tr>
<td>Rey, Sergi</td>
<td>N08-01</td>
</tr>
<tr>
<td>Reyes-San-Martin, Claudia</td>
<td>K06-02</td>
</tr>
<tr>
<td>Reynaud, Emmanuel G.</td>
<td>S04-04</td>
</tr>
<tr>
<td>Rey-Rico, Ana</td>
<td>PS1-10-331</td>
</tr>
<tr>
<td>Rey-Vinolas, Sergi</td>
<td>N02-03</td>
</tr>
<tr>
<td>Rezaei, Azadeh</td>
<td>N09-02</td>
</tr>
<tr>
<td></td>
<td>PS1-14-445</td>
</tr>
<tr>
<td></td>
<td>PS2-06-216</td>
</tr>
<tr>
<td></td>
<td>S08-03</td>
</tr>
<tr>
<td>Rezwan, Kourosh</td>
<td>PS2-06-208</td>
</tr>
<tr>
<td>Rho, Hoon S.</td>
<td>N09-06</td>
</tr>
<tr>
<td></td>
<td>PS1-13-393</td>
</tr>
<tr>
<td>Rhoades, Jonathan</td>
<td>PS1-12-391</td>
</tr>
<tr>
<td>Rial-Hermida, Maria I.</td>
<td>PS2-03-108</td>
</tr>
<tr>
<td>Ribeiro dos Santos, Verónica</td>
<td>PS1-01-013</td>
</tr>
<tr>
<td>Ribeiro, Bruno</td>
<td>PS2-11-354</td>
</tr>
<tr>
<td>Ribeiro, Cristina</td>
<td>N11-07</td>
</tr>
<tr>
<td>Ribeiro, Isabel</td>
<td>PS2-10-326</td>
</tr>
<tr>
<td>Ribeiro, Tiago P.</td>
<td>ESB-SIBB S02-07</td>
</tr>
<tr>
<td>Ribeiro-Machado, Cláudia</td>
<td>N11-07</td>
</tr>
<tr>
<td></td>
<td>S15-03</td>
</tr>
<tr>
<td>Ricco, Pietro</td>
<td>PS2-11-356</td>
</tr>
<tr>
<td>Richards, Geoff</td>
<td>PS2-01-028</td>
</tr>
<tr>
<td>Richter, Richard F.</td>
<td>PS1-01-011</td>
</tr>
<tr>
<td></td>
<td>S08-05</td>
</tr>
<tr>
<td>Ricotti, Leonardo</td>
<td>K07-02</td>
</tr>
<tr>
<td>Author</td>
<td>Page</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>PS1-08-297</td>
<td>1287</td>
</tr>
<tr>
<td>S05-05</td>
<td>274</td>
</tr>
<tr>
<td>Riedel, Stefanie</td>
<td>1736</td>
</tr>
<tr>
<td>PS2-08-272</td>
<td></td>
</tr>
<tr>
<td>Riemann, Sebastian</td>
<td>777</td>
</tr>
<tr>
<td>K08-05</td>
<td></td>
</tr>
<tr>
<td>Rieschel, Merlin</td>
<td>73</td>
</tr>
<tr>
<td>TRS-S01-03</td>
<td></td>
</tr>
<tr>
<td>Rigos, Athanasios E.</td>
<td>1208</td>
</tr>
<tr>
<td>PS1-06-215</td>
<td></td>
</tr>
<tr>
<td>Rikkers, Margot</td>
<td>1402</td>
</tr>
<tr>
<td>PS1-13-403</td>
<td></td>
</tr>
<tr>
<td>Rikmanspoel, Timo</td>
<td>415</td>
</tr>
<tr>
<td>S07-04</td>
<td></td>
</tr>
<tr>
<td>Rimondini, Lia</td>
<td>1451</td>
</tr>
<tr>
<td>PS1-15-451</td>
<td></td>
</tr>
<tr>
<td>PS1-15-455</td>
<td>1454</td>
</tr>
<tr>
<td>PS2-10-338</td>
<td>1803</td>
</tr>
<tr>
<td>Rinoldi, Chiara</td>
<td>1691</td>
</tr>
<tr>
<td>PS2-07-224</td>
<td></td>
</tr>
<tr>
<td>Riol, Martijn</td>
<td>867</td>
</tr>
<tr>
<td>K10-06</td>
<td></td>
</tr>
<tr>
<td>S03-07</td>
<td>125</td>
</tr>
<tr>
<td>Risueño, Ignacio</td>
<td>548</td>
</tr>
<tr>
<td>N10-03</td>
<td></td>
</tr>
<tr>
<td>Ritz, Ulrike</td>
<td>1345</td>
</tr>
<tr>
<td>PS1-11-347</td>
<td></td>
</tr>
<tr>
<td>Riveiro, Antonio</td>
<td>884</td>
</tr>
<tr>
<td>S14-07</td>
<td></td>
</tr>
<tr>
<td>S14-KL01</td>
<td>872</td>
</tr>
<tr>
<td>Rivero, Rebeca</td>
<td>896</td>
</tr>
<tr>
<td>N14-01</td>
<td></td>
</tr>
<tr>
<td>PS1-09-315</td>
<td>1310</td>
</tr>
<tr>
<td>Rizzi, Eleonora</td>
<td>359</td>
</tr>
<tr>
<td>NANOSTEM 03-01</td>
<td></td>
</tr>
<tr>
<td>NANOSTEM 03-02</td>
<td>360</td>
</tr>
<tr>
<td>NANOSTEM 03-03</td>
<td>362</td>
</tr>
<tr>
<td>Rizzo, Antonio</td>
<td>756</td>
</tr>
<tr>
<td>K07-03</td>
<td></td>
</tr>
<tr>
<td>Rizzo, Riccardo</td>
<td>822</td>
</tr>
<tr>
<td>N13-04</td>
<td></td>
</tr>
<tr>
<td>Rizzo, Roberta</td>
<td>117</td>
</tr>
<tr>
<td>S03-03</td>
<td></td>
</tr>
<tr>
<td>Roa, Joan Josep</td>
<td>456</td>
</tr>
<tr>
<td>N08-04</td>
<td></td>
</tr>
<tr>
<td>Robbiani, Baptiste</td>
<td>277</td>
</tr>
<tr>
<td>S05-06</td>
<td></td>
</tr>
<tr>
<td>Robla, Sandra</td>
<td>1170</td>
</tr>
<tr>
<td>PS1-05-181</td>
<td></td>
</tr>
<tr>
<td>Robles Diaz, Diana</td>
<td>1915</td>
</tr>
<tr>
<td>PS2-14-444</td>
<td></td>
</tr>
<tr>
<td>Roca-Cusachs, Pere</td>
<td>464</td>
</tr>
<tr>
<td>N08-08</td>
<td></td>
</tr>
<tr>
<td>Rochetti, Vincenzo</td>
<td>1454</td>
</tr>
<tr>
<td>PS1-15-455</td>
<td></td>
</tr>
<tr>
<td>Rocha, Fernando</td>
<td>1539</td>
</tr>
<tr>
<td>PS2-02-070</td>
<td></td>
</tr>
<tr>
<td>Rocha, Marta A.</td>
<td>1037</td>
</tr>
<tr>
<td>PS1-01-047</td>
<td></td>
</tr>
<tr>
<td>Rocher, Lison</td>
<td>740</td>
</tr>
<tr>
<td>N12-05</td>
<td></td>
</tr>
<tr>
<td>Roderiguez, Brian J.</td>
<td>1500</td>
</tr>
<tr>
<td>PS2-01-026</td>
<td></td>
</tr>
<tr>
<td>Rodilla, Beatriz L.</td>
<td>1722</td>
</tr>
<tr>
<td>PS2-07-254</td>
<td></td>
</tr>
<tr>
<td>Rodrigo, Matilde A.</td>
<td>1637</td>
</tr>
<tr>
<td>PS2-04-174</td>
<td></td>
</tr>
<tr>
<td>Rodrigo-Navarro, Aleixandre</td>
<td>462</td>
</tr>
<tr>
<td>N08-07</td>
<td></td>
</tr>
<tr>
<td>S10-07</td>
<td>692</td>
</tr>
<tr>
<td>Rodrigues, Artur Filipe</td>
<td>699</td>
</tr>
<tr>
<td>S11-04</td>
<td></td>
</tr>
<tr>
<td>K04-07</td>
<td>390</td>
</tr>
<tr>
<td>Rodrigues, Carlos A.V.</td>
<td>332</td>
</tr>
<tr>
<td>N07-03</td>
<td></td>
</tr>
<tr>
<td>Rodrigues, Carlos B.</td>
<td>982</td>
</tr>
<tr>
<td>Biomechanics 04-04</td>
<td></td>
</tr>
<tr>
<td>Rodrigues, Catia R.S.</td>
<td>192</td>
</tr>
<tr>
<td>S04-03</td>
<td></td>
</tr>
<tr>
<td>Rodrigues, João M.</td>
<td>80</td>
</tr>
<tr>
<td>K01-04</td>
<td></td>
</tr>
<tr>
<td>PS2-12-388</td>
<td>1856</td>
</tr>
<tr>
<td>PS2-12-386</td>
<td>1854</td>
</tr>
<tr>
<td>S06-02</td>
<td>395</td>
</tr>
<tr>
<td>Rodrigues, Marco B.</td>
<td>982</td>
</tr>
<tr>
<td>Biomechanics 04-04</td>
<td></td>
</tr>
<tr>
<td>Rodríguez, Lorenzo, Luis M.</td>
<td>255</td>
</tr>
<tr>
<td>K03-02</td>
<td></td>
</tr>
<tr>
<td>Rodriguez, Brian J.</td>
<td>194</td>
</tr>
<tr>
<td>S04-04</td>
<td></td>
</tr>
<tr>
<td>Rodriguez, Daniel</td>
<td>527</td>
</tr>
<tr>
<td>ESB-SIBB S01-06</td>
<td></td>
</tr>
<tr>
<td>Rodriguez, Karla B.</td>
<td>955</td>
</tr>
<tr>
<td>Biomechanics 01-01</td>
<td></td>
</tr>
<tr>
<td>Rodríguez-Cabello, José Carlos</td>
<td>1764</td>
</tr>
<tr>
<td>PS2-08-302</td>
<td></td>
</tr>
<tr>
<td>K05-07</td>
<td>503</td>
</tr>
<tr>
<td>N05-07</td>
<td>299</td>
</tr>
<tr>
<td>N12-02</td>
<td>734</td>
</tr>
<tr>
<td>PS1-14-431</td>
<td>1431</td>
</tr>
<tr>
<td>Rodriguez-Quesada, Laria Fabiola</td>
<td></td>
</tr>
<tr>
<td>AERO InvF-04</td>
<td>625</td>
</tr>
<tr>
<td>Rodriguez-Rius, Daniel</td>
<td>518</td>
</tr>
<tr>
<td>ESB-SIBB S01-02</td>
<td></td>
</tr>
<tr>
<td>Rodriguez-Ruiz, Violeta</td>
<td>442</td>
</tr>
<tr>
<td>ESB-BIOMAT S-05</td>
<td></td>
</tr>
<tr>
<td>Roerig, Josepha</td>
<td>853</td>
</tr>
<tr>
<td>K09-07</td>
<td></td>
</tr>
<tr>
<td>Rohan, Pierre-Yves</td>
<td>270</td>
</tr>
<tr>
<td>S05-03</td>
<td></td>
</tr>
<tr>
<td>Author Index</td>
<td>ESB 2021</td>
</tr>
<tr>
<td>--------------</td>
<td>----------</td>
</tr>
<tr>
<td>Rohringer, Sabrina</td>
<td>PS2-09-314</td>
</tr>
<tr>
<td>Roig, Anna</td>
<td>COST AERoGELS KL-01</td>
</tr>
<tr>
<td></td>
<td>PS1-05-193</td>
</tr>
<tr>
<td></td>
<td>PS2-10-334</td>
</tr>
<tr>
<td>Roig-Sanchez, Soledad</td>
<td>COST AERoGELS KL-01</td>
</tr>
<tr>
<td></td>
<td>PS1-05-193</td>
</tr>
<tr>
<td>Rojas, F. Javier</td>
<td>Biomechanics 03-03</td>
</tr>
<tr>
<td></td>
<td>Biomechanics 04-02</td>
</tr>
<tr>
<td></td>
<td>Rojas-González, Diana M.</td>
</tr>
<tr>
<td>Rolandsson Enes, Sara</td>
<td>PS1-14-431</td>
</tr>
<tr>
<td>Romero-Gavilán, Francisco</td>
<td>PS1-13-401</td>
</tr>
<tr>
<td>Ronan, William</td>
<td>N11-03</td>
</tr>
<tr>
<td>Ronca, Alfredo</td>
<td>N10-09</td>
</tr>
<tr>
<td></td>
<td>PS2-03-118</td>
</tr>
<tr>
<td>Ronsin, Olivier</td>
<td>PS1-10-321</td>
</tr>
<tr>
<td>Roquart, Mailie</td>
<td>ESB-BMSJ S-05</td>
</tr>
<tr>
<td>Rosa, Susana</td>
<td>K07-02</td>
</tr>
<tr>
<td></td>
<td>NANOSTEM 03-03</td>
</tr>
<tr>
<td></td>
<td>NANOSTEM 04-01</td>
</tr>
<tr>
<td>Rosado Balmayor, Elizabeth</td>
<td>PS1-04-157</td>
</tr>
<tr>
<td>Rosatella, Andrea</td>
<td>PS2-05-182</td>
</tr>
<tr>
<td>Rose, Felicity</td>
<td>PS2-04-160</td>
</tr>
<tr>
<td>Rosman, Colin</td>
<td>PS1-15-453</td>
</tr>
<tr>
<td>Rossi, Arianna</td>
<td>N09-05</td>
</tr>
<tr>
<td></td>
<td>PS2-08-282</td>
</tr>
<tr>
<td>Rossi, Filippo</td>
<td>N16-09</td>
</tr>
<tr>
<td>Rossin, Daniela</td>
<td>K07-03</td>
</tr>
<tr>
<td>Rosso, Rachele</td>
<td>K07-03</td>
</tr>
<tr>
<td>Rota, Solène</td>
<td>PS1-04-173</td>
</tr>
<tr>
<td>Rothbauer, Mario</td>
<td>PS2-14-434</td>
</tr>
<tr>
<td>Rother, Sandra</td>
<td>K05-04</td>
</tr>
<tr>
<td>Rottmar, Markus</td>
<td>PS2-02-064</td>
</tr>
<tr>
<td>Rouwkema, Jeroen</td>
<td>N12-07</td>
</tr>
<tr>
<td></td>
<td>N12-09</td>
</tr>
<tr>
<td>Ruetsche, Dominic</td>
<td>N13-04</td>
</tr>
<tr>
<td>Ruiter, Floor</td>
<td>N16-08</td>
</tr>
<tr>
<td>Ruiz-Martínez, Santiago</td>
<td>ESB-SIBB S02-04</td>
</tr>
<tr>
<td>Ruocco, Gerardina</td>
<td>N12-01</td>
</tr>
<tr>
<td>Ruperez, Elisa</td>
<td>ESB-SIBB S01-02</td>
</tr>
<tr>
<td>Rup, Frank</td>
<td>PS1-04-155</td>
</tr>
<tr>
<td></td>
<td>S03-06</td>
</tr>
<tr>
<td>Russell, Stephen J.</td>
<td>N10-01</td>
</tr>
<tr>
<td>Russo, Paola</td>
<td>AERO S01-01</td>
</tr>
<tr>
<td>Rusu, Alina G.</td>
<td>PS1-11-355</td>
</tr>
<tr>
<td></td>
<td>PS2-08-268</td>
</tr>
<tr>
<td></td>
<td>PS2-11-364</td>
</tr>
<tr>
<td>Ryma, Matthias</td>
<td>K03-04</td>
</tr>
<tr>
<td>S</td>
<td>Sabri, Firouzeh</td>
</tr>
<tr>
<td></td>
<td>Sadowska, Joanna M.</td>
</tr>
<tr>
<td></td>
<td>Saenz-Arce, Giovanni</td>
</tr>
<tr>
<td></td>
<td>Sahin, Gozde</td>
</tr>
<tr>
<td></td>
<td>Sahounoue, Meriem</td>
</tr>
<tr>
<td></td>
<td>Saiani, Alberto</td>
</tr>
<tr>
<td></td>
<td>Sajkiewicz, Paweł</td>
</tr>
<tr>
<td></td>
<td>PS2-15-454</td>
</tr>
<tr>
<td></td>
<td>PS2-15-464</td>
</tr>
<tr>
<td>Salatin, Elisa</td>
<td>PS2-11-354</td>
</tr>
<tr>
<td>Saldaña, Sergio M.</td>
<td>N01-05</td>
</tr>
<tr>
<td>Saleh, Yasmin</td>
<td>PS1-08-297</td>
</tr>
</tbody>
</table>

