BIOMAT & MATSAN DAYS
14–19th January 2024
Super-Besse, France

Young Researchers days + Welcome reception and dinner

BIOMAT days

MORNING
8:00 – 8:30: Breakfast
8:30 – 9:15: Keynote: Minno Kellokulli (FI)
9:30 – 10:15: Oral presentations (Session BIOMAT_1)
Chen Oi Lynn Vern, Parhar Vijay Singh, Preiss Laura
10:15 – 11:00: Coffee Break
11:00 – 12:00: Oral presentations (Session BIOMAT_2)
Diaz Colina Andrea Valentina, Lopez Elliot, Dujardin Chloe, Guarneros Goncalves Michel
12:00 – 12:30: Flash presentations (Flash BIOMAT_1)
12:30 – 14:00: Lunch

AFTERNOON
14:00 – 15:00: Oral presentations (Session BIOMAT_3)
Corte Laurent, Hassan Ziad, Chichappara - Flores Henry, Houkqui Amel
15:00 – 15:30: Flash presentations (Flash BIOMAT_2)
15:30 – 16:15: Coffee Break
16:15 – 17:00: Round table BIOMAT – Didier Letournier
17:00 – 18:00: Oral presentations (Session BIOMAT_4)
Martineau, De La Taille Thibault, Alexei, Calderon Rosa
18:00 – 19:00: Biomat General Assembly

Evening
19:00: Dinner
20:30: Conference night: Tal Galesworthy

MatSan days

MORNING
8:00 – 8:30: Breakfast
8:30 – 9:15: Keynote: Matteo d’Este (Ch)
9:30 – 10:15: Oral presentations (Session MATSAN_1)
Barbato, Puse Tozlu, Abajian Alice
10:00 – 10:45: Coffee Break
10:45 – 11:15: Oral presentations (Session MATSAN_3)
Rouge Mouno, Laurence Mariano
11:15 – 11:45: Flash presentations (Flash MATSAN_1)
11:45 – 14:00: Lunch

AFTERNOON
14:00 – 14:45: Keynote: Patrick Schmutz (Ch)
14:45 – 15:30: Oral presentations (MATSAN_3)
Salplatz, Pierre, Safti Daniel
15:30 – 16:15: Coffee Break
16:15 – 17:00: Round table MATSAN
17:00 – 18:00: Oral presentations (Session MATSAN_2)
Batholet Théodore, Delatte Alexis, Dulom Arnaud
18:45 – 18:15: Short break
19:15 – 19:30: Oral presentations (Session MATSAN_2bis)
Barbato, Puse Tozlu, Abajian Alice, Mariano
19:30 – 20:30: Dinner

FREE AFTERNOON!
18:00 – 18:15: Welcome MatSan
18:15 – 19:15: Oral presentations (Session MATSAN_1)
Maaref, Garcia Eva, Carret Emmanuelle, Mathilde MAILLARD

Evening
20:15: Gala Dinner

MORNING
8:00 – 8:30: Breakfast
8:30 – 9:15: Keynote: Donata Iandolo (Fr)
9:15 – 10:00: Oral presentations (Session BIOMAT_5)
Bosquet Hugo, Grossetete Florine, Shaya Shanna
10:00 – 10:45: Coffee Break
10:45 – 11:00: Oral presentations (Session BIOMAT_6)
Wisniewski Nathan, Lemenac Lucas, Ahmed Omar Naima, Chabrilas Emillon, Martin Alice
12:00 – 12:30: Flash presentations (Flash BIOMAT_3)
12:30 – 14:00: Lunch

FREE AFTERNOON!
18:00 – 18:15: Welcome MatSan
18:15 – 19:15: Oral presentations (Session MATSAN_1)
Maaref, Garcia Eva, Carret Emmanuelle, Mathilde MAILLARD

Evening
20:15: Gala Dinner

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HTTPS://BIOMATFRAANCE.COM/
BIOMAT organizing Committee

(BIOMAT Young scientists)

- Joëlle Amédée (BIOTIS, INSERM U1026, Bordeaux)
- Teresa Simon-Yarza (LVTS, UMR_S1148, Paris)
- Astrid Pinzano (IMOPA, UMR 7365, Nancy)
- David Marchat (sainbiose, Mines Saint Etienne, U1059, Saint-Etienne)
- Emmanuel Pauthe (ERRMECe, EA 1391, Cergy)
- Didier Letourneur (LVTS, UMR_S1148, Paris)
- Jérôme Sohier (LBTI, UMR 5305, Lyon)
- Nicolas Blanchemain (ADDS, INSERM U1008, Lille)
- Reine Bareille (Retraité Ingénieur Inserm, Bordeaux)

- Amel Houaoui (CY Cergy Paris Université, Paris)
- Joanna Babilotte (MERLN institute, Maastricht University, The Netherlands)

MatSAN organizing Committee

- Jean-Marie Nedelec (ICCF, UMR 6296 CNRS / UCA, Clermont-Ferrand)
- Christophe Drouet (CIRIMAT, UMR 5085 CNRS UT3 Toulouse-INP, Toulouse)
- Christelle Combes (CIRIMAT, UMR 5085 CNRS UT3 Toulouse-INP, Toulouse)
- Yohann Wittrant (Unité de Nutrition Humaine, UMR1019 INRAE/UCA, Clermont-Ferrand)
- Benoit Ter-Ovanessian (MATEIS, UMR5510, Lyon)
- Jonathan Lao (Laboratoire de Physique de Clermont, UMR 6533, Clermont-Ferrand)
Bienvenue

The ‘Journées BIOMAT and MatSAN’, from January 14 to 19, 2024, aim to bring together academic researchers, industry professionals, clinicians, and students to exchange ideas on biomaterials and medical devices, as well as their evolution over time. This event is co-organized by ‘L’association pour le développement des biomatériaux (BIOMAT)’ and the ‘Commission mixte MatSan (Matériaux pour la Santé)’, involving the scientific societies SF2M, GFC, Cefracor, and Titane. It is also a scientific event supported by the Research Group ‘GdR Réparer l’humain’.

The organizing committee extends its gratitude to all participants!

Over 160 scientists, clinicians, and industry professionals in biomaterials, from France and several countries, are participating in these days. The objective is to bring together students, clinicians, researchers, and companies working in the field of development and evaluation of biomaterials and provide opportunities for networking and exchange. These days focus on European and international cooperation with 9 invited speakers from Europe and France; ii) the training of students and young researchers (more than 70) with a dedicated day for young BIOMAT scientists offering educational opportunities and exchange with experts in medical devices, career choices, funding, and roundtable discussions; iii) interaction with industry professionals in the field (5 present).

All of this, and much more, is integrated into a rich scientific program.

The conference consists of plenary lectures given by eminent European researchers to open 6 BIOMAT sessions, 3 joint sessions between BIOMAT and MatSAN, and 4 MatSAN sessions, spread over 4 days. 52 oral presentations and over 30 flash presentations, highlighted in 4 dedicated sessions, have been selected by the scientific committee. Prizes will be awarded by BIOMAT and MatSAN for the best 2 flash presentations and the best 4 student oral presentations.

We encourage you to interact, discover, and exchange during the Congress, breaks, and free time on Wednesday, January 17th (snowshoeing, igloo building, alpine sports, or leisure).
Simply take the opportunity to learn, meet, share, and discuss science in the beautiful Super-Besse region!

We wish you pleasant ‘Journées BIOMAT and MatSAN’ 2024.

The organizing committee
Invited Speakers

Monday, January 15th, 2024

Dr. Priscila Melo
Newcastle University, Newcastle, United Kingdom

**Session 1 Young Researchers**

“Navigating Academia as an Early Researcher and Woman in Engineering”

Dr. Adrian Seijas-Gamardo
MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, The Netherlands

**Session 1 Young Researchers**

“Introduction to Blender: A Guide to start making 3D Renders for Levelling up your Scientific Figures”

Dr. Francisco Fernandes
Sorbonne Université, LCMCP, Paris

**Session 2 Young Researchers**

“Fundamental vs. Applied Research in the field of Biomaterials: Scientific curiosity, Career strategy and Social impact”

Dr. Emeline Perrier-Groult
INSERM U 1183 - Institut de Médecine Régénératrice et biotherapies, Montpellier

**Session 3 Young Researchers**

“All roads lead to Academia”
Tuesday, January 16th, 2024

Pr. Minna Kellomäki
Tampere University, Finland

Session 1 BIOMAT

Tal Golesworthy
Exstent Ltd, Tewkesbury, United Kingdom

Night Conference
“PEARS: Engineering Surgical Solutions”

Wednesday, January 17th, 2024

Dr. Donata Iandolo,
Ecole des Mines de Saint Etienne, Saint Etienne

Session 5 BIOMAT
“Bioelectricity and bioelectronics for bone tissue engineering”

Thursday, January 18th, 2024

Dr. Matteo D’Este
AO fondation, Davos, Switzerland

Session 2 BIOMATSAN
“Soft biomaterials and composites: a journey from biofabrication to immunomodulation”

Pr. Patrik Schmutz
Empa, Dübendorf, Switzerland

Session 1 MatSAN
“Corrosion of metallic implants: about challenges and innovative therapies”
# Conference Program

## BIOMAT Young Researchers’ Day

### Sunday, January 14th, 2024

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>17:30 - 18:00</td>
<td>Welcome of the participants (Young Researchers) - Belambra Super-Besse Hotel - Le Chambourgut</td>
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<tr>
<td>18:00 - 19:00</td>
<td>Team building activity with aperitive (Young Researchers)</td>
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<tr>
<td>19:00 - 19:30</td>
<td>Dinner</td>
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<td>19:30 - 23:00</td>
<td>Opening Gala (Young Researchers)</td>
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### Monday, January 15th, 2024

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tr>
<td>08:00 - 08:30</td>
<td>Breakfast</td>
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<tr>
<td>08:30 - 08:45</td>
<td>Introduction</td>
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</tbody>
</table>
| 08:45 - 10:15 | Session 1 Young Researchers  
Chair: Amel HOUAOUI                           |
| 08:45 - 09:30 | Navigating Academia as an Early Researcher and Woman in Engineering - Priscila MELO, Newcastle University |
| 09:30 - 10:15 | Introduction to Blender: A Guide to start making 3D Renders for Levelling up your  
Scientific Figures - Adrian SAIJAS-GAMARDO, MERLN Institute for Technology-Inspired  
Regenerative Medicine, Maastricht         |
| 10:15 - 10:45 | Coffee break                                                                           |
| 10:45 - 11:45 | Session 2 Young Researchers  
Chair: Joanna Babilotte                     |
| 10:45 - 11:45 | Fundamental vs. Applied Research in the field of Biomaterials: Scientific curiosity,  
Career strategy and Social impact - Francisco FERNANDES, Sorbonne Université,  
Laboratoire de Chimie de la Matière Condensée de Paris |
| 12:00 - 14:00 | Lunch                                                                                 |
| 14:00 - 15:00 | Session 3 Young Researchers  
Chair: Baptiste CHARBONNIER                  |
| 14:00 - 15:00 | All roads lead to Academia - Emeline PERRIER-GROULT, INSERM U 1183 - Institut de  
Médecine Régénératrice et biothérapies       |
| 15:00 - 15:30 | Coffee break                                                                           |
| 15:30 - 17:00 | Session 4 YR - round table  
Chair: Cyril D’ARROS                          |
| 17:00 - 17:15 | Closing talk                                                                           |
| 18:00 - 19:00 | Welcome of the BIOMAT participants, with aperitive                                     |
| 19:00 - 21:00 | Dinner                                                                                 |
## BIOMAT Days

**Tuesday, January 16th, 2024**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Chairs</th>
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<tbody>
<tr>
<td>08:00 - 08:30</td>
<td>Breakfast</td>
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<tr>
<td>08:30 - 08:45</td>
<td>Introduction</td>
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<tr>
<td>08:45 - 10:15</td>
<td>Session BIOMAT_1</td>
<td>Didier LETOURNEUR – Rosa CALDERON</td>
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<tr>
<td>08:45 - 09:30</td>
<td>Keynote - Minna Kellomäki, Biomaterials and Tissue Engineering Group, Faculty of Medicine and Health Technology, Tampere University, Finland</td>
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<tr>
<td>09:30 - 09:45</td>
<td>Effect of surface functionalization and dispersion milieu on NMR relaxation properties of iron oxide nanoparticles - Lyns Verel CHE DJI, Cristallographie, Résonance Magnétique et Modélisations, Institut Jean Lamour</td>
<td></td>
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<tr>
<td>09:45 - 10:00</td>
<td>Phase controlled gallol functionalized hyaluronic acid-base hydrogel with improved injectability and self healing properties - Vijay Singh PARIHAR, Biomaterials and Tissue Engineering Group, Faculty of Medicine and Health Technology, Tampere University, Tampere</td>
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<tr>
<td>10:00 - 10:15</td>
<td>On the potential of µ-CT on the observation and interpretation of bone behavior around dental implants - Laura PREISS, Matériaux, ingénierie et science, Villeurbanne</td>
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<td>10:15 - 11:00</td>
<td>Coffee break</td>
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<tr>
<td>11:00 - 12:00</td>
<td>Session BIOMAT_2</td>
<td>Christèle COMBES - Bruno PAIVA</td>
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<td>11:00 - 11:15</td>
<td>Mechanics of anisotropic polyvinylalcohol hydrogel fibers for osteoarticular tissue reconstruction - Andrea Valentina DIAZ COLINA, Centre des Matériaux, Chimie Moléculaire, Macromoléculaire et Matériaux (UMR7167)</td>
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<tr>
<td>11:15 - 11:30</td>
<td>Growth and study of tumor spheroids behavior in a biomimetic vascularized platform - Elliot LOPEZ, Macromolécules et Microsystèmes en Biologie et Médecine, UMR 168, Institut Curie, Institut Pierre Gilles de Gennes, Université Paris Cité, Université Sorbonne Paris Nord, LVTS Inserm U1148</td>
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<tr>
<td>11:30 - 11:45</td>
<td>A pre-vascularized structured hydrogel membrane mimicking the outer blood-retinal barrier to rescue the retinal pigment epithelium in retinal dystrophies - Chloé DUIJARDIN, Laboratoire de Recherche Vasculaire Translationnelle</td>
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<tr>
<td>11:45 - 12:00</td>
<td>Phosphorus-based films for actinide decontamination - Mickael GUERREIRO GONCALVES, Institut des Biomolécules Max Mousseron [Pôle Chimie Balard], Institut Charles Gerhardt</td>
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<tr>
<td>12:00 - 12:30</td>
<td>FLASH BIOMAT_1</td>
<td>Amel HOUAOUI</td>
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<td>12:00 - 12:03</td>
<td>WAVE-INSPIRED MEW SCAFFOLDS FOR ENHANCED LIGAMENT TISSUE REGENERATION - Joanna BABILOTTE, Complex Tissue Regeneration department, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University</td>
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</table>
12:03 - 12:06  Pre-vascularization with SHED stem cells to engineer a functional vascular network - **Caroline GORIN**, URP2496, Pathologies, imagerie et biothérapies oro-faciales, Services de médecine bucco-dentaire, GH Paris Nord

12:06 - 12:09  BORONATE ESTER HYDROGELS: PROMISING MATERIALS FOR BIOMEDICAL APPLICATIONS - **Lea TERRIAC**, RMeS - Skeletal Physiopathology and Joint Regenerative Medicine - UMR 1229

12:09 - 12:12  Enhancing Aneurysm Healing through Fucoïdane-Coated Coils - **Emilie ROCH**, CEA Saclay, Balt extrusion

12:12 - 12:15  COLLAGEN / POLYESTER-POLYURETHANE POROUS SCAFFOLDS FOR USE IN MENISCAL REPAIR - **Benjamin NOTTELET**, Institut des Biomolécules Max Mousseron, Pôle Chimie Balard

12:15 - 12:18  Supercritical CO2-Enabled Aerogels of Platelet Lysate and Collagen for Tissue Repair: Natural Biomaterials and Growth Factor Delivery - **Fahd TIBOURTINE**, Centre interuniversitaire de recherche et dingenierie des matériaux

12:18 - 12:21  CRYOPRESERVATION OF RED BLOOD CELLS IN ABSENCE OF TOXIC CRYOPROTECTANTS VIA ICE TEMPLATING - **Francisco FERNANDES**, Laboratoire de Chimie de la Matière Condensée de Paris

12:21 - 12:24  Develop an implantable medical device based on a dehydrated, sterile, viro-inactivated membrane derived from perinatal tissues. - **Lorinne ADAM**, Laboratoire BIOS, EA4691, BIOBank

12:30 - 14:00  Lunch

14:00 - 15:00  Session BIOMAT_3  
**Chairs: Astrid PINZANO - Nicolas TOUYA**

14:00 - 14:15  Adhesion to internal tissues: slippery when wet - **Laurent CORTE**, Chimie Moléculaire, Macromoléculaire et Matériaux (UMR7167), Centre des Matériaux

14:15 - 14:30  SMILING, PRE-CLINICAL EVALUATION OF ATISSUE-ENGINEERED VASCULAR SUBSTITUTE CONSTRUCTED FROM HUMAN UMBILICAL CORD, IN PIGS AS A BIG ANIMAL MODEL - **Ziad HASSAN**, CHRU, MTinov, UTCT

14:30 - 14:45  From in vitro to in vivo assessment of an active viscosupplement hydrogel for intra-articular injection in temporomandibular joint disorders - **Henry CHIJCHEAPAZA-FLORES**, University of Lille, INSERM, U1008 - Advanced Drug Delivery Systems, F-59000 Lille, France

14:45 - 15:00  3D-PRINTED LUMINESCENT BIOACTIVE GLASS SCAFFOLDS FOR BONE BIOENGINEERING - **Amel HOUAOUI**, CY Cergy Paris Université

15:00 - 15:30  FLASH BIOMAT_2  
**Chair: Astrid PINZANO**

15:00 - 15:03  Osteogenic Effect of Fisetin Doping in Bioactive Glass/Poly(caprolactone) Hybrid Scaffolds - **Yohann WITTRANT**, Unité de Nutrition Humaine
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<tr>
<th>Time</th>
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<tbody>
<tr>
<td>15:03 - 15:06</td>
<td>Amphiphilic PCL-g-Dex nanoparticles: Towards hybrid hydrogel/nanoparticles dual-cancer drug delivery system - Benjamin Nottelet, Institut des Biomolécules Max Mousseron - <em>Hélène VAN DEN BERGHE, Institut des Biomolécules Max Mousseron</em></td>
</tr>
<tr>
<td>15:06 - 15:09</td>
<td>DEVELOPMENT OF A 3D BIOMATERIALAS A PRO-RESOLVING CELL SUPPORT FOR THE HEALING AND TREATMENT OF CHRONIC WOUNDS - <em>Ali SANOUJI, RESTORE, CIRIMAT</em></td>
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<tr>
<td>15:09 - 15:12</td>
<td>STENTS COVERED WITH POLYCAPROLACTONE-CIPROFLOXACIN ELECTROSPUN MEMBRANES: CHARACTERIZATION AND KINETIC RELEASE STUDY - <em>Mickaël MATON, U1008, Advance Drug Delivery System, Lille</em></td>
</tr>
<tr>
<td>15:12 - 15:15</td>
<td>Skin decontamination: development and validation of film-forming formulations on skin mice against chemical warfare agents. - <em>Magaly MISBACH, Laboratoire de Biologie Tissulaire et d’Ingénierie Thérapeutique</em></td>
</tr>
<tr>
<td>15:15 - 15:18</td>
<td>TOWARDS SAFE SILICONE IMPLANTS ENGINEERING - <em>Eve RANDRIANARIDERA, Institut de Science des Matériaux de Mulhouse</em></td>
</tr>
<tr>
<td>15:18 - 15:21</td>
<td>An all-in-one biocompatible Collagen/Tannic Acid tough and adhesive hydrogel with antibacterial, antioxidant and anti-tumor biological activities. - <em>Anaïs LARVAND, Biomatériaux et inflammation en site osseux - EA 4691</em></td>
</tr>
<tr>
<td>15:21 - 15:24</td>
<td>Dense collagen hydrogels loaded with anti-sclerostin antibodies as biomaterials for critical size calvaria defect repair. - <em>Ludovic SICARD, EA2496</em></td>
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<tr>
<td>15:30 - 16:15</td>
<td>Coffee break</td>
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<tr>
<td>16:15 - 17:00</td>
<td>BIOMAT round table - Ethique et Fraude Scientifique - <em>Didier LETOURNEUR</em></td>
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<tr>
<td>17:00 - 18:00</td>
<td><strong>Session BIOMAT 4</strong>&lt;br&gt;<em>Chairs: Teresa SIMON YARZA – LUCAS LEMARIE</em></td>
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<tr>
<td>17:00 - 17:15</td>
<td>BIOMIMETIC COLLAGEN-BASED MATERIALS FOR VASCULAR REPLACEMENT - <em>Isabelle MARTINIER, Laboratoire de Chimie de la Matière Condensée de Paris</em></td>
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<tr>
<td>17:15 - 17:30</td>
<td>Functionalized polysaccharide nanoparticles for a targeted treatment of ischemic strokes - <em>Thibault DE LA TAILLE, Laboratoire de Recherche Vasculaire Translationnelle</em></td>
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<tr>
<td>17:30 - 17:45</td>
<td>Salt-Compacted Albumin Materials for Anti-Tumor Drug Delivery: Integrating Localized and Targeted Approaches for Enhanced Cancer Treatment - <em>Eya ALOUI, INSERM UMR 1121 Biomaterials and Bioengineering, Conception et application de molécules bioactives</em></td>
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<tr>
<td>17:45 - 18:00</td>
<td>INCORPORATION OF NATURALACTIVE COMPOUNDS IN LAYER-BY-LAYER FILMS - <em>Rosa CALDERON, Laboratoire ERRMECe</em></td>
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<tr>
<td>18:00 - 19:00</td>
<td>BIOMAT General assembly</td>
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<td>19:00 - 19:30</td>
<td>Dinner</td>
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<td>19:30 - 20:30</td>
<td><strong>Night conference</strong> - PEARs: Engineering Surgical Solutions - <em>Tal Golesworthy, Exstent Ltd, Tewkesbury, United Kingdom</em></td>
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## Wednesday, January 17th, 2024