Page 2002 of 2028
<table>
<thead>
<tr>
<th>Author Index</th>
<th>ESB 2021</th>
<th>Abstract Book</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salema Oom, Madalena</td>
<td>PS1-07-245</td>
<td>1237</td>
</tr>
<tr>
<td>Sales, M. Goret F.</td>
<td>K06-KL01</td>
<td>651</td>
</tr>
<tr>
<td>Salgado, Christiane</td>
<td>PS1-14-427</td>
<td>1425</td>
</tr>
<tr>
<td>Salgado, Christiane L.</td>
<td>PS2-02-084</td>
<td>1555</td>
</tr>
<tr>
<td>Salicio-Paz, Asier</td>
<td>PS1-04-139</td>
<td>1130</td>
</tr>
<tr>
<td>Salma-Ancane, Kristine</td>
<td>PS1-08-281</td>
<td>1273</td>
</tr>
<tr>
<td>Salmeron-Sanchez, Manuel</td>
<td>PS1-08-301</td>
<td>1291</td>
</tr>
<tr>
<td>Salvado, Isabel Margarida M.</td>
<td>PS2-09-318</td>
<td>1782</td>
</tr>
<tr>
<td>Salvatore, Luca</td>
<td>PS2-05-200</td>
<td>1666</td>
</tr>
<tr>
<td>Samitier, Josep</td>
<td>PS1-03-109</td>
<td>1097</td>
</tr>
<tr>
<td>San Felix, Ana</td>
<td>N08-03</td>
<td>454</td>
</tr>
<tr>
<td>San Juan Ferrer, Alejandro</td>
<td>Biomechanics 03-05</td>
<td>969</td>
</tr>
<tr>
<td>San Martín, Sebastián</td>
<td>PS1-07-257</td>
<td>1248</td>
</tr>
<tr>
<td>San Roman, Julio</td>
<td>ESB-SIBB S02-01</td>
<td>705</td>
</tr>
<tr>
<td>San Román, Julio</td>
<td>N05-01</td>
<td>290</td>
</tr>
<tr>
<td>Sanai, Nader</td>
<td>PS2-01-034</td>
<td>1506</td>
</tr>
<tr>
<td>Sancakli, Aykut</td>
<td>PS2-07-224</td>
<td>1691</td>
</tr>
<tr>
<td>Santos, Susana G.</td>
<td>PS1-08-294</td>
<td>1757</td>
</tr>
<tr>
<td>Sánchez, Irene</td>
<td>TRS-S02-05</td>
<td>172</td>
</tr>
<tr>
<td>Sanchez-Kopper, Andrés</td>
<td>AERO InVF-07</td>
<td>633</td>
</tr>
<tr>
<td>Sanchez-Rubio, Alvaro</td>
<td>K03-01</td>
<td>253</td>
</tr>
<tr>
<td>Sandeman, Susan</td>
<td>PS1-13-405</td>
<td>1403</td>
</tr>
<tr>
<td>Sandri, Monica</td>
<td>PS2-13-394</td>
<td>1863</td>
</tr>
<tr>
<td>Sandström, Corine</td>
<td>S10-03</td>
<td>684</td>
</tr>
<tr>
<td>Sangiorgi, Nicola</td>
<td>K10-05</td>
<td>865</td>
</tr>
<tr>
<td>Sankaran, Shrikrishnan</td>
<td>PS2-05-200</td>
<td>1666</td>
</tr>
<tr>
<td>Santinetti, Marida</td>
<td>K07-04</td>
<td>759</td>
</tr>
<tr>
<td>Santillano-Gonzalez, Carla</td>
<td>PS2-01-014</td>
<td>1486</td>
</tr>
<tr>
<td>Santin, Matteo</td>
<td>ESB-CSBM S-04</td>
<td>203</td>
</tr>
<tr>
<td>Santorelli, Filippo M.</td>
<td>S12-06</td>
<td>794</td>
</tr>
<tr>
<td>Santos, Catarina</td>
<td>PS2-10-326</td>
<td>1791</td>
</tr>
<tr>
<td>Santos Beato, Patricia</td>
<td>PS2-09-312</td>
<td>1777</td>
</tr>
<tr>
<td>Santos, Lúcia F.</td>
<td>ESB-BMSJ S-03</td>
<td>724</td>
</tr>
<tr>
<td>Santos, Nuno C.</td>
<td>S11-03</td>
<td>697</td>
</tr>
<tr>
<td>Santos, Sara C.</td>
<td>K05-05</td>
<td>499</td>
</tr>
<tr>
<td>Santos, Simão C.</td>
<td>S02-04</td>
<td>108</td>
</tr>
<tr>
<td>Santos, Sofia D.</td>
<td>K07-02</td>
<td>753</td>
</tr>
<tr>
<td>Santos, Susana G.</td>
<td>K05-03</td>
<td>495</td>
</tr>
<tr>
<td>Sankaran, Shrikrishnan</td>
<td>K10-05</td>
<td>865</td>
</tr>
<tr>
<td>Sannino, Alessandro</td>
<td>PS2-05-200</td>
<td>1666</td>
</tr>
<tr>
<td>Sano, Alessandra</td>
<td>S10-03</td>
<td>684</td>
</tr>
<tr>
<td>Sano, Alessandra</td>
<td>S10-03</td>
<td>684</td>
</tr>
<tr>
<td>Santos, Susana G.</td>
<td>PS1-08-318</td>
<td>1782</td>
</tr>
<tr>
<td>San Roman, Julio</td>
<td>ESB-SIBB S02-01</td>
<td>705</td>
</tr>
<tr>
<td>San Román, Julio</td>
<td>N05-01</td>
<td>290</td>
</tr>
<tr>
<td>Sanai, Nader</td>
<td>PS2-01-034</td>
<td>1506</td>
</tr>
<tr>
<td>Sancakli, Aykut</td>
<td>PS2-08-294</td>
<td>1757</td>
</tr>
<tr>
<td>Sánchez, Irene</td>
<td>TRS-S02-05</td>
<td>172</td>
</tr>
<tr>
<td>Sanchez-Kopper, Andrés</td>
<td>AERO InVF-07</td>
<td>633</td>
</tr>
<tr>
<td>Sanchez-Rubio, Alvaro</td>
<td>K03-01</td>
<td>253</td>
</tr>
<tr>
<td>Sandeman, Susan</td>
<td>PS1-13-405</td>
<td>1403</td>
</tr>
<tr>
<td>Sandri, Monica</td>
<td>PS2-13-394</td>
<td>1863</td>
</tr>
<tr>
<td>Sannino, Alessandro</td>
<td>PS2-05-200</td>
<td>1666</td>
</tr>
<tr>
<td>San Roman, Julio</td>
<td>ESB-SIBB S02-01</td>
<td>705</td>
</tr>
<tr>
<td>San Román, Julio</td>
<td>N05-01</td>
<td>290</td>
</tr>
<tr>
<td>Sanai, Nader</td>
<td>PS2-01-034</td>
<td>1506</td>
</tr>
<tr>
<td>Sancakli, Aykut</td>
<td>PS2-08-294</td>
<td>1757</td>
</tr>
</tbody>
</table>

Page 2003 of 2028
<table>
<thead>
<tr>
<th>Author Name</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Santos, Tiago</td>
<td>PS1-05-191</td>
</tr>
<tr>
<td>Santos-Rosasales, Víctor</td>
<td>AERO S01-03</td>
</tr>
<tr>
<td>Saracino, Emanuela</td>
<td>S10-03</td>
</tr>
<tr>
<td>Sarafidou, Katia</td>
<td>PS1-04-169</td>
</tr>
<tr>
<td>Saraiva, Catarina A.</td>
<td>PS2-03-108</td>
</tr>
<tr>
<td>Saraiva, Jorge A.</td>
<td>PS1-07-247</td>
</tr>
<tr>
<td>Saramago, Benilde</td>
<td>PS1-07-245</td>
</tr>
<tr>
<td>Sarasua, Jose R.</td>
<td>N15-06</td>
</tr>
<tr>
<td>Sarasua, Jose-Ramon</td>
<td>K04-05</td>
</tr>
<tr>
<td>Sardelli, Lorenzo</td>
<td>N08-09</td>
</tr>
<tr>
<td>Sargento-Freitas, João</td>
<td>NANOSTEM 03-05</td>
</tr>
<tr>
<td>Sargioti, Nikoletta</td>
<td>N06-02</td>
</tr>
<tr>
<td>Sarmento, Bruno</td>
<td>ESB-CRS S-05</td>
</tr>
<tr>
<td>Sarrigiannidis, Stylianos</td>
<td>K03-01</td>
</tr>
<tr>
<td>Sarrigiannidis, Stylianos O.</td>
<td>S10-07</td>
</tr>
<tr>
<td>Sartori, Susanna</td>
<td>N06-09</td>
</tr>
<tr>
<td>Sasson, Enrico</td>
<td>PS1-05-189</td>
</tr>
<tr>
<td>Sautou, Valérie</td>
<td>PS2-10-320</td>
</tr>
<tr>
<td>Scaini, Denis</td>
<td>PS2-07-254</td>
</tr>
<tr>
<td>Scaini, Denis</td>
<td>PS1-02-091</td>
</tr>
<tr>
<td>Scalia, Alessandro</td>
<td>PS1-15-451</td>
</tr>
<tr>
<td>Scalzone, Annachiara</td>
<td>N04-07</td>
</tr>
<tr>
<td>Schaefer, Natasha</td>
<td>N14-04</td>
</tr>
<tr>
<td>Schaefer, Tim</td>
<td>PS1-14-445</td>
</tr>
<tr>
<td>Schaefer, Natasa</td>
<td>PS2-06-216</td>
</tr>
<tr>
<td>Schäfer, Natascha</td>
<td>PS2-01-050</td>
</tr>
<tr>
<td>Schätzlein, Eva</td>
<td>PS2-01-006</td>
</tr>
<tr>
<td>Schenke-Layland, Katja</td>
<td>PS1-13-409</td>
</tr>
<tr>
<td>Schier, Arne</td>
<td>PS2-10-340</td>
</tr>
<tr>
<td>Schierz, Arne K.</td>
<td>PS1-07-251</td>
</tr>
<tr>
<td>Schiller, Laura</td>
<td>K09-07</td>
</tr>
<tr>
<td>Schilling, Franz</td>
<td>PS1-03-129</td>
</tr>
<tr>
<td>Schirhagl, Romana</td>
<td>K06-02</td>
</tr>
<tr>
<td>Schlaepfer, David D.</td>
<td>S09-02</td>
</tr>
<tr>
<td>Schlosser, Michael</td>
<td>PS1-15-457</td>
</tr>
<tr>
<td>Schlosser, Michael</td>
<td>PS2-04-148</td>
</tr>
<tr>
<td>Schlund, Matthias</td>
<td>PS1-02-085</td>
</tr>
<tr>
<td>Schmal, Hagen</td>
<td>PS2-10-332</td>
</tr>
<tr>
<td>Schmelzer, Christian</td>
<td>ESB-SLABO S-05</td>
</tr>
<tr>
<td>Schmelzer, Christian E.H.</td>
<td>ESB-BIOMAT S-04</td>
</tr>
<tr>
<td>Schmid, Rafael</td>
<td>PS1-14-443</td>
</tr>
<tr>
<td>Schmid, Ruth</td>
<td>S12-02</td>
</tr>
<tr>
<td>Schmidt, Sonja K</td>
<td>PS1-14-443</td>
</tr>
<tr>
<td>Author Name</td>
<td>Page Numbers</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Schmitz, Katja</td>
<td>PS1-11-347.............1345</td>
</tr>
<tr>
<td>Schmitz, Moniek G.</td>
<td>S03-07................125</td>
</tr>
<tr>
<td>Schnabelrauch, Matthias</td>
<td>K05-04................497</td>
</tr>
<tr>
<td></td>
<td>N10-08................558</td>
</tr>
<tr>
<td></td>
<td>PS2-15-462...............1936</td>
</tr>
<tr>
<td>Schneider, Marc</td>
<td>NANOSTEM 02-02.........352</td>
</tr>
<tr>
<td></td>
<td>NANOSTEM 03-02.........360</td>
</tr>
<tr>
<td>Schneider, Verena</td>
<td>PS1-01-033.............1025</td>
</tr>
<tr>
<td>Schneidereit, Dominik</td>
<td>PS1-09-317.............1311</td>
</tr>
<tr>
<td></td>
<td>PS2-14-442.............1914</td>
</tr>
<tr>
<td>Schönberg, Antje</td>
<td>N09-01................469</td>
</tr>
<tr>
<td>Schorle, Hannah</td>
<td>K08-06................779</td>
</tr>
<tr>
<td>Schot, Maik R.</td>
<td>PS1-01-021.............1013</td>
</tr>
<tr>
<td></td>
<td>S01-06................98</td>
</tr>
<tr>
<td>Schräder, Philipp</td>
<td>PS2-01-008.............1479</td>
</tr>
<tr>
<td>Schreurs, Yvonne W.</td>
<td>PS2-14-430.............1901</td>
</tr>
<tr>
<td>Schröter, Lena</td>
<td>PS2-05-198.............1664</td>
</tr>
<tr>
<td>Schreuer, Stefan</td>
<td>PS1-02-095.............1085</td>
</tr>
<tr>
<td>Schrüfer, Stefan</td>
<td>PS1-14-443.............1444</td>
</tr>
<tr>
<td></td>
<td>PS2-14-442.............1914</td>
</tr>
<tr>
<td>Schubert, Dirk W.</td>
<td>PS1-14-443.............1444</td>
</tr>
<tr>
<td>Schubert, Dirk W.</td>
<td>N14-09.................913</td>
</tr>
<tr>
<td></td>
<td>PS1-01-023.............1015</td>
</tr>
<tr>
<td></td>
<td>PS1-01-053.............1044</td>
</tr>
<tr>
<td></td>
<td>PS1-02-095.............1085</td>
</tr>
<tr>
<td></td>
<td>PS2-14-442.............1914</td>
</tr>
<tr>
<td></td>
<td>S10-04................686</td>
</tr>
<tr>
<td>Schulz, Matthias C.</td>
<td>N09-01................469</td>
</tr>
<tr>
<td>Schulze, Agnes</td>
<td>PS2-08-272.............1736</td>
</tr>
<tr>
<td>Schulze, Hendrik A.</td>
<td>PS2-10-340.............1806</td>
</tr>
<tr>
<td>Schulze, Sabine</td>
<td>PS1-04-149.............1138</td>
</tr>
<tr>
<td>Schulze, Thomas</td>
<td>N09-07................480</td>
</tr>
<tr>
<td>Schulz-Siegmund, Michaela</td>
<td>K09-07................853</td>
</tr>
<tr>
<td>Schumacher, Matthias</td>
<td>PS2-15-462...............1936</td>
</tr>
<tr>
<td>Schurgers, Leon</td>
<td>PS2-14-426...............1898</td>
</tr>
<tr>
<td>Schuster, Karin</td>
<td>PS1-15-457...............1457</td>
</tr>
<tr>
<td>Schütz, Kathleen</td>
<td>N09-07................480</td>
</tr>
<tr>
<td>Schuurmann, Richte</td>
<td>PS2-09-308...............1773</td>
</tr>
<tr>
<td>Schwab, Andrea</td>
<td>N13-05................824</td>
</tr>
<tr>
<td>Schwabe, Kerstin</td>
<td>PS2-15-446...............1917</td>
</tr>
<tr>
<td>Schwaminger, Sebastian P.</td>
<td>PS1-03-129...............1117</td>
</tr>
<tr>
<td>Schwartz Jr., Simo</td>
<td>ESB-CRS S-05...........429</td>
</tr>
<tr>
<td>Schwarz, Hans-Christoph</td>
<td>PS2-10-340...............1806</td>
</tr>
<tr>
<td>Schwarz, Romana</td>
<td>PS2-09-310...............1775</td>
</tr>
<tr>
<td>Scialla, Stefania</td>
<td>PS2-03-118...............1586</td>
</tr>
<tr>
<td>Scordo, Giorgio</td>
<td>PS1-14-435...............1435</td>
</tr>
<tr>
<td></td>
<td>PS2-14-438...............1910</td>
</tr>
<tr>
<td>Seabra, Catarina L.</td>
<td>K10-04................863</td>
</tr>
<tr>
<td></td>
<td>N15-07................929</td>
</tr>
<tr>
<td>Seabra, Cátia</td>
<td>N06-03................311</td>
</tr>
<tr>
<td>Seegers, Monika</td>
<td>PS2-04-138...............1606</td>
</tr>
<tr>
<td>Seibert, Franz</td>
<td>N11-06................575</td>
</tr>
<tr>
<td>Seidelmann, Max</td>
<td>PS1-14-433.............1434</td>
</tr>
<tr>
<td>Seidenstuecker, Michael</td>
<td>PS2-10-332...............1797</td>
</tr>
<tr>
<td>Seifried, Bernhard</td>
<td>AERO S03-KL03...........637</td>
</tr>
<tr>
<td>Seijas-Gamardo, Adrian</td>
<td>N05-09................304</td>
</tr>
<tr>
<td>Seitz, Hermann</td>
<td>PS1-09-317...............1311</td>
</tr>
<tr>
<td>Seixas, Duarte</td>
<td>K06-03................655</td>
</tr>
<tr>
<td>Selam, Edanur</td>
<td>PS1-07-253...............1244</td>
</tr>
<tr>
<td>Selaru, Aida</td>
<td>PS1-07-219...............1214</td>
</tr>
<tr>
<td>Selegård, Robert</td>
<td>PS2-03-110...............1580</td>
</tr>
<tr>
<td>Author Name</td>
<td>Page Numbers</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Selicato, Nora</td>
<td>1801</td>
</tr>
<tr>
<td>Sembdner, Philipp</td>
<td>469</td>
</tr>
<tr>
<td>Semitela, Angela</td>
<td>1474</td>
</tr>
<tr>
<td>Sen, Ozlem</td>
<td>794</td>
</tr>
<tr>
<td>Şen, Ozlem</td>
<td>1845</td>
</tr>
<tr>
<td>Sener Raman, Tugce</td>
<td>1736</td>
</tr>
<tr>
<td>Senger, Bernard</td>
<td>869</td>
</tr>
<tr>
<td>Şenses, Erkan</td>
<td>639</td>
</tr>
<tr>
<td>Serafin, Andrada</td>
<td>292</td>
</tr>
<tr>
<td>Serafin, Aleksandra</td>
<td>337</td>
</tr>
<tr>
<td>Serbu-Franzos, Joaquin</td>
<td>705</td>
</tr>
<tr>
<td>Serban, Alexandru</td>
<td>1352</td>
</tr>
<tr>
<td>Sergi, Francesca</td>
<td>756</td>
</tr>
<tr>
<td>Serra, Julia</td>
<td>1561</td>
</tr>
<tr>
<td>Serrano Ruiz, Manuel</td>
<td>1593</td>
</tr>
<tr>
<td>Serrano, Juana</td>
<td>781</td>
</tr>
<tr>
<td>Serrano, Maria C.</td>
<td>1849</td>
</tr>
<tr>
<td>Serre, Karine</td>
<td>1722</td>
</tr>
<tr>
<td>Serro, Ana Paula</td>
<td>815</td>
</tr>
<tr>
<td>Sgarfinato, Viola</td>
<td>362</td>
</tr>
<tr>
<td>Sgarminato, Viola</td>
<td>1435</td>
</tr>
<tr>
<td>Sevastre, Bogdan</td>
<td>1558</td>
</tr>
<tr>
<td>Sevin, Emmanuel</td>
<td>323</td>
</tr>
<tr>
<td>Shapko, Kaveh</td>
<td>508</td>
</tr>
<tr>
<td>Shan, Junwen</td>
<td>1046</td>
</tr>
<tr>
<td>Sharipova, Aliya</td>
<td>581</td>
</tr>
<tr>
<td>Sharma, Aarushi</td>
<td>1630</td>
</tr>
<tr>
<td>Sharma, Prashant K.</td>
<td>1052</td>
</tr>
<tr>
<td>Sharpe, Paul T.</td>
<td>682</td>
</tr>
<tr>
<td>Sheng, Christina</td>
<td>898</td>
</tr>
<tr>
<td>Shin, Su R.</td>
<td>772</td>
</tr>
<tr>
<td>Shipp, Christopher</td>
<td>1408</td>
</tr>
<tr>
<td>Sidious, Robin</td>
<td>792</td>
</tr>
<tr>
<td>Siamidi, Aggeliki</td>
<td>1808</td>
</tr>
<tr>
<td>Siarampi, Eleni</td>
<td>1632</td>
</tr>
<tr>
<td>Sibiliano, Teresa</td>
<td>1666</td>
</tr>
<tr>
<td>Sicard, Ludovic</td>
<td>1161</td>
</tr>
<tr>
<td>Siculella, Luisa</td>
<td>1149</td>
</tr>
<tr>
<td>Siibennmorgen, Clio</td>
<td>1255</td>
</tr>
<tr>
<td>Sieber, Maciej</td>
<td>1452</td>
</tr>
<tr>
<td>Siegel, Anna</td>
<td>1285</td>
</tr>
<tr>
<td>Sierakowski, Maciej</td>
<td>1338</td>
</tr>
<tr>
<td>Sievers, Jana</td>
<td>1342</td>
</tr>
<tr>
<td>Sigaeva, Alina</td>
<td>653</td>
</tr>
<tr>
<td>Sigurjonssohn, Olafur E.</td>
<td>1838</td>
</tr>
<tr>
<td>Silva, A. Sofia</td>
<td>215</td>
</tr>
<tr>
<td>Silva, Ana Francisco G.</td>
<td>1215</td>
</tr>
<tr>
<td>Silva, Ana S.</td>
<td>724</td>
</tr>
<tr>
<td>Silva, Carla</td>
<td>239</td>
</tr>
<tr>
<td>Silva, Carla</td>
<td>89</td>
</tr>
<tr>
<td>Silva, Carla</td>
<td>1350</td>
</tr>
<tr>
<td>Silva, Diana</td>
<td>1237</td>
</tr>
</tbody>
</table>

Page 2006 of 2028
Author Index

Silva, Eduardo A.
K07-06 ......................................................... 763
Silva, Fernando
NANOSTEM 03-05 ......................................... 365
Silva, Filipe S.
PS1-05-195 ..................................................... 1184
Silva, Jorge C.
PS1-03-117 ..................................................... 1102
PS1-08-291 ..................................................... 1282
Silva, Samira
ESB-SIBB S01-07 ............................................. 530
Silva-Correia, Joana
PS2-12-382 ..................................................... 1851
Silva-Herdade, Ana S.
PS2-07-238 ..................................................... 1708
Silvani, Sara
S09-04 .......................................................... 670
Silva-Reis, Sara C.
K09-04 .......................................................... 846
N01-06 .......................................................... 139
Silveira, João
PS2-07-256 ..................................................... 1724
Silvestri, Teresa
S06-03 .......................................................... 398
Simões, Susana
N06-05 .......................................................... 316
NANOSTEM 03-03 ............................................. 362
NANOSTEM 04-03 ............................................. 371
Simões, Susana M.N.
NANOSTEM 04-01 ............................................. 368
Simon, Jan C.
K05-04 .......................................................... 497
Simón, Valeska
PS1-07-257 ..................................................... 1248
Simón-Vázquez, Rosana
AERO InvF-06 .................................................. 630
Simon-Yarza, Teresa
N12-08 .......................................................... 745
PS1-08-271 ..................................................... 1263
PS1-03-125 ..................................................... 1112
Simpson, Christopher R.
PS2-08-284 ..................................................... 1747
Sims-Mourtada, Jennifer
PS2-14-432 ..................................................... 1903
Singer, Verena
PS2-01-036 ..................................................... 1508
Singh, Krutika
PS2-01-026 ..................................................... 1500
S04-04 .......................................................... 194
Sinha, Ravi
PS2-04-132 ..................................................... 1601
Six, Milan
K02-07 .......................................................... 186
Sjollema, Jelmer
PS1-15-453 ..................................................... 1452
Skadins, Ingus
PS1-06-203 ..................................................... 1195
PS1-10-337 ..................................................... 1334
Skibiński, Szymon
PS1-03-111 ..................................................... 1098
PS1-04-147 ..................................................... 1136
PS2-03-112 ..................................................... 1582
Skirtach, Andre
ESB-BIOMAT S-07 ........................................... 447
Skjöldebrand, Charlotte
S13-03 .......................................................... 803
Skrinda, Marite
PS1-10-327 ..................................................... 1324
Slámečka, Karel
PS2-02-080 ..................................................... 1550
PS2-05-188 ..................................................... 1652
Ślósarczyk, Anna
N02-02 .......................................................... 151
PS1-03-111 ..................................................... 1098
PS1-04-147 ..................................................... 1136
PS2-03-112 ..................................................... 1582
PS2-04-146 ..................................................... 1610
Smeets, Bert
PS2-01-008 ..................................................... 1479
Smink, Alexandra M.
K08-03 .......................................................... 772
N11-05 .......................................................... 701
Smirnova, Irina
AERO S01-02 ................................................... 590
Smit, Ineke
N04-03 .......................................................... 234
Soares, Paula
PS2-08-288 ..................................................... 1752
Soares, Paula I.
PS1-07-243 ..................................................... 1235
Soares, Paula J.P.
N01-03 .......................................................... 133
PS1-03-117 ..................................................... 1102
PS1-08-291 ..................................................... 1282
PS2-08-280 ..................................................... 1743
Soares, Raquel
N10-05 .......................................................... 552
Sobczak, Klaudia O.
PS2-11-356 ..................................................... 1823
Sobociński, Jonathan
PS2-01-002 ..................................................... 1472
Söderlund, Zackarias
PS1-14-431 ..................................................... 1431
Sohier, Jerome
ESB-BIOMAT S-06 ........................................... 444
Sohier, Jérôme
S05-06 .......................................................... 277
Söhling, Nicolas
PS2-01-006 ..................................................... 1477
Page 2007 of 2028
<table>
<thead>
<tr>
<th>Author/Name</th>
<th>Index/No</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Espinosa, Xavi</td>
<td>N16-06</td>
<td>941</td>
</tr>
<tr>
<td>Solimei, Luca</td>
<td>PS1-06-209</td>
<td>1202</td>
</tr>
<tr>
<td></td>
<td>PS1-06-213</td>
<td>1206</td>
</tr>
<tr>
<td></td>
<td>PS2-04-152</td>
<td>1616</td>
</tr>
<tr>
<td>Somby, Karin</td>
<td>PS2-08-270</td>
<td>1735</td>
</tr>
<tr>
<td>Song, Wenhui</td>
<td>PS2-12-384</td>
<td>1853</td>
</tr>
<tr>
<td>Sonnleitner, David</td>
<td>PS2-01-050</td>
<td>1520</td>
</tr>
<tr>
<td>Sonntag, Frank</td>
<td>K06-05</td>
<td>659</td>
</tr>
<tr>
<td>Soria, Federico</td>
<td>ESB-SIBB S01-KL01</td>
<td>516</td>
</tr>
<tr>
<td>Soriente, Alessandra</td>
<td>K08-07</td>
<td>781</td>
</tr>
<tr>
<td></td>
<td>N01-08</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td>PS2-03-118</td>
<td>1586</td>
</tr>
<tr>
<td></td>
<td>PS2-07-244</td>
<td>1712</td>
</tr>
<tr>
<td></td>
<td>PS2-07-246</td>
<td>1714</td>
</tr>
<tr>
<td>Sort, Jordi</td>
<td>PS1-04-139</td>
<td>1130</td>
</tr>
<tr>
<td>Sosnik, Alejandro</td>
<td>N11-09</td>
<td>581</td>
</tr>
<tr>
<td>Soulé, Jérémmy</td>
<td>N04-02</td>
<td>232</td>
</tr>
<tr>
<td></td>
<td>PS1-06-205</td>
<td>1198</td>
</tr>
<tr>
<td></td>
<td>S12-05</td>
<td>792</td>
</tr>
<tr>
<td>Sousa, Aureliana</td>
<td>PS2-07-256</td>
<td>1724</td>
</tr>
<tr>
<td>Sousa, Beatriz</td>
<td>PS2-04-156</td>
<td>1621</td>
</tr>
<tr>
<td>Sousa, Cristiana F.</td>
<td>PS2-03-108</td>
<td>1578</td>
</tr>
<tr>
<td></td>
<td>S02-03</td>
<td>106</td>
</tr>
<tr>
<td>Sousa, Flávia</td>
<td>N02-09</td>
<td>167</td>
</tr>
<tr>
<td>Sousa, Joana Patrícia M</td>
<td>K04-06</td>
<td>388</td>
</tr>
<tr>
<td>Souza, João A.</td>
<td>NANOSTEM 03-05</td>
<td>365</td>
</tr>
<tr>
<td>Sousa, Rui A.</td>
<td>TRS-S01-01</td>
<td>73</td>
</tr>
<tr>
<td>Southall, Maria</td>
<td>YSF-AERogels WS-02</td>
<td>616</td>
</tr>
<tr>
<td>Souto-Lopes, Mariana</td>
<td>PS2-02-084</td>
<td>1555</td>
</tr>
<tr>
<td>Spangenberg, Janina</td>
<td>PS1-01-011</td>
<td>1004</td>
</tr>
<tr>
<td></td>
<td>PS1-11-351</td>
<td>1348</td>
</tr>
<tr>
<td>Spedicati, Mattia</td>
<td>N12-01</td>
<td>732</td>
</tr>
<tr>
<td>Spratt, Joel</td>
<td>NANOSTEM 03-04</td>
<td>364</td>
</tr>
<tr>
<td>Sprecher, Christoph</td>
<td>PS1-01-001</td>
<td>994</td>
</tr>
<tr>
<td></td>
<td>PS2-01-028</td>
<td>1502</td>
</tr>
<tr>
<td>Spreitzer, Matjaž</td>
<td>N07-07</td>
<td>341</td>
</tr>
<tr>
<td>Spreitzer, Matjaž</td>
<td>PS2-11-352</td>
<td>1819</td>
</tr>
<tr>
<td>Spriano, Silvia</td>
<td>PS1-13-399</td>
<td>1398</td>
</tr>
<tr>
<td></td>
<td>PS1-15-451</td>
<td>1451</td>
</tr>
<tr>
<td></td>
<td>PS2-15-460</td>
<td>1934</td>
</tr>
<tr>
<td>Sprio, Simone</td>
<td>N09-05</td>
<td>476</td>
</tr>
<tr>
<td></td>
<td>PS2-04-150</td>
<td>1614</td>
</tr>
<tr>
<td></td>
<td>PS2-04-162</td>
<td>1624</td>
</tr>
<tr>
<td>Syropoulos, Konstantinos</td>
<td>PS2-02-088</td>
<td>1560</td>
</tr>
<tr>
<td>Srinivasan, Sumithra Y.</td>
<td>AERO PS01-03</td>
<td>601</td>
</tr>
<tr>
<td></td>
<td>AERO PS01-04</td>
<td>603</td>
</tr>
<tr>
<td>Sroczyk, Ewa A.</td>
<td>PS1-07-225</td>
<td>1219</td>
</tr>
<tr>
<td>Stachewicz, Urszula</td>
<td>N14-08</td>
<td>912</td>
</tr>
<tr>
<td></td>
<td>PS1-07-225</td>
<td>1219</td>
</tr>
<tr>
<td></td>
<td>PS1-07-229</td>
<td>1223</td>
</tr>
<tr>
<td></td>
<td>PS1-13-397</td>
<td>1396</td>
</tr>
<tr>
<td></td>
<td>PS2-04-140</td>
<td>1608</td>
</tr>
<tr>
<td>Stachowicz, Marie-Laure</td>
<td>S14-05</td>
<td>880</td>
</tr>
<tr>
<td></td>
<td>PS1-01-061</td>
<td>1048</td>
</tr>
<tr>
<td></td>
<td>PS2-01-052</td>
<td>1523</td>
</tr>
<tr>
<td></td>
<td>S01-03</td>
<td>92</td>
</tr>
<tr>
<td>Staelens, Jean-Noël</td>
<td>PS1-07-223</td>
<td>1217</td>
</tr>
<tr>
<td>Stamboroski, Stephani</td>
<td>S12-04</td>
<td>790</td>
</tr>
<tr>
<td>Stamboulis, Artemis</td>
<td>PS2-11-356</td>
<td>1823</td>
</tr>
<tr>
<td>Stanca, Eleonora</td>
<td>PS1-04-159</td>
<td>1149</td>
</tr>
<tr>
<td>Stancu, Izabela</td>
<td>PS1-07-219</td>
<td>1214</td>
</tr>
<tr>
<td>Stancu, Izabela C.</td>
<td>N05-02</td>
<td>292</td>
</tr>
<tr>
<td>Stankovic, Tijana</td>
<td>PS1-14-437</td>
<td>1438</td>
</tr>
</tbody>
</table>
Author Index

Starbird-Perez, Ricardo  
AERO InvF-04 ........................................... 625  
AERO InvF-07 ........................................... 633

Stark, Robert W.  
K02-02 .................................................... 175

Stasiwicz, Martyna  
K05-02 .................................................... 492

Staubli, Flurina  
N13-05 .................................................... 824

Steenackers, Hans P.  
PS2-13-410 ............................................. 1881

Stein, Svenja  
PS2-05-198 ............................................. 1664

Steiner, Dominik  
PS2-14-442 ............................................. 1914

Steinke, Nathalie  
PS1-02-075 ............................................. 1063

Stella, Barbara  
PS2-12-372 ............................................. 1840

Stepanova, Valentina  
PS2-06-214 ............................................. 1681

Stępień, Karolina  
N02-02 .................................................... 151

Stevanovic, Milena  
PS1-14-439 ............................................. 1440

Stevens, Molly M.  
S14-04 .................................................... 878

Stiesch, Meike  
PS2-04-138 ............................................. 1606  
PS2-08-258 ............................................. 1727

Stipniece, Liga  
PS1-06-203 ............................................. 1195  
PS2-06-204 ............................................. 1671

Stoddart, Martin  
N02-06 .................................................... 160

Stoddart, Martin J.  
N13-06 .................................................... 827  
PS1-01-039 ............................................. 1031

Stojkovska, Jasmina  
PS1-14-437 ............................................. 1438  
PS1-14-439 ............................................. 1440

Stoppe, Thomas  
S07-05 .................................................... 417

Stötzel, Sabine  
PS1-02-067 ............................................. 1057

Strauß, Tim-Joshua  
PS2-10-340 ............................................. 1806

Strick, Reiner  
K08-06 .................................................... 779  
N14-04 .................................................... 902

Stricker, Stefan H.  
N06-05 .................................................... 316  
NANOSTEM 04-02 ..................................... 370

Strissel, Pamela L.  
K08-06 .................................................... 779  
N14-04 .................................................... 902

Stromme, Maria  
N10-04 .................................................... 550

Strüder, Daniel  
PS2-04-148 ............................................. 1612

Stühn, Lukas  
K02-02 .................................................... 175

Su, Yingchun  
ESB-CSBM S-05 ...................................... 205

Suarato, Giulia  
PS2-07-232 ............................................. 1701

Suarez Vargas, Natalia  
PS2-14-440 ............................................. 1912

Suay, Julio  
N08-06 .................................................... 460  
PS1-13-401 ............................................. 1400

Suba, Gilles  
PS1-01-027 ............................................. 1020

Sung, Baecckkyoung  
PS2-13-398 ............................................. 1868

Surmiak, Marcin  
PS2-09-310 ............................................. 1775

Suter, Naiana  
S12-04 .................................................... 790

Swartz Jr, Simo  
ESB-SIBB S02-01 .................................... 705

Świergosz, Tomasz  
PS1-02-097 ............................................. 1087

Święszkowski, Wojciech  
K10-06 .................................................... 867

Swiklo, Stephen  
PS1-01-041 ............................................. 1033

Szabó, Anna  
PS2-02-072 ............................................. 1542

Szeman, Gabor N.  
AERO S02-03 ........................................... 613

Szewczyk, Piotr  
PS1-13-397 ............................................. 1396

T

Tabary, Nicolas  
PS1-07-223 ............................................. 1217  
PS2-01-002 ............................................. 1472

Taebnia, Nayere  
PS1-08-275 ............................................. 1267

Tahmasebi Birgani, Niloofar Z.  
PS2-13-406 ............................................. 1876  
N09-06 .................................................... 478  
PS1-13-393 ............................................. 1391  
PS2-14-426 ............................................. 1898
Author Index