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<tr>
<th>Time</th>
<th>Session</th>
<th>Description</th>
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<tbody>
<tr>
<td>08:00 - 08:30</td>
<td>Breakfast</td>
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<tr>
<td>08:30 - 10:00</td>
<td>Session BIOMAT_5</td>
<td><strong>Chairs:</strong> Nicolas BLANCHEMAIN – Micaela ROQUE</td>
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<tr>
<td>08:30 - 09:15</td>
<td>Keynote</td>
<td>Bioelectricity and bioelectronics for bone tissue engineering - <strong>Donata IANDOLO</strong>, Ecole des Mines de Saint Etienne</td>
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<tr>
<td>09:15 - 09:30</td>
<td>Development</td>
<td>of an Electrospun membranes for the reconstruction of critical size segmental bone loss - <strong>Hugo BOSSUT</strong>, Unité Matériaux et Transformations - UMR 8207</td>
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<tr>
<td>09:30 - 09:45</td>
<td>EXOSOME IMPREGNATION OF A HUMAN UMBILICAL CORD-DERIVED BIOMATERIAL AND PROGRESSIVE RELEASE OVER TIME - <strong>Florine GROSSETETE</strong>, TBF - Tissue Engineering, 69780 Mions, France</td>
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<td>09:45 - 10:00</td>
<td>Development</td>
<td>of a 3D in vitro mineralized bone model to reproduce the osseointegration process of dental implants - <strong>Ghannaa SHAYYA</strong>, Inserm BioTis, Laboratory for the Bioengineering of Tissues, University of Bordeaux, Bordeaux, France</td>
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<td>10:00 - 10:45</td>
<td>Coffee break</td>
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<tr>
<td>10:45 - 12:00</td>
<td>Session BIOMAT_6</td>
<td><strong>Chairs:</strong> Emmanuel PAUTHE – Romane LESIEUR</td>
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<td>10:45 - 11:00</td>
<td>HARVESTING EXTRACELLULAR MATRIX OF WHARTON'S JELLY BY EXTRACTION OR DECELLULARIZATION PROCESS: A PROMISING MATERIAL FOR CARDIOVASCULAR TISSUE ENGINEERING - <strong>Nathan WISNIEWSKI</strong>, Ingénierie Moléculaire et Physiopathologie Articulaire</td>
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<tr>
<td>11:00 - 11:15</td>
<td>3D PRINTABLE ALGINATE- GELATIN HYDROGELS WITH VARIABLE VISCOELASTIC PROPERTIES AS SOLE DIFFERENTIATION FACTOR OF INDUCED PLURIPOTENT STEM CELLS FOR TISSUE ENGINEERING - <strong>Lucas LEMARIE</strong>, Laboratoire de Biologie Tissulaire et d’ingénierie Thérapeutique UMR 5305, SEGULA Technologies, Institut de Chimie et Biochimie Moléculaires et Supramoléculaires</td>
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<tr>
<td>11:15 - 11:30</td>
<td>Development</td>
<td>of Novel Polysaccharide Membranes for Guided Bone Regeneration: In Vitro and In Vivo Evaluations - <strong>Naïma AHMED OMAR</strong>, U1026 Biotis Inserm</td>
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<tr>
<td>11:30 - 11:45</td>
<td>BIODEGRADABLE IMPLANTABLE WOUND DRESSING BASED ON BIOPOLYMERS: STERILIZATION METHOD AND IN VIVO BIODEGRADATION - <strong>Emilien CHABRILLAC</strong>, Centre interuniversitaire de recherche et d’ingenierie des matériaux, Institut Universitaire du Cancer de Toulouse - Oncopole</td>
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<td>11:45 - 12:00</td>
<td>IN VITRO TOXICITY ASSESSMENT OF PARTICULATE EMISSIONS FROM BRAKING SYSTEMS - <strong>Alice MIRAILLER</strong>, Laboratoire de Mécanique des Contacts et des Structures, Villeurbanne</td>
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<tr>
<td>12:00 - 12:30</td>
<td>FLASH BIOMAT_3</td>
<td><strong>Chair:</strong> Emmanuel PAUTHE</td>
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<tr>
<td>12:00 - 12:03</td>
<td>HYBRID HYDROGEL TO CONTROL CELL RESPONSE AND REGENERATE DENTAL PULP - <strong>Jerome SOHIER</strong>, Laboratoire de Biologie Tissulaire et d’ingénierie Thérapeutique UMR 5305</td>
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<tr>
<td>12:03 - 12:06</td>
<td><strong>DYNAMIC AND DEGRADABLE NETWORKS FOR 3D-PRINTING OF ELASTOMERIC SELF-HEALABLE DEVICES</strong> - Benjamin NOTTELET, Institut des Biomolécules Max Mousseron, Pôle Chimie Balard</td>
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<tr>
<td>12:06 - 12:09</td>
<td><strong>BIOMATERIAL FUNCTIONALIZATION WITH TRIPLE-HELICAL PEPTIDES FOR CARTILAGE TISSUE ENGINEERING</strong> - Audrey ZIVEREC, Laboratoire de Biologie Tissulaire et d’ingénierie Thérapeutique UMR 5305</td>
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<td>12:09 - 12:12</td>
<td><strong>COMPETITIVE BINDING ENABLES THE FAST DISSOLUTION OF BORONATE ESTER HYDROGELS FOR THE EXTRACTION AND BIOLOGICAL ANALYSIS OF ENCAPSULATED CELLS</strong> - Garance SAINT-PE, RMeS - Skeletal Physiopathology and Joint Regenerative Medicine</td>
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<td>12:12 - 12:15</td>
<td><strong>SYNTHESIS OF THREE-DIMENSIONAL NETWORK HYDROGELS (PAA ,PEG 200) OR (PAA,PEG 400) AND EVALUATION OF THEIR PROPERTIES FOR POTENTIAL BIOMEDICAL APPLICATIONS</strong> - Fetta AIT AHSENE-AISSAT, unité de recherche Matériaux Procédés Environnement URMPE</td>
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<td>12:15 - 12:18</td>
<td>Unravelling the roles of texture and basal lamina composition on the endothelialization of biomimetic type I collagen matrices - Minaine BOUABDALLAH, Laboratoire de Chimie de la Matière Condensée de Paris</td>
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<td>12:18 - 12:21</td>
<td><strong>DEVELOPMENT OF ELECTROSPUN SCAFFOLD AS BIOMIMETIC DURAL SUBSTITUTES</strong> - Nathalia ODERICH MUNIZ, Biomécanique et Bioingénierie</td>
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<td>12:21 - 12:24</td>
<td>Silicon effects on human dental pulp stem cells seeded in 3D dense collagen hydrogel - Daline MBITTA AKOA, Laboratoire de Chimie de la Matière Condensée de Paris</td>
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<tr>
<td>12:30 - 14:00</td>
<td>Lunch</td>
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<tr>
<td>14:00 - 18:00</td>
<td>Free afternoon or activities</td>
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<tr>
<td>18:00 - 18:15</td>
<td><strong>Welcome of the MatSAN participants</strong></td>
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### BIOMATSAN Days

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<td>Session BIOMATSAN_1</td>
<td>Jerome SOHIER - Isabelle MARTINIER</td>
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<td>18:15 - 18:30</td>
<td>MODIFICATION OF ZIRCONIA DENTAL IMPLANTS TO PROMOTE SOFT TISSUE ADHESION AND LIMIT BACTERIA COLONIZATION</td>
<td>Fryni SKORDA, MATEIS, Villeurbanne</td>
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<tr>
<td>18:30 - 18:45</td>
<td>Functionalization of Zirconia for Enhanced Osseointegration in Dental Implants</td>
<td>Eva GARCIA, MATEIS, Villeurbanne</td>
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<td>18:45 - 19:00</td>
<td>BIOFABRICATION OF A 3D IN VITRO MODEL RECAPITULATING THE OVINE IVD STRUCTURE FOR EVALUATING THE EFFICIENCY OF NOVEL THERAPIES.</td>
<td>Emmaëlle CARROT, RMes - Skeletal Physiopathology and Joint Regenerative Medicine</td>
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<td>19:00 - 19:15</td>
<td>DEVELOPMENT OF POLYSACCHARIDE BASED MEMBRANE FOR GUIDED BONE REGENERATION: MECHANICAL, IN VITRO AND IN VIVO ASSESSMENT</td>
<td>Mathilde MAILLARD, Laboratoire de Recherche Vasculaire Translationnelle</td>
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<td>19:15 - 23:00</td>
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**Thursday, January 18th, 2024**

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<td>08:00 - 08:30</td>
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<td>08:30 - 10:00</td>
<td>Session BIOMATSAN_2</td>
<td>Joelle AMEDEE – Ludovic SICARD</td>
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<td>08:30 - 09:15</td>
<td>Keynote</td>
<td>Matteo D’ESTE, AO fondation, Davos</td>
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<td>09:15 - 09:30</td>
<td>3D-PRINTED GELATIN-PLURONIC SCAFFOLDS PROMOTE GLIAL CELLS ALIGNMENT FOR SPINAL CORD REGENERATION</td>
<td>SACHA BARBERAT, Institut Charles Gerhardt Montpellier</td>
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<td>09:30 - 10:00</td>
<td>Biomimetic apatite and m-CPPD crystals-based materials as models to improve ex vivo osteoarticular calcifications diagnosis</td>
<td>Louize TOUATI, Centre interuniversitaire de recherche et d’ingenierie des materiaux</td>
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<td>09:45 - 10:00</td>
<td>NEW APPROACH TO EVALUATE THE INFLUENCE OF BIOCERAMIC COMPOSITION ON THE BEHAVIOR OF CELLS INVOLVED IN BONE REGENERATION</td>
<td>Alice ABELANET, Institut de Recherche sur les CERamiques</td>
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<td>10:00 - 10:45</td>
<td>Coffee break</td>
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<tr>
<td>10:45 - 11:15</td>
<td>Session BIOMATSAN_3</td>
<td>David MARCHAT - Mathilde MAILLARD</td>
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<td>10:45 - 11:00</td>
<td>A COMPOSITE ELASTIN DERIVATIVE-BASED HYDROGEL DESIGNED FOR PROMOTING BONE FORMATION, VASCULARIZATION, AND INNERVATION: IN VIVO EVALUATION IN ECTOPIC AND HETEROTOPIC MODELS</td>
<td>Micaela ROQUE, BioTis - U1026</td>
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11:00 - 11:15 Investigation of the early apical release from endodontic hydrogels: a 3D printed model - Marianne LEVEQUE, Laboratoire de Biologie Tissulaire et ingénierie Thérapeutique UMR 5305

11:15 - 11:45 FLASH BIOMATSAN_1
Chair: David MARCHAT

11:15 - 11:18 PHOTOCROSSLINKABLE, DEGRADABLE AND BIOACTIVE POLYMERIC INK FOR MENISCUS REGENERATION BY 3D PRINTING - Coline PINESE, Institut des Biomolécules Max Mousseron, Pôle Chimie Balard

11:18 - 11:21 MULTI-DOPED BIOCERAMICS FOR LARGE BONE DEFECTS REGENERATION - Alice SZMYTKO, Institut de Chimie de Clermont-Ferrand

11:21 - 11:24 Influence of macroscopic confinement on tissue organization and osteogenesis - Alexis ROMERO, Santé Ingénierie Biologie Saint-Etienne

11:24 - 11:27 PATIENT SPECIFIC RECONSTRUCTION OF CLEFT PALATE DEFORMITIES IN DOG WITH FLEXIBLE 3D-PRINTED ORGANO-MINERAL CEMENTS - Nicolas TOUYA, Regenerative Medicine and Skeleton

11:27 - 11:30 Patient specific scaffolds for osteo-gingival regeneration - Priscilla QUENUM, Nantes Université, Oniris, Univ Angers, CHU Nantes, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229

11:30 - 11:33 CHARACTERIZATION OF THE OSTEOCHONDRAJON FOR THE DEVELOPMENT OF BIO-INKS DEDICATED TO THE REPRODUCTION OF THIS ANCHORING ZONE IN THE TREATMENT OF FOCAL OSTEOCHONDRAL LESIONS BY TISSUE ENGINEERING - Ghita SEKKAT, Ingénierie Moléculaire et Physiopathologie Articulaire

11:45 - 14:00 Lunch
MatSAN Days

14:00 - 15:30  Session MATSAN_1  
*Chairs: Jonathan LAO - Nathalia ODERICH MUNIZ*

14:00 - 14:45  **Keynote** - CORROSION OF METALLIC IMPLANTS: ABOUT CHALLENGES AND INNOVATIVE THERAPIES  - Patrik Schmutz, Empa, Dübendorf, Switzerland

14:45 - 15:00  AMINO-BASED COATINGS FOR IMPROVING IMPLANTS' TISSUE INTEGRATION - Hernando SALAPARE, Institut de Science des Matériaux de Mulhouse

15:00 - 15:15  HYBRID PARTICLES FOR THE PHYSICAL TREATMENT OF THROMBOTIC DISEASES  - Pierre SARFATI, Laboratoire de Recherche Vasculaire Translationnelle

15:15 - 15:30  ATP/COLLAGEN COACERVATES AS NEW PRECURSORS FOR BIOPRINTED DENSE COLLAGEN MATRICES - Daniel A. BLAGA, Laboratoire de Chimie de la Matière Condensée de Paris

15:30 - 16:15  Coffee break

16:15 - 17:00  MATSAN round table - Durability of medical devices: from ex vivo evaluation to clinical outcomes  - Patrik Schmutz, Benoit Ter Ovanessian, Ana Sfarghiu, Christophe Drouet

17:00 - 18:15  Session MATSAN_2  
*Chairs: Yohann WITTRANT - Marjorie DUFAUD*

17:00 - 17:15  Investigating the influence of bioactive glass 92S6 P123 on 3D-Printed scaffold fabrication  - Théodore BERTHELOT, Univ Rennes, CNRS, ISCR - UMR 6226, Rennes, France

17:15 - 17:30  Design of a biodegradable bone cement for the treatment of bone fractures induced by breast cancer metastases - Alexia Delnatte, Institut Européen des membranes

17:30 - 17:45  APPLICATIVE POTENTIAL OF BIOPOLYMER PRODUCED BY MICROALGAE IN BONE REGENERATION  - Arnaud DULOM, RMes UMR INSERM 1229

17:45 – 18:15  Short Break

18:15 - 19:15  Session MATSAN_2bis  
*Chairs: Benoit TER OVANESSIAN - Sacha BARBERAT*

18:15 - 18:30  Antimicrobial and antibiofilm activity of peptide-functionalized hydrogels - Ines Ben HADJI KADDOUR, Inserm UMR S 1121 Biomatériaux et bioingénierie, SPARTHA Medical

18:30 - 18:45  DEVELOPMENT OF A TOPICAL PLATFORM FOR THE DELIVERY OF NATURAL BIOACTIVE COMPOUNDS AND STAPHYLOCOCCUS AUREUS PHAGES FOR DERMAL APPLICATIONS  - Carole ARMAL, CY Cergy Paris Université - ERRMECe Laby, Biomaterials for Health Group

18:45 – 19:00  SITE-SPECIFIC DRUG DELIVERY: INNOVATIVE DEVICE FOR MITIGATING PELVIC RADIOTHERAPY SIDE EFFECTS IN THE COLON - Diego AMAARANTE SILVA, Laboratoire de Radiobiologie des expositions médicales

19:00 - 19:15  AN INNOVATIVE PROCESS TO REVEAL WHARTON'S JELLY POTENTIAL - Charlotte GAROT, TBF - Tissue Engineering, 69780 Mions, France

19:15 - 21:00  Dinner
**Friday, January 19, 2024**

08:00 - 08:30 Coffee break

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<td>08:30 - 08:45</td>
<td>PROCESSED INVERTED HUMAN UMBILICAL VESSELS NERVE REGENERATION CONDUIT IN THE TREATMENT OF HAND NERVE SECTION - Laurence BARNOUIN, TBF - Tissue Engineering, 69780 Mions, France</td>
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<tr>
<td>08:45 - 09:00</td>
<td>POLY(BETA-AMINO)ESTER ELECTROSPUN WOUND DRESSING WITH MODULATED DEGRADATION KINETICS FOR CHRONIC WOUNDS TREATMENT - Liam AYADEN, Unité Matériaux et Transformations - UMR 8207</td>
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<td>09:00 - 09:15</td>
<td>Development of a Biphasic Osteochondral Model for Joint Tissue Repair using Extrusion-based 3D Bioprinting of a Natural Composite Hydrogel - Marjorie DUFAUD, Cellules Souches, Plasticité Cellulaire, Médecine Régénératrice et Immunothérapies (IRMB)</td>
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<td>09:15 - 09:30</td>
<td>CHITOSAN-GRAFTED-FIBRONECTIN FOR BIOACTIVE THERMOSENSITIVE HYDROGELS - Pierre MARQUAILLE, Chimie Moléculaire, Macromoléculaire et Matériaux (UMR7167), Equipe de recherche sur les relations matrice extracellulaire-cellules</td>
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<td>09:30 - 09:45</td>
<td>A degradable nanofibrous scaffold of poly(ε-caprolactone-co-lactide) for intervertebral disc regeneration - Mansoor CHAABAN, RMeS - Regenerative Medicine and Skeleton</td>
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<td>09:45 - 10:00</td>
<td>Régénération diaphragmatique par bioimpression de microparticules pré-cellularisés - Mélissa LANGLOIS, Biomatiériaux et Bioingénierie</td>
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<td>10:00 - 10:45</td>
<td>Coffee break</td>
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<td>10:45 - 11:30</td>
<td><strong>Session MATSAN_4</strong>&lt;br&gt;<strong>Chairs: Ana SFARGHIU - Mansoor CHAABAN</strong></td>
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<td>10:45 - 11:00</td>
<td>SURFACE MODIFICATION OF A 3D-PRINTED POLYURETHANE STENT-GRAFT FOR ANTI-THROMBOTIC PROPERTIES - Kim VAN DEN BROECK, Inserm U1008 - Advanced Drug Delivery Systems (ADDS), Unité Matériaux et Transformations - UMR 8207</td>
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<td>11:00 - 11:15</td>
<td>Reactive CO2 laser sintering of powders to produce bioactive glassy scaffolds - Ronan LEBULLENGER, Institut des Sciences Chimiques de Rennes</td>
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<td>11:15 - 11:30</td>
<td>FORMULATION OF BIO-INKS DEDICATED TO SPECIFIC CARTILAGE LAYERS USING COLLAGEN AND HYALURONIC ACID ADDITIVES - Christel HENRIONNET, Ingénierie Moléculaire et Physiopathologie Articulaire</td>
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<td>Awards and Closing ceremony</td>
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<td>11:45 - 14:00</td>
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Abstracts

Oral and Flash presentations
Monday, January 15\textsuperscript{th}, 2024

BIOMAT Young Researchers’ Day

Chairs:
Amel HOUAOU, Joanna BABILOTTE,
Baptiste CHARBONNIER, Cyril D’ARROS
Navigating Academia as an Early Researcher and Woman in Engineering

Priscila Melo *† 1

1 Newcastle University – Royanne-Uni

Dr. Priscila Melo: Pioneering Biomedical Engineer and Advocate for Women in STEM
Dr. Priscila Melo, a distinguished Portuguese biomedical engineer, is renowned for her expertise in additive manufacturing (AM) and biofabrication of biomaterials, focusing on tissue engineering and regenerative medicine.

Her academic journey began with a Bachelor’s degree in biomedical engineering and continued with a Master’s degree in biomaterials and medical devices. Driven by her passion for tissue engineering, she pursued a ground-breaking PhD at Newcastle University in the UK, where she explored the use of bioglass-ceramics and composites to create scaffolds for bone growth and stem cell differentiation, revolutionizing our understanding of material-biological interactions. Throughout her career, Dr. Melo expanded her research to include bioprinting and the development of in vitro disease models.

Despite facing early challenges as a lecturer, she co-leads the ECR network in biomedical engineering at Newcastle, striving to create a supportive academic environment for early-career researchers.

As a woman in engineering, Dr. Melo is a prominent advocate for women in STEM. She works to eliminate bias and promote diversity within academia, actively engaging in outreach programs to inspire the next generation of scientists and engineers.

In summary, Dr. Priscila Melo’s remarkable career is marked by innovative research in tissue engineering and her dedication to advancing diversity and inclusion in STEM. Her journey serves as an inspiration to aspiring scientists and underscores the importance of perseverance and advocacy in the pursuit of excellence in STEM fields.

Mots-Clés: STEM

*Intervenant
†Auteur correspondant:
Introduction to Blender: A Guide to start making 3D Renders for Levelling up your Scientific Figures

Adrian Seijas-Gamardo *† 1

1 MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht – Pays-Bas

Adrián Seijas-Gamardo received his BSc in Biotechnology (2017) from University of León (Spain) and his MSc in Biomedical Sciences (2019) from Maastricht University (The Netherlands). Currently he is a PhD candidate at the MERLN Institute for Technology-Inspired Regenerative Medicine under the supervision of Dr. Paul Wieringa and Prof. Lorenzo Moroni. His research focuses on the generation of complex in vitro models, with a special focus on tissue innervation, using diverse biofabrication technologies, 3D co-culture systems, human iPSCs and organoids.

Mots-Clés: Blender

*Intervenant
†Auteur correspondant:
Fundamental vs. Applied Research in the field of Biomaterials: Scientific curiosity, Career strategy and Social impact

Francisco Fernandes *† 1

1 Sorbonne Université, Laboratoire de Chimie de la Matière Condensée de Paris – Laboratoire de Chimie de la Matière Condensée de Paris, Sorbonne Université, UMR 7574, Paris, France – France

Francisco obtained a degree in Applied Chemistry and a MSci in Environmental Sciences from the University of Minho in Braga, Portugal. He has then joined Prof. Ruiz-Hitzky’s group at the Materials Science Institute of Madrid (CSIC) to pursue a PhD in Applied Physical Chemistry, focusing on the interface between biopolymers and clay minerals in bionanocomposite materials. In 2011, he integrated the Laboratory of Condensed Matter Chemistry (LCMCP) at Sorbonne Université as a post-doctoral fellow, under the guidance of Dr. N. Nassif, where he developed collagen-based materials by spray drying.

Francisco was appointed assistant professor in 2013 at Sorbonne Université (Materials & Biology team at the LCMCP), and passed his Habilitation à Diriger des Recherches in 2022. In 2016 Francisco held a position of Visiting Lecturer at the Department of Physics at King’s College London. His current research interests revolve around the transformation of "classical" materials fabrication techniques into biofabrication techniques. In particular, he has developed a recognized expertise on ice templating applied to biological entities-from biomolecules up to living cells. Francisco has authored over 40 research papers, 8 book chapters and is the co-inventor of 7 international patents. Since 2023 he became member of the editorial board of Advanced Biology.

Mots-Clés: À venir
All roads lead to Academia

Emeline Perrier-Groult *† 1

1 INSERM U 1183 - Institut de Médecine Régénératrice et biothérapies – INSERM U1183 Montpellier – France

Emeline obtained her PhD on the origins of electrophysiological alterations in cardiac remodeling and failure in 2004 from INSERM U637, Pr. Sylvain Richard (Montpellier, France) and Laboratory of endocrinology and diabetology at the Cantonal University Hospital of Geneva, Pr. Michel Rossier (Geneva, Switzerland). After a two-year post-doctoral fellowship in the cardiovascular division of the Research Institute Servier (Suresnes, France), she joined the CNRS group of Dr. Frédéric Mallein-Gerin (Biologie et Ingénierie du Cartilage, IBCP) in Lyon, France where she was recruited in 2009 to work on the influence of three-dimensional architecture and various biomaterials on the phenotype of chondrocytes. Then, in 2021, she moved back to Montpellier to join INSERM UMR1183, Dr. Danièle Noël to develop articular organoids from MSCs or MSCs derived from iPSCs (induced pluripotent stem cells) and to evaluate the impact of different physical and/or biological stimuli on the development of osteoarticular pathologies. Emeline has published more than 30 papers in renowned international journals.

Mots-Clés: A venir
Tuesday, January 16th, 2024

BIOMAT Session 1

Chairs:
Didier LETOURNEUR, Rosa CALDERON
Minna Kellomäki *† 1

1 Biomaterials and Tissue Engineering Group, Faculty of Medicine and Health Technology, Tampere University – Finland

*Intervenant
†Auteur correspondant:
Effect of surface functionalization and dispersion milieu on NMR relaxation properties of iron oxide nanoparticles

Lyns Verel Che Dji *† 1,2, Amel Cherraj 2, Thomas Girardet 2, Solenne Fleutot 2, Sabine Bouquet-Bonnet 1

1 Cristallographie, Résonance Magnétique et Modélisations – Université de Lorraine, Centre National de la Recherche Scientifique – France
2 Institut Jean Lamour – Université de Lorraine, Centre National de la Recherche Scientifique, Centre National de la Recherche Scientifique : UMR7198 – France

Iron oxide nanoparticles possess a unique magnetic property called superparamagnetism, enabling their use as contrast agents in magnetic resonance imaging, a diagnostic tool in the medical field. However, this requires a well-optimized synthesis method to yield highly crystalline and surface-functionalized nanoparticles. Surface functionalization increases biocompatibility, colloidal stability, targeting, and size control. This work aimed to synthesize nanoparticles with different capping agents and dispersion in different solvents. They were synthesized by the microwave approach. Capping agents such as citric acid, pentetic acid, and methylene phosphonic acid were chosen because they contain phosphate and carboxylic functional groups that show a tight bond to the nanoparticle surface. Nanoparticle synthesis was carried out in ultra-pure water and those functionalized by citric acid were subsequently dispersed in phosphate-buffered saline and physiological serum, both of which contain essential electrolytes for proper functioning of the human body. They were subjected to methodical analyses to determine their physicochemical and magnetic properties. Nuclear magnetic resonance relaxation measurements were also conducted to evaluate their efficacy also known as relaxivity at medically relevant magnetic field strengths. The results showed high relaxivity values, implying less dosage needed to produce high-quality images. Relaxivity is field-dependent and the analysis(i) of nuclear magnetic resonance dispersion (NMRD) profiles also allowed us to obtain physicochemical parameters consistent with the ones obtained by other methods.


Keywords: Capping agent, superparamagnetic iron oxide nanoparticles, relaxivity

*Speaker
†Corresponding author: lyns-verel.che-dji@univ-lorraine.fr
PHASE CONTROLLED GALLOL FUNCTIONALIZED HYALURONIC ACID-BASED HYDROGEL WITH IMPROVED INJECTABILITY AND SELF HEALING PROPERTIES

Vijay Singh Parihar *† 1, Hatai Jongprasitkul 1, Minna Kellomäki 1

1 Biomaterials and Tissue Engineering Group, Faculty of Medicine and Health Technology, Tampere University, Tampere – Finland

Self-healing biomaterials, particularly injectable hydrogels, are broadening the applications from basic tissue engineering to organ-on-chip technology. These hydrogels can temporarily transform under stress and then regain their original properties due to reversible chemistry.1 The combination of injectability and self-healing properties in hydrogels offers high tunability for precise delivery with a narrow syringe. Hyaluronic acid (HA) is a natural glycosaminoglycan, containing free carboxylic group and hydroxyl groups for various chemical functionalization. In this work, we demonstrated the phase-controlled behaviour of catechol-modified HA hydrogels through pH adjustments. The hydrogen bonding and metal (Fe3+) coordination of catechol groups provide injectable and self-healing properties to the hydrogels. In our previous work we utilised the dual crosslinking approach to improve the structural integrity and tissue adhesion of hydrogels.2,3

The methacrylated HA (HAMA) was functionalized with gallol (GA) using EDC coupling chemistry (Figure 1). 1H-NMR spectroscopy confirmed the degree of methacrylation and conjugation of GA to the HA backbone. The results show that the weak crosslinking of HAMA-GA-FeCl3 gels at acidic pH displayed a viscous behaviour in dynamic oscillatory measurement and shear-thinning behaviour in flow measurement, supporting non-Newtonian behaviour for injectability. The storage modulus was also increased by adding higher concentrated of FeCl3 attributed to the strong catechol-metal complexation. In addition, HAMA-GA-FeCl3 gels at neutral and basic pH underwent enhanced crosslinking due to bis- and tris-complex formation in hydrogel network. This increased complexation resulted in more elasticity (G’ > G”); subsequently, leading to a high yield stress, which were not suitable for injecting. The combination of catechol-metal complexes and photocrosslinking can rapidly reform after breaking, providing the self-healing properties and injectability (Figure 2). The self-healing properties of Fe3+ induced HAMA-GA were assessed by the oscillatory amplitude sweep and time sweep. The strain was applied until it reached the critical strain and then allowed to recover under 0.1% of strain. The coordinate bond plays a supporting role under high strain and reorganized the network quickly due to the elasticity of covalent bonds, leading to rapid re-formation of gels.

*Speaker
†Corresponding author: vijay.parihar@tuni.fi
HAMA-GA-FeCl\textsubscript{3} gels exhibit improved adhesion and cohesion with higher pH and FeCl\textsubscript{3} concentration. This mussel-inspired approach expands their uses in bioadhesive, stimuli-responsive hydrogels, and bioinks, owing to their flexible network and viscoelasticity.

References:


2. H. Jongprasitkul, S. Turunen, V. S. Parihar, M. Kellomaki; Biomacromolecules, 2022, 24, 1, 502–514.


Keywords: Injectable, Catechol, Metal Coordination

**Figure 1:** Synthesis of gallol functionalized hyaluronic acid methacrylate (HAMA-GA).

**Figure 2:** The HAMA-GA gel formation under the influence of Fe\textsuperscript{3+}, providing injectability and self-healing properties during injection followed by photocrosslinking.
On the potential of $\mu$-CT on the observation and interpretation of bone behavior around dental implants

Laura Preiss *, Rémy Gauthier 1, Richard Hervé 2, Loic Courtois 3, Nicolas Courtois 2, Jérôme Chevalier 1

1 Matériaux, ingénierie et science [Villeurbanne] – Université Claude Bernard Lyon 1, Institut National des Sciences Appliquées de Lyon, Centre National de la Recherche Scientifique, Centre National de la Recherche Scientifique : UMR5510 – France
2 Anthogyr SAS – Straumann Group – France
3 3DMagination – United Kingdom

Primary stability is one of the key factors in the osseointegration process of an oral implant. The methods that exist for estimating this primary stability (insertion torque, resonance measurement) are global methods that take into account neither the geometry of the implant nor the type of bone implanted. The method that has been developed and that will be presented will make it possible to quantify both the deformations and the bone densifications induced by the insertion of a dental implant. Based on these factors, the authors believe that primary stability will be better described, reliably and quantitatively, for a variety of implant designs.

The method involves inserting a Ti-6Al4V dental implant into a sample of porcine bone; in situ in a tomograph. A volume is acquired before and after implantation (and a video is radiographed during the implant insertion). Thanks to image analysis and DVC (Digital Volume Correlation), the fraction of additional bone around the implant and the deformations induced can be determined. Two types of implants were inserted, one of a ‘regular’ design (Axim REG by Anthogyr SAS), and one with a self-tapping design (Axiom X3 by Anthogyr SAS), and 5 of each were implanted into porcine bone (hips harvested from butcher). The insertion torque and ISQ were reported for each case for comparison.

The results show that the method developed enables the primary stability of several different implants to be better described on similar bone models, with very different volumes of bone affected depending on whether or not the implant has a self-tapping design. The method therefore appears to be of interest as a complement to the more global methods of measuring insertion torque and ISQ. To go further, particularly in terms of ‘continuous’ DVC, the same measurements were made at the synchrotron, and will be the subject of a forthcoming study.

Keywords: Bone/implant interaction, dental implant, bone behavior, primary stability, osseointegration

*Speaker
Tuesday, January 16\textsuperscript{th}, 2024

BIOMAT Session 2

Chairs:
Christèle COMBES, Bruno PAIVA
MECHANICS OF ANISOTROPIC POLYVINYL ALCOHOL HYDROGEL FIBERS FOR OSTEOARTICULAR TISSUE RECONSTRUCTION

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Synthetic polyvinyl alcohol (PVA) hydrogels exhibit a combination of biocompatible and mechanical properties that are potentially suitable for the reconstruction of non-mineralized osteoarticular tissues (1,2). The tensile response of these hydrogels can be greatly enhanced by orienting the semi-crystalline network of PVA (3-5). In particular, strongly anisotropic PVA hydrogels can be prepared in the form of fibers and assemblies of such fibers reproduce the water content, dimensions, and tensile response of the human ligament (6). For this application, a high fatigue resistance is required to maintain the functionality over a large number of loading cycles (> 10^8 cycles). Here, we investigate the role of the network anisotropy in the cyclic response of PVA hydrogels under clinically relevant loading conditions. For that, we compare anisotropic PVA hydrogel fibers (AHF) to isotropic PVA hydrogel films (IHF) having similar crystallinity (50 ± 2%) and swelling ratio (2.5 ± 0.2). Uniaxial tensile tests were performed on single AHF and IHF in water at different temperatures (figure 1a). Both systems exhibited very different non-linear viscoelastic behaviors with AHF showing significantly higher tensile strength values at 37°C (25.4 ± 2.4 MPa), close to those of the human anterior cruciate ligament (22-38 MPa) (7,8). Moreover, cyclic loading tests show that individual AHFs subjected to a maximum strain of 35% undergo a softening after the first cycle but fully recover their original behavior after 2h of rest (figure 1b). These softening and self-recovery are well explained by the dissociation and realignment of weak hydrogen-bonds in the swollen amorphous phase of the PVA hydrogel. Such fibers constitute promising building blocks to design durable tissue substitutes able to sustain physiological fatigue loadings representative of ligament biomechanics.

Keywords: Polyvinyl alcohol, hydrogel fibers, ligament reconstruction

*Speaker
Figure 1. (a) Tensile strength of individual anisotropic PVA hydrogel fibers and isotropic PVA hydrogel films in water at different temperatures, (b) uniaxial cyclic loading for one PVA hydrogel fiber before and after 2 h of resting time.
Growth and study of tumor spheroids behavior in a biomimetic vascularized platform

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Cancer remains a global health challenge and asks for more accurate models for drug development. Indeed, conventional monolayer cultures fail to recapitulate the complex tumor microenvironment (TME). Notably, they do not incorporate a vascular compartment, which plays a pivotal role in cancer development and drug testing. Conversely, in vivo models exhibit limited tunability and present escalating financial and ethical issues. Therefore, three-dimensional biomimetic systems emerge as promising candidates for mimicking the pathophysiological complexity of a tumor by integrating biochemical and mechanical cues as well as enabling coculture in highly tuneable matrices. Besides, they hold the possibility to add liquid microenvironments to integrate the vascular or lymphatic system. Although vascular bed is most of the time induced by mixing cancer and endothelial cells in a disorganized manner, precise engineering of blood vessels by 3D printing or template moulding is now more widespread and allows better control of the vascularized compartment. Models that take this into account while keeping ease of manipulation and high throughput are thus of major help for applications in cancer development and invasion, and for the evaluation of drug delivery systems for example. Herein, we have developed a polysaccharide-based platform, composed of two compartments: first, a microwells network of controllable stiffness, porosity, and geometry, in which cells are seeded and rapidly aggregate into cancer spheroids after 2 to 3 days of culture. It has been validated by testing two chemotherapeutic drugs on various cancer cell lines, pinpointing a 2 to 10-fold increase in resistance for spheroids versus monolayers in most conditions. The enrichment of this TME with cancer-associated fibroblasts within the pores of the hydrogel will next allow studying the interplays between cancer and stromal cells in culture, monitored by microscopy. This part is easy to produce, reproducible, and gives birth to a battery of biologically unique spheroids that accounts for biological disparities, especially important when dealing with cancer treatment. The second compartment of the platform consists in hollow microchannels in a gel of similar composition, made porous by an additional freeze-drying step. This gel is then layered by endothelial cells that are matured to form tubular constructs and sprouting towards the pores. This gel can also be refined by loading fibroblasts or mesenchymal stromal cells in the pores to foster its vascularization. It is designed to be combined with the spheroids network. After one to seven days of incubation, we observe the effects of the vascularization on the development of the spheroids, as well as the effects caused by the presence of cancer cells on the

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nearby endothelial structures. Overall, our system provides an innovative co-culture platform integrating stromal, cancer, and endothelial cells in a biomimetic substrate to investigate interplays between spheroids and a vascularized TME. As a perspective, this coupled hydrogel could also be integrated into a microfluidic chip to investigate the influence of the flow as well as potential perfusion capabilities using soluble factors or drug delivery systems.

**Keywords:** cancer, tumor, vascularization, biomaterials, spheroids

(A) A network of microwells is produced by moulding of polysaccharides (stained with FITC-dextran). (B) Scheme of the vascularized cancer model, with hydrogel 1 (in green) used to produce spheroids and hydrogel 2 (in yellow) used to grow blood vessels. (C) A porous gel with a central channel is produced with polysaccharides. (D) A673 cancer cells are deposited in the hydrogel shown in (A) and aggregate into spheroids after 3-6 days. (E) After seeding, the spheroids are treated with doxorubicin and their survival is assessed by resazurin assay. (F) Gel shown in (C) is seeded with HUVECS that reorganize into a tubular monolayer after 3 days of incubation.
3D bioprinting of a human respiratory epithelial tissue model

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Respiratory diseases represent the third global death cause, with more than 5 million deaths in 2019 (WHO, 2022). The development of treatments against chronic diseases or the reaction against epidemics are greatly delayed by the poor understanding of respiratory tissue physiological functioning and immunity. Animal models are limited in resembling human tissue. Human 2D culture models remain too simplistic and fail to recreate the native alveolar architecture. To overcome those issues, human 3D models allow greater biological complexity with the possibility to combine an epithelial layer with a basal compartment, but they still missing elaborated geometrical design.

The goal of this study is to use bioprinting technologies to produce a complex and functional 3D respiratory epithelial tissue model with primary cells and bronchial structures.

With this aim, we bioprinted 3D models encapsulating human primary fibroblasts (NHLF) and endothelial cells (HMEC-1, HUVEC or HMVEC-L) using micro-extrusion printing. After 14 days of maturation, the basal compartment was top-seeded with human epithelial cells (Calu-3 or primary lung epithelial cells). Ink-jet printing was evaluated to standardize the seeding process further.

Around day 28 of liquid-liquid culture, the formation of an epithelial monolayer was confirmed before placing the models at the air-liquid interface (ALI) for 14 days to fully mature. All along culture periods, cell viability, metabolic activity and tissue organization were characterized using calcein assay, lactate quantification, and hematoxylin & eosin staining. Immunohistochemical colorations of collagen I, EN4 and Muc1 were used to identify fibroblasts, endothelial cells, and epithelial cells, respectively.

In this work, we validated the feasibility of using micro-extrusion to print a respiratory basal compartment composed of primary fibroblasts and 3 different types of endothelial cells. All mod-

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els showed homogenous cell distribution, stable cell viability over time and increasing metabolic activity. The ink-jet epithelial cell seeding demonstrated full surface coverage and formation of an organized and polarized epithelial monolayer. Collagen I secretion by the fibroblast was found to be resembling the native tissue distribution. EN4 staining allowed the identification of small endothelial structures. An epithelial mono-layer-producing mucus has been highlighted by Muc1 staining.

To further improve this model, human primary lung endothelial and epithelial cells will be deeply characterized to fabricate a full primary 3D model. Maturation, differentiation and healthy behavior (ciliation, mucus secretion, and formation of tight junctions) of primary epithelial cells will be verified. Finally, this full primary 3D model will be printed with complex geometries.

**Keywords:** Micro, extrusion bioprinting, human respiratory epithelium, 3D tissue model, lung primary cells, ink, jet printing
A pre-vascularized structured hydrogel membrane mimicking the outer blood-retinal barrier to rescue the retinal pigment epithelium in retinal dystrophies

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The outer blood-retina barrier (oBRB), composed of the retinal pigment epithelium (RPE), on the Bruch’s membrane (BM) and overlying the vascularized choroid, is disrupted in several retinal dystrophies. The main current therapeutic strategy is based on the implantation of a RPE monolayer on a biomaterial mimicking the BM and does not include the choroid which will alter the graft integration and survival upon implantation. Here, we aim to develop a 3D structured membrane, for cellular therapy, mimicking the entire oBRB and co-cultured with RPE cells and endothelial cells (ECs) to pre-vascularize the graft.

Membrane synthesis. A polysaccharide hydrogel was synthesized from a pullulan-dextran solution and freeze-dried (FD) to tailor its porosity. After FD optimization, we obtained 200 µm thick membranes with, on one side, a porous surface connected to the inner porosity for the pre-vascularization and, on the other side, a smooth non-porous surface intended for the RPE monolayer.

RPE monoculture seeding. RPE cells derived from human induced pluripotent stem cells were seeded on the smooth side and cultured for up to three weeks. The cells adhered and proliferated on the smooth side, forming a tight monolayer within a week, without entering the porous network (Fig.1.A). RPE cells expressed typical markers such as bestrophin-1 (Fig.1.B) and secreted growth factors such as VEGF.

ECs monoculture seeding. Human retinal microvascular ECs (HRMVEC) were seeded on the membrane porous side and cultured for up to two weeks. Results showed that the ECs entered the inner structure, where they adhered, proliferated and formed tight junctions showed by VE-Cadherin immunostaining (Fig.1.C).

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Co-culture seeding. For co-culture, RPE were first seeded on the smooth side, the HRMVEC being added after 10 days on the porous side. Results showed that each cell type was located on its dedicated side and survived for up to two weeks (Fig. 2). The expression of typical EC and RPE markers increased in co-culture, showing beneficial interactions between the two cell types.

In conclusion, we designed a membrane mimicking the oBRB structure and suitable for co-culture with precise localization of each cell type. Co-culture experiments showed positive interactions between RPE and ECs, thus demonstrating the importance of including the choroid in an oBRB model. Support cells are currently being added in the porous network to form a more complex pre-vascularization and in vivo experiments are on-going to study the benefits of the ECs presence on the graft vascularization.

Keywords: Hydrogel, Tissue engineering, Retina, Cellular therapy

**Figure 1:** Expression of typical markers by the retinal pigment epithelial (RPE) cells cultivated on the smooth side (A: tight junctions and B: Bestrophin-1) and by the human retinal microvascular endothelial cells cultivated in the porous structure (C: tight junctions) after 2 weeks in monoculture. In the cross-sections, the smooth side is located on top. Scale bar: 200μm.

**Figure 2:** 3D reconstruction of the co-culture. The RPE cells are visible in blue, forming a monolayer on the smooth side while the endothelial cells, pre-stained in red before the seeding, are visible in the porous structure. Confocal imaging conducted after 3 days of co-culture.
Phosphorus-based films for actinide decontamination

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Actinides are radioactive elements carrying a large amount of energy, finding utility across diverse industries from energy to medicine. Nevertheless, drawbacks are the potential accidents and impacts on both environment and health. When people experience contamination, either internally or externally, their organs suffer damage, potentially leading to death. As a result, developing solutions allowing effective decontamination of individuals (decoration) is essential to ensure their safety.

Out of all the potential options, using chelating materials for actinide decoration emerged as an appealing approach for human decontamination. However, solutions are still rare, expensive, and relatively ineffective. Among all possibilities, recent studies have displayed that functional polymeric materials are of interest, as they proved to be able to efficiently complex both lanthanides and actinides (1,2), mainly due to the high density of chelating sites along the polymer backbone (3).

In that context, the aim of this project is to formulate a cream easily spreadable on the skin based on photosensitive phosphorus-based polymers. When exposed to UV light, these polymers can crosslink and form a film easily removable from the skin. For such purpose, the target polymer is composed of a polymethacrylate backbone bearing pendent bispophonic acid functions. So, a series of copolymers were synthesized by RAFT polymerization of oligo(ethylene glycol) methyl ether methacrylate (OEGMEMA), methacrylic acid (MAA) and hydroxyethyl methacrylate (HEMA) (Scheme). Photosensitive and bispophonic acid moieties were introduced by post-polymerization modifications.

Sorption/desorption studies were carried out with Neodymium (Nd), using Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES) and Isothermal Titration Calorimetry (ITC). Bispophonic based polymers developed, showed good results for actinide surrogate (Nd) sorption. Desorption was also efficient, proving that these materials are promising candidates for actinide recovery. Finally, aqueous formulations of this polymer were able to form films under UV light, while complexing Nd and keeping it trapped into the polymer matrix.

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Tuesday, January 16th, 2024

BIOMAT Flash Session 1

Chair:
Amel HOUAOUI
WAVE-INSPIRED MEW SCAFFOLDS FOR ENHANCED LIGAMENT TISSUE REGENERATION

Joanna Babilotte * 1, Cecilia Avventi 2, Monize Caiado Decarli 1, Paul Wieringa 1, Vladimiro Vida 2, Lorenzo Moroni 1

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Melt-electrowriting (MEW) is a 3D fabrication technique known for its ability to create highly organized fibrous structures with submicrometric resolution, closely resembling native tissue structures. This technology has gained interest in tissue engineering, especially for complex tissue applications like ligaments, where the unique wavy structure and organization of collagen fibers are intrinsically linked to their function. Polycaprolactone (PCL) is a commonly used material in MEW due to its advantageous low viscosity and stability properties. However, it lacks the necessary elasticity required for a wide range of applications. Polylactic acid (PLA) offers impressive tensile strength but lacks extension capacity. PCL and PLA combination results in copolymers with suitable mechanical properties for the regeneration of soft, though resilient, tissues like ligaments. This project aimed to explore the potential of using these copolymers with MEW to fabricate scaffolds specifically tailored for ligament tissue regeneration.

Using MEW, we successfully fabricated fibrous structures that closely mimicked the wavy collagen fiber arrangement found in ligaments. By adjusting design and printing parameters, we were able to create a range of wave patterns by varying amplitudes (from 0.2 to 1 mm) and lengths (from 1 to 4 mm), with fiber diameters ranging from 10-30 μm, demonstrating the versatility of MEW. Regarding mechanical characteristics, we observed that PCL-PLA exhibited higher elasticity when compared to PCL, indicating distinct mechanical behaviors between these materials. Moreover, varying wave amplitudes and lengths also impacted tensile properties. The in vitro investigations showed that ligament cells attached, spread along the scaffolds, and proliferated, with a clear influence of the scaffold structure.

Our results demonstrated new possibilities for producing fibrous scaffolds using MEW with highly viscous polymers that provide high resilience and flexibility to the final fabricated structures. Further investigations are ongoing to better correlate cell activity to scaffold structures to guide tissue regeneration.

Keywords: Ligament, Melt, electrowriting

*Speaker
Pre-vascularization with SHED stem cells to engineer a functional vascular network

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Lack of sufficient vascular supply is a major hindrance for the regeneration of the orofacial tissues. Implantation of vessels engineered in vitro is thus a promising strategy for rapid Anastomosis between the graft and host vasculature. Previously, we described pre-vascularized constructs using human umbilical vein endothelial cells (HUVEC) and dental pulp stem cells from human exfoliated deciduous teeth (SHED) due to their angiogenic potential and pericyte function. Here, our objective was to investigate the ability of SHED to produce functional pre-vascularized constructs in vivo, by analyzing with multi-modal imaging the maturation of implanted vessels bearing SHED. Two pre-vascularized hydrogels seeded in polylactic acid grid (PLA) - SHED with HUVEC (SHED-HUVEC) and HUVEC in the conditioned medium of SHED (CM-HUVEC) - were implanted ectopically in athymic mice, compared to a non-pre-vascularized control group (SHED). After 10 days, using positron emission tomography, we first demonstrated an increased angiogenic process within pre-vascularized SHED-HUVEC constructs compared to the SHED group. After in vivo injection of species-specific lectins and a fluorescent Dextran, multiple anastomoses of human implanted vessels with the host’s vessels were detected, and no vascular leakage was observed. The SHED-HUVEC pre-vascularized constructs produced a well-interconnected vessel network of superior vascular density, length and innervation, whereas CM-HUVEC vessels network showed signs of vessel regression. SHED stem cells expressing a pericyte marker were consistently found around human vessels in vivo, confirming their role in vessel maturation and their sustainability in time. Moreover, quantitative analyses of the vascular perfusion of a contrast agent obtained by Micro-CT acquisitions revealed a statistically significant increase in the total vascular volume in the SHED-HUVEC PLA constructs compared to all other groups at one-month post-implantation. This study highlights SHED stem cells as an interesting player in vessel functionality, and their interest for orofacial tissue engineering.

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Keywords: Prevascularization, hydrogel, SHED, HUVEC
BORONATE ESTER HYDROGELS: PROMISING MATERIALS FOR BIOMEDICAL APPLICATIONS

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Hydrogels are widely used for biomedical applications. Among the different classes of hydrogels, dynamic covalent hydrogels, which are viscoelastic materials based on reversible molecular interactions, are attracting increasing attention. They offer interesting properties of injectability, malleability, and self-healing. Dynamic covalent hydrogels can be obtained using boronate ester (BE) crosslinking, which results from the reversible reaction between a phenylboronic acid (PBA) and a diol. Although promising, BE hydrogels are difficult to obtain at physiological pH, and often degrade within hours to days. This observation has prompted the scientific community, including our team, to develop new BE crosslinking strategies based on advanced molecular design.

We recently screened tens of combinations of PBAs and diols. For this purpose, we grafted the PBAs and diols of interest onto hyaluronic acid using the well-described DMTMM mediated amidation method. We further compared all combinations regarding their hydrogel formation ability and their rheological properties. This study enabled us to identify an innovative crosslinking couple composed of ortho-aminomethyl-PBA and glucamine. Using rheometry, we demonstrated that the resulting hydrogels are self-healing and fast relaxing with a half relaxation time in the order of a second. More importantly, optimizing their composition (i.e., polymer MW, polymer content, degrees of substitution, molar ratio), we were able to obtain two BE hydrogel formulations with distinct storage moduli (G’1Hz of 100 vs 1000 Pa) and that are minimally swelling and stable for at least two months in both phosphate buffer and culture medium. We also showed that these viscoelastic hydrogels can be manually injected through a 26G-needle. Finally, by performing viability test (live/dead staining), metabolic assay (CCK-8), and DNA quantification (Picogreen) on two cells types (i.e. L929 fibroblasts and hASCs), we confirmed cell viability after seven days of culture. In light of our new understanding of the key parameters in BE chemistry, we further developed a second generation of BE hydrogels with a much slower

*Speaker
relaxation (\(\tau_{1/2}\) of 70 s) compared to the first generation, while maintaining a comparable storage modulus (\(G'\) of 500 Pa). These results pave the way for the use of BE hydrogels with tunable viscoelastic properties for a breadth of biomedical applications including 3D cell culture, bioprinting and drug delivery. During this presentation, we will briefly present an overview of the main challenges and opportunities in BE hydrogel design for biomedical applications, before discussing our most recent work on the physicochemistry of BE hydrogels.