Tampieri, Anna
N09-05 ............................................. 476
PS2-01-018 .................................... 1491
PS2-04-150 .................................... 1614
PS2-04-162 .................................... 1624
S10-03 ........................................... 684
Tampieri, Francesco
N16-06 ........................................... 941
Tandon, Biranche
N07-04 ........................................... 335
N14-04 ........................................... 902
Tang, Junmei
PS1-07-247 ..................................... 1239
Tang, Qiao
PS1-12-375 ..................................... 1372
Tanner, Kathleen E.
PS2-04-166 ..................................... 1628
Tarricone, Giulia
PS2-12-372 ..................................... 1840
Tarsini, Paolo
PS1-15-455 ..................................... 1454
Tartau-Mititelu, Liliana
PS2-08-268 ..................................... 1734
Tassi, Natália
S03-02 ........................................... 115
Tausescu, Marian
PS1-05-177 ..................................... 1166
PS2-02-086 ..................................... 1558
Tavares, Tânia D.
ESB-SIBB S01-05 ................................ 524
PS1-11-353 ..................................... 1350
PS2-07-226 ..................................... 1693
Tavoni, Marta
PS2-04-150 ..................................... 1614
PS2-04-162 ..................................... 1624
Taymour, Rania
N13-07 ........................................... 829
Tayyem, May A.
PS2-11-362 ..................................... 1829
Teixeira, Ana
NANOSTEM 03-04 ................................ 364
Teixeira, Cátia
K10-04 ........................................... 863
PS1-11-359 ..................................... 1356
S03-02 ........................................... 115
Teixeira, Liliana
PS2-14-424 ..................................... 1896
YSF WS-05 ..................................... 66
Teixeira, Marta
PS1-07-221 ..................................... 1215
Teixeira, Marta A.
ESB-SIBB S01-05 ................................ 524
N10-06 ........................................... 554
S12-03 ........................................... 788
Teixeira, Marta O.
ESB-SIBB S01-05 ................................ 524
Teixeira, Simao P.B.
N15-01 ........................................... 916
Temelli, Feral
AERO S03-KL03 ................................ 637
ten Den, Simone
K07-07 ........................................... 765
Tenje, Maria
PS1-04-153 ..................................... 1143
S13-04 ........................................... 805
Terpstra, Margo L.
S01-04 ........................................... 94
Terraciano, Cesare
K07-02 ........................................... 753
Terzi, Alberta
PS2-05-200 ..................................... 1666
Terzopoulou, Zoe
PS2-01-058 ..................................... 1526
Teske, Michael
PS1-15-461 ..................................... 1463
Teßmar, Jörg
K08-02 ........................................... 770
PS1-01-059 ..................................... 1046
PS1-07-233 ..................................... 1226
PS2-01-042 ..................................... 1514
Texier, Isabelle
N07-02 ........................................... 330
Texier-Nogues, Isabelle
S12-02 ........................................... 786
Tey, Marc
Biomechanics 01-04 ................................ 955
Teyssier, Catherine
PS1-01-049 ..................................... 1039
Theocharidou, Anna
PS1-06-215 ..................................... 1208
PS1-12-391 ..................................... 1388
Thienpont, Hugo
ESB-ISBF S-04 ................................... 537
Thievessen, Ingo
PS1-14-443 ..................................... 1444
PS2-01-050 ..................................... 1520
PS2-14-442 ..................................... 1914
Thijssen, Quinten
PS2-05-180 ..................................... 1645
Thomann, Jean-Sebastien
PS1-12-381 ..................................... 1378
Thompson, Keith
N02-06 ........................................... 160
Tiainen, Hanna
ESB-SIBB S01-04 ................................ 522
PS2-15-448 ..................................... 1919
Tiainen, Laura K.
PS1-05-195 ..................................... 1184
Tibbitt, Mark W.  
K03-03 ........................................ 257

Tien, Nguyen D.  
PS1-07-231 .................................. 1224

Tina, Vermendon  
N09-08 ........................................ 482

Tironi, Matteo  
S09-04 ........................................ 670

Tkachenko, Serhii  
PS2-05-188 .................................. 1652

Todea, Milica  
PS1-02-071 .................................. 1058
PS1-05-177 .................................. 1166

Toffoletto, Nadia  
PS1-07-245 .................................. 1237
PS1-07-247 .................................. 1239
PS2-07-238 .................................. 1708

Toftdal, Mette S.  
PS1-08-275 .................................. 1267

Tohi, Shafagh D.  
N10-06 ........................................ 554
S12-03 ........................................ 788

Tokhadze, Nicolas  
PS2-10-320 .................................. 1784

Toma, Corina  
PS2-02-086 .................................. 1558

Tomás, Helena  
K01-04 ........................................ 80

Tomás, Helena M.  
PS2-12-386 .................................. 1854

Tomasin, Clarissa  
K01-02 ........................................ 76
S14-03 ........................................ 876

Tomasin, Claudia  
PS1-06-211 .................................. 1204

Tomatis, Francesca  
N06-05 ........................................ 316
NANOSTEM 03-03 ................................ 362
NANOSTEM 04-01 ................................ 368

Tonda-Turo, Chiara  
K08-04 ........................................ 775
PS1-01-017 .................................. 1011
PS1-14-435 .................................. 1435
PS2-01-030 .................................. 1504
PS2-14-438 .................................. 1910

Tophete, Ana  
PS1-07-247 .................................. 1239

Topping, Geoffrey J.  
PS1-03-129 .................................. 1117

Torchio, Alessandro  
N06-09 ........................................ 325

Torii, Ryo  
PS2-01-048 .................................. 1518

Tornin, Juan  
K09-05 ........................................ 848

Torrado, Marília  
N01-06 ........................................ 139
S02-03 ........................................ 106

Torregrossa, Marta  
K05-04 ........................................ 497

Torres Sanchez, Ruben  
PS2-01-010 .................................. 1481

Torres, Ana L.  
K07-06 ........................................ 763
S09-07 ........................................ 676

Torres, Gonzalo  
Biomechanics 03-05 ................................ 969

Torres, Paula Maria C.  
PS2-05-202 .................................. 1669

Torres, Yoann  
N12-03 ........................................ 736

Torricelli, Paola  
PS1-06-211 .................................. 1204

Tóth, Zsejke R.  
PS1-02-071 .................................. 1058
PS2-02-072 .................................. 1542

Toth, Zsejke-Reka  
PS2-02-086 .................................. 1558

Tournier, Pierre  
N16-05 ........................................ 940

Tracuma, Eliza  
PS1-10-327 .................................. 1324

Traldi, Federico  
NANOSTEM 01-05 ................................ 350
NANOSTEM 03-03 ................................ 362
PS1-12-373 .................................. 1371

Trapella, Claudio  
S03-03 ........................................ 117

Trávníčková, Martina  
PS1-02-097 .................................. 1087

Treacy, Niall  
S04-04 ........................................ 194

Triacca, Valentina  
TRRS-S01-02 ................................ 73

Trichet, Léa  
S02-05 ........................................ 110

Trikalitis, Vasileios  
N12-09 ........................................ 747

Trmka, Julien  
S05-03 ........................................ 270

Trombetta, Marcella  
N16-09 ........................................ 948

Trotier, Alexandre  
N01-05 ........................................ 137

Troçiño, Sonia R.  
PS2-10-330 .................................. 1795

Trucco, Diego  
PS1-08-297 .................................. 1287
PS2-04-168 .................................. 1630
Author Index

Truckenmüller, Roman
N02-07 ........................................... 162
PS2-13-406 ..................................... 1876
PS2-14-424 ..................................... 1896
PS2-14-426 ..................................... 1898
PS2-15-456 ..................................... 1930
Trujillo Miranda, Mairon
PS2-01-022 ...................................... 1496
Trujillo, Sara
N08-03 ........................................... 454
Tryfonidou, Marianna A.
S15-KL02 ........................................ 888
Tsamesidis, Ioannis
PS1-06-215 ..................................... 1208
PS1-12-391 ..................................... 1388
Tufan, Yiqithan
PS1-06-207 ..................................... 1200
PS2-03-120 ..................................... 1588
Tuna, Taskin
PS1-02-075 ...................................... 1063
Türel, Akif E.
NANOSTEM 02-02 ............................. 352
NANOSTEM 03-02 ............................. 360
Türel, Nazende G.
ESB-CRS S-03 ................................. 424
Turłybekuly, Amanzhol
PS2-04-134 ...................................... 1603
Turner, Joel
N09-02 ........................................... 471
N10-07 ........................................... 556
PS1-14-445 ..................................... 1445
PS2-06-216 ..................................... 1684
PS2-12-374 ..................................... 1842
S08-03 ........................................... 508
Tuzlakoglu, Kadriye
PS1-07-253 ...................................... 1244
Tzagiollari, Antzela
PS2-04-154 ...................................... 1618
Tzanakakis, Emmanouil-George C.
PS1-06-215 ..................................... 1208
Tzoutzas, Ioannis
PS1-06-215 ..................................... 1208
U
Uboldi, Marco
S04-05 ............................................ 196
Udovc, Lea
N07-07 ............................................ 341
Ugolini, Giovanni S.
PS2-14-444 ..................................... 1915
Ubu, Mai
AERO PS01-02 .................................. 599
Uludağ, Hasan
PS2-10-346 ..................................... 1812
Ulalan, Irem
PS1-11-349 ..................................... 1347
Unal, Ufuk
K04-05 ............................................ 386
PS2-07-228 ..................................... 1695
Unune, Deepak Rajendra
PS1-02-099 ...................................... 1089
Ura, Daniel
PS1-13-397 ..................................... 1396
Urbanek, Olga
PS2-07-224 ..................................... 1691
Urzi, Christian
PS2-05-194 ..................................... 1659
Usseglio, Julie
PS1-03-119 ..................................... 1105
Uzuner Demir, Aysegul
PS2-08-294 ..................................... 1757
V
Vadivel, Dhanalakshmi
PS1-02-091 ...................................... 1080
Val d’Oleiros e Silva, Rodrigo
N11-07 ........................................... 577
Vale, Ana C.
N11-08 ........................................... 579
PS1-15-449 ..................................... 1449
Valencia, Leticia
N10-03 ........................................... 548
Valente, Joana
PS1-01-015 ...................................... 1009
Valenti, Joan
Biomechanics 01-02 ........................... 955
Valiokas, Ramūnas
PS1-01-051 ...................................... 1041
Vallejo-Giraldo, Catalina
N01-01 ........................................... 128
Vallet-Regi, Maria
K01-02 ............................................ 76
PS2-12-380 ..................................... 1849
Vallittu, Pekka
GW AW02-01 .................................. 648
Vallchanov, Hristo
PS1-09-319 ..................................... 1313
Valtin, Juliane
K06-05 ........................................... 659
van Blitterswijk, Clemens A.
PS2-13-406 ..................................... 1876
KdG AW02-01 .................................. 649
Van Charante, Frits
K10-06 ........................................... 867
### Author Index

**van Damme, Lana**  
ESB-BIOMAT S-07 .............................. 447  
ESB-ISBF S-04 .............................. 537  
N01-07 ................................... 141  
PS2-07-222 .................................. 1689  
S05-02 ..................................... 267  
S10-KL01 ..................................... 679

**Van Den Bogerd, Bert**  
N03-09 ....................................... 227

**van der Boon, Torben A.B.**  
PS1-13-395 .................................... 1394

**Van der Eycken, Erik V.**  
PS2-13-410 .................................... 1881

**Van der Gucht, Marie**  
N03-01 ....................................... 208

**van der Heide, Daphne**  
N13-06 ......................................... 827  
PS1-01-039 ..................................... 1031

**Van der Meeren, Louis**  
ESB-BIOMAT S-07 .............................. 447

**van der Spoel, Mikkey**  
N05-09 ....................................... 304

**Van Der Straeten, Catherine**  
PS1-03-123 ..................................... 1109

**Van Dijck, Patrick**  
ESB-CRS S-04 .................................. 427

**van Duijn, Joost**  
N14-02 ......................................... 898  
N14-05 ......................................... 904

**Van Erps, Jürgen**  
ESB-ISBF S-04 .................................. 537

**van Gaalen, Kerstin**  
PS2-02-068 ..................................... 1537

**Van Hoorick, Jasper**  
ESB-ISBF S-04 .................................. 537  
ESB-SLABO S-03 .................................. 283  
N01-07 ......................................... 141  
N03-09 ......................................... 227  
S10-KL01 ..................................... 679  
YSF WS-08 ..................................... 67

**Van Hoven, Inge**  
ESB-SLABO S-03 .................................. 283

**van Kampen, Kenny**  
PS1-09-315 ...................................... 1310

**van Kooten, Theo**  
PS1-15-453 ...................................... 1452

**van Loo, Bas**  
K07-07 ......................................... 765  
S11-05 ......................................... 701

**van Rijen, Mattie**  
N08-02 ....................................... 452

**van Rijn, Patrick**  
PS1-08-263 ...................................... 1255  
PS1-15-453 ...................................... 1452  
PS2-09-308 ...................................... 1773

**Van Rijt, Sabine**  
PS2-10-322 ...................................... 1787

**van Tienen, Florence**  
PS2-01-008 ...................................... 1479

**van Uden, Sebastião**  
PS2-14-440 ...................................... 1912

**Van Vlierberghe, Hans**  
ESB-BIOMAT S-07 .............................. 447

**Van Vlierberghe, Sandra**  
ESB-BIOMAT S-07 .................................. 447  
ESB-ISBF S-04 .................................. 537  
ESB-SLABO S-03 .................................. 283  
K08-05 ......................................... 777  
N01-07 ......................................... 141  
N03-09 ......................................... 227  
PS1-04-135 ...................................... 1124  
PS2-07-222 ...................................... 1689  
PS2-08-276 ...................................... 1741  
S10-KL01 ..................................... 679  
S05-02 ....................................... 267

**van Weerd, Jasper**  
PS1-15-463 ..................................... 1465

**van Weeren, Rene**  
N04-03 ....................................... 234

**Vandenhaute, Elodie**  
NANOSTEM 03-02 .................................. 360

**Vandesteene, Marie**  
PS2-07-250 ...................................... 1718

**Vannozzi, Lorenzo**  
S05-05 ......................................... 274

**Vaporidou, Nefeli**  
PS1-14-445 ...................................... 1445  
PS2-06-216 ...................................... 1684

**Varas, Juan**  
PS1-07-257 ...................................... 1248

**Varela-Calviño, Rubén**  
PS1-05-181 ...................................... 1170

**Varoni, Elena Maria**  
PS2-10-338 ...................................... 1803  
YSF Opening .................................. 66

**Vasconcelos, Daniela P.**  
K07-06 ......................................... 763  
PS2-04-156 ...................................... 1621

**Vasques-Nóvoa, Francisco**  
K07-02 ......................................... 753

**Vasquez-Sancho, Fabian**  
AERO InvF-04 ..................................... 625

**Vasudevan, Praveen**  
PS1-09-317 ...................................... 1311

**Vaughan, Ted J.**  
PS1-09-311 ...................................... 1304  
PS2-02-068 ...................................... 1537
<table>
<thead>
<tr>
<th>Author</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vautier, Dominique</td>
<td>869</td>
</tr>
<tr>
<td>Vázquez Rodriguez, Augusto</td>
<td>867</td>
</tr>
<tr>
<td>Vázquez-Lasa, Blanca</td>
<td>1211</td>
</tr>
<tr>
<td>Vázquez-Lasa, Blanca</td>
<td>1506</td>
</tr>
<tr>
<td>Vdovchenko, Alena</td>
<td>349</td>
</tr>
<tr>
<td>Veciana, Jaume</td>
<td>1735</td>
</tr>
<tr>
<td>Vecstaudzka, Jana</td>
<td>1681</td>
</tr>
<tr>
<td>Vedaraman, Sitara</td>
<td>1289</td>
</tr>
<tr>
<td>Velga, Anabela</td>
<td>1539</td>
</tr>
<tr>
<td>Velga, Santiago</td>
<td>958</td>
</tr>
<tr>
<td>Velasco, Diego</td>
<td>548</td>
</tr>
<tr>
<td>Veljovic, Djordje</td>
<td>1440</td>
</tr>
<tr>
<td>Ventura, João O.</td>
<td>192</td>
</tr>
<tr>
<td>Vergnaud, Florestan</td>
<td>1385</td>
</tr>
<tr>
<td>Vermaelen, Karim</td>
<td>815</td>
</tr>
<tr>
<td>Vermeulen, Steven</td>
<td>162</td>
</tr>
<tr>
<td>Vermondten, Tina</td>
<td>318</td>
</tr>
<tr>
<td>Verné, Enrica</td>
<td>1584</td>
</tr>
<tr>
<td>Vesković, Ana</td>
<td>1934</td>
</tr>
<tr>
<td>Vial, Julie</td>
<td>1246</td>
</tr>
<tr>
<td>Viateau, Véronique</td>
<td>1718</td>
</tr>
<tr>
<td>Vibert, Eric</td>
<td>729</td>
</tr>
<tr>
<td>Vichery, Charlotte</td>
<td>1385</td>
</tr>
<tr>
<td>Vidal, Elia</td>
<td>518</td>
</tr>
<tr>
<td>Vidal, Ricardo</td>
<td>192</td>
</tr>
<tr>
<td>Vieira, Sandra I.</td>
<td>215</td>
</tr>
<tr>
<td>Vigué, Annabelle</td>
<td>869</td>
</tr>
<tr>
<td>Vilaça, Adriana</td>
<td>759</td>
</tr>
<tr>
<td>Vilaça, Andreia</td>
<td>956</td>
</tr>
<tr>
<td>Vilela, Carla</td>
<td>1777</td>
</tr>
<tr>
<td>Villa, Riccardo</td>
<td>466</td>
</tr>
<tr>
<td>Villagomez-Galindo, Miguel</td>
<td>1574</td>
</tr>
<tr>
<td>Villano, Anthea</td>
<td>756</td>
</tr>
<tr>
<td>Villapun Puzas, Victor M.</td>
<td>1448</td>
</tr>
<tr>
<td>Villatoro, Antonio</td>
<td>1706</td>
</tr>
<tr>
<td>Villiou, Maria</td>
<td>415</td>
</tr>
<tr>
<td>Villmann, Carmen</td>
<td>779</td>
</tr>
<tr>
<td>Visai, Livia</td>
<td>482</td>
</tr>
<tr>
<td>Vermeulen, Steven</td>
<td>162</td>
</tr>
<tr>
<td>Vesper, Jonathan</td>
<td>1706</td>
</tr>
<tr>
<td>Vissie, Rick</td>
<td>1915</td>
</tr>
<tr>
<td>Vissiannon, Cica</td>
<td>853</td>
</tr>
<tr>
<td>Vitale Brovarone, Chiara</td>
<td>76</td>
</tr>
<tr>
<td>Vite, Anna</td>
<td>241</td>
</tr>
<tr>
<td>Viti, Alessandra</td>
<td>1568</td>
</tr>
<tr>
<td>Vite, Enrico</td>
<td>1398</td>
</tr>
</tbody>
</table>