**Keywords:** Dynamic covalent chemistry, hydrogels, biomedical application
Enhancing Aneurysm Healing through Fucoïdane-Coated Coils

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Strokes represent the second leading cause of global mortality and the third leading cause of combined mortality and disability worldwide. Presently, 101 million individuals live with the repercussions of a stroke, a number that has doubled in the past three decades. (1) Among those strokes, 15% are hemorrhagic, stemming from arterial wall elasticity loss, leading to intracranial aneurysm (IA) formation. (2) The introduction of endovascular metallic coils, termed "coils", for occlusion within aneurysms has reduced the demand for highly invasive and dangerous open cranial surgeries to treat IAs. However, up to 30% of IAs treated with this approach recur within a year, necessitating frequent re-interventions and elevating the risk of rupture and associated mortality. To address the challenging issue of aneurysm recanalization (Figure 1.) following coil embolization, we propose an innovative approach to counter the lack of cell proliferation and aid aneurysm healing, by grafting a bioactive molecule on coil surface. (3) (4) (5) (6)

Figure 1 : Schematic representations of (A) an aneurysm, (B) treated by coiling, which (C) progresses to recanalization and (D) finally ruptures.

Bioactive endovascular devices (BED) able to stimulate a quick healing of IA were prepared by coating the coils with fucoidan, a polysaccharide extracted from the extracellular matrix of brown algae. Fucoidan exhibits various biological activities, including the ability to bind to vascular growth factors promoting the proliferation of the cells implied in the healing process, as well as pro/anti-thrombotic and anti-inflammatory properties to both strengthen the thrombus formation within the aneurysm and limit the inflammatory response (ref 7). (7)

We present the fucoidan grafting strategies used, the characterizations of the grafted coils and some of the main in vivo results obtained, which are patented. (8) Strategies of fucoidan grafting onto the coils were implemented to get BED which were evaluated in a rabbit model of IA. (5) In vivo results evidenced significant disparities between untreated coils and fucoidan-coated coils,

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with a marked improvement in histological indices, an increased presence of collagen fibers, and enhanced tissue organization in the coated coils group. These findings suggest the potential of fucoidan-coated coils to enhance aneurysm healing and reduce recurrence rates, thereby improving patient outcomes in the management of intracranial aneurysms.

References

Keywords: Aneurysm, Grafting, Coils

Figure 1 : Schematic representations of (A) an aneurysm, (B) treated by coiling, which (C) progresses to recanalization and (D) finally ruptures.
COLLAGEN /
POLYESTER-POLYURETHANE POROUS SCAFFOLDS FOR USE IN MENISCAL REPAIR

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Focusing on the repair and regeneration of damaged knee meniscus, and with aim to combine suitable mechanical properties with enhanced biological integration, we designed a hybrid scaffold made of a poly(ester-urethane) (PEU) foam whose pores are coated with collagen. To ensure biocompatibility and degradability, the degradable PEU was prepared from a poly(ε-caprolactone), L-lysine diisocyanate prepolymer (PCL di-NCO) and poly(lactic-co-glycolic acid) diol (PLGA). The PEU (Mn =52,000 g/mol) was obtained by polyaddition and thoroughly characterized before use in the foam preparation. Porous scaffolds were processed using the solvent casting (SC) / particle leaching (PL) method at an optimized salt/PEU weight ratio of 5:1. The morphology, pore size and porosity of the foam were evaluated using SEM showing interconnected pores with a uniform size around 150 μm, whereas mechanical properties were found close to the ones of the human meniscus (Ey ~ 0.6 MPa). To enhance the biological properties of the foam, collagen type 1 was infiltrated into the foam and the resulting scaffolds were characterized by SEM-EDX and X-ray tomography to confirm the morphology and highlight the efficient and homogeneous coating of the pores with an average of 0.3 wt% collagen in the scaffolds. Finally, in vitro L929 cell assays confirmed higher cell proliferation and an improved cellular affinity towards the proposed scaffolds compared to culture plates and a gold standard commercial meniscal implant.

Keywords: Porous scaffold, Poly(ester, urethane), Collagen
Overview of the evaluation of porous (poly(lactide-co-glycolide)-co-(ε-caprolactone)) polyurethane coated with collagen.
Supercritical CO2-Enabled Aerogels of Platelet Lysate and Collagen for Tissue Repair: Natural Biomaterials and Growth Factor Delivery

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Natural biomaterials have a unique appeal for tissue engineering. They offer a synergy of biocompatibility, biodegradability, hydration properties and the ability to promote cell adhesion, proliferation and differentiation (1). The combination of type I collagen and fibrin derived from platelet lysate is a compelling choice for the production of porous three-dimensional matrices (2). Platelet lysate, which is naturally rich in growth factors and bioactive molecules, actively stimulates cellular processes that are critical for tissue repair (3). At the same time, type I collagen mimics the three-dimensional structure of the extracellular matrix and exhibits impressive mechanical properties, particularly in terms of tensile strength (4).

Despite the clinical potential of these biomaterials in the form of hydrogels, they have their limitations in practice, e.g. in terms of handling and limited stability and durability. To overcome these challenges, our research focuses on the design and production of aerogels derived from type I collagen and platelet lysate hydrogels using an environmentally friendly process utilizing supercritical CO2 (5).

We have developed a highly reproducible and efficient moulding protocol that ensures the formation of porous aerogels that maintain their dimensional structure while exhibiting exceptionally advantageous mechanical properties (Figure1).

Microscopic examinations using scanning electron microscopy (Figure 2) and FTIR analysis confirm that both the physical structure and chemical composition are preserved after the shaping

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process. Release studies show that the developed aerogels enable a sustained and biologically functional release of bioactive molecules.

In addition, stability studies show that these aerogels can be stored significantly longer compared to their hydrogel counterparts. Importantly, these aerogels are of great importance for clinical applications as they retain their 3D structure and allow for longer storage. When used, they release bioactive molecules that actively support tissue repair and represent a promising avenue for advanced regenerative solutions in the field of tissue engineering.

This extensive research is a significant step towards the development of advanced biomaterials with improved regenerative potential that contribute to innovative solutions in the fields of wound healing and tissue engineering.

References:


Keywords: Platelet Lysate, Type I Collagen, supercritical CO2
CRYOPRESERVATION OF RED BLOOD CELLS IN ABSENCE OF TOXIC CRYOPROTECTANTS VIA ICE TEMPLATING

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In most conditions freezing threatens the integrity and the viability of biological entities. Paradoxically, cryopreservation is the single available solution to extend the lifespan of living cells and to preserve tissues and biomolecules. In recent years, the field of cryopreservation has increasingly relied upon toxic cryoprotectant agents (CPAs) such as dimethyl sulfoxide (DMSO) or glycerol1. However, the limitations imposed by toxic CPAs still hamper the widespread use of cryopreservation in blood products for most transfusion purposes. In this communication, we discuss the relevance of a materials science strategy, ice templating (or directional freezing), to obtain high yield of functional red blood cells (RBCs) after freezing and thawing in absence of toxic CPAs, a strategy that could dramatically change cell therapies.

Ice templating has evolved from a materials processing technique to an increasingly relevant technique in the domain of biomaterials due to the simple yet effective control over ice growth2,3. Using a home-built ice templating setup4 (Fig. 1a) we were able to control the ice front velocity of sheep RBC suspensions in BSA isotonic solutions. After freezing, cell samples were kept at -80°C for days 1 and 100 to assess cell recovery by hemolysis, flow cytometry (esterase activity by calcein), scanning electron microscopy (SEM) and by 3D confocal microscopy images.

Physiological and sub-physiological concentrations of RBCs were frozen using ice templating between 10 and 100 μm s⁻¹ to determine the optimal cryopreservation conditions in absence of toxic CPAs. Higher ice front velocities and BSA contents provided the best cell recovery rates (c.a. 90%, Fig. 1d) and esterase activity fractions measured under flow cytometry. Results between 1 and 100 days storage at -80°C showed no significant difference in cell activity and integrity, suggesting the method is adapted for long-term cell preservation.

Because of control over the cell environment formed within the interstitial space formed by ice crystals, ice templating of living cells allows addressing the challenges of cryopreservation under a new light. Beyond RBCs, these results are expected to translate to the preservation of other cell types and application settings, from fundamental biology research labs up to the clinic.

Keywords: Ice templating, Cryopreservation, Red, blood cells

*Speaker
Develop an implantable medical device based on a dehydrated, sterile, viro-inactivated membrane derived from perinatal tissues.

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In medicine, autografting is considered the gold standard because of the host’s unresponsiveness, but sampling is traumatic and availability is often very limited. Perinatal tissues are highly advantageous as allografts because they are immune-naïve and therefore do not cause host rejection. Used since the 1st World War to treat deep wounds, interest in them continues to grow, particularly in the ocular and dermal fields. To date, methods of preserving perinatal tissues have failed to maintain the structural and mechanical quality of the extracellular matrix (ECM). The aim of this study is to investigate the possibility of treating perinatal tissues with supercritical carbon dioxide (CO2sc). CO2sc is obtained by heating CO2 to 31°C, a green process that requires no chemical solvents. Combined with co-solvents, the CO2sc process produces a sterile, viro-inactivating allograft that can be stored at room temperature. Treated with CO2sc, perinatal tissues are devitalized (residual DNA > 50 ng/μg dry tissue) with improved hemostatic properties. Combined with co-solvent treatment, CO2sc protects the integrity of the ECM from potential degradation. The result showed that CO2sc treated perinatal tissues are non-cytotoxic, porous, non-cross-linked and capable of rehydration with enhanced bioactivity. However, this bioactivity needs to be confirmed by proteomic analysis and cell activation tests (neutrophil, monocyte/macrophage and epithelial cell activation).

Keywords: Perinatal tissues, supercritical carbon dioxide, extracellular matrix, implantable medical device
Tuesday, January 16th, 2024

BIOMAT Session 3

Chairs:
Astrid PINZANO - Nicolas TOUYA
Adhesion to internal tissues: slippery when wet

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The adhesion of biomaterials and devices onto internal tissues is strongly impeded by the presence of fluids wetting the surface of organs. Here, we explore how the transport of fluids occurring at the interface between tissues and soft polymer films plays a role in the creation of an adhesive contact. For that, ex vivo and in vivo experiments were devised to measure the adhesion between model poly(ethylene glycol) (PEG) films and the surface of biological tissues. We show that a transition from a lubricated contact to an adhesive contact results from the draining of the interfacial liquid by the PEG films. This transition is well described by a simple model taking into account the microanatomy of tissues. Furthermore, by occluding in vivo the vascularization of liver lobes, we reduce temporarily the flux of fluids transuding at the liver surface. This decrease in transudation flux produces a great enhancement in adhesion strength and explains the large differences in adhesion observed between ex vivo and in vivo conditions. In addition, we find that in the presence of blood, coatings of procoagulant silica nanoparticles provide a way to enhance the adhesion strength by inducing the rapid formation of a clot at the interface. These methods and results shed a new light on the design of predictive bioadhesion tests and on the strategies to control the fixation and biointegration of hydrogel based-devices.

Keywords: Tissue adhesion, polymer, hydrogel, in vivo testing
SMILING, PRE-CLINICAL EVALUATION OF A TISSUE-ENGINEERED VASCULAR SUBSTITUTE CONSTRUCTED FROM HUMAN UMBILICAL CORD, IN PIGS AS A BIG ANIMAL MODEL

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Cardiovascular diseases are responsible for nearly one-third of global deaths. Among vascular treatments, arterial bypass surgery remains the most effective treatment for long-term revascularization. Autologous vessels remain the "gold standard" for bypass surgeries, but their recovery involves invasive procedures, they may be unavailable due to previous use or can be of poor quality. Synthetic vascular prostheses, while available, are unsuitable for replacing small vessels and may lead to complications, such as thrombosis, probably due to a poor/lack of functionalization of their inner surface.

To address this challenge and offer a potential therapeutic alternative, we developed "SMILING", an innovative small diameter vascular graft whose luminal surface is functionalized with a cellular component. This graft is created by vascular tissue engineering, using a decellularized human umbilical artery as a scaffold. Its luminal surface is coated with an extracellular matrix extracted from Wharton’s jelly (WJ-ECM) and then cellularized with mesenchymal stem cells derived from this same fetal tissue. This process is optimized using an innovative "inside-out" method enhancing access to the luminal surface of the artery. The entire production process adheres to good manufacturing practice (GMP) conditions including quality control examinations for advanced therapeutic medicinal products. Mechanical studies confirm the graft’s suitability for vascular applications, and that reversing the artery has no effect on its mechanical properties.

Previous in vitro and in vivo studies have demonstrated the SMILING cytocompatibility, efficacy and permeability up to 3 weeks in a carotid bypass model in rabbits and led to an ANR financial support in order to move to the next phase of this study.

To progress toward clinical trials, these promising results, require validation in a large animal, leading us to the next step in our project which involves studying the graft use as coronary arterial bypass in pigs, evaluating its integration capacity, immunogenicity and short/medium-term patency. Although the in vivo study is still preliminary, our graft could present an attractive therapeutic solution by offering a ready-to-use product, that can be grafted by minimally inva-
sive robotic techniques avoiding the need for highly invasive surgery and long patient recovery periods.

**Keywords:** Vascular tissue engineering, Wharton’s Jelly, Vascular graft, mesenchymal stem cells
From in vitro to in vivo assessment of an active viscosupplement hydrogel for intra-articular injection in temporomandibular joint disorders

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Temporomandibular disorders (TMD) are a group of diseases that affect the temporomandibular joint (TMJ) and its associated tissues in 31% of the world’s population. Temporomandibular joint osteoarthritis (TMJOA) is a form of TMD that is partially caused by the degradation of synovial fluid. One of the main treatments consists of intra-articular injections, such as viscosupplementation (VS, 1% hyaluronic acid solution), and oral administration of non-steroidal anti-inflammatory drugs (NSAID). However, therapeutic effectiveness is rarely attained due to the rapid biodegradation of VS and the side effects of NSAIDs in long-term treatment (kidney injury, gastric ulcers, and hypertension). This study aimed to develop and assess in vivo an innovative VS hydrogel composed of chitosan (CHT) and a polymer of cyclodextrin (PCD) for the intra-articular release of naproxen (NX, NSAID drug).

Hydrogels were prepared by co-milling the powders of CHT and the PCD-NX complex previously prepared by wet granulation. The powder was first mixed with water and then with lactic acid (final concentration, 1% v/v) in an interconnected system of syringes. A rheological study confirmed the formation of a viscoelastic physical hydrogel (tan δ=0.51) for the formulation CHT/PCD/NX and the fluid/liquid behavior of a commercial product used as a control, Ostenil® (tan δ=0.95). The tribological assessment proved the lubricant effect of the hydrogel compared to a saline (NaCl 0.9%) solution. Additionally, the NX release study showed maximum drug release over 48h. The in vivo evaluation of the hydrogels in a monoiodoacetate (MIA)-induced TMJ OA model in rats (Von Frey Method) was performed for 30 days. The results demonstrated a significantly lower pain sensitivity value (p<0.05) for rats randomly treated with the CHT/PCD/NX hydrogel compared to placebo (saline solution) and control

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(Ostenil®).
These results allowed us to obtain a proof of concept, and a future study will be performed using a larger animal model.

**Keywords:** active viscosupplementation, osteoarticular diseases, hydrogels
3D-PRINTED LUMINESCENT BIOACTIVE GLASS SCAFFOLDS FOR BONE BIOENGINEERING

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Complex and critical-size bone defects require innovative devices presenting osteoproperties: from osteocompatibility to osteogenesis. Due to its ability to release ions in solution and trigger signaling pathways leading to ”ostecompotent responses”, bioactive glass (BAG) appears as a pertinent solution to face the needs in bone engineering. 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). However, there is a need for control release of therapeutic agents as antimicrobial molecules. For such application, the proposed alternative is to use persistent luminescence (PeL) microparticles (MPs) which would allow to photolocate photoresponsive drug in a specific temporal manner (2). Thus, it is crucial to ensure that PeL MPs are cytocompatible and do not degrade during the bone healing process.

The porous scaffolds combining 13-93B20 BAG and PeL MPs (turquoise, blue, green, or red) were obtained by robocasting with a ratio of 90/10 (weight %) respectively. The effect of these PeL MPs in vitro on Human Fat Stem Cells (HFSCs) under subsequent excitation and emission were studied. Critical bilateral defects of 5mm diameter were created on 20 rats calvaria to perform in vivo studies.

After 3D-printing, the luminescence of PeL MPs in the scaffolds remains, showing that the fabrication method does not affect PeL MPs properties (Figure 1).

Figure 1: Luminescence properties of the scaffolds after 3D-printing

Cytocompatibility tests with HFSCs show the cytotoxicity of the red PeL MPs and the highest proliferation rate with turquoise PeL MPs which were chosen for the in vivo study.

After 3 months implantation in rats calvaria, X-ray micro-computed tomography imaging show bone formation, confirmed by Von Kossa and Sirius Red histological stainings, and the persistence of the turquoise particles' luminescence (Figure 2).

Figure 2: A) Bone formation observed by X-ray micro-computed tomography after 3 months

*Speaker
implantation (Scale bar 5mm). B) Conservation of luminescence properties after 3 months implantation observed by excitation with UV light.
Our results confirm that turquoise PeL MPs are cytompatible. In vitro and in vivo experiments reveal promising behavior toward the potential use of these scaffolds in hard tissue regeneration and mineralization. This work not only opens the path to bioimaging but also trends to improve their use in the release of photosensitive molecules for therapeutic interest.

Keywords: Bioactive glass 3D scaffold, Persistent luminescent particles, Bone bioengineering

Figure 1: Luminescence properties of the scaffolds after 3D-printing

Figure 2: A) Bone formation observed by X-ray micro-computed tomography after 3 months implantation (Scale bar 5mm). B) Conservation of luminescence properties after 3 months implantation observed by excitation with UV light.
Tuesday, January 16th, 2024

BIOMAT Flash Session 2

Chair:
Astrid PINZANO
Osteogenic Effect of Fisetin Doping in Bioactive Glass/Poly(caprolactone) Hybrid Scaffolds

Yohann Wittrant * 1


Treating large bone defects or fragile patients may require enhancing the bone regeneration rate to overcome a weak contribution from the body. This work investigates the osteogenic potential of nutrient fisetin, a flavonoid found in fruits and vegetables, as a doping agent inside the structure of a SiO2-CaO bioactive glass-poly(caprolactone) (BG-PCL) hybrid scaffold. Embedded in the full mass of the BG-PCL hybrid during one-pot synthesis, we demonstrate fisetin to be delivered sustainably; the release follows a first-order kinetics with active fisetin concentration being delivered for more than 1 month (36 days). The biological effect of BG-PCL-fisetin-doped scaffolds (BG-PCL-Fis) has been highlighted by in vitro and in vivo studies. A positive impact is demonstrated on the adhesion and the differentiation of rat primary osteoblasts, without an adverse cytotoxic effect. Implantation in critical-size mouse calvaria defects shows bone remodeling characteristics and remarkable enhancement of bone regeneration for fisetin-doped scaffolds, with the regenerated bone volume being twofold that of nondoped scaffolds and fourfold that of a commercial trabecular bovine bone substitute. Such highly bioactive materials could stand as competitive alternative strategies involving biomaterials loaded with growth factors, the use of the latter being the subject of growing concerns.

Keywords: Nutrients, Organic doping, Hybrid scaffold, Bioactive glass
Amphiphilic PCL-g-Dex nanoparticles: Towards hybrid hydrogel/nanoparticles dual-cancer drug delivery system

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Triple-negative breast cancer (TNBC) is a prevalent and challenging subtype of cancer due to the absence of targeted therapy options, its aggressive nature, and its high recurrence risk. Researchers are exploring combination treatments using multiple modalities to enhance anticancer therapies and developing innovative dual-action drug delivery systems to improve efficacy, reduce side effects, and overcome drug resistance. This project focuses on designing a novel biodegradable polymeric hydrogel/nanoparticle hybrid system as a dual-drug delivery system of hydrophobic anticancer drugs and hydrophilic antibodies for enhanced cancer treatment. Hydrogel and nanoparticles (NPs) are based on an amphiphilic graft copolymer of the same nature, poly(caprolactone)-g-dextran (PCL-g-Dex), with different hydrophilic-lipophilic balance to ensure a more homogenous system and a more controlled release.

In this contribution, we focus on the design of paclitaxel (PTX)-loaded PCL-g-Dex NPs. First, PCL-g-Dex copolymers are synthesized by an azide-alkyne Huisgen’s cycloaddition between propargylated PCL (PCL-yne) (1) and an azido-dextran (Dex-N3) (2) varying Dex/CL ratios and Dex/PCL molar masses. The copolymers were used to prepare PTX-loaded NPs through a nanoprecipitation process. NPs were characterized in terms of size, drug loading and encapsulation efficiency. PTX release kinetics and in vitro biological evaluations of the nanosystem are currently under investigation.

References


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Oral presentations BIOMAT Flash Session 2 - Tuesday, January 16th, 2024


Keywords: polyester, polysaccharide, amphiphilic copolymers, nanoparticles, anti, cancer agents, dual drug delivery

Figure 1: Polymeric hybrid hydrogel/nanoparticles for dual-cancer drug delivery system
DEVELOPMENT OF A 3D BIOMATERIAL AS A PRO-RESOLVING CELL SUPPORT FOR THE HEALING AND TREATMENT OF CHRONIC WOUNDS

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Chronic wounds represent a major public health problem with no current effective treatment. These wounds are associated with chronic inflammation due to a lack of pro-resolving macrophages in the lesion. The aim of this project is therefore to develop a 3D scaffold that will allow us to bring, in the lesion, functional pro-resolutive cells such as macrophages and mesenchymal stromal cells that will decrease inflammation and favorize tissue repair. In this context, we developed a 3D-scaffold composed of a polyelectrolyte complex (PEC) of alginate and chitosan, which already demonstrated his performance for soft tissues cell therapy.

To perform this, various irradiation conditions were applied, and their effect on scaffolds 3D structure, mechanical properties and stability to rehydration were studied. This step could be deleterious for scaffold 3D structure and stability, but may have a potential interest via the beneficial generation of oligosaccharides. Our results show that an accurate choice of irradiation conditions can prevent bacterial growth without notably modifying the modulus of elasticity as well as the stability of the matrix upon rehydration. The maintenance of the 3D architecture of the scaffold was assessed by electronic microscopy (SEM) and confocal microscopy was used to study the seeded pro-resolving cells viability. Our results clearly show that UV irradiation promotes the viability of pro-resolving cells.

In a second step, we studied the effect of the irradiated scaffold on the pro resolutive and pro healing functions of cells seeded in its structure. To this end, we studied the effect of the embedded-pro-resolutive cells in the 3D matrix on the proliferation of fibroblasts, cells that play a central role in wound healing. We demonstrated that the association of two different pro-resolutive cells cultivated within the 3D scaffold increased fibroblast proliferation in contrast to pro-resolutive cells alone. We were also able to compare the secretory profile of pro-resolving cells seeded or not within our biomaterial, and found that cells embedded in the matrix secreted

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more pro-resolving factors than cells alone. Therefore, we demonstrated that our 3D-scaffold can support pro-resolutive cells and have positive effect on their therapeutic functions.

**Keywords:** Cell therapy, biopolymer, based 3D, scaffold, wound healing
STENTS COVERED WITH POLYCAPROLACTONE-
CIPROFLOXACIN ELECTROSPUN MEMBRANES: CHARACTERIZATION AND KINETIC RELEASE STUDY

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INTRODUCTION
Polycaprolactone (PCL) is a biocompatible and biodegradable polymer widely used in medical device (1). Electrospinning is a process to obtain loaded non-woven membranes with drugs for drug delivery application. The aim of this work is to optimized and characterized stents covered with PCL electrospun membranes with ciprofloxacin (CFX) to prevent infections and like a drug model.

EXPERIMENTAL METHODS
Electrospinning solutions were prepared by solubilization of 12% PCL in formic/acetic acid mixture (50/50,v/v)(2), without or with 1%, 3% or 5% of CFX. The electrospinning of these solutions on stents allows to obtain different stents covered with PCL-CFX membranes, respectively PCL/CFX-0%, PCL/CFX-1%, PCL/CFX-3%, PCL/CFX-5%. A morphological analysis was performed by scanning electron microscopy to determine the impact of CFX concentration on fibers morphology. The total amount of CFX was determined by HPLC-DAD at 278 nm, after solubilization of electrospun membranes. Release profile were determined for each membrane under static conditions in PBS (pH7.4, 80 rpm) and by agar diffusion method. Briefly, the static release process was realized with 11mm diameter samples in 1mL of PBS. At 0.5h, 1h, 2h, 3h, 4h, 6h, and 24h, PBS was collected and replaced by fresh media, and analysed by HPLC-DAD. Diffusion agar gel release was realized with 0.6% agar gel, at the same timepoints as static release method, 0.6mm of gel samples removed each centimeter were taken. CFX was extracted and analysed by HPLC-DAD. The dynamic release was realized with CE7-smart apparatus with stents deployed in 0.6% agar gel (Fig. 1), in 80mL of PBS, and analysed by HPLC-DAD.

*Speaker
RESULTS

The SEM analysis shows homogenous nanofibers for all membranes with diameter of 146nm; 152nm; 145nm; 116nm respectively for PCL/CFX-0%; PCL/CFX-1%; PCL/CFX-3%; PCL/CFX-5%. MEB observations shows limited impact of crimping process on electrospun membranes. Release profiles determined under dynamic conditions shows an increasing concentration of CFX in release media with CFX rate on stents, respectively around 108μg/cm², 237μg/cm² and 558μg/cm² after 24h, for PCL/CFX-1%, PCL/CFX-3% and PCL/CFX-5% after 24h (Fig2). Quantification of diffused CFX in agar gel during the dynamic release show after extraction of CFX a concentration of CFX around 4.12μg/g, 9.26μg/g and 21.60μg/g of gel respectively for PCL/CFX-1%, PCL/CFX-3% and PCL/CFX-5%.

CONCLUSION

To conclude, the electrospinning process permit to obtain stents covered with PCL membranes with different CFX quantities. Kinetic profiles show a sustained release in release media, and a good diffusion in agar gel under dynamic conditions.

REFERENCES


Keywords: Electrospinning, Drug delivery, Stents
Fig_1_Through_flow_cell_with_agar_gel_for_diffusion_during_dynamic_release

Fig_2_Dynamic_CFX_kinetic_release_35mL/min_for_PCL_CFX_1__CFX_3__CFX_5_in_g.g_of_gel
Skin decontamination: development and validation of film-forming formulations on skin mice against chemical warfare agents.

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Introduction and purpose
Skin decontamination is crucial to prevent irreversible effects and death against chemical warfare agents (CWA). Fuller’s Earth (FE), a phyllosilicate, is commonly used as a powder for healthy skin decontamination. However, this method has poor handling present a risk of cross-contamination and cannot be applied on damaged skin.

Therefore we developed an innovative decontamination method based on the film-forming capacity of polyvinyl-alcohol and the adsorption capacity of FE, which facilitates its use while avoiding possible cross-contamination. These composite film-forming formulations (FFF) enable the deposition and peel-off of films, demonstrating effective decontamination of skin (achieving more than 95% on an ex vivo model) and sequestration of paraoxon-ethyl (POX), a simulant of the VX agent.

These promising results incited us to evaluate the cytotoxicity of the FFF, as well as their safety and decontamination efficiency in in vivo mice models.

Materials and methods
Cytotoxicity was evaluated on normal human dermal fibroblast (NHDF) with metabolic test (ISO-standard 10993-5). 8-weeks old SKH1 mice were used in in vivo experiments. FFF toxicity was assessed by applying 50μL of each formulation on the back mice. Once dried (20 minutes), FFF were peeled-off and the condition of the mice’s skin was assessed macroscopically. Interleukins 6, 1/β and TNF alpha, characteristic of an inflammatory response, were measured by ELISA. Decontamination tests involved the application of the contaminant paraoxon-ethyl (POX, a VX simulant) and FFF covering after 5 minutes of contamination. The overall condition of the mice was measured, as was the level of acetylcholinesterase (AChE, an enzyme inhibited during contamination by nerve chemicals). These results were compared with 2 control groups: with PBS decontamination and with Reactive Skin Decontamination Lotion Kit (RSDL), the current gold standard for skin decontamination.

Results and conclusions

*Speaker
None of the tested FFF compositions, with and without FE, showed cytotoxic effect on NHDF (viability > 90%, data not shown). Furthermore, application and removal of FFF left the stratum corneum intact and showed no inflammatory reaction on the mice’s skin (Figure 1), either macroscopically (redness) or molecularly (no increase in inflammatory cytokine levels), as did the control groups (PBS and RSDL).

However, mice decontaminated with FFF died after an average of 3 h (Figure 2A), unlike the PBS and RSDL-treated mice, which remained alive even after decontamination. The incomplete decontamination of FFF was as well indicated in the reduction of AChE levels (0 mU/mL), while RSDL group maintained normal levels (6000 mU/mL). These results seem to stem from the POX spreading phenomenon that occurs when FFF is applied on the contaminant, which increases its exposure surface area, unlike the RSDL sponge, which allows contaminant absorption without prior spreading. Further ongoing experiments aiming at harmonizing contaminant surface areas on mice skin clearly show an improvement of the FFF decontamination efficiency, with a survival of all contaminated mice after 72 hours.

**Keywords:** Film, forming formulations, decontamination, skin mice

![Image](image_url)

**Figure 1.** Applying, removing and toxicity of FFF on skin mice. (A) Easy application and withdrawal of FFF on mice back. (B) Scoring of the mice’s skin condition, 0 corresponding to normal skin and 3 to a major skin reaction. (n=5)
Figure 2. Survival and AChE levels of mice, 72 h after POX contamination and decontamination with FFF (light and dark green), RSDL (blue) and PBS (black). (A) Scoring of mice condition ranging from 0 (survival, no symptom) to 10 (death), taking into account the various symptoms defining a cholinergic crisis due to exposure to organophosphorus compounds. (n=5) (B) AChE levels before contamination, 1 h post contamination and 72 h post contamination. (n=5)
TOWARDS SAFE SILICONE IMPLANTS ENGINEERING

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Silicone breast implants for cosmetic or reconstructive purposes are among the most used medical devices. Their world market size was valued at $3.07 billion in 2022 and is projected to grow from $3.27 billion in 2023 to $5.34 billion by 20301. Despite an ongoing debate on implant biocompatibility, it is now widely accepted that silicone implants may cause a broad spectrum of chronic inflammatory diseases2,3. Breast implants trigger a cascade of stereotype immune events so called Foreign Body Reaction (FBR) leading to the formation of a capsule surrounding and isolating the implant from the rest of the body. Capsule and thereby immune cells patrolling on it, then becomes the first recipient of silicone substances released by the implant, including silicone oils permeating from the gel through the shell and eroded shell debris (Figure 1).
This poster illustrates the strategy developed in my thesis to support the transition to safer silicone implants based on 3 axes:

1) Identification of material characteristics that could impact protein conformation - The silicone shell material characteristics such as chemical composition, hardness, heavy metal content or surface topography may affect silicone permeation through the shell and sustain inflammatory response.

2) Modification/altered in a protein model conformation: the fibrinogen – 822 proteins adsorb onto implant surface during the first phase of the FBR4. Among them, fibrinogen is one of the most abundant and known to tune macrophage response to biomaterial such that we chose fibrinogen as a protein model for in vitro experiments.

3) Macrophage polarization after silicone exposure - Macrophages are key immune cells and predominate during each step of FBR. At any stage, they can mediate silicone-induced adverse responses. Non-polarized macrophages (M0) have the ability to switch between a pro-inflammatory (M1) and a pro-healing (M2) state in a process known as polarization. M0, M1 and M2 macrophages in contact with fibrinogen/silicone will be characterized upon their specific cytokine released signatures.

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Keywords: silicone implant, Foreign Body Reaction, Macrophage
An all-in-one biocompatible Collagen/Tannic Acid tough and adhesive hydrogel with antibacterial, antioxidant and anti-tumor biological activities.

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New multifunctional hydrogels are today needed to meet the modern challenges in wound healing and tissue regeneration. One popular approach to generate bioactive hydrogels that try to mimic these tissues’ characteristics is to use decellularized tissues. The self-assembled fibrillar structure of decellularized matrix is challenging to handle and is mechanically weak and chemically unstable against reactive oxygen species, limiting the application of such hydrogels in regenerative applications. Tannic acid (TA), a naturally occurring polyphenol, can engage in hydrogen bonding with a wide range of macromolecules including collagen. Based on the biological potential of TA, we report some results focused on several peculiar aspects, mainly regarding both the characterization of TA–collagen (TA-Coll) hydrogel and the biological activities of the released TA in vitro and in vivo. The nanotomography transversal views revealed the presence of a sheet-like structure within the TA-Coll hydrogel while the uncross-linked showed a loose structure. TA-Coll hydrogel showed an increase in gel fraction, in elastic modulus and in resistance to the collagenase degradation in comparison with the uncross-linked hydrogel. TA diffused through the hydrogels, exerting therefore its biological effects. Indeed, in comparison with uncross-linked hydrogels, the TA-Coll hydrogel showed (1) antibacterial activities against Gram positive (S. aureus) and Gram negative (P. aeruginosa) strains, decreasing the bacteria planctonik growth and biofilm formation, (2) antioxidant activities, avoiding the increase in the intracellular accumulation of ROS in H2O2 stimulated fibroblasts, (3) anticancer activities, inducing osteosarcoma (SaOS2) cell apoptosis and (4) immunomodulatory and osteogenic properties leading to an increase in parietal bone volume after 12 weeks of implantation. Taken together, these results showed the osteo-biocompatibility and the great potentials of TA-Coll hydrogels for bone regenerative medicine.

*Speaker
Keywords: Collagen, polyphenols, antioxidant, antibacterial, bone regeneration
Dense collagen hydrogels loaded with anti-sclerostin antibodies as biomaterials for critical size calvaria defect repair.

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Objectives: A tissue engineering strategy based on dense collagen hydrogels (DCH) is a promising approach to restore bone defects especially in combination with bioactive molecules or mesenchymal stem cells. Here, we aimed at developing DCH-derived scaffolds enclosing and delivering anti-Sclerostin antibody. The addition of dental pulp stem cells (mDPSC) was evaluated to improve further bone reparation. Ultimately, the best condition was compared with intra-veinous (IV) injections of the antibody.

Methods: DCH scaffolds were prepared using the plastic compression process with two different concentrations of anti-sclerostin antibody (Scl-ab, 0.2mg/mL and 2 mg/mL), seeded or not with 2 million mDPSC. They were studied by micro-X-ray, computed tomography, rheology and scanning electron microscopy. Antibody release kinetics were studied by ELISA. In vivo (APAFIS agreement # 24.297), a 3.5 mm critical size defect was surgically created in the parietal bone of 10-week-old male WT mice with defects subjected to the following conditions: left empty, DCH +/- anti-Scl Ab, +/- mDPSC. A similar experiment was realised comparing the condition showing the best result with the implantation of a DCH alone associated with weekly IV injection of the antibody (50mg/kg). Bone formation was assessed by Micro-CT at 1 and 2 months and by immuno-chemistry. Statistical analyses were performed using non-parametric tests. Significance was defined as a p-value lower than 0.05.

Results: Significantly improved bone formation was observed in mice at 1 and 2 months with antibody loaded DHC when compared with no DCH or DCH alone (p< 0.05). Adding mDPSC didn’t improve further bone formation. Bone reparation is similar to IV injections associated with DCH alone.

Conclusion: Anti-sclerostin antibody loaded collagen-based hydrogels are promising biomaterials to repair critical-size bone defects. Despite their biological properties, mDPSC didn’t improve in vivo efficiency compared with anti-sclerostin antibody alone.

Keywords: collagen, hydrogel, antibody, bone

*Speaker
Tuesday, January 16th, 2024

BIOMAT Session 4

Chairs:
Teresa SIMON YARZA - Lucas LEMARIE
BIOMIMETIC COLLAGEN-BASED MATERIALS FOR VASCULAR REPLACEMENT

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Arteries and especially small-sized arteries, lack of biocompatible replacement solutions (1). Despite providing similar shape and mechanical properties to native tissues, current grafts fail to reach their complex biological composition and organization. The arterial wall is composed of an assembly of glycosaminoglycans and proteins, mainly type I collagen (2), which form the extra-cellular matrix (ECM) surrounding cells. If decellularized, the ECM reveals a porous 3D collagenous structure, whose reconstitution has remained elusive despite the advances in biofabrication.

Our group has developed a successful method to control the hierarchical structure of 3D dense collagen materials using ice templating (2,3), allowing for the subsequent self-assembly of collagen molecules into native-like fibrils. Such biomaterials recapitulate the structural, biological and functional features of cellular microenvironment, without using any cross-linkers or harsh chemicals.

Collagen solutions were ice templated to induce the segregation of the protein in between the growing ice crystals, resulting in a locally high collagen concentration. The obtained frozen monoliths were thawed at low temperature to reveal the porous network. Simultaneously, collagen self-assembly was induced by ammonia vapors to obtain macroporous dense fibrillar materials (Figure 1). Versatile structures can be obtained by tailoring the freezing parameters and fibrillogenesis pathway, resulting in various mechanical and biological properties.

Here we report the use of ice nucleation and growth as tools to shape collagen-based biomaterials. Our approach enables to tailor: the textural aspects of the materials interfaces to direct its interactions with relevant cells (endothelial cells and mesenchymal stem cells), the number of structural layers to reproduce the native architecture of arteries (from one and up to three layers), and the various mechanical features (compliance and axial moduli) necessary for a functional arterial replacement (Figure 2). These results open an exciting pathway to tackle the current limitations of small diameter arterial replacement using this new family of biomimetic matrices. Preclinical large animal model experiments are expected in the upcoming months (4).


*Speaker


**Keywords:** collagen, vascular, self, assembly

*Figure 1.* Macroporous fibrillar gels obtained by ice-templating and topotactic fibrillogenesis.

*Figure 2.* Mimicking arteries ECM through ice-templating and innovative collagen self-assembly.
Functionalized polysaccharide nanoparticles for a targeted treatment of ischemic strokes

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Cardiovascular diseases are held responsible for a third of all deaths worldwide, leading with heart attacks and strokes, among which the latter is responsible for over 12.2 million cases each year (1). Notably, 80% of strokes are of ischemic nature, and the recommended care for this pathological blood clot (thrombus) is the quick systemic injection of a fibrinolytic drug, rtPA, which suffers from severe side effects, offers a short therapeutic window, and does not guarantee recanalization (2). It was also shown that the more complex nature of thrombi impedes their lysis (3). Our approach is therefore to design a biocompatible nanosystem targeting the thrombus, to deliver drugs for improved blood clot bursting and improved recovery. The technology consists in a nanoparticle made from a polysaccharide hydrogel, crosslinked after formation of a water-in-oil nanoemulsion. The resulting nanogels contain a sulfated polysaccharide, fucoidan, which was demonstrated to have high affinity to P-selectin, a marker of activated platelets and endothelial cells and can thus be used for thrombus targeting. Then they are loaded with drugs (such as rtPA) by controlled adsorption to provoke the thrombolysis through localized fibrinolysis at the clot site without exerting systemic side effects.

Currently, the particles’ one-pot synthesis has been optimized with critical downsizing from micro- to 300 nm nanoparticles, by concurring improvements to the nanoemulsion and crosslinking process. These objects were fully characterized, and the loading of chosen therapeutic agents was assessed. The loaded nanoparticles are tested in vitro for thrombus targeting, drug release, efficacy, and cytotoxicity; then they will be assayed in vivo for thrombolytic efficacy, safety profile and biodistribution.


*Speaker
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**Keywords:** Nanosystem, thrombolysis, hydrogel

*Figure:* (left to right) TEM image of nanoparticles. In flow targeting assay showing only the targeted NPs (red) adhering to activated platelet aggregates (green).
Salt-Compacted Albumin Materials for Anti-Tumor Drug Delivery: Integrating Localized and Targeted Approaches for Enhanced Cancer Treatment

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Albumin emerges as a promising candidate for the development of drug delivery systems tailored for cancer treatment. This versatile protein serves as a native ligand carrier, accommodating both hydrophilic and hydrophobic drugs. Additionally, albumin facilitates tumor targeting through the Enhanced Permeability and Retention (EPR) effect, as well as via the overexpression of albumin-related receptors by cancer cells. In this context, the utilization of biomaterials that release albumin binding an antitumor agent holds potential to enhance cancer treatment (1).

Our contribution to this field involves the creation of highly biocompatible biomaterials exclusively composed of albumin, achieved through an innovative and patented technology known as ALBUPAD technology. These materials are generated through the salt-assisted compaction of albumin, offering customizable features such as shape, size, and porosity. This study specifically focuses on adapting the Albupad technology (2) for the delivery of anti-tumor molecules.

Doxorubicin, a DNA intercalant recognized as a reference treatment for various cancers (e.g., breast cancer), exhibits a narrow therapeutic index due to strong systemic toxicity and a lack of specificity. Consequently, it serves as an excellent candidate for albumin-bound delivery (3).

Recently, our process was adapted to produce injectable drug delivery systems. From our macroscopic materials, stable albumin microparticles were formed entirely in an aqueous phase without physical or chemical crosslinking, employing a top-down approach. Loading assays of doxorubicin demonstrated an 85% loading efficiency and a drug-loading rate of up to 10% (w/w, Doxo/albumin). An in vivo proof of concept was conducted on a subcutaneous tumor model in NMRI mice, and the most effective formulation led to complete tumor regression post-implantation of the materials.

An in vivo pharmacokinetic study revealed sustained release of Doxo from the materials without a burst effect. Moreover, the use of these materials resulted in a higher concentration of Doxo in the tumor, as well as a plasmatic bioavailability of Doxo that is more consistent with less fluctuation than Doxo administered intravenously.

*Speaker
The subsequent phase of our research involves expanding this development to construct new materials that facilitate both sustained and targeted delivery of antitumor drugs.

References:


Keywords: Albumin, targeting, localized delivery, antitumor, drug delivery, sustained release
INCORPORATION OF NATURAL ACTIVE COMPOUNDS IN LAYER-BY-LAYER FILMS

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The long-term success of osseointegrated trans-tissular medical devices, such as dental implants and intrasosseous transtcutaneous amputation prosthesis, is usually compromised by a poor fixation between material and surrounding soft tissue, leading to a breach open to bacterial colonization and infection. These complications have been associated with increased morbidity and high medical costs, making this condition a major public health issue. Thin films formed by the Layer-by-Layer (LbL) technique are an attractive surface functionalization approach, since they may act as reservoir of bioactive molecules that guide cellular behavior on the material surface. In this regard, our works consisted in the development of bifunctional biomimetic LbL coatings, able to enhance soft tissue integration while providing bactericidal activity to trans-tissular implants.

LbL films have been built using the extra-cellular matrix glycoprotein fibronectin (Fn), due to its pro-adhesive properties, and a low molecular weight α-poly-L-lysine (30 residues, PLL30), for its intrinsic antimicrobial properties. The Fn-enriched PLL30-Fn films promoted human gingival fibroblasts (HGF) adhesion and proliferation but presented weak antibiofilm effect against Staphylococcus aureus (S. aureus) and Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans), and a moderate action towards Streptococcus mutans (S. mutans) (2.8 log10 reduction).

The easy accessibility and good pharmacological potential of natural products, containing bioactive compounds with antimicrobial properties, as well as anti-oxidant and anti-inflammatory effects, may constitute a promising approach for facing bacterial biofilms development. Our previous works showed the ability of some natural compounds to eradicate biofilms and may therefore represent an efficient alternative for enhancing the antibiofilm properties of PLL-Fn films.

(PLL30-Fn)10 multi-layered films have incorporated PLL30 as antimicrobial compound directly during film assembly. However, these systems could serve as a vehicle to load natural active compounds. Our approach consisted in the introduction of a second antimicrobial molecule into the films which, together with PLL, could generate a synergistic effect that enhances the

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antibacterial properties of the coating.
Different strategies were used to incorporate curcumin and carvacrol as natural active compounds while still allowing the formation of the LbL. Among these, an emulsion formulated between PLL and carvacrol, an essential oil, presented interesting results. Depending on the PLL used, emulsions of different sizes were obtained. When using higher molecular weight PLL (PLL70-150KDa) during emulsion formulation, bigger nanoparticles were formed (256 nm) compared to carvacrol-PLL30 emulsions (178 nm). These emulsions exerted strong efficiency against S. aureus biofilms, particularly when a high molecular weight PLL (70-150 KDa) was utilized (5.7 log10).

Keywords: Layer, by, Layer, natural compounds, antibiofilm
Tuesday, January 16th, 2024

BIOMAT Night conference
PEARS: Engineering Surgical Solutions

Tal Golesworthy *

1 Existent – United Kingdom

A Chartered Engineer, Member of the Energy Institute and the Royal Society of Chemistry with 44 years Research & Development experience in combustion and Air Pollution Control, 17 years at the National Coal Board's Coal Research Establishment, including 3 years training as a patent agent, and 28 years a director of Environmental Development Technology Ltd running R&D projects in combustion and flue gas cleaning/Air Pollution Control.

As a Marfan Syndrome patient with aortic dilation, Tal was unimpressed with the options of a Total Root Replacement (TRR or Bentall) or Valve Sparing Root Replacement (VSRR) so set about engineering an alternative. 19 years after receiving the first personalised external aortic root support (PEARS) himself, he is now working to extend this surgical option to a wider group of patients with aortic dilation.

With NICE IPAC approval, 40+ surgical centres up and running, 900+ patients successfully treated, and a collective 3,000+ post operative patient years, PEARs continues to progress.

March 2014 saw the TED talk on the PEARs project break 1,000,000 viewings (https://www.ted.com/)

July 2015 saw Tal receive one of the first Patient Innovation Awards in Lisbon (http://us3.campaign-archive1.com/?u=7ed42017b5a721b88e1910afb&id=2d6c3fee79)

PEARs is now written up in 50+ peer reviewed journal papers and has been presented at various major international conferences. (www.existent.com)

Married for 43 years to Teresa, Tal has some time for clay pigeon shooting, deer stalking, music, and 20 century European history.

*Speaker
†Corresponding author:
Wednesday, January 17th, 2024

BIOMAT Session 5

Chairs:
Nicolas BLANCHEMAIN, Micaela ROQUE,
Bioelectricity and bioelectronics for bone tissue engineering

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"What is the role of bioelectricity under physiological and pathological conditions? Can we harness bioelectricity to influence cell fate?

To answer these questions, I will give an overview of my work to date on the use of organic electronic materials and devices. I will present some of the work done on the detection of metabolites released by cells undergoing osteogenic differentiation and on the development of 3D architectures to host and monitor stem cells. These substrates can also be used as an interface with cells for their electrical stimulation.

Keywords: biosensor, cell, biomaterial interaction, electrical stimulation

*Speaker
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Development of an Electrospun membranes for the reconstruction of critical size segmental bone loss

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2 Médicaments et biomatériaux à libération contrôlée: mécanismes et optimisation - Advanced Drug Delivery Systems - U 1008 – Institut National de la Santé et de la Recherche Médicale, Université de Lille, Centre Hospitalier Régional Universitaire [Lille] – France

Critical bone loss can be managed in orthopedic and trauma surgery by the induced membrane technique developed by Masquelet. At first a PMMA spacer is cast into the bone defect. This spacer will be fully covered by an induced membrane (IM) through a foreign-body reaction. This IM serve during the second step as a vascularized receptacle for a bone autograft. Despite the high success rate (up to 90%) of this technique, the IM is formed over several weeks before bone grafting, and it requires two surgical interventions followed by prolonged hospitalizations. On this basis, this project focuses on the development of an electrospun membrane (EM) that would accelerate the IM formation. Finally, this EM could be stuffed with bone autograft, allowing a one-step Masquelet procedure.