Page 2014 of 2028
<table>
<thead>
<tr>
<th>Author Name</th>
<th>Index</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitale-Brovarone, Chiara</td>
<td>N09-03</td>
<td>473</td>
</tr>
<tr>
<td></td>
<td>S08-04</td>
<td>510</td>
</tr>
<tr>
<td></td>
<td>S10-06</td>
<td>690</td>
</tr>
<tr>
<td></td>
<td>S14-03</td>
<td>876</td>
</tr>
<tr>
<td>Vitiello, Giuseppe</td>
<td>N15-04</td>
<td>923</td>
</tr>
<tr>
<td></td>
<td>S03-05</td>
<td>121</td>
</tr>
<tr>
<td>Vivero-Lopez, Maria</td>
<td>N16-01</td>
<td>932</td>
</tr>
<tr>
<td>Vivero-Lopez, Maria</td>
<td>PS1-08-285</td>
<td>1277</td>
</tr>
<tr>
<td>Vlachou, Marilena</td>
<td>PS2-10-342</td>
<td>1808</td>
</tr>
<tr>
<td>Vlasceanu, George M.</td>
<td>K04-04</td>
<td>385</td>
</tr>
<tr>
<td>Vlierberghe, Sandra V.</td>
<td>PS2-05-180</td>
<td>1645</td>
</tr>
<tr>
<td>Vodnar, Dan C.</td>
<td>PS1-02-071</td>
<td>1058</td>
</tr>
<tr>
<td>Voigt, Diana</td>
<td>PS2-10-332</td>
<td>1797</td>
</tr>
<tr>
<td>Vollaire, Julien</td>
<td>N02-01</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td>PS1-04-165</td>
<td>1153</td>
</tr>
<tr>
<td>Volpini, Cristina</td>
<td>PS2-07-252</td>
<td>1720</td>
</tr>
<tr>
<td>von Witzleben, Max</td>
<td>PS2-01-040</td>
<td>1512</td>
</tr>
<tr>
<td>Vonk, Lucienne</td>
<td>PS1-13-403</td>
<td>1402</td>
</tr>
<tr>
<td>Vorndran, Elke</td>
<td>K03-06</td>
<td>263</td>
</tr>
<tr>
<td>Vozzi, Giovanni</td>
<td>N11-05</td>
<td>573</td>
</tr>
<tr>
<td>Vrana, Nihal E.</td>
<td>PS2-13-400</td>
<td>1871</td>
</tr>
<tr>
<td>Vranckx, Cedric</td>
<td>N03-07</td>
<td>222</td>
</tr>
<tr>
<td>Vukomanović, Marija</td>
<td>N07-07</td>
<td>341</td>
</tr>
<tr>
<td></td>
<td>PS2-11-352</td>
<td>1819</td>
</tr>
<tr>
<td>Walczak, Piotr</td>
<td>PS2-12-382</td>
<td>1851</td>
</tr>
<tr>
<td>Walczyk, Anna</td>
<td>PS1-08-303</td>
<td>1293</td>
</tr>
<tr>
<td>Waletzko-Hellwig, Janine</td>
<td>PS2-04-148</td>
<td>1612</td>
</tr>
<tr>
<td>Walschus, Uwe</td>
<td>PS1-15-457</td>
<td>1457</td>
</tr>
<tr>
<td>Walsh, Pamela</td>
<td>PS2-04-164</td>
<td>1626</td>
</tr>
<tr>
<td>Wang, Meng</td>
<td>PS1-08-293</td>
<td>1284</td>
</tr>
<tr>
<td>Wang, Rong</td>
<td>N13-03</td>
<td>820</td>
</tr>
<tr>
<td>Wang, Xiao N.</td>
<td>N04-08</td>
<td>245</td>
</tr>
<tr>
<td></td>
<td>S01-07</td>
<td>101</td>
</tr>
<tr>
<td>Wang, Xiao Nong</td>
<td>N04-07</td>
<td>243</td>
</tr>
<tr>
<td>Wang, Xiaolin</td>
<td>PS1-10-321</td>
<td>1316</td>
</tr>
<tr>
<td>Ward, Danny</td>
<td>PS2-05-196</td>
<td>1662</td>
</tr>
<tr>
<td>Warnecke, Athanasia</td>
<td>PS2-10-340</td>
<td>1806</td>
</tr>
<tr>
<td>Warwas, Dawid P.</td>
<td>PS2-10-340</td>
<td>1806</td>
</tr>
<tr>
<td>Wasserberg, Dorothee</td>
<td>PS1-15-463</td>
<td>1465</td>
</tr>
<tr>
<td>Weber, Florian</td>
<td>PS2-15-448</td>
<td>1919</td>
</tr>
<tr>
<td>Weber, Josefin</td>
<td>PS2-01-060</td>
<td>1528</td>
</tr>
<tr>
<td></td>
<td>PS2-13-420</td>
<td>1892</td>
</tr>
<tr>
<td>Weichhold, Jan</td>
<td>PS2-05-198</td>
<td>1664</td>
</tr>
<tr>
<td>Weigel, Tobias</td>
<td>PS1-01-033</td>
<td>1025</td>
</tr>
<tr>
<td></td>
<td>PS1-05-185</td>
<td>1174</td>
</tr>
<tr>
<td>Weinans, Harrie</td>
<td>K03-06</td>
<td>263</td>
</tr>
<tr>
<td>Weiss, Martin</td>
<td>PS1-13-409</td>
<td>1408</td>
</tr>
<tr>
<td>Weiss, Pierre</td>
<td>PS2-08-292</td>
<td>1755</td>
</tr>
<tr>
<td></td>
<td>S05-06</td>
<td>277</td>
</tr>
<tr>
<td>Wellings, Don</td>
<td>K02-06</td>
<td>184</td>
</tr>
<tr>
<td>Welzel, Petra B.</td>
<td>PS1-10-345</td>
<td>1342</td>
</tr>
<tr>
<td>Weng, Xisheng</td>
<td>ESB-CSBM S-03</td>
<td>201</td>
</tr>
</tbody>
</table>

Page 2015 of 2028
<table>
<thead>
<tr>
<th>Author Name</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Werner, Carsten</td>
<td>K06-05</td>
</tr>
<tr>
<td></td>
<td>PS1-08-295</td>
</tr>
<tr>
<td></td>
<td>PS1-10-345</td>
</tr>
<tr>
<td>Werner, Consuelo</td>
<td>PS1-07-257</td>
</tr>
<tr>
<td>Westergren-Thorsson, Gunilla</td>
<td>PS1-14-431</td>
</tr>
<tr>
<td>Więcek, Justyna</td>
<td>PS2-09-310</td>
</tr>
<tr>
<td>Wieland, Annalena</td>
<td>K08-06</td>
</tr>
<tr>
<td></td>
<td>N14-04</td>
</tr>
<tr>
<td>Wieland, Florian</td>
<td>PS1-02-067</td>
</tr>
<tr>
<td>Wieringa, Paul</td>
<td>N05-09</td>
</tr>
<tr>
<td></td>
<td>N14-03</td>
</tr>
<tr>
<td></td>
<td>PS1-02-085</td>
</tr>
<tr>
<td>Wiesbeck, Maximillian F.</td>
<td>N03-06</td>
</tr>
<tr>
<td>Wiesmann, Hans-Peter</td>
<td>K03-06</td>
</tr>
<tr>
<td>Willemen, Niels G.</td>
<td>K08-03</td>
</tr>
<tr>
<td>Willemse, Koen</td>
<td>K03-06</td>
</tr>
<tr>
<td>Williams, Rachel</td>
<td>K02-06</td>
</tr>
<tr>
<td>Windisch, Johannes</td>
<td>K03-05</td>
</tr>
<tr>
<td>Winkel, Louise</td>
<td>PS1-08-275</td>
</tr>
<tr>
<td>Winning, Danielle</td>
<td>PS1-08-261</td>
</tr>
<tr>
<td></td>
<td>S04-04</td>
</tr>
<tr>
<td>Witjes, Max</td>
<td>PS1-15-453</td>
</tr>
<tr>
<td>Witko, Malgorzata</td>
<td>PS1-08-303</td>
</tr>
<tr>
<td>Wittrant, Yohann</td>
<td>N06-01</td>
</tr>
<tr>
<td>Włodarczyk-Biegun, Malgorzata</td>
<td>S07-04</td>
</tr>
<tr>
<td></td>
<td>PS2-01-034</td>
</tr>
<tr>
<td>Woelk, Christian</td>
<td>ESB-SLABO S-05</td>
</tr>
<tr>
<td>Wolf, Eckhard</td>
<td>PS2-07-220</td>
</tr>
<tr>
<td>Wolfart, Stefan</td>
<td>PS1-02-075</td>
</tr>
<tr>
<td>Wölker, Christian</td>
<td>K09-07</td>
</tr>
<tr>
<td></td>
<td>N04-09</td>
</tr>
<tr>
<td>Woods, Ian U.</td>
<td>K04-02</td>
</tr>
<tr>
<td></td>
<td>K05-02</td>
</tr>
<tr>
<td>Wróbel, Andrzej</td>
<td>PS1-08-303</td>
</tr>
<tr>
<td>Wu, Chengtie</td>
<td>N10-07</td>
</tr>
<tr>
<td></td>
<td>PS1-01-011</td>
</tr>
<tr>
<td>Wu, Lei</td>
<td>PS1-08-293</td>
</tr>
<tr>
<td>Wu, Xixi</td>
<td>S07-04</td>
</tr>
<tr>
<td>Wüllmann, Maximilian</td>
<td>PS1-01-033</td>
</tr>
<tr>
<td>Wychowanicz, Jacek K.</td>
<td>PS1-08-261</td>
</tr>
<tr>
<td></td>
<td>PS1-13-421</td>
</tr>
<tr>
<td></td>
<td>PS2-01-026</td>
</tr>
<tr>
<td></td>
<td>S04-04</td>
</tr>
<tr>
<td>Wylie, Matthew</td>
<td>PS1-11-363</td>
</tr>
<tr>
<td></td>
<td>PS1-11-348</td>
</tr>
<tr>
<td>Xia, Wei</td>
<td>ESB-CSBM S-03</td>
</tr>
<tr>
<td></td>
<td>Xie, Ruoxiao</td>
</tr>
<tr>
<td></td>
<td>Xie, Yujie</td>
</tr>
<tr>
<td></td>
<td>PS1-07-235</td>
</tr>
<tr>
<td>Xu, Zeqian</td>
<td>S03-06</td>
</tr>
<tr>
<td>Yamamoto, Koji</td>
<td>N03-02</td>
</tr>
<tr>
<td></td>
<td>N03-08</td>
</tr>
<tr>
<td></td>
<td>PS1-02-081</td>
</tr>
<tr>
<td></td>
<td>PS1-03-113</td>
</tr>
<tr>
<td>Yang, Chunrcheng</td>
<td>PS1-04-155</td>
</tr>
<tr>
<td>Yang, Fang</td>
<td>S06-04</td>
</tr>
<tr>
<td>Yang, Xiao</td>
<td>ESB-CSBM S-04</td>
</tr>
<tr>
<td>Yanik, Tulin</td>
<td>PS2-13-402</td>
</tr>
<tr>
<td>Yao, Tianyu</td>
<td>N14-01</td>
</tr>
<tr>
<td>Yépez, Byron</td>
<td>AERO S03-KL03</td>
</tr>
</tbody>
</table>

Page 2016 of 2028
<table>
<thead>
<tr>
<th>Author Index</th>
<th>ESB 2021</th>
<th>Abstract Book</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yilgor Huri, Pinar</td>
<td>PS1-07-253</td>
<td>1244</td>
</tr>
<tr>
<td>Yoo, James</td>
<td>N01-04</td>
<td>136</td>
</tr>
<tr>
<td>Yousefi-Mashouf, Hamid</td>
<td>ESB-BIOMAT S-06</td>
<td>444</td>
</tr>
<tr>
<td>Yu, Tracy</td>
<td>K09-03</td>
<td>844</td>
</tr>
<tr>
<td>Yuan, Huipin</td>
<td>N13-06</td>
<td>827</td>
</tr>
<tr>
<td>Yuan, Yichen</td>
<td>PS01-03-121</td>
<td>1107</td>
</tr>
<tr>
<td>Yuste-Hidalgo, Francisco</td>
<td>Biomechanics 04-02</td>
<td>976</td>
</tr>
<tr>
<td>Zaat, Sebastian A.</td>
<td>S03-07</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>S03-KL01</td>
<td>113</td>
</tr>
<tr>
<td>Zaat, Sebastian A.J.</td>
<td>K10-06</td>
<td>867</td>
</tr>
<tr>
<td>Zabila, Yevhen</td>
<td>PS2-15-466</td>
<td>1940</td>
</tr>
<tr>
<td>Zadpoor, Amir A.</td>
<td>PS1-01-043</td>
<td>1035</td>
</tr>
<tr>
<td>Zager, Paula</td>
<td>K05-04</td>
<td>497</td>
</tr>
<tr>
<td>Zaliskas, Saskia</td>
<td>PS02-08-258</td>
<td>1727</td>
</tr>
<tr>
<td></td>
<td>PS02-10-340</td>
<td>1806</td>
</tr>
<tr>
<td>Zalite, Vita</td>
<td>PS02-06-212</td>
<td>1679</td>
</tr>
<tr>
<td>Zamboulis, Alexandra</td>
<td>PS2-10-342</td>
<td>1808</td>
</tr>
<tr>
<td>Zamora-Sequeira, Roy</td>
<td>AERO InvF-04</td>
<td>625</td>
</tr>
<tr>
<td>Zanfardino, Anna</td>
<td>N15-04</td>
<td>923</td>
</tr>
<tr>
<td></td>
<td>S03-05</td>
<td>121</td>
</tr>
<tr>
<td>Zapata, Osnat</td>
<td>AERO PS01-04</td>
<td>603</td>
</tr>
<tr>
<td>Zare, Mohadeseh</td>
<td>PS2-11-356</td>
<td>1823</td>
</tr>
<tr>
<td>Zargarzadeh, Mehrzad</td>
<td>N16-03</td>
<td>936</td>
</tr>
<tr>
<td>Zarzycki, Arkadiusz</td>
<td>PS2-15-466</td>
<td>1940</td>
</tr>
<tr>
<td>Zavan, Barbara</td>
<td>N10-09</td>
<td>560</td>
</tr>
<tr>
<td>Zderic, Ivan</td>
<td>N02-06</td>
<td>160</td>
</tr>
<tr>
<td>Zegre, Miguel</td>
<td>PS2-10-326</td>
<td>1791</td>
</tr>
<tr>
<td>Zeidler-Rentzsch, Ines</td>
<td>N14-06</td>
<td>906</td>
</tr>
<tr>
<td>Zeiter, Stephan</td>
<td>N02-06</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>PS2-13-410</td>
<td>1881</td>
</tr>
<tr>
<td>Zema, Lucia</td>
<td>S04-05</td>
<td>196</td>
</tr>
<tr>
<td>Zembrzycki, Krzysztof</td>
<td>PS02-07-224</td>
<td>1691</td>
</tr>
<tr>
<td>Zeng, Kui</td>
<td>PS01-08-283</td>
<td>1275</td>
</tr>
<tr>
<td>Zenobi-Wong, Marcy</td>
<td>ESB-ISBF S-KL01</td>
<td>533</td>
</tr>
<tr>
<td></td>
<td>ESB-ISBF S-KL02</td>
<td>534</td>
</tr>
<tr>
<td></td>
<td>N13-04</td>
<td>822</td>
</tr>
<tr>
<td>Zhai, Dong</td>
<td>N10-07</td>
<td>556</td>
</tr>
<tr>
<td>Zhang, Huijie L.</td>
<td>K05-06</td>
<td>501</td>
</tr>
<tr>
<td>Zhang, Jiena</td>
<td>N12-07</td>
<td>744</td>
</tr>
<tr>
<td>Zhang, Kai</td>
<td>PS01-08-283</td>
<td>1275</td>
</tr>
<tr>
<td>Zhang, Ruichen</td>
<td>PS02-09-308</td>
<td>1773</td>
</tr>
<tr>
<td>Zhang, Xingdong</td>
<td>ESB-CSBM S-04</td>
<td>203</td>
</tr>
<tr>
<td>Zhang, Yanping</td>
<td>PS01-01-025</td>
<td>1018</td>
</tr>
<tr>
<td>Zhang, Yecheng</td>
<td>Biomechanics 04-03</td>
<td>979</td>
</tr>
<tr>
<td>Zhang, Yue</td>
<td>K06-02</td>
<td>653</td>
</tr>
<tr>
<td>Zhang, Yujie</td>
<td>ESB-BIOMAT S-02</td>
<td>434</td>
</tr>
<tr>
<td>Zhao, Xingchen</td>
<td>N10-07</td>
<td>556</td>
</tr>
<tr>
<td>Zheng, Jibao</td>
<td>PS01-04-155</td>
<td>1145</td>
</tr>
<tr>
<td>Zheng, Kai</td>
<td>PS02-01-054</td>
<td>1525</td>
</tr>
<tr>
<td></td>
<td>PS02-15-462</td>
<td>1936</td>
</tr>
<tr>
<td></td>
<td>S03-06</td>
<td>123</td>
</tr>
<tr>
<td>Zhou, Dennis W.</td>
<td>S09-02</td>
<td>665</td>
</tr>
<tr>
<td>Zhou, Jie</td>
<td>PS01-01-043</td>
<td>1035</td>
</tr>
<tr>
<td>Zhu, Wei</td>
<td>ESB-CSBM S-03</td>
<td>201</td>
</tr>
<tr>
<td>Zhu, Yutao</td>
<td>N03-02</td>
<td>210</td>
</tr>
<tr>
<td>Ziccarelli, Anna</td>
<td>PS2-14-440</td>
<td>1912</td>
</tr>
<tr>
<td>Zieilinski, Piotr</td>
<td>S07-04</td>
<td>415</td>
</tr>
<tr>
<td>Ziemann, Christina</td>
<td>PS01-05-185</td>
<td>1174</td>
</tr>
<tr>
<td>Author</td>
<td>Reference</td>
<td>Page</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>--------------------</td>
<td>------</td>
</tr>
<tr>
<td>Zima, Aneta</td>
<td>N02-02</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>PS1-03-111</td>
<td>1098</td>
</tr>
<tr>
<td></td>
<td>PS1-04-147</td>
<td>1136</td>
</tr>
<tr>
<td></td>
<td>PS2-03-112</td>
<td>1582</td>
</tr>
<tr>
<td></td>
<td>PS2-04-146</td>
<td>1610</td>
</tr>
<tr>
<td>Ziminska, Monika</td>
<td>N11-01</td>
<td>563</td>
</tr>
<tr>
<td>Zimmermann, Ralf</td>
<td>PS1-10-345</td>
<td>1342</td>
</tr>
<tr>
<td>Zoccola, Mayte M.</td>
<td>AERO PS01-03-601</td>
<td>601</td>
</tr>
<tr>
<td>Zoetebier Liszka, Barbara</td>
<td>S11-05</td>
<td>701</td>
</tr>
<tr>
<td>Zonnari, Alessandra</td>
<td>N06-03</td>
<td>311</td>
</tr>
<tr>
<td>Zoso, Alice</td>
<td>N12-01</td>
<td>732</td>
</tr>
<tr>
<td>Zou, Younzhe</td>
<td>PS1-03-129</td>
<td>1117</td>
</tr>
<tr>
<td>Zu, Guangyue</td>
<td>PS1-08-263</td>
<td>1255</td>
</tr>
<tr>
<td></td>
<td>PS1-15-453</td>
<td>1452</td>
</tr>
<tr>
<td>Zubko, Mikhail K.</td>
<td>PS2-05-196</td>
<td>1662</td>
</tr>
<tr>
<td>Zuza, Ester</td>
<td>N15-06</td>
<td>927</td>
</tr>
<tr>
<td></td>
<td>PS1-02-079</td>
<td>1067</td>
</tr>
<tr>
<td>Žvab, Klara</td>
<td>AERO S02-02-612</td>
<td>612</td>
</tr>
<tr>
<td>Zivcic, Jovana</td>
<td>PS1-14-439</td>
<td>1440</td>
</tr>
</tbody>
</table>
Topic Index