First, a multi-parameter study was carried out on the electrofilling of a mixture of thermoplastic polyurethane (TPU) and PMMA, with a view to using the same material as for Masquelet surgery and obtaining mechanical properties adapted to the desired application. Polymers were dissolved in DMSO/DMF and electrospun through an homemade device. The resulting membranes were observed by scanning electron microscopy (FLEXSEM 1000, Hitachi®) and Fiber diameters were quantified using ImageJ (v1.42q, NIH). Resorption kinetic was studied in PBS, and the mechanical properties of the membranes were studied. Thanks to this, we could choose materials that would meet our specifications. The in vitro cytotoxicity of the fibers was assessed on a MC3T3-E1 pre-osteoblastic cell line, in accordance with ISO 10993-5, using the Alamar-Blue® Assay extraction method. Very soon, the first in-vivo tests on rats will be carried out to see whether natural induced membrane formation can be achieved with subcutaneous implantation of our materials.

Keywords: Electrospinning, critical Bone loss, Masquelet

Figure 1: Scheme of the use of the artificial membrane
EXOSOME IMPREGNATION OF A HUMAN UMBILICAL CORD-DERIVED BIOMATERIAL AND PROGRESSIVE RELEASE OVER TIME

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We developed an allograft from human umbilical cord lining, that is virally inactivated, ground in a ball mill, moulded and dehydrated by freeze-drying, and finally sterilized by gamma-irradiation (1). Before freeze-drying, the product is a gel with a viscous texture, that allows to mould it into different shapes. The umbilical cord lining is made of an amnion and Wharton jelly, which is rich in glycosaminoglycans, collagen fibers, growth factors and proteoglycans. Proteoglycans conformation gives the product the property to absorb liquids. Thus, we wanted to evaluate the ability of this biomaterial to absorb a solution containing exosomes before or after freeze-drying, then observe the release kinetics (2). Exosomes can be resistant to the freeze-drying process.

Exosomes were purified from C2C12 cell culture medium using ultra centrifugal filters. Purified exomes were loaded on the biomaterial after freeze-drying. Then, the loaded products were placed in 0.9% NaCl as an extraction medium. The extraction medium was removed and replaced at different time points: 30 min, 1h, 2h, 6h, 24h and 48h. The removed medium was transferred in a collection tube and stored at +4°C until the end of the experiment. As the raw material can also contain exosomes, a negative control has been performed with a non-loaded biomaterial. Then, exosomes in extraction medium from the loaded and non-loaded samples, and in the initial solution were quantified using an ELISA kit based on CD63 protein binding. An observation by transmission electron microscopy (TEM) with 0.1% uranyl acetate as a staining agent has also been performed on the initial solution and on the 6th hour extract to check the exosome integrity.

The quantitative analysis showed that the biomaterial allows a progressive release over the 48 hours of the experiment (Figure 1). The observation of the initial solution and the extract at 6th hour showed integer microvesicles from different ranges of sizes (Figure 2). The density of these microvesicles was more important in the initial solution than in the extract at 6th hour.

Figure 1. Cumulative release of exosomes over time

Figure 2. Staining of the extract collected after 6h extraction

*Speaker
This biomaterial could be moulded into different forms and thus used in various medical applications, depending on the type of exosome it would be loaded with. As the product is not immediately degraded in the human body, it could allow the protection and the progressive dispensation of specific exosomes directly in the interest zone.

References:


Keywords: Exosome, extracellular matrix, allograft

**Figure 1.** Cumulative release of exosomes over time

**Figure 2.** Staining of the extract collected after 6h extraction
Development of a 3D in vitro mineralized bone model to reproduce the osseointegration process of dental implants

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Background: Dental implants success depends on a biological seal with the gingiva (soft tissue integration) and the bone (osseointegration). The breakage of this seal causes the leakage of bacteria into underlying bone causing a destructive inflammation and implant loss, a common complication called peri-implantitis. There is thus a need to develop new implants biomaterials and new research model for screening those biomaterials

Aim: The aim of our study was to fabricate, using biofabrication methods a 3D in vitro osseointegration model around a titanium implant to study the implant integration process.

Methods: The model consists of: 1) PLA scaffold 3D printed by Fused deposition modeling, 2) titanium implant, 3) Cell-laden gel (Figure1). The gel candidates tested were methacrylated gelatin (GelMA), collagen (ColMA), or collagen with hyaluronic acid (ColMA+HAMA). Cell candidates (SAOS2, MC3T3-E1, and immortalized MSCs) were cultured in the gels to examine their survival, metabolic activity, and proliferation. We also assessed cells’ osteogenic differentiation over 5 weeks through calcium deposition evaluation (Alizarin red staining, o-cresolphthalein complexone assay, longitudinal follow-up by xylenol orange, scanning electron microscopy and energy dispersive X-ray spectroscopy).

Results: Regarding the gel choice, GelMA was eliminated as low number of cells persisted till the end of 5-week culture period in this gel. ColMA on the other hand showed better properties as cells remained metabolically active and were homogenously distributed within this gel for 5 weeks. However, ColMA alone was not stable and retracted over time so ColMA+HAMA was used instead which maintained both cellular survival and gel stability. With respect to the cell line, SAOS2 cells could not attach to ColMA+HAMA and maintained a round shape while both MC3T3-E1 and immortalized MSCs exhibited spread morphology. Therefore, MSCs and MC3T3-E1 were further investigated for their behavior in ColMA+HAMA. When those two cell lines were cultured in differentiation medium, they deposited calcium within the gel to form a densely mineralized matrix. Since immortalized MSCs are human cells and could be

*Speaker
used to develop human model, we concentrated on those cells and showed that spherical minerals deposited by them had high calcium and phosphate content suggesting that the mineral deposited could be hydroxyapatite. Based on those results, the final components of our system were immortalized MSCs embedded in ColMA+HAMA.

Conclusions and Future experiments: We optimized 3D culture conditions to obtain mineralized bone tissue in vitro. Ongoing experiments include insertion of the titanium implants to evaluate the mineralized matrix / dental implant connection.

Keywords: in vitro model, 3D culture, Mineralization
Wednesday, January 17th, 2024

BIOMAT Session 6

Chairs:
Emmanuel PAUTHE, Romane LESIEUR
HARVESTING EXTRACELLULAR MATRIX OF WHARTON’S JELLY BY EXTRACTION OR DECELLULARIZATION PROCESS: A PROMISING MATERIAL FOR CARDIOVASCULAR TISSUE ENGINEERING

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Cardiovascular diseases such as coronary artery disease (CAD) are the leading cause of death worldwide. CAD most often results from the development of atherosclerosis, leading to total or partial occlusion of one or more coronary arteries. Depending on the case’s severity the patient may require the replacement of one or more coronary arteries. To address this issue, vascular grafts of natural or synthetic origin have been successfully developed to restore blood flow. However, their applicability for the replacement of small diameter (< 6 mm) blood vessels is limited. Therefore, we are developing a 100% human-derived vascular graft of small diameter using tissue-engineering based on the umbilical cord. This graft will be acellular to avoid immune reaction when implanted and to facilitate storage.

To achieve this, we use decellularized umbilical artery as a scaffold that will be coated extracellular matrix (ECM) from Wharton’s Jelly. ECM is harvested using either an extraction or a decellularization process found in the literature1.2. Our results indicate that the extraction process is much more efficient to collect different ECM components than decellularization process. Moreover, the addition of an ultrasound step3 to the extraction protocol allows for greater yield of collagens and proteins, increase the variety of angiogenesis factors retrieved and lower the residual quantity of DNA, compared to the decellularization process. Thus, this material could be of great interest for cardiovascular applications and in particular to coat small diameter vascular grafts to attain better performance and permeability.

References


*Speaker


**Keywords:** Cardiovascular diseases, extracellular matrix, Wharton’s Jelly
3D PRINTABLE ALGINATE-GELATIN HYDROGELS WITH VARIABLE VISCOELASTIC PROPERTIES AS SOLE DIFFERENTIATION FACTOR OF INDUCED PLURIPOTENT STEM CELLS FOR TISSUE ENGINEERING

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Introduction and purpose
Stem cells, particularly human iPSC, constitute a powerful tool for tissue engineering, notably through spheroid and organoid models (1). While the sensitivity of stem cells to the viscoelastic properties of their direct microenvironment is well described (2), such a link has never been investigated for hiPSC. As a result, hiPSC growth and differentiation still relies on supplementation of biochemical factors during culture (3).

Our aim is therefore to investigate the role of the viscoelastic properties of hiPSC spheroids direct environment on their fate. To ensure that cell growth and differentiation can be driven only by mechanical interactions, bioprintable alginate-gelatin hydrogels with significantly different viscoelastic properties were utilized in factor-free culture medium.

Materials and methods
3 alginate-gelatin hydrogels (AG-nX, n=1,3,5) with an increasing concentration were prepared by dissolution in DPBS. Hydrogels porosity was induced by liquid-liquid emulsion of AG formulations with polyethylene oxyde. hiPSC spheroids from two different cell lines were prepared by aggregation (=100 μm, n≥ 1x104). After spheroids inclusion, culture was maintained in DMEM + ITS for 14 days. Transversal culture (adjacent spheroids-laden AG-nX) was also performed. Phase contrast, immunofluorescence, confocal microscopy and qPCR were used to determine spheroid growth/morphology and gene/protein expression of pluripotency and differentiation markers expression.

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Results and conclusions

The AG-1, 3, 5X formulations demonstrated Young’s modulus of 1k, 40k and 100k Pa, as well as rheological properties enabling printability. Spheroids embedded within dense hydrogels exhibited limited growth, irrespective of formulation. Contrarily, in porous hydrogels, the study revealed a direct correlation between the growth of spheroids and the viscoelastic characteristics of the three-dimensional (3D) environment. Additionally, during transverse culture involving the bioprinting of different AG-nX* hydrogels within a single construct, these growth phenomena persisted (Figure 1). This indicates that the growth observed is independent of any unanticipated diffusion or biochemical-related effects within the hydrogels. Regarding viscoelastic-induced cell fate, all spheroids embedded in porous hydrogels showed a spontaneous loss of pluripotency (Figure 2). Interestingly, the different hydrogel environments induced the spheroid towards distinct germ layers, establishing for the first time an indisputable correlation between environmental viscoelastic properties and fate of hIPSC stem cells in 3D culture. Current experiments are further determining the precise temporal and spatial expression of multiple differentiation and mechanotransduction markers (YAP/TAZ activity, integrin/cadherin, FAK and vinculin expression, actin cytoskeleton tensegrity), as function of each viscoelastic conditions.

Keywords: hIPS – Viscoelastic properties, Bioprinting

Figure 1. Impact of viscoelastic properties of AG-nX hydrogels (n=1,3,5) on the growth of hIPSC spheroids in transversal culture at D14. (a) Representation of alternating hydrogels in transverse culture (AG-1X, AG-3X and AG-5X, extruded 3 times). (b) AG08C5 spheroids deposited in the same well at D14. (c) SCTI-003 spheroids deposited in the same well at D14. Scale bar = 500μm.
Figure 2. Pluripotency markers (Oct3/4, SSEA-4 and SOX2) expression after 14 days of maturation in DMEM-ITS in the formulations AG-nX* (n=1,3,5). (a) Embryoid bodies at D0 before inclusion in AG-nX* (b) EB in AG-1X* after 14 days (c) EB in AG-3X* after 14 days (d) EB in AG-5X* after 14 days. Scale bar = 10μm.
Development of Novel Polysaccharide Membranes for Guided Bone Regeneration: In Vitro and In Vivo Evaluations

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Due to the complexity of new bone formation in orthopedic and maxillofacial surgeries, developing a guided bone regeneration (GBR) membrane to support bone ingrowth remains challenging. Specific criteria are required to reach clinical outcomes of GBR membranes such as biocompatibility, biodegradability, porosity, mechanical properties, and biological activity. Natural polymers like pullulan and dextran have interesting biological and physicochemical properties that can be used for bone tissue engineering. They have already been tested as bone filling materials, however, these two polymers have never been used simultaneously for GBR procedures. The present study aimed to develop and characterize two novel polysaccharide-derived membranes for GBR.

Two membrane formulations made from pullulan and dextran were produced: one containing hydroxyapatite (Mb + HA) and one without (Mb). Their characterization was performed by scanning electron microscopy and nanoindentation. In addition, cytotoxicity on human bone marrow mesenchymal stem cells was evaluated according to the ISO standard 10993-5. In vivo, subcutaneous implantation in rats was performed to evaluate their biocompatibility and their resorption at 1, 4, and 16 weeks post-surgery. Moreover, bone femoral defects were created to compare the two pullulan/dextran-based membranes (Mb and Mb + HA) with a commercial collagen membrane (Bio-Gide®). After 1, 2, and 4 weeks, the bone repair was analyzed using micro-computed tomography (micro-CT) and histology.

Using scanning electron microscopy, both membranes presented a porous structure with two distinct sides – rough and smooth. Their mechanical properties were assessed by nanoindentation showing comparable Young’s moduli compared to Bio-Gide®. The two polysaccharide membranes were proven to be cytocompatible and biocompatible by in vitro and in vivo studies. Subcutaneous implantation also demonstrated that the formulation containing HA showed

*Speaker
the slowest resorption rate. Micro-CT analysis of the bone femoral defects revealed that bone regeneration occurred significantly faster for Mb + HA compared to the commercially available membrane two weeks after implantation. Similar effects were observed among the three membranes in terms of bone regeneration one month post-surgery. Histomorphometric measurements of newly formed bone corroborated micro-CT analysis.

In this study, two promising pullulan/dextran-based membranes were developed for GBR procedures. They evidenced biocompatibility without interfering with bone regeneration and maturation. Furthermore, incorporating hydroxyapatite enhanced their potential for GBR procedures by promoting early bone regeneration and offering adequate mechanical support compared to the commercial membrane.

**Keywords:** polysaccharides, membrane, guided bone regeneration
BIODEGRADABLE IMPLANTABLE WOUND DRESSING BASED ON BIOPOLYMERS: STERILIZATION METHOD AND IN VIVO BIODEGRADATION

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Polysaccharide-based wound dressings have been widely investigated in tissue engineering, in particular, alginate and chitosan as biocompatible biopolymers. Alginate presents good swelling abilities and is already used as wound dressing1. It accelerates wound healing by eliminating wound fluids, maintaining a favorable moist environment and by the calcium-dependent activation of cells involved in tissue repair, hemostasis and immunity. Chitosan is biodegradable and presents good mechanical and antimicrobial properties2. The combination of alginate and chitosan as polyelectrolyte complexes (PEC) results in absorbent matrices (PEC matrices) with good mechanical properties3.

The aim of this study was to determine the best sterilization method for the PEC matrices in order to allow to maintain their initial physico-chemical properties and absence of cytotoxicity. This implantable biomaterial was designed for surgical applications, intended to be absorbable and feature wound healing properties alongside a high swelling ability, biodhesion and mechanical properties allowing a suture to the wound bed. This implantable biomaterial needed to be sterile and not cytotoxic.

The strategy was to use mannnuronic alginate and to compare two different alginate/chitosan mass ratios. In this study, we evaluated the swelling ratio, the *in vitro* degradation, the *in vitro* cytotoxicity, the mechanical properties and the *in vivo* biodegradation of PEC matrices. These elements were studied before and after various doses of gamma-radiation sterilization and

*Speaker
temperatures.

All aforementioned biomaterial characteristics were altered by sterilization. As the radiation dose increases, the biomaterials were more rapidly degraded and exhibited decreased swelling properties in NaCl 0.9% and phosphate buffered saline. After sterilization, while the PEC matrices presented an increased Young’s modulus and a decreased elongation at break, they maintained suitable physico-chemical characteristics for their surgical application. Before sterilization, the alginate/chitosan ratio with a higher quantity of chitosan was cytotoxic while the other one was not. After sterilization at room temperature, with any dose of radiation, our results showed that the biomaterials were cytotoxic. However, the PEC matrices were non-cytotoxic when performing a low-temperature sterilization. The PEC matrix featuring the best properties has been tested in vivo for biointegration, bioadhesion and biodegradability. Ten mice were implanted subcutaneously. No animal presented with sign of infection. Pathological analysis of explants 15 and 30 days after implantation showed an incomplete resorption of the biomaterial.

Implantable PEC matrices based on alginate and chitosan were elaborated. We herein determined the best sterilization method to maintain suitable properties for the purpose of wound healing improvement.

**Keywords:** biopolymers, sterilization, implantable biomaterial

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*Figure 1: Process of elaboration of sterilized PEC Matrices*
IN VITRO TOXICITY ASSESSMENT OF PARTICULATE EMISSIONS FROM BRAKING SYSTEMS

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Air pollution causes around 4.2 million premature deaths worldwide every year (1). Several studies have demonstrated the harmful effects of poor air quality on health: onset of respiratory diseases (2), cardiovascular problems (3) and neurological disorders (4). Faced with this health challenge, the World Health Organization sets standards for particulate emissions (particles smaller than 2.5μm = PM2.5), of which road traffic is one of the main emitters. However, while exhaust emissions are widely studied, this is not the case for non-exhaust emissions (brakes, tires, pavement, vehicle components and re-suspension phenomena), which account for the majority of particulate emissions (5).

In order to characterize the non-exhaust emissions and to evaluate their toxicity, braking tests were carried out in the laboratory as a part of the INSA - VOLVO Chair collaborative project. We collected particles resulting directly from braking, either on the cell culture medium or on biomimetic lung surfactant (a new device developed under the Pulsalys DPPA project). PM2.5 were collected using ELPI particle counter, and their chemical identification were performed by SEM-EDX analysis. We further cultured cells (RAW264 macrophages) in the presence of 10% collection media. We assessed cell cytotoxicity via Live/Dead tests. We observed no significant differences in cell viability. Nevertheless, our results confirmed a reduced proliferation rate of cells in the presence of braking particles with respect to control conditions (fig 1). In order to better understand the interaction between the pollutants and the cells, we used a membrane fluidity marker (DIOLL (6)) to detect changes in cell metabolism and found that cells in the presence of particles became more rigid (fig 2b). On the contrary, pulmonary surfactant with the presence of particles became more fluid (fig 2a). To conclude, our study has shown a significant disturbance in cell metabolism, despite good cell viability. This could be an early marker of chronic pathologies (cancer, pulmonary fibrosis, etc.).


(2) Cailland et al, "Outdoor pollution and its effects on lung health in France,” Revue des

*Speaker


Keywords: Air pollution, transport, brakes, health impact

Figure 1: Macrophage cells observed under confocal microscope, a) without particles b) with braking particles.

Figure 2: Comparison of GPs after addition of air or brake particles on a) biomimetic pulmonary surfactant, b) macrophage cells after 72h of culture.
Wednesday, January 17\textsuperscript{th}, 2024

BIOMAT Flash Session 3

Chair:
Emmanuel PAUTHE,
HYBRID HYDROGEL TO CONTROL CELL RESPONSE AND REGENERATE DENTAL PULP

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Caries is a common chronic oral bacterial disease that gradually destroys dental tissues(1). In the absence of early treatment, this leads to the invasion of the endodontic space by bacteria and ultimately, the necrosis of dental pulp requiring subsequent disinfection and obturation. The current treatment that results in the devitalization of the tooth, which loses the ability to detect and protect itself from a new bacterial invasion, is not satisfactory. Promising alternatives aim to regenerate the dental pulp by injecting hydrogels (fibrin) associated with mesenchymal stem cells (MSCs) in the endodontic space(2). However, these regeneration strategies still exhibit heterogeneous success rates in clinical applications, as fibrin gels shrink considerably, sometimes forcing them to detach from the dental wall. Meanwhile, a synthetic and injectable hydrogel composed of lysine dendrimers and poly(ethylene glycol) (DGL/PEG) offers great versatility in mechanical properties while allowing the culture of different differentiated cells without contraction phenomena (3,4). However, this hydrogel does not promote the adhesion and culture of undifferentiated stem cells.

The goal of the project was therefore to explore the possibility of developing a hybrid hydrogel that provides three-dimensional stability during the culture of human dental pulp stem cells (hDPSC) to maximize the benefits of each type of hydrogel, with a pro-regenerative aim.

First, several hybrid formulations were explored to determine the compatibility of the two systems by determination of cross-linking time, demonstrating the suitability of fibrinogen and/or fibrin with DGL/PEG. The impact of the combination of the two hydrogel systems on the final mechanical properties confirmed a reciprocal integration. The benefit for adhesion and proliferation of hDPSC on the resulting hybrid hydrogels of different compositions was then quantitatively evaluated on two-dimensional hydrogels through image analyses of the morphology of fluorescently labelled cells (figure 1). These results confirmed the ability for fibrin or fibrinogen to provide interaction capabilities with stem cells to the DGL/PEG hydrogels. In view of three-dimensional cell culture, the possibility of formulating porous hybrid hydrogels by an effervescent approach (4) was validated and the effect of the incorporation of fibrinogen on pores size and interconnection quantified (figure 2). Finally, the ability to seed hDPSC in

*Speaker
the porous and injectable hydrogels was confirmed in a specifically designed dental canal model produced by 3D printing, as visualized by light sheet acquisitions. Ongoing studies will help define cellular behaviour within these innovative structures.

**Keywords:** Hydrogels, stem cells, dental pulp regeneration

**Figure 1:** Effect of fibrinogen and fibrin incorporation in DGL/PEG hydrogels on cell attachment and morphology

**Figure 2:** Appearance and pore size of DGL/PEG and fibrinogen hybrid injectable and porous hydrogel, and migration of hDPSC over 5 culture days
DYNAMIC AND DEGRADABLE NETWORKS FOR 3D-PRINTING OF ELASTOMERIC SELF-HEALABLE DEVICES

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Self-healable degradable networks encounter a growing popularity for biomedical applications due to their ability to recover their properties after damage.1,2 Self-healable hydrogels dominate with applications in tissue engineering and drug delivery. On the opposite and despite their potential for medical devices, self-healable elastomers remain scarce, especially if they must be compatible with the popular fused deposition modeling (FDM) 3D-printing. This unmet challenge is addressed in the present work with degradable elastomeric networks based on dynamic imine bonds that display efficient self-healing properties at 37°C and can be processed via FDM.3 To this end, multi(aldehyde) and multi(amine) hydrophobic PEG-PLA star-shaped copolymers are combined. The star topology of these copolymers is the key feature of our strategy as it allows the design of multifunctional high molecular weights pre-polymers that ensure an efficient dynamic chemical crosslinking while guaranteeing access to the FDM process generally restricted to thermoplastics. The proposed elastomeric networks combine high self-healing efficiencies (> 97 %) with mechanical properties compatible with soft tissues and a linear degradation profile. Their FDM processing to produce self-healable tubular devices is demonstrated. Finally, their cytocompatibility is assessed and confirm their potential as biodegradable elastomeric networks to be used for the design of self-healable 3D-printed devices.

Keywords: self, healing, degradable network, 3D, printing, medical device

*Speaker
BIOMATERIAL FUNCTIONALIZATION WITH TRIPLE-HELICAL PEPTIDES FOR CARTILAGE TISSUE ENGINEERING

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The supply of adequate biological cues to cells seeded on biomaterials designed for tissue reconstruction is a major challenge in regenerative medicine. Collagen, the main constituent of the extracellular matrix (ECM), naturally provides a structural and biological support for cells, and is frequently advocated as a prominent material in tissue engineering. Understanding cellular interactions with ECM components, and in particular collagen, is key to elicit cellular processes associated with tissue repair.

To direct cell response on biomaterials, we have employed triple-helical peptides (THPs), which adopt the characteristic triple-helix structure of collagen. THPs were designed to include a recognition motif for collagen-binding receptors or secreted proteins, notably collagen-binding integrins (α1β1, α2β1, α10β1 and α11β1), Discoidin Domain Receptors and von Willebrand Factor. Such THPs constitute biomimetic tools to replicate cell-collagen interactions in biologically inert biomaterials, both for the fabrication of engineered tissue constructs for regenerative medicine (1), and the fundamental understanding of the role of collagen-binding receptors in cell behaviour (2).

We have applied THP functionalization for the repair of cartilage, which possesses poor intrinsic healing properties. Cartilage is characterized by a dense ECM network rich in collagen, that notably interacts with chondrocytes through the α10β1 integrin. This receptor is the most abundant integrin in chondrocytes, is key to cartilage integrity and mechanotransduction, and is expressed in chondrogenic mesenchymal stem cells (MSCs) (3). We have therefore functionalized biologically inert substrates with THPs that are ligands for collagen-binding receptors with selective affinities, including for α10β1. These peptides mediated the adhesion, spreading and chondrogenic marker expression of adipose MSCs, chosen for their availability, proliferative ability and differentiation potential. Our results highlight the role of collagen-binding receptors in MSC function and the potential of THPs in the production of engineered cartilage constructs for tissue engineering applications.

*Speaker
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(1) J.-D. Malcor et al, Collagen scaffolds functionalized with triple-helical peptides support 3D HUVEC culture, Regenerative Biomaterials 7(5) (2020) 471-482.


**Keywords:** Tissue repair, Collagen biomaterials, Triple, helical peptides
COMPETITIVE BINDING ENABLES THE FAST DISSOLUTION OF BORONATE ESTER HYDROGELS FOR THE EXTRACTION AND BIOLOGICAL ANALYSIS OF ENCAPSULATED CELLS

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3D cell culture in biomaterials allows cells to grow in an environment that best mimics the native extracellular matrix (ECM). In particular, hydrogels can mimic the mechanical and biochemical properties of the ECM, with many applications in tissue engineering. However, hydrogels commonly used for 3D cell culture do not allow for the extraction of intact cells after encapsulation, which limits our ability to further characterize the encapsulated cell phenotype or use the cells for biomedical applications. In this context, using dynamic covalent hydrogels recently developed by our team, we sought to develop a new dissolution method to extract live cells from hydrogels in a most straightforward manner.

We recently reported a new type of boronate ester hydrogels based on the reversible crosslinking of the ortho-aminomethyl-phenylboronic acid (o-AM-PBA) and glucamine. Using the dynamic nature of these boronate ester hydrogels, we developed a dissolution technique based on the principle of competitive binding. The method consists in introducing a competitive reactive molecule to alter the crosslinking equilibrium and dissolve the hydrogel. First, we tested a series of diol molecules as potential competitive binders. Their cytocompatibility was evaluated on human chondrocyte models (TC28a2) and bone marrow mesenchymal stromal cells using a live/dead assay. Our results highlighted the cytotoxicity of some molecules (i.e., pyrogallol and pinanediol), which were therefore excluded. Then, we investigated the feasibility of hydrogel dissolution with the remaining diols using rheometry. This experiment allowed us to identify sorbitol as the best molecule for dissolution owing to its excellent cytocompatibility (> 98% at 0.5 M) and its ability to dissolve our hydrogels within minutes. After optimization, we showed that 1 mL of sorbitol at 0.4 M completely dissolved 100 µL of different hydrogel formulations within 5 to 13 min without manual intervention. We investigated the cell extraction yield (cell counting experiments) as well as the dissolution process cytocompatibility (live/dead assay) on encapsulated TC28a2 cells. We successfully recovered a majority of encapsulated cells (> 80%) with high viability (> 90%). Along with the effect of the hydrogel dissolution on the metabolic activity and proliferation of encapsulated cells, we are now investigating the use of this method.

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for the biological evaluation of 3D-cultured chondrocytes.

In conclusion, we developed a dissolution technique based on competitive binding that allows us to extract live cells from boronate ester hydrogels within minutes. This new method will allow for a better analysis of encapsulated cell phenotype for advanced cell-material interaction investigations.

**Keywords:** dynamic covalent hydrogel, competitive binding, 3D cell culture
SYNTHESIS OF THREE-DIMENSIONAL NETWORK HYDROGELS (PAA, PEG 200) OR (PAA, PEG 400) AND EVALUATION OF THEIR PROPERTIES FOR POTENTIAL BIOMEDICAL APPLICATIONS

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We have synthesized a multi-network hydrogel which is a material with vast biomedical applications, due to its optimal biological property and physical characteristic. This triple network hydrogel is synthesized from two cross-linked polymers (cross-linking reaction) of (PAA, PEG), whose property is their total solubility in water as well as their biodegradability and biocompatibility, which consequently implies that of the hydrogel.

To select our hydrogel, we carried out different syntheses of these two polymers in the absence and presence of an acid catalyst with different biosolvents.

Confirmation of the modification was demonstrated qualitatively and quantitatively by FTIR spectral analysis and NMR analysis, with an absorption rate of up to 9000% by weight and good stability at a temperature of 30°C. In addition, the results of cell viability tests indicated adequate cell viability and non-toxicity. This hydrogel is a highly promising material for the medical field.

Keywords: Hydrogel, Cross, Linking reaction, FTIR

*Speaker
Unravelling the roles of texture and basal lamina composition on the endothelialization of biomimetic type I collagen matrices

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Understanding cell-surface interactions on the luminal surface of arteries is key for the development of vascular grafts, as proper endothelialization is critical to hinder thrombosis. In vivo, the basal lamina lines the luminal surface of arteries and is mainly composed of two intertwined collagen IV and laminin networks. It provides biological and mechanical cues favoring endothelial cells’ adhesion, proliferation, and activity, essential for blood vessels’ integrity. Beyond composition, the nanometer or micrometer scale topography of basal lamina also regulates cell morphology and activity (1,2).

Here, highly concentrated macroporous type I collagen constructs mimicking accurately the extracellular matrix of blood vessels were produced through ice templating followed by topotactic fibrillogenesis (3,4). We used this new biomimetic model of the arterial wall extracellular matrix to evaluate the combined effect of composition and topography on the surface of biologically relevant substrates (5). To that end, we have fabricated materials that feature two different topographies: a smooth one and a textured one with ridges and grooves. Each of these surfaces was coated with basal lamina proteins laminin, fibronectin, or collagen IV. Surfaces and coating deposition were characterized by scanning electron microscopy and confocal microscopy. To gain insights on cell-surface interactions, materials seeded with bovine aortic endothelial cells were observed with fluorescent and confocal microscopy. We observe endothelial cells’ adhesion on the surfaces, and their higher ability to produce VE-Cadherin and therefore form a tight monolayer on a smooth surface, regardless of the coating. Besides, we notice that unidirectional micrometer scale patterns induce a preferential orientation of the cells. Quantitative results on cell density, metabolic activity and proliferation over time enable to decorrelate the role of topography and composition and suggest that biomimetic coatings favor endothelial cells’ metabolism. The obtained results are of great interest to enhance endothelialization of biomimetic materials for vascular tissue engineering and gain control over colonization kinetics regulated by topography.


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**Keywords:** arterial wall model, basal lamina, endothelialization
DEVELOPMENT OF ELECTROSPUN SCAFFOLD AS BIOMIMETIC DURAL SUBSTITUTES

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Dura mater is the most external and strongest layer of the meninges, a half-rigid membrane crucial for protecting the brain and spinal cord. Its biomechanical behavior is vital, as any alterations can compromise biological functions. When injured or removed, artificial substitutes are commonly used but complications are still frequent (up to 40% of cerebrospinal fluid leakage with bovine pericardium grafts). Based on the morphological similarities between the native dural extracellular matrix and polymer fibers produced by electrospinning, we aim to develop a novel multiphasic biomimetic electrospun dural mater substitute. We expect it to be more bioactive by mimicking the mechanical and architectural native tissue structure) and more functional (by adding specific layers that provide advanced properties such as watertightness), leading to fewer postoperative complications.

Electrospun fibers were produced from 10% and 12% w/v polycaprolactone (PCL) solution, pure or combined with hydroxyapatite nanoparticles to promote bone development and silk fibroin to promote dural cells development. Also, an electrospun film of PCL was obtained to create a watertightness layer. Random and aligned fibers were produced and their mechanical properties (elastic modulus, ultimate tensile strength, elongation at break) were compared. The degradation behavior in vitro was also analyzed. To assess biocompatibility and bioactivity, primary human dural fibroblasts or immortalized adipose-derived stem cells were cultured on the scaffolds (1 week) before measuring metabolic activity (Alamar Blue, MTS) and viability (Live/Dead).

PCL scaffolds showed lower mechanical properties than native dura according to literature (for instance 8.61 (PCL) VS 68.1 MPa (dura) for elastic modulus), but all modifications performed on the scaffolds led to increased mechanical properties mimicking better the actual tissue. Silk fibroin increased the elastic modulus of PCL fibers while the addition of hydroxyapatite decreased it. From the biological point of view, both cell types were able to attach, spread and proliferate on all scaffolds with high viability.

Therefore, future studies will investigate multiphasic structures to approach the optimal mechanical behavior. Biological functionality (matrix composition, stem cell differentiation) will also be evaluated in the longer term before moving on to in vivo implantations of the most promising scaffolds.

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Keywords: Mechanical characterization, electrospinning, dural graft
Silicon effects on human dental pulp stem cells seeded in 3D dense collagen hydrogel

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Previous studies revealed that dietary silicon (Si) absorbed in the form of silicic acid (Si(OH)4), as well as Si(OH)4-releasing biomaterials, are beneficial for bone formation (1). The optimal dose of Si(OH)4 likely to favor mineralization varies between cell types, from physiological (10 µM) to supraphysiological doses. Although Si-based compounds are also widely used for tooth restoration (2), how Si affects dental tissue formation at the cellular level is not well addressed. Here, we investigated the in vitro effects of Si(OH)4 on human dental pulp stem cells (hDPSCs) seeded in 3D dense collagen hydrogels. Si(OH)4 concentrations of 10 and 100 µM were added to the mineralizing medium to grow hDPSCs. Control (Si-0 µM) and Si-treated groups showed similar cell viability, with few dead cells on day 24 of culture. The expression of bone- or tooth-related markers in hDPSCs was analysed with RT-qPCR. ALP, OCN, BSP and Col1 genes were expressed in hDPSCs cultured for 1, 7, 14 and 21 days. Increasing silicon content to 100 µM resulted in a 1.5-fold lower OCN gene expression compared to the other groups (Si-0, Si-10 µM) at day 7. Additionally, immunohistochemistry staining demonstrated lower osteopontin protein expression in the presence of Si-100 µM. Histology with alizarin red staining showed significantly higher mineralization in both control and Si-10 µM groups. Furthermore, structural analysis of cell-seeded gels by SHG revealed that exposure to Si impacted collagen fiber alignment. Expression levels of collagen type 1 increased over time, with a peak at day 14 in the absence of Si (thereafter remained constant), while continuing to increase until day 21 in the presence of Si. This suggests a direct interaction between Si and the matrix. These findings reveal that supraphysiological concentrations of Si, albeit subtoxic, negatively affect the mineralization function of hDPSCs. Ongoing research on Si(OH)4-releasing biomaterials will provide further insights.

References


Keywords: Silicon/Silicic acid, dental pulp stem cells, mineralization

*Speaker
Wednesday, January 17th, 2024

BIOMATSAN Session 1

Chairs:
Jerome SOHIER, Isabelle MARTINIER
MODIFICATION OF ZIRCONIA DENTAL IMPLANTS TO PROMOTE SOFT TISSUE ADHESION AND LIMIT BACTERIA COLONIZATION

Fryni Skorda *† 1, Eva Garcia 1, Yann Chevolot 2, Jérôme Chevalier 1, Emmanuella Laurenceau 2, Laurent Gremillard 1

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Within the NOMAD project (Horizon Europe project 101091669), our primary mission is to contribute to the development of cutting-edge, resilient, and highly effective dental implants. The overarching goal is to ensure the sustained clinical success of dental implants to improve zirconia osseointegration, while simultaneously mitigating the incidence of peri-implant diseases. One part of our group’s specific focus lies in enhancing the ZrO2 implant bioactive surface modifications with the aim of fostering the effective and fast sealing of soft tissue around the abutment.

The prevalence of peri-implant mucositis and peri-implantitis, affecting 43% and 22% of treated individuals, respectively, underscores the importance of our research. While peri-implant mucositis is easily treatable if detected early, its potential progression to peri-implantitis poses significant risks, including bone destruction and implant failure. Surgical intervention for peri-implantitis is a complex and unpredictable process, making prevention and early detection pivotal in contemporary dentistry. Our emphasis on microbial biofilm control and soft-tissue sealing presents critical challenges, met by innovative surface modifications at the abutment level.

Traditionally, Titanium (Ti) has been a widely employed dental implant material due to its robust osseointegration capabilities and cost-effectiveness. However, the emergence of high-performance ceramic materials, such as zirconia (ZrO2), has gained prominence. Zirconia, distinguished by its tooth-like color, high mechanical strength and excellent soft tissue integration, has spurred an exploration of bioactive and osteogenic surface modifications. Our team is functionalizing the ZrO2 surface through the covalent bonding of specific peptides, utilizing an intermediate layer of organo-silanes or organo-phosphonic acids bearing a chemically active functional group at the ω-position.

The covalent biofunctionalization process unfolds in two steps. First, a covalent bonding of a layer of organosilanes or phosphonic acids with a carboxylic acid, amine or maleimide end group is established. Subsequently, since grafting peptides directly on the material is challenging, peptide grafting occurs on this first layer, with coupling performed through click chemistry

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or NHS ester/EDC chemistry. In the present contribution, to minimize surface contamination and activate the oxide’s surface, samples underwent pre-treatment techniques, including ozone treatment under UV exposure and/or immersion in different acids (i.e. piranha solution H2SO4/H2O2). The resulting surfaces were characterized using Water Contact Angle (WCA) measurements, X-ray Photoelectron Spectroscopy (XPS), and Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy (ATR-FTIR). Next, the grafted layers of organophosphonic acid and organo-silane molecules were optimized. The efficacy of the grafting was ascertain using WCA, colorimetric assays (Toluidine blue, ADECA titration) and XPS. The zirconia surface pre-treatment and preparation will be discussed.

**Keywords:** Zirconia  Covalent Biofunctionalization  Peptide Grafting
Functionalization of Zirconia for Enhanced Osseointegration in Dental Implants

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This project is part of the Nomad European Project (Horizon Europe project 101091669) which aims to develop the next generation of dental implants with a focus on preventing peri-implantitis, consequently enhancing the long-term success of implants. Peri-implantitis is a major contributor to dental implant failures.

One of the specific aim is to stimulate bone tissue growth on zirconia implants. Zirconia is a promising material for dental implant due to its excellent aesthetic properties, such as opacity and color. Zirconia has other advantages such as biocompatibility and very low inflammatory response when it comes in contact with tissues which again make it a possible alternative to titanium which may cause allergic reactions.

The overall strategy is to functionalize zirconia with ECM components. To do so, a first organic layer is attached to zirconia by chemical functionalization of the surface. This first layer aims to facilitate the grafting of biochemically active molecules (such as extracellular matrix components and growth factors, peptides) recognized by osteoblasts and therefore promoting their growth. As we aim to enhance osseo-integration, zirconia surface was sandblasted to obtain a surface roughness that promotes adhesion and proliferation of osteoblasts. In the present contribution, we focus on zirconia surface preparation (sandblasting, surface cleaning, and surface activation) and on the impact it has on the grafting of the first organic layer. The surface roughness, composition, chemistry and the material crystallinity were characterized using surface analysis techniques, including X ray diffraction

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(XRD)
Water contact angle (WCA), Scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS) and attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR). This study gave us information on the impact of the various treatment and on the efficacy of surface cleaning. The efficiency of the subsequent grafting of organo silane and organophosphonic acids is addressed.

**Keywords:** zirconia, surface functionalization, dental implants
BIOFABRICATION OF A 3D IN VITRO MODEL RECAPITULATING THE OVINE IVD STRUCTURE FOR EVALUATING THE EFFICIENCY OF NOVEL THERAPIES.

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The intervertebral disc (IVD) is a fibrocartilaginous structure composed of an outer fibrous ring of collagen (Annulus Fibrosus, AF) surrounding a soft gelatinous core (Nucleus Pulposus, NP). IVD degeneration (IVDD) is a widespread cause of lower back pain, affecting 40-60 % of the population. Unfortunately, the current management of IVDD is symptomatic, and new therapies to clinically treat IVDD and regenerate the IVD are needed. In vitro models are commonly used to assess the efficiency of these therapies; however they are often simplistic and fail to recapitulate the complex IVD organization. Furthermore, ethical regulations restrict the use of animal models, limiting research possibilities. In this context, we sought to develop an ovine in vitro model that would reproduce the spatial organization of cells and matrix, thus mimicking both the fibrous and gelatinous structures.

To that aim, the NP core of the construct was formed by encapsulating ovine NP cells (2 x 106 cells/mL) in methacrylated alginate hydrogel (MAH). Once cross-linked, MAH exhibited a stiffness (4 kPa) similar to that of native NP tissue (Figure 1A). This was achieved by optimizing MAH concentration (2 %) and UV crosslinking dose (10 mJ/mm²) without compromising cell viability of embedded cells. The AF part is composed of polycaprolactone (PCL) fibers, generated through melt-electrowriting (Figure 1B). These PCL fibers, each measuring 20 μm in diameter, were thoughtfully organized into thirteen concentric circles, forming an interlamellar structure. The space between the circles was filled with 2 % MAH, loaded with ovine AF cells (2 x 106 cells/mL) (Figure 1C). This strategic design enabled AF cells to adhere and proliferate along the PCL fibers surface, resembling the architecture observed in IVD (Figure 1D). Once assembled, this model was stable in culture for up to 28 days, with good cell viability (over 80 %, Figure 1E) and no cell sedimentation. Currently, Histological, biochemical, and transcriptomic

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analyses are under way to assess matrix content and cell organisation and phenotype. The fidelity of the bioprinting process, with the spatial distribution of the cells mimicking an IVD architecture, opens the way for the next phase: simulating IVD degeneration to evaluate the effectiveness of novel therapeutics. This advancement holds great promise in the search for a more effective treatment for IVD degeneration.

**Keywords:** Melt, electrowriting, Bioprinting, Intervertebral disc

Figure 1. Development of a 3D in vitro model recapitulating the ovine IVD structure. (A) Young modulus of methacrylated alginate hydrogel (MAH) according to culture time in medium culture at 37°C, N=1, n=7. (B) Image of Meltelectrowriting Polycaprolactone (PCL) scaffold forming the Annulus fibrosus (AF) part of the model. (C) Radial plan of AF part of the model showing alternance between PCL circles and MAH containing cells. (D) Cytoskeleton labelling of AF cells adherents on PCL fibers. (C) Cell viability of AF cells encapsulated into the model at different days of culture, N=1, n=4.
DEVELOPMENT OF POLYSACCHARIDE BASED MEMBRANE FOR GUIDED BONE REGENERATION: MECHANICAL, IN VITRO AND IN VIVO ASSESSMENT

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Guided bone regeneration (GBR) using membranes is widely used in oral and maxillofacial surgery in order to promote and/or support the bone reconstruction. The primary challenge of this type of membrane is the ability of surgeons to manipulate and secure it in the desired location without breaking it and avoiding displacement, while maintaining the biological performance needed for the bone regeneration.

In this work, we describe how to develop a natural polymer-based membrane, made with polysaccharides, as well as its physico-chemical, mechanical and biological properties (Ref). The membrane was obtained through optimization of the composition and manufacturing using freeze-drying. The swelling, degradation rate, cytocompatibility, and barrier function in vitro were evaluated. Stiffness and strain were enhanced by combining covalent crosslinking of pullulan and dextran (Ref) with electrostatic crosslinking with alginate to create an interpenetrating polymer network. This physical crosslinking allows obtaining reversible/dynamic bond formation and also shows the ability to dissipate energy when breaks and reformation occur (Ref). For surgical requirements, this mechanical improvement allowed to suture the membrane without damaging it. Formation of this interpenetrating network accelerated the degradation rate as sought for in vivo experiments. Other physical features of the membrane, including porosity (pore size, interconnected pores, gradient of pores) were studied due to their influence on cell fate such as viability and cell penetration. Finally, preliminary in vivo experiments have been carried out to study the impact of sterilisation using gamma-radiation on the biodegradation and tissue response to this material.

This work was funded by the H2020-NMBP-HUBS-2018 TBMED project: Grant Agreement No. 814439; and by the ANR (ANR-20-CE18-0034).


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**Keywords:** Hydrogels, Bone regeneration, Medical device
Thursday, January 18th, 2024

BIOMATSAN Session 2

Chairs:
Joelle AMEDEE, Ludovic SICARD
Soft biomaterials and composites: a journey from biofabrication to immunomodulation

Matteo D’este *† 1

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Soft biomaterials from pristine or semi-synthetic biopolymers derivatives are essential in biomedicine, and their combination into composites expands our opportunities to modulate their properties and to engineer tissue architectures. This talk will illustrate our research on semisynthetic derivatives of hyaluronic acid describing a series of applications in biofabrication with emphasis on musculoskeletal tissues. The combination with collagen will be illustrated as a mean to give the obtained constructs controlled microscopic architecture and cell-instructive properties. Physical, chemical, and biological properties of biopolymers are also key to biomaterial immunomodulatory properties. Although most of the research in this field has investigated macrophages as key mediators, the role of neutrophils in immunomodulation needs better understanding.

The second part of the talk will describe methods to decipher the role of neutrophils in determining the fate of inflammation, better characterize their interaction with biomaterials, and the significance of this interaction for tissue healing. In vitro models of biomaterials immunomodulation can be used to reveal mechanisms and to predict outcomes, thereby contributing to complement, or even to refine and replace in vivo experiments. Studying biomaterials from the immunomodulation perspective will help the field to design materials harnessing the body healing capability for tissue regeneration.

Mots-Clés: À venir

*Intervenant
†Auteur correspondant:
3D-PRINTED GELATIN-PLURONIC SCAFFOLDS PROMOTE GLIAL CELLS ALIGNMENT FOR SPINAL CORD REGENERATION

Sacha Barberat *† 1, Nagarajan Sakthivel‡ 1, Sébastien Blanquer§ 1, Anne Aubert-Pouëssel¶ 1, Marie-Noëlle Labour 1

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Spinal cord injuries (SCI) affect approximately 0.5 million people worldwide every year and result in total or partial loss of motor and sensory functions (1). The combination of biomimetic materials to neural or glial cells is particularly promising for reconnecting injured axons to their original target. Our objective is to develop a 3D printed biomimetic scaffold that favors Schwann cell alignment to promote directional axonal growth. The strategy consists in printing Nerve Guides (NGs) using high-resolution stereolithography to produce tubular structure presenting an anisotropic architecture and topography to promote Schwann cells alignment. To this aim, we used layer by layer assembly of a combination of gelatin and pluronic to produce a scaffold favouring cell adhesion and presenting adapted mechanical properties.

Photosensitive gelatin and pluronic were obtained using methacrylation chemistry (2) and combined with various ratios and polymer concentrations to print multichannel NGs with different channel diameters fitting design dimensions (Figure 1).

The scaffolds present tunable mechanical properties in particular linked to pluronic micellar structure that brings flexibility (3) while keeping a low young modulus close to neural tissues favourable for neural cells adhesion (4). Compressive and rheological tests show that gelatin-pluronic scaffolds are sufficiently rigid and flexible to withstand handling compared to fragile and brittle gelatin scaffolds. The microstructure and degradation properties were also characterized. In particular, electron microscopy observations highlighted an internal microtopography following the channel direction that is linked to the printing process.

Primary Schwann cells (pSCs) extracted from sciatic nerve and purified were efficiently and

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homogeneously seeded within the channels using various methods. Cellular analysis showed efficient cells adhesion, coverage of the scaffold’s channels and a high viability. Most importantly, Schwann cells align towards the channel direction independently of the channel diameter due to the microtopography (Figure 2). Finally, these pSCs colonized NGs should promote directional axonal growth which is promising for nerve regeneration(5).

REFERENCES


Keywords: 3D printing, Nerve Guides, Schwann cell

Figure 1Macroscopic view of the NGs

Figure 2Confocal images of actin stained pSCs in NGs channels
Biomimetic apatite and m-CPPD crystals-based materials as models to improve ex vivo osteoarticular calcifications diagnosis

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Osteoarthritis (OA) is the most common adult joint disease in the world.(1) The presence of intra-articular calcification was identified as one of the aggravating factors associated with synovial inflammation and destruction of the cartilage, suggesting a direct pathogenic role of crystals in the disease development. Calcium phosphate microcrystals are identified in cartilage calcification as carbonated apatite (CA) in 90 % of cases which are in 20 % of cases also associated with m- or t-CPPD calcium pyrophosphate dihydrate (m-CPPD and t-CPPD).(2) Currently, the diagnosis is performed with non-invasive imaging techniques at a quite late stage of the disease development and have no capacity to differentiate CPPD from CA crystals. The goal of the project is to provide standard materials to radiologists and engineers in view of developing multi-energy spectral CT scanner (SPCCT) protocol to differentiate CPPD and CA crystals in joints ex vivo and improve patientcare. To achieve this final goal, we developed two cement routes to obtain monoliths of agglomerated CPPD or CA crystals as standard materials for analysis with SPCCT scanner.