A

A-01 - 3D and 4D (bio)printing
ESB-ISBF S-03 ........................................ 535
ESB-ISBF S-05 ........................................ 539
ESB-SIBB S01-02 .................................... 518
K02-03 .................................................. 177
K03-01 .................................................. 253
K03-02 .................................................. 255
K03-05 .................................................. 261
K03-06 .................................................. 263
N13-02 .................................................. 818
N14-05 .................................................. 904
PS1-01-001 ........................................... 994
PS1-01-005 ........................................... 998
PS1-01-009 .......................................... 1003
PS1-01-021 .......................................... 1013
PS1-01-031 .......................................... 1023
PS1-01-037 .......................................... 1029
PS1-01-043 .......................................... 1035
PS1-01-047 .......................................... 1037
PS1-01-051 .......................................... 1041
PS1-01-061 .......................................... 1048
PS2-01-002 .......................................... 1472
PS2-01-006 .......................................... 1477
PS2-01-010 .......................................... 1481
PS2-01-018 .......................................... 1491
PS2-01-024 .......................................... 1498
PS2-01-026 .......................................... 1500
PS2-01-030 .......................................... 1504
PS2-01-038 .......................................... 1509
PS2-01-048 .......................................... 1518
PS2-01-052 .......................................... 1523
PS2-01-058 .......................................... 1526
PS2-11-366 ......................................... 1832
S14-06 ................................................. 882

A-02 - Biofabrication
K02-04 .................................................. 180
K02-05 .................................................. 182
K02-06 .................................................. 184
K07-05 .................................................. 761
N13-04 .................................................. 822
N13-07 .................................................. 829
N14-03 .................................................. 900
N14-04 .................................................. 902
PS1-01-007 .......................................... 1001
PS1-01-035 .......................................... 1027
PS1-01-049 .......................................... 1039
PS1-01-059 .......................................... 1046
PS2-01-008 .......................................... 1479
PS2-01-012 .......................................... 1484
PS2-01-042 .......................................... 1514
PS2-01-050 .......................................... 1520
S01-02 .................................................. 89
S01-03 .................................................. 92
S01-04 .................................................. 94
S01-05 .................................................. 96
S01-06 .................................................. 98
S07-03 .................................................. 412
S07-04 .................................................. 415
S07-05 .................................................. 417

A-03 - Bioinks
ESB-ISBF S-04 ........................................ 537
N13-05 .................................................. 824
N14-02 .................................................. 898
PS1-01-011 .......................................... 1004
PS1-01-029 .......................................... 1022
PS1-01-039 .......................................... 1031
PS1-01-041 .......................................... 1033
PS2-01-014 .......................................... 1486
PS2-01-020 .......................................... 1493
PS2-01-036 .......................................... 1508
PS2-01-040 .......................................... 1512
PS2-01-046 .......................................... 1516
PS2-04-174 .......................................... 1637
S01-07 .................................................. 101
S14-04 ................................................. 878

A-04 - Electrosprinning
N14-08 .................................................. 912
N15-04 .................................................. 923
PS1-01-013 .......................................... 1007
PS1-01-015 .......................................... 1009
PS1-01-017 .......................................... 1011
PS1-01-023 .......................................... 1015
PS1-01-025 .......................................... 1018
PS1-01-027 .......................................... 1020
PS1-01-033 .......................................... 1025
PS1-01-045 .......................................... 1036
PS1-01-053 .......................................... 1044
PS2-01-004 .......................................... 1474
PS2-01-016 .......................................... 1488
PS2-01-022 .......................................... 1496
PS2-01-054 .......................................... 1525
S14-03 ................................................. 876

A-05 - Laser-based AM technologies
K02-02 .................................................. 175
N13-08 .................................................. 831
PS1-01-003 .......................................... 996
S14-05 .................................................. 880
S14-07 .................................................. 884

Page 2019 of 2028
A-06 - Novel AM technologies and tools
K03-03 ........................................... 257
K03-04 ........................................... 259
PS2-01-028 ................................... 1502
PS2-01-034 ................................... 1506
PS2-01-060 ................................... 1528
AERO - AERoGELS COST Action
AERO InvF-01 ................................... 618
AERO InvF-02 ................................... 621
AERO InvF-03 ................................... 623
AERO InvF-04 ................................... 625
AERO InvF-05 ................................... 628
AERO InvF-06 ................................... 630
AERO InvF-07 ................................... 633
AERO PS01-01 ................................... 597
AERO PS01-02 ................................... 599
AERO PS01-03 ................................... 601
AERO PS01-04 ................................... 603
AERO PS01-05 ................................... 605
AERO S01-01 ................................... 588
AERO S01-02 ................................... 590
AERO S01-03 ................................... 592
AERO S01-04 ................................... 594
AERO S02-01 ................................... 609
AERO S02-02 ................................... 612
AERO S02-03 ................................... 613
AERO S02-KL02 ................................ 607
AERO S03-01 ................................... 639
AERO S03-02 ................................... 641
AERO S03-03 ................................... 643
AERO S03-04 ................................... 645
AERO S03-KL03 ................................ 637
COST AERoGELS KL-01 ...................... 587

B-05 - In silico testing
N11-05 ........................................... 573
PS1-04-153 .................................... 1143
PS1-09-319 .................................... 1313

B-06 - In vitro testing
K09-07 ........................................... 853
PS1-02-071 .................................... 1058
PS1-02-073 .................................... 1060
PS1-02-075 .................................... 1063
PS1-02-077 .................................... 1065
PS2-02-064 .................................... 1532
PS2-02-066 .................................... 1534
PS2-02-072 .................................... 1542
PS2-02-092 .................................... 1563
PS2-02-098 .................................... 1570
S12-05 ........................................... 792
S13-04 ........................................... 805

B-07 - In vivo testing
ESB-SFB S-04 ................................... 813
N04-03 ........................................... 234
PS1-02-065 .................................... 1054
PS1-02-085 .................................... 1074
PS2-02-086 .................................... 1558
PS2-02-100 .................................... 1572

B-08 - Mechanical characterisation
N05-07 ........................................... 299
N12-06 ........................................... 742
PS1-02-063 .................................... 1052
PS1-02-079 .................................... 1067
PS1-02-081 .................................... 1070
PS1-02-091 .................................... 1080
PS2-02-078 .................................... 1548
PS2-02-080 .................................... 1550

B-09 - Modelling of material properties
ESB-SLABO S-04 .............................. 285
N05-02 ........................................... 292
PS1-02-089 .................................... 1079
PS1-02-093 .................................... 1082
PS1-02-095 .................................... 1085
PS2-02-070 .................................... 1539
PS2-02-074 .................................... 1544
PS2-02-094 .................................... 1566

BIO - Biomechanics in sports and in
Biomedicine
Biomechanics 03-02 ............................ 960
Biomechanics 03-03 ............................ 963
Biomechanics 03-04 ............................ 966
Biomechanics 03-05 ............................ 969
Biomechanics 03-06 ............................ 971
Biomechanics 04-01 ............................ 974
Biomechanics 04-02 ............................ 976
Biomechanics 04-03 ............................ 979
Biomechanics 04-04 ............................ 982
Biomechanics 04-05 ............................ 985
Biomechanics 04-06 ............................ 988
<table>
<thead>
<tr>
<th>Topic Index</th>
<th>ESB 2021</th>
<th>Abstract Book</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>PS1-08-259</td>
<td>1251</td>
</tr>
<tr>
<td></td>
<td>PS1-08-263</td>
<td>1255</td>
</tr>
<tr>
<td></td>
<td>PS1-08-267</td>
<td>1259</td>
</tr>
<tr>
<td></td>
<td>PS1-08-269</td>
<td>1261</td>
</tr>
<tr>
<td></td>
<td>PS1-08-271</td>
<td>1263</td>
</tr>
<tr>
<td></td>
<td>PS1-08-273</td>
<td>1265</td>
</tr>
<tr>
<td></td>
<td>PS1-08-275</td>
<td>1267</td>
</tr>
<tr>
<td></td>
<td>PS1-08-277</td>
<td>1269</td>
</tr>
<tr>
<td></td>
<td>PS1-08-279</td>
<td>1271</td>
</tr>
<tr>
<td></td>
<td>PS1-08-281</td>
<td>1273</td>
</tr>
<tr>
<td></td>
<td>PS1-08-285</td>
<td>1277</td>
</tr>
<tr>
<td></td>
<td>PS1-08-287</td>
<td>1278</td>
</tr>
<tr>
<td></td>
<td>PS1-08-289</td>
<td>1280</td>
</tr>
<tr>
<td></td>
<td>PS1-08-299</td>
<td>1289</td>
</tr>
<tr>
<td></td>
<td>PS1-08-301</td>
<td>1291</td>
</tr>
<tr>
<td></td>
<td>PS1-08-303</td>
<td>1293</td>
</tr>
<tr>
<td></td>
<td>PS2-08-260</td>
<td>1728</td>
</tr>
<tr>
<td></td>
<td>PS2-08-262</td>
<td>1730</td>
</tr>
<tr>
<td></td>
<td>PS2-08-266</td>
<td>1732</td>
</tr>
<tr>
<td></td>
<td>PS2-08-268</td>
<td>1734</td>
</tr>
<tr>
<td></td>
<td>PS2-08-272</td>
<td>1736</td>
</tr>
<tr>
<td></td>
<td>PS2-08-276</td>
<td>1741</td>
</tr>
<tr>
<td></td>
<td>PS2-08-282</td>
<td>1745</td>
</tr>
<tr>
<td></td>
<td>PS2-08-284</td>
<td>1747</td>
</tr>
<tr>
<td></td>
<td>PS2-08-286</td>
<td>1750</td>
</tr>
<tr>
<td></td>
<td>PS2-08-290</td>
<td>1753</td>
</tr>
<tr>
<td></td>
<td>PS2-08-292</td>
<td>1755</td>
</tr>
<tr>
<td></td>
<td>PS2-08-294</td>
<td>1757</td>
</tr>
<tr>
<td></td>
<td>PS2-08-296</td>
<td>1758</td>
</tr>
<tr>
<td></td>
<td>PS2-08-298</td>
<td>1760</td>
</tr>
<tr>
<td></td>
<td>PS2-08-300</td>
<td>1762</td>
</tr>
<tr>
<td></td>
<td>S09-07</td>
<td>676</td>
</tr>
<tr>
<td></td>
<td>S10-02</td>
<td>682</td>
</tr>
<tr>
<td></td>
<td>S10-03</td>
<td>684</td>
</tr>
<tr>
<td>C-03 - Ceramics</td>
<td>K05-03</td>
<td>.495</td>
</tr>
<tr>
<td>ESB-CSBM S-03</td>
<td>N09-06</td>
<td>478</td>
</tr>
<tr>
<td></td>
<td>N09-07</td>
<td>480</td>
</tr>
<tr>
<td></td>
<td>PS1-06-203</td>
<td>1195</td>
</tr>
<tr>
<td></td>
<td>PS1-06-205</td>
<td>1198</td>
</tr>
<tr>
<td></td>
<td>PS1-06-207</td>
<td>1200</td>
</tr>
<tr>
<td></td>
<td>PS1-06-209</td>
<td>1202</td>
</tr>
<tr>
<td></td>
<td>PS1-06-211</td>
<td>1204</td>
</tr>
<tr>
<td></td>
<td>PS1-06-213</td>
<td>1206</td>
</tr>
<tr>
<td></td>
<td>PS1-06-215</td>
<td>1208</td>
</tr>
<tr>
<td></td>
<td>PS2-06-204</td>
<td>1671</td>
</tr>
<tr>
<td></td>
<td>PS2-06-206</td>
<td>1672</td>
</tr>
<tr>
<td></td>
<td>PS2-06-208</td>
<td>1675</td>
</tr>
<tr>
<td></td>
<td>PS2-06-210</td>
<td>1677</td>
</tr>
<tr>
<td></td>
<td>PS2-06-212</td>
<td>1679</td>
</tr>
<tr>
<td></td>
<td>PS2-06-214</td>
<td>1681</td>
</tr>
<tr>
<td></td>
<td>PS2-06-216</td>
<td>1684</td>
</tr>
<tr>
<td>C-04 - Composites</td>
<td>N10-01</td>
<td>544</td>
</tr>
<tr>
<td>ESB-BIOMAT S-06</td>
<td>PS1-05-177</td>
<td>1166</td>
</tr>
<tr>
<td></td>
<td>PS1-05-183</td>
<td>1172</td>
</tr>
<tr>
<td></td>
<td>PS1-05-191</td>
<td>1180</td>
</tr>
<tr>
<td></td>
<td>PS1-05-195</td>
<td>1184</td>
</tr>
<tr>
<td></td>
<td>PS1-05-201</td>
<td>1191</td>
</tr>
<tr>
<td></td>
<td>PS2-05-176</td>
<td>1640</td>
</tr>
<tr>
<td></td>
<td>PS2-05-188</td>
<td>1652</td>
</tr>
<tr>
<td></td>
<td>PS2-05-190</td>
<td>1654</td>
</tr>
<tr>
<td></td>
<td>PS2-05-202</td>
<td>1669</td>
</tr>
<tr>
<td>S08-05</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>C-05 - Decellularized extracellular matrix</td>
<td>N09-03</td>
<td>294</td>
</tr>
<tr>
<td>N02-08</td>
<td>164</td>
<td></td>
</tr>
<tr>
<td>N16-01</td>
<td>932</td>
<td></td>
</tr>
<tr>
<td>N16-02</td>
<td>934</td>
<td></td>
</tr>
<tr>
<td>N16-03</td>
<td>936</td>
<td></td>
</tr>
<tr>
<td>N16-04</td>
<td>938</td>
<td></td>
</tr>
<tr>
<td>N16-05</td>
<td>940</td>
<td></td>
</tr>
<tr>
<td>N16-06</td>
<td>941</td>
<td></td>
</tr>
<tr>
<td>N16-07</td>
<td>944</td>
<td></td>
</tr>
<tr>
<td>N16-08</td>
<td>946</td>
<td></td>
</tr>
<tr>
<td>C-06 - Hydrogels</td>
<td>K04-05</td>
<td>386</td>
</tr>
<tr>
<td>K05-06</td>
<td>501</td>
<td></td>
</tr>
<tr>
<td>K05-01</td>
<td>290</td>
<td></td>
</tr>
<tr>
<td>N09-08</td>
<td>482</td>
<td></td>
</tr>
<tr>
<td>N11-08</td>
<td>579</td>
<td></td>
</tr>
<tr>
<td>PS1-05-181</td>
<td>1170</td>
<td></td>
</tr>
<tr>
<td>PS1-05-187</td>
<td>1176</td>
<td></td>
</tr>
<tr>
<td>PS1-05-189</td>
<td>1178</td>
<td></td>
</tr>
<tr>
<td>PS2-05-09</td>
<td>1645</td>
<td></td>
</tr>
<tr>
<td>PS2-05-186</td>
<td>1651</td>
<td></td>
</tr>
<tr>
<td>PS2-05-192</td>
<td>1657</td>
<td></td>
</tr>
<tr>
<td>PS2-05-198</td>
<td>1664</td>
<td></td>
</tr>
</tbody>
</table>

Page 2021 of 2028
<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS1-05-193</td>
<td>1182</td>
</tr>
<tr>
<td>PS1-05-197</td>
<td>1187</td>
</tr>
<tr>
<td>PS2-05-184</td>
<td>1649</td>
</tr>
<tr>
<td>PS2-05-194</td>
<td>1659</td>
</tr>
<tr>
<td>PS2-05-196</td>
<td>1662</td>
</tr>
<tr>
<td>PS2-05-200</td>
<td>1666</td>
</tr>
<tr>
<td>C-11 - Nucleic acid-based</td>
<td></td>
</tr>
<tr>
<td>S11-03</td>
<td>697</td>
</tr>
<tr>
<td>C-12 - Peptide-based</td>
<td></td>
</tr>
<tr>
<td>ESB-SFB S-03</td>
<td>810</td>
</tr>
<tr>
<td>PS1-03-121</td>
<td>1107</td>
</tr>
<tr>
<td>S02-03</td>
<td>106</td>
</tr>
<tr>
<td>S14-02</td>
<td>874</td>
</tr>
<tr>
<td>C-13 - Polymers</td>
<td></td>
</tr>
<tr>
<td>N06-09</td>
<td>325</td>
</tr>
<tr>
<td>N13-03</td>
<td>820</td>
</tr>
<tr>
<td>N14-01</td>
<td>896</td>
</tr>
<tr>
<td>PS1-10-329</td>
<td>1326</td>
</tr>
<tr>
<td>PS2-05-178</td>
<td>1643</td>
</tr>
<tr>
<td>PS2-05-182</td>
<td>1647</td>
</tr>
<tr>
<td>PS2-11-358</td>
<td>1825</td>
</tr>
<tr>
<td>S03-03</td>
<td>117</td>
</tr>
<tr>
<td>C-14 - Porous</td>
<td></td>
</tr>
<tr>
<td>N04-02</td>
<td>232</td>
</tr>
<tr>
<td>N09-09</td>
<td>484</td>
</tr>
<tr>
<td>PS1-05-199</td>
<td>1189</td>
</tr>
<tr>
<td>C-15 - Self-assembled</td>
<td></td>
</tr>
<tr>
<td>PS1-10-331</td>
<td>1328</td>
</tr>
<tr>
<td>PS1-11-355</td>
<td>1352</td>
</tr>
<tr>
<td>S06-04</td>
<td>400</td>
</tr>
<tr>
<td>S06-05</td>
<td>402</td>
</tr>
<tr>
<td>S06-06</td>
<td>404</td>
</tr>
<tr>
<td>S06-07</td>
<td>406</td>
</tr>
<tr>
<td>C-16 - Stimuli-responsive (smart)</td>
<td></td>
</tr>
<tr>
<td>K01-02</td>
<td>76</td>
</tr>
<tr>
<td>PS1-08-261</td>
<td>1253</td>
</tr>
<tr>
<td>PS1-08-265</td>
<td>1257</td>
</tr>
<tr>
<td>PS1-08-283</td>
<td>1275</td>
</tr>
<tr>
<td>PS1-08-291</td>
<td>1282</td>
</tr>
<tr>
<td>PS1-08-293</td>
<td>1284</td>
</tr>
<tr>
<td>PS1-08-297</td>
<td>1287</td>
</tr>
<tr>
<td>PS2-08-258</td>
<td>1727</td>
</tr>
<tr>
<td>PS2-08-270</td>
<td>1735</td>
</tr>
<tr>
<td>PS2-08-274</td>
<td>1739</td>
</tr>
<tr>
<td>PS2-08-280</td>
<td>1743</td>
</tr>
<tr>
<td>PS2-08-288</td>
<td>1752</td>
</tr>
<tr>
<td>S04-04</td>
<td>194</td>
</tr>
<tr>
<td>S04-05</td>
<td>196</td>
</tr>
<tr>
<td>S05-05</td>
<td>274</td>
</tr>
<tr>
<td>S09-06</td>
<td>674</td>
</tr>
<tr>
<td>C-17 - Textile and fibre-based</td>
<td></td>
</tr>
<tr>
<td>PS1-11-353</td>
<td>1350</td>
</tr>
</tbody>
</table>

**D**

- **D-01 - Adipose tissue**
  - PS2-07-222 .................................. 1689

- **D-02 - Antibacterial**
  - ESB-SIIB S01-03 ................................ 520
  - ESB-SIIB S01-05 ................................ 524
  - ESB-SIIB S01-06 ................................ 527
  - K10-06 ......................................... 867
  - PS1-11-347 ..................................... 1345
  - PS1-11-349 ..................................... 1347
  - PS1-11-351 ..................................... 1348
  - PS1-11-357 ..................................... 1354
  - PS1-11-359 ..................................... 1356
  - PS1-11-361 ..................................... 1358
  - PS1-11-363 ..................................... 1360
  - PS1-11-365 ..................................... 1362
  - PS2-11-348 ..................................... 1815
  - PS2-11-350 ..................................... 1817
  - PS2-11-352 ..................................... 1819
  - PS2-11-354 ..................................... 1821
  - PS2-11-356 ..................................... 1823
  - PS2-11-362 ..................................... 1829
  - PS2-11-364 ..................................... 1831
  - S03-02 .......................................... 115
  - S03-04 .......................................... 119
  - S03-06 .......................................... 123
  - S03-07 .......................................... 125

- **D-05 - Biosensors**
  - K06-02 .......................................... 653

- **D-06 - Biomaterials for electrical stimulation**
  - K04-02 .......................................... 380
  - K04-07 .......................................... 390
  - N07-01 .......................................... 328
  - N07-02 .......................................... 330
  - N07-03 .......................................... 332
  - N07-04 .......................................... 335
  - N07-06 .......................................... 339
  - N07-07 .......................................... 341
  - PS1-04-139 ..................................... 1130
  - PS1-07-251 ..................................... 1241
  - PS1-07-253 ..................................... 1244
  - PS1-12-375 ..................................... 1372
  - PS2-07-254 ..................................... 1722

- **D-07 - Biomaterials for internet of things**
  - S04-03 .......................................... 192

- **D-08 - Biomaterials for magnetic stimulation**
  - K01-05 .......................................... 82
  - PS2-12-378 ..................................... 1847

- **D-10 - Bone tissue**
  - ESB-CSBM S-04 .................................. 203
  - K04-04 .......................................... 385
  - K09-06 .......................................... 850
  - K10-02 .......................................... 858
  - N02-01 .......................................... 148
<table>
<thead>
<tr>
<th>Topic Index</th>
<th>Page 23 of 2028</th>
</tr>
</thead>
<tbody>
<tr>
<td>N02-02 ........................................</td>
<td>151</td>
</tr>
<tr>
<td>N02-04 ........................................</td>
<td>156</td>
</tr>
<tr>
<td>N04-05 .........................................</td>
<td>239</td>
</tr>
<tr>
<td>N04-06 .........................................</td>
<td>241</td>
</tr>
<tr>
<td>N09-02 .........................................</td>
<td>471</td>
</tr>
<tr>
<td>N09-03 .........................................</td>
<td>473</td>
</tr>
<tr>
<td>N09-04 .........................................</td>
<td>475</td>
</tr>
<tr>
<td>N11-04 .........................................</td>
<td>571</td>
</tr>
<tr>
<td>N13-06 .........................................</td>
<td>827</td>
</tr>
<tr>
<td>PS1-04-131 ....................................</td>
<td>1119</td>
</tr>
<tr>
<td>PS1-04-133 ....................................</td>
<td>1122</td>
</tr>
<tr>
<td>PS1-04-135 ....................................</td>
<td>1124</td>
</tr>
<tr>
<td>PS1-04-137 ....................................</td>
<td>1127</td>
</tr>
<tr>
<td>PS1-04-143 ....................................</td>
<td>1132</td>
</tr>
<tr>
<td>PS1-04-145 ....................................</td>
<td>1134</td>
</tr>
<tr>
<td>PS1-04-147 ....................................</td>
<td>1136</td>
</tr>
<tr>
<td>PS1-04-149 ....................................</td>
<td>1138</td>
</tr>
<tr>
<td>PS1-04-155 ....................................</td>
<td>1145</td>
</tr>
<tr>
<td>PS1-04-157 ....................................</td>
<td>1148</td>
</tr>
<tr>
<td>PS1-04-159 ....................................</td>
<td>1149</td>
</tr>
<tr>
<td>PS1-04-165 ....................................</td>
<td>1153</td>
</tr>
<tr>
<td>PS1-04-167 ....................................</td>
<td>1156</td>
</tr>
<tr>
<td>PS1-04-173 ....................................</td>
<td>1161</td>
</tr>
<tr>
<td>PS1-04-175 ....................................</td>
<td>1162</td>
</tr>
<tr>
<td>PS2-04-134 ....................................</td>
<td>1603</td>
</tr>
<tr>
<td>PS2-04-136 ....................................</td>
<td>1605</td>
</tr>
<tr>
<td>PS2-04-138 ....................................</td>
<td>1606</td>
</tr>
<tr>
<td>PS2-04-140 ....................................</td>
<td>1608</td>
</tr>
<tr>
<td>PS2-04-146 ....................................</td>
<td>1610</td>
</tr>
<tr>
<td>PS2-04-150 ....................................</td>
<td>1614</td>
</tr>
<tr>
<td>PS2-04-154 ....................................</td>
<td>1618</td>
</tr>
<tr>
<td>PS2-04-160 ....................................</td>
<td>1622</td>
</tr>
<tr>
<td>PS2-04-162 ....................................</td>
<td>1624</td>
</tr>
<tr>
<td>PS2-04-164 ....................................</td>
<td>1626</td>
</tr>
<tr>
<td>PS2-04-166 ....................................</td>
<td>1628</td>
</tr>
<tr>
<td>S08-03 .........................................</td>
<td>508</td>
</tr>
<tr>
<td>S08-04 .........................................</td>
<td>510</td>
</tr>
<tr>
<td>S10-06 .........................................</td>
<td>690</td>
</tr>
<tr>
<td>S10-07 .........................................</td>
<td>692</td>
</tr>
<tr>
<td>D-11 - Cartilage and osteochondral tissue</td>
<td></td>
</tr>
<tr>
<td>ESB-SLABO S-03 ...............................</td>
<td>283</td>
</tr>
<tr>
<td>ESB-SLABO S-05 ...............................</td>
<td>287</td>
</tr>
<tr>
<td>N02-05 .........................................</td>
<td>158</td>
</tr>
<tr>
<td>N04-04 .........................................</td>
<td>237</td>
</tr>
<tr>
<td>PS1-04-151 ....................................</td>
<td>1140</td>
</tr>
<tr>
<td>PS1-04-163 ....................................</td>
<td>1151</td>
</tr>
<tr>
<td>PS2-04-132 ....................................</td>
<td>1601</td>
</tr>
<tr>
<td>PS2-04-156 ....................................</td>
<td>1621</td>
</tr>
<tr>
<td>PS2-04-168 ....................................</td>
<td>1630</td>
</tr>
<tr>
<td>D-12 - Cancer</td>
<td></td>
</tr>
<tr>
<td>ESB-BIOMAT S-03 ................................</td>
<td>436</td>
</tr>
<tr>
<td>ESB-BMSJ S-04 ................................</td>
<td>727</td>
</tr>
<tr>
<td>ESB-SFB S-05 ..................................</td>
<td>815</td>
</tr>
<tr>
<td>ESB-SIBB S02-03 ................................</td>
<td>709</td>
</tr>
<tr>
<td>ESB-SIBB S02-04 ................................</td>
<td>711</td>
</tr>
<tr>
<td>ESB-SIBB S02-06 ................................</td>
<td>715</td>
</tr>
<tr>
<td>K08-07 .........................................</td>
<td>781</td>
</tr>
<tr>
<td>N04-01 .........................................</td>
<td>230</td>
</tr>
<tr>
<td>N06-06 .........................................</td>
<td>318</td>
</tr>
<tr>
<td>PS1-07-227 ....................................</td>
<td>1221</td>
</tr>
<tr>
<td>PS1-07-243 ....................................</td>
<td>1235</td>
</tr>
<tr>
<td>PS1-07-255 ....................................</td>
<td>1246</td>
</tr>
<tr>
<td>PS2-07-244 ....................................</td>
<td>1712</td>
</tr>
<tr>
<td>PS2-07-252 ....................................</td>
<td>1720</td>
</tr>
<tr>
<td>D-13 - Cardiovascular</td>
<td></td>
</tr>
<tr>
<td>K07-02 .........................................</td>
<td>753</td>
</tr>
<tr>
<td>N12-02 .........................................</td>
<td>734</td>
</tr>
<tr>
<td>N12-03 .........................................</td>
<td>736</td>
</tr>
<tr>
<td>N12-05 .........................................</td>
<td>740</td>
</tr>
<tr>
<td>PS1-09-305 ....................................</td>
<td>1296</td>
</tr>
<tr>
<td>PS1-09-307 ....................................</td>
<td>1298</td>
</tr>
<tr>
<td>PS1-09-309 ....................................</td>
<td>1301</td>
</tr>
<tr>
<td>PS1-09-311 ....................................</td>
<td>1304</td>
</tr>
<tr>
<td>PS1-09-313 ....................................</td>
<td>1307</td>
</tr>
<tr>
<td>PS1-09-315 ....................................</td>
<td>1310</td>
</tr>
<tr>
<td>PS1-09-317 ....................................</td>
<td>1311</td>
</tr>
<tr>
<td>PS2-09-304 ....................................</td>
<td>1767</td>
</tr>
<tr>
<td>PS2-09-308 ....................................</td>
<td>1773</td>
</tr>
<tr>
<td>PS2-09-310 ....................................</td>
<td>1775</td>
</tr>
<tr>
<td>PS2-09-312 ....................................</td>
<td>1777</td>
</tr>
<tr>
<td>PS2-09-314 ....................................</td>
<td>1779</td>
</tr>
<tr>
<td>PS2-09-316 ....................................</td>
<td>1780</td>
</tr>
<tr>
<td>PS2-09-318 ....................................</td>
<td>1782</td>
</tr>
<tr>
<td>D-14 - Dental and maxillofacial</td>
<td></td>
</tr>
<tr>
<td>N02-03 .........................................</td>
<td>154</td>
</tr>
<tr>
<td>N09-01 .........................................</td>
<td>469</td>
</tr>
<tr>
<td>N11-07 .........................................</td>
<td>577</td>
</tr>
<tr>
<td>PS1-04-169 ....................................</td>
<td>1159</td>
</tr>
<tr>
<td>PS2-04-152 ....................................</td>
<td>1616</td>
</tr>
<tr>
<td>PS2-04-170 ....................................</td>
<td>1632</td>
</tr>
<tr>
<td>PS2-04-172 ....................................</td>
<td>1635</td>
</tr>
<tr>
<td>D-16 - Drug delivery</td>
<td></td>
</tr>
<tr>
<td>ESB-BIOMAT S-05 ................................</td>
<td>442</td>
</tr>
<tr>
<td>ESB-BMSJ S-03 ................................</td>
<td>724</td>
</tr>
<tr>
<td>ESB-CRS S-03 ..................................</td>
<td>424</td>
</tr>
<tr>
<td>ESB-CRS S-04 ..................................</td>
<td>427</td>
</tr>
<tr>
<td>ESB-CRS S-05 ..................................</td>
<td>429</td>
</tr>
<tr>
<td>K07-04 .........................................</td>
<td>759</td>
</tr>
<tr>
<td>N04-09 .........................................</td>
<td>247</td>
</tr>
<tr>
<td>N06-01 .........................................</td>
<td>307</td>
</tr>
<tr>
<td>N06-02 .........................................</td>
<td>309</td>
</tr>
<tr>
<td>N16-09 .........................................</td>
<td>948</td>
</tr>
<tr>
<td>PS1-10-321 ....................................</td>
<td>1316</td>
</tr>
<tr>
<td>PS1-10-323 ....................................</td>
<td>1319</td>
</tr>
<tr>
<td>PS1-10-325 ....................................</td>
<td>1321</td>
</tr>
<tr>
<td>PS1-10-327 ....................................</td>
<td>1324</td>
</tr>
<tr>
<td>PS1-10-335 ....................................</td>
<td>1331</td>
</tr>
<tr>
<td>PS1-10-337 ....................................</td>
<td>1334</td>
</tr>
<tr>
<td>PS1-10-339 ....................................</td>
<td>1336</td>
</tr>
<tr>
<td>PS1-10-341 ....................................</td>
<td>1338</td>
</tr>
<tr>
<td>PS1-10-343</td>
<td>1340</td>
</tr>
<tr>
<td>PS1-10-345</td>
<td>1342</td>
</tr>
<tr>
<td>PS2-10-320</td>
<td>1784</td>
</tr>
<tr>
<td>PS2-10-322</td>
<td>1787</td>
</tr>
<tr>
<td>PS2-10-324</td>
<td>1789</td>
</tr>
<tr>
<td>PS2-10-326</td>
<td>1791</td>
</tr>
<tr>
<td>PS2-10-332</td>
<td>1797</td>
</tr>
<tr>
<td>PS2-10-334</td>
<td>1799</td>
</tr>
<tr>
<td>PS2-10-338</td>
<td>1803</td>
</tr>
<tr>
<td>PS2-10-340</td>
<td>1806</td>
</tr>
<tr>
<td>PS2-10-342</td>
<td>1808</td>
</tr>
<tr>
<td>PS2-10-344</td>
<td>1810</td>
</tr>
<tr>
<td>PS1-07-225</td>
<td>1219</td>
</tr>
<tr>
<td>PS1-07-229</td>
<td>1223</td>
</tr>
<tr>
<td>PS1-07-235</td>
<td>1227</td>
</tr>
<tr>
<td>PS2-07-242</td>
<td>1710</td>
</tr>
<tr>
<td>D-26 - Tendon and ligament</td>
<td></td>
</tr>
<tr>
<td>PS2-07-250</td>
<td>1718</td>
</tr>
<tr>
<td>S05-02</td>
<td>267</td>
</tr>
<tr>
<td>S05-03</td>
<td>270</td>
</tr>
<tr>
<td>S05-04</td>
<td>272</td>
</tr>
<tr>
<td>D-27 - Tissue adhesives and anti-adhesives</td>
<td></td>
</tr>
<tr>
<td>ESB-BMSJ S-05</td>
<td>729</td>
</tr>
<tr>
<td>D-28 - Wound healing</td>
<td></td>
</tr>
<tr>
<td>K05-04</td>
<td>497</td>
</tr>
<tr>
<td>N10-04</td>
<td>550</td>
</tr>
<tr>
<td>N10-05</td>
<td>552</td>
</tr>
<tr>
<td>N10-06</td>
<td>554</td>
</tr>
<tr>
<td>N10-07</td>
<td>556</td>
</tr>
<tr>
<td>N10-08</td>
<td>558</td>
</tr>
<tr>
<td>N14-07</td>
<td>909</td>
</tr>
<tr>
<td>PS1-07-221</td>
<td>1215</td>
</tr>
<tr>
<td>PS1-07-223</td>
<td>1217</td>
</tr>
<tr>
<td>PS1-07-231</td>
<td>1224</td>
</tr>
<tr>
<td>PS2-07-226</td>
<td>1693</td>
</tr>
<tr>
<td>PS2-07-230</td>
<td>1698</td>
</tr>
<tr>
<td>PS2-07-232</td>
<td>1701</td>
</tr>
<tr>
<td>PS2-07-236</td>
<td>1706</td>
</tr>
<tr>
<td>PS2-07-246</td>
<td>1714</td>
</tr>
<tr>
<td>PS2-07-248</td>
<td>1716</td>
</tr>
<tr>
<td>PS2-07-256</td>
<td>1724</td>
</tr>
<tr>
<td>S12-03</td>
<td>788</td>
</tr>
</tbody>
</table>

**E**

<p>| E-01 - 3D scaffolds |
| ESB-BIOMAT S-07 | 447 |
| K05-05 | 499 |
| N10-09 | 560 |
| N11-01 | 563 |
| N11-03 | 568 |
| N12-01 | 732 |
| N14-06 | 906 |
| PS1-03-105 | 1094 |
| PS1-03-109 | 1097 |
| PS1-03-111 | 1098 |
| PS1-03-117 | 1102 |
| PS1-03-123 | 1109 |
| PS1-03-127 | 1114 |
| PS2-03-106 | 1576 |
| PS2-03-108 | 1578 |
| PS2-03-110 | 1580 |
| PS2-03-112 | 1582 |
| PS2-03-116 | 1584 |
| PS2-03-118 | 1586 |
| PS2-03-120 | 1588 |
| PS2-03-122 | 1591 |</p>
<table>
<thead>
<tr>
<th>Topic Index</th>
<th>ESB 2021</th>
<th>Abstract Book</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS2-03-124 ......................................................</td>
<td>1593</td>
<td></td>
</tr>
<tr>
<td>PS2-03-128 ......................................................</td>
<td>1595</td>
<td></td>
</tr>
<tr>
<td>PS2-03-130 ......................................................</td>
<td>1598</td>
<td></td>
</tr>
<tr>
<td>PS2-08-302 ......................................................</td>
<td>1764</td>
<td></td>
</tr>
<tr>
<td>S05-06 ............................................................</td>
<td>277</td>
<td></td>
</tr>
<tr>
<td>S10-05 ............................................................</td>
<td>688</td>
<td></td>
</tr>
<tr>
<td>E-02 - Bioreactors, including physical stimulation of TE constructs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS1-03-113 ......................................................</td>
<td>1100</td>
<td></td>
</tr>
<tr>
<td>E-03 - Innervation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N01-04 ............................................................</td>
<td>136</td>
<td></td>
</tr>
<tr>
<td>E-04 - Vascularisation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K05-07 ............................................................</td>
<td>503</td>
<td></td>
</tr>
<tr>
<td>K07-06 ............................................................</td>
<td>763</td>
<td></td>
</tr>
<tr>
<td>N12-07 ............................................................</td>
<td>744</td>
<td></td>
</tr>
<tr>
<td>N12-08 ............................................................</td>
<td>745</td>
<td></td>
</tr>
<tr>
<td>N12-09 ............................................................</td>
<td>747</td>
<td></td>
</tr>
<tr>
<td>PS1-03-119 ......................................................</td>
<td>1105</td>
<td></td>
</tr>
<tr>
<td>PS1-03-125 ......................................................</td>
<td>1112</td>
<td></td>
</tr>
</tbody>
</table>

**F**

| F-01 - Bacteria-biomaterial interactions | |
| K04-03 ............................................................ | 383 |
| K06-04 ............................................................ | 657 |
| K06-05 ............................................................ | 659 |
| K10-04 ............................................................ | 863 |
| K10-05 ............................................................ | 865 |
| K10-07 ............................................................ | 869 |
| PS1-13-413 .................................................... | 1410 |
| PS1-13-415 .................................................... | 1412 |
| PS1-13-417 .................................................... | 1414 |

| F-02 - Biofilm studies | |
| PS2-13-410 .................................................... | 1881 |

| F-03 - Cell adhesion, migration, proliferation and differentiation | |
| ESB-BIOMAT S-02 .................................................. | 434 |
| K06-06 ............................................................ | 661 |
| N08-01 ............................................................ | 450 |
| N08-02 ............................................................ | 452 |
| N08-03 ............................................................ | 454 |
| N08-04 ............................................................ | 456 |
| N08-05 ............................................................ | 458 |
| PS1-13-395 .................................................... | 1394 |
| PS1-13-397 .................................................... | 1396 |
| PS1-13-405 .................................................... | 1403 |
| PS1-13-409 .................................................... | 1408 |
| PS1-13-411 .................................................... | 1409 |
| PS1-13-421 .................................................... | 1418 |
| PS1-13-423 .................................................... | 1420 |
| PS2-13-394 .................................................... | 1863 |
| PS2-13-400 .................................................... | 1871 |
| PS2-13-402 .................................................... | 1874 |
| PS2-13-414 .................................................... | 1886 |
| S10-04 ............................................................ | 686 |

| F-04 - Cell-particle interactions | |
| PS2-13-398 .................................................... | 1868 |
| PS2-13-406 .................................................... | 1876 |
| S06-02 ............................................................ | 395 |
| S06-03 ............................................................ | 398 |
| F-05 - Fungus-biomaterial interactions | |
| K10-03 ............................................................ | 860 |

| F-06 - Protein-biomaterial interactions | |
| N08-06 ............................................................ | 460 |
| PS1-13-399 .................................................... | 1398 |
| PS1-13-401 .................................................... | 1400 |
| PS2-13-412 .................................................... | 1884 |
| PS2-13-416 .................................................... | 1888 |
| PS2-13-418 .................................................... | 1890 |

| F-07 - Mechano-sensing | |
| N08-08 ............................................................ | 464 |
| PS1-13-419 .................................................... | 1416 |
| PS2-02-104 ..................................................... | 1574 |
| PS2-13-396 .................................................... | 1866 |
| PS2-13-422 .................................................... | 1893 |
| PS2-13-408 .................................................... | 1878 |
| PS2-13-420 .................................................... | 1892 |

**G**

| G-01 - Biomaterial-related clinical problems | |
| N11-02 ............................................................ | 565 |
| PS1-05-179 ..................................................... | 1168 |
| PS2-11-360 ..................................................... | 1827 |
| S12-07 ........................................................... | 796 |

| G-02 - Clinical trials | |
| N11-06 ............................................................ | 575 |
| PS1-07-257 ..................................................... | 1248 |

**H**

| H-03 - Translational research | |
| S02-04 ............................................................ | 108 |

**I**

| I-02 - Lab-on-a-chip | |
| K06-03 ............................................................ | 655 |
| PS2-10-336 ..................................................... | 1801 |

| I-03 - Robotics | |
| PS1-08-295 ..................................................... | 1285 |

**K**

<p>| K-01 - Nanoparticles | |
| ESB-SIBB S02-01 ................................................ | 705 |
| ESB-SIBB S02-07 ................................................ | 717 |</p>
<table>
<thead>
<tr>
<th>Topic Index</th>
<th>ESB 2021</th>
<th>Abstract Book</th>
</tr>
</thead>
</table>

| K01-03 | .......................................................... | 78 |
| K01-06 | .......................................................... | 85 |
| K07-03 | .......................................................... | 756 |
| N01-02 | .......................................................... | 131 |
| N01-03 | .......................................................... | 133 |
| N05-08 | .......................................................... | 301 |
| N06-03 | .......................................................... | 311 |
| N06-05 | .......................................................... | 316 |
| N06-08 | .......................................................... | 323 |
| N15-02 | .......................................................... | 918 |
| N15-03 | .......................................................... | 921 |
| N15-07 | .......................................................... | 929 |
| PS1-12-369 | ......................................................... | 1367 |
| PS1-12-371 | ......................................................... | 1369 |
| PS1-12-377 | ......................................................... | 1374 |
| PS1-12-379 | ......................................................... | 1376 |
| PS1-12-381 | ......................................................... | 1378 |
| PS1-12-383 | ......................................................... | 1380 |
| PS1-12-385 | ......................................................... | 1382 |
| PS1-12-387 | ......................................................... | 1383 |
| PS1-12-389 | ......................................................... | 1385 |
| PS1-12-391 | ......................................................... | 1388 |
| PS2-12-368 | ......................................................... | 1836 |
| PS2-12-370 | ......................................................... | 1838 |
| PS2-12-372 | ......................................................... | 1840 |
| PS2-12-376 | ......................................................... | 1845 |
| PS2-12-380 | ......................................................... | 1849 |
| PS2-12-382 | ......................................................... | 1851 |
| PS2-12-384 | ......................................................... | 1853 |
| PS2-12-386 | ......................................................... | 1854 |
| PS2-12-388 | ......................................................... | 1856 |
| PS2-12-390 | ......................................................... | 1858 |
| PS2-12-392 | ......................................................... | 1860 |
| S03-05 | .......................................................... | 121 |
| S12-06 | .......................................................... | 794 |

<table>
<thead>
<tr>
<th>K-02 - Protein corona</th>
</tr>
</thead>
<tbody>
<tr>
<td>N06-04</td>
</tr>
<tr>
<td>N15-01</td>
</tr>
<tr>
<td>PS1-12-373</td>
</tr>
<tr>
<td>PS2-12-374</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>KS - Keynote Lecture</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESB-CRS S-KL02</td>
</tr>
<tr>
<td>K01-KL01</td>
</tr>
<tr>
<td>K02-KL01</td>
</tr>
<tr>
<td>K03-KL01</td>
</tr>
<tr>
<td>K04-KL01</td>
</tr>
<tr>
<td>K05-KL01</td>
</tr>
<tr>
<td>K06-KL01</td>
</tr>
<tr>
<td>K07-KL01</td>
</tr>
<tr>
<td>K08-KL01</td>
</tr>
<tr>
<td>K09-KL01</td>
</tr>
<tr>
<td>K10-KL01</td>
</tr>
<tr>
<td>S07-KL01</td>
</tr>
<tr>
<td>S13-KL01</td>
</tr>
<tr>
<td>S15-KL01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-01 - Coatings</td>
</tr>
<tr>
<td>ESB-SIBB S01-04</td>
</tr>
<tr>
<td>N03-01</td>
</tr>
<tr>
<td>N03-02</td>
</tr>
<tr>
<td>N03-03</td>
</tr>
<tr>
<td>PS1-15-449</td>
</tr>
<tr>
<td>PS1-15-451</td>
</tr>
<tr>
<td>PS1-15-453</td>
</tr>
<tr>
<td>PS1-15-457</td>
</tr>
<tr>
<td>PS1-15-461</td>
</tr>
<tr>
<td>PS1-15-463</td>
</tr>
<tr>
<td>PS2-15-446</td>
</tr>
<tr>
<td>PS2-15-448</td>
</tr>
<tr>
<td>PS2-15-450</td>
</tr>
<tr>
<td>PS2-15-452</td>
</tr>
<tr>
<td>PS2-15-454</td>
</tr>
<tr>
<td>PS2-15-458</td>
</tr>
<tr>
<td>PS2-15-462</td>
</tr>
<tr>
<td>PS2-15-464</td>
</tr>
<tr>
<td>PS2-15-466</td>
</tr>
<tr>
<td>S13-03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>L-02 - Micro- and nanopatterning</th>
</tr>
</thead>
<tbody>
<tr>
<td>N03-04</td>
</tr>
<tr>
<td>N03-05</td>
</tr>
<tr>
<td>N03-06</td>
</tr>
<tr>
<td>PS1-15-447</td>
</tr>
<tr>
<td>PS1-15-459</td>
</tr>
<tr>
<td>PS2-15-456</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>L-03 - Self-assembled monolayers</th>
</tr>
</thead>
<tbody>
<tr>
<td>N03-07</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>L-04 - Surface characterisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>N03-08</td>
</tr>
<tr>
<td>PS1-02-103</td>
</tr>
<tr>
<td>PS1-15-455</td>
</tr>
<tr>
<td>PS1-15-465</td>
</tr>
<tr>
<td>PS2-15-460</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-01 - 3D cell culture</td>
</tr>
<tr>
<td>ESB-CSBM S-05</td>
</tr>
<tr>
<td>N04-07</td>
</tr>
<tr>
<td>N05-06</td>
</tr>
<tr>
<td>N05-09</td>
</tr>
<tr>
<td>N06-07</td>
</tr>
<tr>
<td>N08-09</td>
</tr>
<tr>
<td>PS1-14-431</td>
</tr>
<tr>
<td>PS1-14-441</td>
</tr>
<tr>
<td>PS1-14-445</td>
</tr>
<tr>
<td>PS2-14-432</td>
</tr>
<tr>
<td>PS2-14-440</td>
</tr>
<tr>
<td>S02-05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>M-02 - Cancer models</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESB-SIBB S02-05</td>
</tr>
</tbody>
</table>
Topic Index

ESB-SIBB S02-08.......................... 719
K08-02..................................... 770
K08-05..................................... 777
K08-06..................................... 779
K09-02..................................... 842
K09-05..................................... 848
N09-05..................................... 476
PS1-14-429............................... 1428
PS1-14-433............................... 1434
PS1-14-437............................... 1438
PS1-14-439............................... 1440
PS1-14-443............................... 1444
PS2-14-424............................... 1896
PS2-14-442............................... 1914
M-03 - Microfluidics
K07-07..................................... 765
N15-05..................................... 925
PS2-14-430............................... 1901
S11-05..................................... 701
M-04 - Organ-on-a-chip
K02-07..................................... 186
PS1-14-425............................... 1423
PS1-14-435............................... 1435
PS2-14-426............................... 1898
PS2-14-434............................... 1906
PS2-14-436............................... 1908
PS2-14-438............................... 1910
PS2-14-444............................... 1915
M-05 - Organoids and spheroids
K09-03..................................... 844
N02-07..................................... 162
N04-08..................................... 245
PS1-14-427............................... 1425
M-06 - Organotypic cultures
S15-05..................................... 893
N
N-01 - SARS-COV-2
ESB-SIBB S01-07.......................... 530
NANO - NANO STEM Meeting
NANOSTEM 01-03.......................... 347
NANOSTEM 01-04.......................... 349
NANOSTEM 01-05.......................... 350
NANOSTEM 02-02.......................... 352
NANOSTEM 02-03.......................... 354
NANOSTEM 02-04.......................... 356
NANOSTEM 03-01.......................... 359
NANOSTEM 03-02.......................... 360
NANOSTEM 03-03.......................... 362
NANOSTEM 03-04.......................... 364
NANOSTEM 03-05.......................... 365
NANOSTEM 04-01.......................... 368
NANOSTEM 04-02.......................... 370
NANOSTEM 04-03.......................... 371
P
PL - Plenary Lecture
GW AW01-01............................... 70
GW AW02-01............................... 648
Int AW-01................................. 833
JL AW01-01................................. 71
JL AW02-01................................. 376
KdG AW01-01............................... 374
PL1-01.......................... 343
PL2-01.......................... 583
PL3-01.......................... 836
S
SS - Special Symposia Keynote Lecture
ESB-BIOMAT S-KL01.......................... 432
ESB-Biomaterials Science-KL01........ 722
ESB-Biomaterials Science-KL02........ 723
ESB-CRS S-KL01.......................... 421
ESB-CSBM S-KL01.......................... 199
ESB-CSBM S-KL02.......................... 200
ESB-ISBF S-KL01.......................... 533
ESB-ISBF S-KL02.......................... 534
ESB-SFB S-KL01.......................... 808
ESB-SFB S-KL02.......................... 809
ESB-SIBB S01-KL01......................... 516
ESB-SLABO S-KL01......................... 281
ESB-SLABO S-KL02......................... 282
LS-01.......................... 488
S01-KL01.......................... 88
S02-KL01.......................... 104
S02-KL02.......................... 105
S03-KL01.......................... 113
S04-KL01.......................... 189
S04-KL02.......................... 191
S05-KL01.......................... 266
S06-KL01.......................... 393
S07-KL02.......................... 411
S08-KL01.......................... 505
S08-KL02.......................... 507
S09-KL01.......................... 664
S10-KL01.......................... 679
S11-KL02.......................... 696
S12-KL01.......................... 785
S14-KL01.......................... 872
S15-KL02.......................... 888
Time of printing
September 03, 2021, subject to change

This program shows the current status at time of printing. Changes and updates will be displayed on site and published at the website www.esb2021.org/scientific-program/

Responsibility in terms of content
Ana Paula Pêgo.
Principal Investigator
nBTT - nanoBiomaterials for Targeted Therapies Group
INEB - Instituto de Engenharia Biomédica (www.ineb.up.pt)
and
i3S - Instituto de Investigação e Inovação em Saúde (www.i3s.up.pt)
Porto, Portugal

Conference Secretary
K.I.T. Group GmbH Dresden
Bautzner Strasse 117-119 l 01099 Dresden l Germany
E-Mail: info@esb2021.org