Cement route is based on a mixture of reactive powders in which a liquid phase is incorporated to obtain a self-setting paste. Reactive powders were synthesized by double decomposition (3)(4) in aqueous solution. The resulting paste was placed in a mould and, after curing for two days at 37°C in a wet atmosphere and then drying at 37°C, leading to cohesive m-CPPD or CA cement. Powders and cements were fully characterized by XRD, Raman and FTIR spectroscopy, SEM and TGA. m-CPPD cement formulations were optimized by varying powders’ reactivities, liquid composition and by using some crystal seeds to reach the material specifications provided by the radiologist. On a fundamental point of view, in vitro cell study on the agglomerated CA and m-CPPD crystals is in progress to compare their inflammatory potential to that of isolated crystals in relation to OA disease development.

*Speaker


**Keywords:** Osteoarthritis, calcium phosphate, cement
NEW APPROACH TO EVALUATE THE INFLUENCE OF BIOCERAMIC COMPOSITION ON THE BEHAVIOR OF CELLS INVOLVED IN BONE REGENERATION

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The introduction of chemical species in the calcium phosphate lattice such as Mg2+, Mn2+, Sr2+, Cu2+ etc. is widely investigated to improve the biological properties of ceramic for bone regenerative medicine. Therefore, screening the effect of such species in solution on the living cells may help the optimization of effective doped-bioceramics. This work aims to monitor the impact of bioceramic chemical composition on human cells involved in bone regeneration, using a diagnostic tool based on an electromagnetic microsystem. This microsystem uses the principle of ultra-high frequency dielectrophoresis (UHF-DEP) to investigate biological behaviour, e.g. the differentiation state of cells. This technique allows the measurement of the cell’s second crossover frequency f x02 enabling the exploration of distinctive traits in the intracellular content of the cells (1). Therefore, by combining UHF-DEP cell signatures and conventional bioassays, our objective is to demonstrate the feasibility of establishing a pioneering, non-destructive and label-free tool based on an innovative cell characterization method. This sensor was used to monitor the biological behavior of Human mesenchymal stem cells (hMSCs) in the context of the development of a bioceramic, active support for bone regeneration. The UHF-DEP signature of hMSC differentiation was measured after their culture in a growth medium (GM), unable to induce cell differentiation, or in an osteogenic differentiation medium (ODM) for 48h, 7, 15 and 21 days. Conventional biology was used to validate cellular behaviours and fluorescence staining to study cellular density, structure and morphology. Early changes were identified during in vitro osteogenic differentiation. After day 7, cells cultured in ODM showed higher cellular density, increased nuclear overlap, decreased nuclear average area and roundness compared to GM cultured cells. Clear differences in distribution pattern of Collagen I expression was consistently observed between GM and ODM conditions. Preliminary results using UHF-DEP indicate that cells cultured in ODM exhibit an overall decrease of crossover frequencies f x02 in comparison
with GM cultured cells after 7 days. Interestingly, the UHF-DEP results highlighted the existing heterogeneity in hMSCs differentiation ability which opens perspectives as a quality control method.

Those results reveal that UHF-DEP could be a promising method to identify biological changes associated with bone regeneration. Efforts are now made to investigate the impact of bioceramic chemical composition on biological responses by applying dose ranging of CuSO4 and MgSO4 on hMSC and explore their UHF-DEP signature together with a complete analysis of their physiological status.


Keywords: Mesenchymal Stem cells, Bioceramic, Ultra High Frequency Dielectrophoresis
Thursday, January 18th, 2024

BIOMATSAN Session 3

Chairs:
David MARCHAT, Mathilde MAILLARD
A COMPOSITE ELASTIN DERIVATIVE-BASED HYDROGEL DESIGNED FOR PROMOTING BONE FORMATION, VASCULARIZATION, AND INNERVATION: IN VIVO EVALUATION IN ECTOPIC AND HETEROTOPIC MODELS

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Despite continuous progress in bone tissue engineering, the successful reconstruction of large bone losses remains a major unsolved problem in the clinical sector. This project focuses on developing an innovative biomaterial that allows the recruitment of bone cells, endothelial cells and neuronal fibers within the same matrix, allowing the regeneration of bone tissue. This bioactive hydrogel is based on a matrix of recombinant Elastin-Like Polypeptides (ELPs), chemically modified to allow the graft of laminin-derived peptides intended to stimulate angiogenesis and innervation, combined with calcium phosphate particles to promote bone repair. After its physicochemical characterization, the objective of this work was to evaluate the effectiveness of this functionalized ELPs-based hydrogel in vivo in murine preclinical studies. The functionalized ELPs-based hydrogel was first implanted ectopically in a mice subcutaneous model. Micro-CT analysis confirmed the formation of ectopic mineralized tissue within the implants, which increased over time. Histological sections of the hydrogel showed cell colonization, formation of osteoid tissue, no significant fibrosis, and normal recruitment of immune cells. Nerves were always present in the periphery of the material, and vascularization started at 1 week after implantation, moreover, 1 month after, the gel was fully vascularized. The ELPs-based hydrogel was therefore tested in a femoral condyle defect model in rat. This hydrogel, a negative control (empty defect) and a positive control (Collapat®) were followed by longitudinal micro-CT, showing that the volume of mineralized tissue formed after 2 months was not significantly higher in empty defects nor in Collapat®, but there was a significant increase in hydrogels. For lesions filled with the hydrogels, a network of blood vessels was formed after 2 weeks, and nerve fibers could be seen inside the lesions. To evaluate the use of this hydrogel for craniofacial bone reconstruction, we created a critical mandibular bone defect model in rat. The same conditions were tested. Interestingly, a partial reconstruction was observed in the periphery of the defect.

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after 1 month only for the ELPs-based hydrogel. Moreover, after 2 weeks the lesions filled with ELP-based hydrogels were vascularized and innervated. The cell-free and growth factor-free hydrogel is biocompatible, capable of inducing mineralized tissue, angiogenesis and nerve sprouting. Such a holistic approach represents a technological breakthrough contrasting with current strategies based solely on bone and endothelial cells. These results are very encouraging, and we are currently pursuing further in vivo testing in mini-pigs for pre-clinical evaluation.

**Keywords:** Hydrogel, Bone lesions, in vivo
Investigation of the early apical release from endodontic hydrogels: a 3D printed model

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Background: Regenerative Endodontic Procedures (REP) using new materials such as hydrogels aim to replace current endodontic treatments, but numerous limitations are to overcome. Mainly, the parameters of the hydrogel are little explored. Apical release especially, was little explored in previous studies, for hydrogels that incorporate molecules, such as growth factors and antibiotics. Apical release is a key mechanism in achieving regeneration, as it could potentially regulate disinfection or cell colonisation. Few models exist for the apical release, limiting the transfer of these devices from bench to bedside.

Aim: The aim of the study was to design a simple and standardised model to identify parameters that influence the apical early release kinetic of molecules from endodontic hydrogels.

Methodology: Endodontic Release Models (ERMs) were designed to mimic the situation of an immature incisor using three different diameters (Ø 0,5 mm; Ø 1mm; Ø 2 mm), and to allow the study of the early release from a hydrogel in a 96-well plate. ERMs were produced with a 3D printing machine. The kinetic release was investigated using one fluorescent hydrophobic (BDP500) and one hydrophilic (Fluorescein) molecules. Release was studied from fibrin hydrogels at different concentrations and from agarose hydrogels, and in various media (PBS or human serum). The release kinetics were estimated by measuring the fluorescence in a 96-well plate at different (1, 3, 5 and 24 hours).

Results: Three different models were designed, produced using a 3D printer, and tested by exploring various parameters influencing diffusion from a hydrogel into a release media. ERMs use made it possible to report that apical diameters, apical composition, solubility of molecules, and physico-chemical properties of scaffolds all significantly influenced the early kinetic of apical release.

Conclusion: The use of ERMs enables to investigate the parameters influencing the release kinetics from endodontic hydrogels. These models will help further investigations exploring the interactions of these parameters with each other, and will ease the transition towards animal models and clinical practice.

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Clinical Significance: This study demonstrates that there are at least 6 parameters that significantly influence the release kinetics of molecules from endodontic hydrogels, especially physico-chemical properties of molecules and scaffolds, and apical diameter.

Keywords: Hydrogels, Endodontie, Matériaux
Thursday, January 18\textsuperscript{th}, 2024

BIOMATSAN Flash Session 1

Chair:
David MARCHAT
PHOTOCROSSLINKABLE, DEGRADABLE AND BIOACTIVE POLYMERIC INK FOR MENISCUS REGENERATION BY 3D PRINTING

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3D printing techniques are giving rise to new processing strategies. Photopolymerisation offers new advances with high-definition techniques like 2 photon polymerization (2PP), which allows photo-triggered CAD-customized implants with micro- to nanometric resolution, an ideal solution to reproduce physiologically microstructured tissues like meniscus. Besides structuration, such implants require photoactive biomaterials with a good balance between mechanical properties and bioactivity to enhance the regeneration process. To this extend, we developed a new photo-crosslinkable ink which combines bioactive peptides and both natural and synthetic degradable polymers. Star shaped poly(D,L lactide) (PLA) as well as gelatin (GEL) were synthesized and functionalized with tyramine (Tyr), a biocompatible photocrosslinker derived from tyrosine amino acid. Photo-resins containing Tyr-PLA/Tyr-GEL at different ratios were crosslinked via 2PP or LED irradiation using green light (λ = 525 nm) to yield polymeric networks. The ink containing Tyr-PLA/Tyr-GEL at 50:50 was selected based on its printability, mechanical properties and the high proliferation of human fibroblasts on the resulting network.

In a second step, to further improve bioactivity, we synthesized tyrosine (Y) photocrosslinkable peptides containing HAVDI sequence, well known to enhance differentiation of mesenchymal stem cells in chondrocytes, i.e. the cells of meniscus tissue. The peptides were introduced in the selected 50:50 ink to generate networks presenting bioactive peptides as pending moieties (HAVDYG) or incorporated in the network (YGGHAVDYG). The response of mesenchymal stem cell towards these bioactive networks was evaluated in terms of adhesion, proliferation and chondrocyte differentiation. Subsequently, we studied the mechanical and structural properties of 2PP 3D printed objects with SEM, AFM and fluorescence microscopy. Finally, we studied the behaviour of mesenchymal stem cells on printed objects mimicking meniscus microstructures. Overall, we report on a bioactive, degradable and 2PP 3D printable ink with both good mechanical properties and good cell response, that holds potential to help regenerating tissues.

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Keywords: 3D printing ink, hybrid material, tissue regeneration
MULTI-DOPED BIOCERAMICS FOR LARGE BONE DEFECTS REGENERATION

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Following arthroplasty, trauma, infection, or tumor resection, a critical bone defect can appear, preventing the tissues from repairing spontaneously. The defect thus must be filled using an autograft, an allograft, or a xenograft, but this solution is limited by the lack of raw material or the risk of infection.

One of the emerging solutions is using synthetic materials such as calcium phosphate ceramics. Hydroxyapatite (HAp), β-tricalcium-phosphate (β-TCP), and α-tricalcium-phosphate (α-TCP) are already marketed because of their osteoconduction properties. Bone regeneration is a very slow process that can take weeks to months depending on the initial defect. Indeed, stoichiometric HAp ceramics are well incorporated after 5 years, but 15 years may be necessary for new bone to replace it. Also, to avoid soft tissue invasion, bone-filling material degradability needs to be finely tuned, matching neo-bone formation speed, in order to keep a constant volume occupancy inside the bone cavity. Although a combination of multiple CaP phases could solve this issue, the CaP materials available on the market do not limit the risk of bacterial infection and do not promote osteogenesis.

To this end, a doped biphasic calcium phosphate powder was synthesized by coprecipitation. Copper, zinc, and strontium nitrate were used to dope the ceramic matrix, with the aim of controlling inflammation and bacterial growth and stimulating bone cell activity. Playing on different parameters such as dopant quantity, synthesis parameters such as temperature or pH, and sintering temperature, allowed to control the composition and the phases present in the final powder.

The obtained biphasic calcium phosphate samples were finely characterized (crystal structure, chemical composition, morphology) using X-ray diffraction, scanning electron microscopy, atomic emission spectroscopy, and infrared spectroscopy. Thereafter, copper, zinc, and strontium ionic release kinetics were studied after powder immersion in simulated body fluid at 37°C.


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**Keywords:** bioceramics, ionic dopin, bone regeneration
Influence of macroscopic confinement on tissue organization and osteogenesis

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Biomaterials for bone regeneration are ephemeral structures that serve as support for the development of new functional vascularized bone tissue. The limits of available commercial products have shown that a new generation of biomaterials intended for the regeneration of large bone defects (> 1cm3) needs to be developed. These biomaterials need to be more than a support for tissue ingrowth, they have to be bioinstructive. Today, it is accepted that cells are able to sense geometrical cues larger than their own size (> 100μm). Individually, cell response to supra-cellular topographic patterns is regulated by the interaction of the surrounding extracellular matrix, the cytoskeleton and the nucleus. The objective of this in vitro project is to understand the influence of the biomaterial architecture at the supra-cellular scale on intra and extracellular biomechanics, especially the collective dynamics of the nucleus and stress fibres under these confining conditions, and their key role on osteogenesis. To reach this objective, human mesenchymal stem cells will be cultivated on hydroxyapatite-based bioceramics with designs adapted to the scientific questions. We will study, after seeding, the effect of cell-cell contacts on cellular behaviour and the extracellular matrix production according to the macroarchitecture. Then we will analyse extracellular matrix and cell biomechanics as a function of its confinement. Finally, we will study the consequences of the cell’s state of tension on osteogenic differentiation. This study will enable us to develop an in vitro 3D osteogenic culture model and a bioinstructive material for the regeneration of large bone defects.

Keywords: osteogenesis, macrotopography, bioceramics
PATIENT SPECIFIC RECONSTRUCTION OF CLEFT PALATE DEFORMITIES IN DOG WITH FLEXIBLE 3D-PRINTED ORGANO-MINERAL CEMENTS

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Introduction: Developments in the field of additive manufacturing have allowed significant improvements in the design and production of calcium phosphate scaffolds with biologically relevant features to treat bone defects. Unfortunately, these scaffolds are often brittle and fragile, uneasing their handling by surgeons, with significant risks of fracture during their insertion in the defect. Consequently, we developed organo-mineral cementitious scaffolds displaying evolutive mechanical properties to treat maxillofacial bone deformities in veterinary clinics.

Methods: In vitro physico-chemical and biological characterizations of scaffolds formulation were performed. Treatment of dog patients with spontaneous cleft palate/lip deformities was approved by ethic and welfare committees (CERVO-2022-14-V). To date, 8 puppies received the following treatment procedure: Two weeks prior surgery, CT-scan of patient’s skull was performed. Organo-mineral pastes were formulated by mixing cement precursor (α-Ca3(PO4)2) to a self-reticulating hydrogel (silanized hyaluronic acid) supplemented with a viscosifier (hydroxymethylpropylcellulose). Patient-specific scaffolds were 3D-printed by robocasting and disinfected in absolute ethanol for 3 days prior to surgery. Before implantation, the scaffolds were rehydrated ~30min in saline, then soaked with autologous bone marrow. Surgical intervention included both the reconstruction of soft tissues and the insertion of the soaked scaffold. Bone formation was assessed 3 and 6 months after reconstruction via microcomputed tomography, and a biopsy was also performed at 6 months.

Results: Scaffolds displayed great handling properties and were inserted within bone cleft without significant issue. Osteointegration of the scaffolds was observed after 3 months, and regeneration of the cleft at 6 months seemed quite promising. In several cases, dehiscence occurred two weeks post-surgery.

Discussion: Preliminary results have demonstrated a potential of the set-up strategy to treat cleft lip/palate deformities in clinical practice. Surgery strategy was turned into two operations to match human clinic procedure (soft tissues reconstruction, then cleft filling with personalized scaffold). Translation of these innovative scaffolds to orthopaedics is planned for a near future.

*Speaker

Keywords: calcium phosphate, hyaluronic acid, bone
Patient specific scaffolds for osteo-gingival regeneration

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Tooth loss represents a significant oral health issue impacting both physiological functions and aesthetics. Dental cavity and periodontal diseases such as gingivitis and periodontitis are major causes of tooth loss. Risks include both extrinsic (e.g., oral hygiene, smoking) and intrinsic (e.g., age, genetics) factors. Standard of care to replace a missing tooth rely on dental implants, which are widely recognized as a highly effective solution for restoring complete oral functions. Unfortunately, if tooth replacement cannot be performed in due time, the absence of mechanical stimulation leads to physiological bone resorption and gum recession. In such cases, osteo-gingival augmentation becomes necessary. Although various clinical solutions exist, they often present significant drawbacks hampering their widespread application.

To improve current bone augmentation procedures, we aim in the development of a personalized 3D-printed bi-materials osteo-gingival scaffold. An innovative organo-mineral bone cementitious scaffold (“bone compartment”) will be placed in apposition with the atrophied bone. Its architecture and composition will be optimized to set up a bone substitute that hardens in-situ in order to guide and stimulate bone formation. The ”gingival” compartment, cohesive to the synthetic bone scaffold, will act as a protective barrier, shielding the surgical site from the buccal environment and supporting the closure of gingival tissue.

This research project, including my Ph.D thesis, goes from physico-chemistry to in vivo assays, with the final aim of proposing clinically relevant proof of concept to treat mandibular atrophy.

Keywords: osteo-gingival regeneration, additive manufacturing, multimaterials

*Speaker
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CHARACTERIZATION OF THE OSTEOCHONDRAL JUNCTION FOR THE DEVELOPMENT OF BIO-INKS DEDICATED TO THE REPRODUCTION OF THIS ANCHORING ZONE IN THE TREATMENT OF FOCAL OSTEOCHONDRAL LESIONS BY TISSUE ENGINEERING

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Context. Spontaneous tissue healing of focal osteochondral defects does not provide tissue with the same properties as the native one, which makes the repair non-durable. Tissue engineering aims to repair these injured tissues. One of the pitfalls is the difficulty of reproducing a full implant considering this tissue’s composition and architecture (non-calcified cartilage, calcified cartilage, and subchondral bone). Newly developed implants must integrate the complexity of this tissue and the pivot zone with the subchondral bone to promote successful grafting. The multi-layered biomaterial will be obtained by bio-extrusion’s 3D printing. This process enables the implant to be printed in a single piece and allows the composition of the bio-ink to be topographically adapted. To better prepare the composition of the bio-inks, we decided to further characterize the organization and composition of this osteochondral junction (OJ) from patients undergoing knee surgery.

Methods. Osteochondral plugs from the patella, femoral condyle, and tibial plateau have been collected and characterized, with a special attention to the OJ. The histological assessment has been compared to radiologic scores and an analysis of the layers forming the OJ was performed. Based on the histological analysis, we could propose bio-inks enriched with ECM molecules, or minerals, as well as biological properties.

Results. Work was initiated on ten knees using histologic staining. The results indicated a

*Speaker
good correlation between the Kellgren-Lawrence score, and the modified Mankin’s score (MS). For similar features in the OJ, we have not noticed an apparent correlation between the MS and the changes in the OJ. However, the criteria will be refined to make it easier to identify common modifications.

Concerning the bio-inks and based on these observations, we have been able to develop bio-inks dedicated to produce the subchondral bone plate-calcified cartilage junction zone (SBP-CCJZ), which can induce a specific biochemical behavior of MSCs embedded in the bio-ink. Scaffolds were analyzed with histological staining/immunostaining (collagen II), and significant changes were observed in ECM biosynthesis, according to culture conditions. **Conclusions.** The MS provides some evidence that the organization of the cartilage and subchondral bone does not correlate with the pathological evolution of OJ. However, a correlation is evident between the Kellgren-Lawrence score and the MS. In the context of reproducing SBP-CCJZ, the results indicate that a detailed analysis of OJ is required to design bio-inks to produce a suitable anchoring zone for treating focal osteochondral defects.

**Keywords:** Osteochondral joint, 3D printing, biomaterials
Thursday, January 18th, 2024

MatSAN Session 1

Chairs:
Jonathan LAO, Nathalia ODERICH MUNIZ
CORROSION OF METALLIC IMPLANTS: ABOUT CHALLENGES AND INNOVATIVE THERAPIES

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Keywords: Biocorrosion, implants, surface oxides

ABSTRACT

Interactions of implanted materials and devices with the human body are still largely unknown and their detrimental effect on the material stability and body responses are sometimes clearly underestimated, evident from a raising number of failure reports. On the other hand, the medical field relies increasingly on the use of medical materials for various therapies ranging from implants for fracture fixation or cardiovascular intervention to progressively advanced medical devices including those for monitoring and sensing. In fact, considering the current understanding of the material-biology interface interactions, it is evident that macroscopic aspects such as fractured implants or material-particle release, are already documented. There is, however, a clear lack of understanding of the processes that underlie the formation of micro- and nanoscale corrosion products and ionic leaching, as well as their consequences (see Figure 1).

In this overview presentation, we will start with examples of materials of concern that still require special attention in terms of understanding their corrosion mechanisms in physiological environments. The use of Co-Cr-Mo alloys has already been restricted for some larger implant applications where considerable wear is expected, but the risk of ionic leaching is still mostly disregarded for other implantable applications. Reliable testing protocols, discussed throughout the presentation, are required that reflect the dynamic, aggressive local chemical environment that cells and tissues create (e.g. large pH modulation in wound and inflammation conditions). These testing protocols need to provide the required level of detail, i.e. specification of the relevant sensitivity and magnification at which material dissolution may occur.

Not only bulk materials (e.g. synthesized through new production methods such as additive manufacturing) might still be problematic, but also implant coatings need to be designed properly. The example of Diamond like Carbon (DLC) coating will be presented with the focus on interface degradation and in particular of the chemical stability of silicon, a topic also relevant for the silicon based implanted device. Finally, in the field of degradable implants, the positive use of metallic materials (Mg and its alloys) corrosion will be presented and discussed for various applications. The whole presentation is organized around future topics of a task force of the European Federation of Corrosion (EFC) that will briefly be introduced.
**Figure 1:** Scientific questions needing to be addressed in relation with implant and devices materials corrosion. Link to the European Federation of Corrosion (EFC) task force: [https://efcweb.org/TF_Corrosion_of_Medical_Implants_and_Devices.html](https://efcweb.org/TF_Corrosion_of_Medical_Implants_and_Devices.html)
AMINO-BASED COATINGS FOR IMPROVING IMPLANTS’ TISSUE INTEGRATION

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In industrialized nations, osteoporosis is becoming increasingly widespread. Therefore, it is critical to design bioactive orthopedic implants that can better integrate into osteoporotic bone tissue. Coating materials with an amino-rich layer is one of the methods utilized to create bioactive implants (1-4). In this study, we examined the response of MG-63 osteoblast-like cells and the adsorption of proteins onto different amino-based coatings. We prepared three types of amino-based coatings and they are listed as follows: (1) positively-charged trimethoxysilylpropyl modified poly(ethyleneimine) (Ti-TMS-PEI) on titanium (2), (2) self-assembled monolayers with different and methyl densities on 1 x 1 cm silicon wafer substrates fabricated using a protocol that involves silanization using bromine-terminated silane, SN2 substitution of bromine to azide, and reduction of azide to amine (3), and (3) thin films prepared by plasma polymerization of allylamine on 1 x 1 cm titanium-coated flat and micro-grooved silicon wafer substrates (4-6). The physico-chemical properties of the amino-based coatings were characterized by water contact angle measurements, Zeta potential, and X-ray photoelectron spectroscopy (XPS). Cell spreading and cellular abrogation assay and protein adsorption experiment were performed by immersing the samples in aqueous solutions containing BSA, then the adsorbed BSA on the surface were desorbed using phosphate buffered saline (PBS) and sodium dodecyl sulfate (SDS). The solutions from the adsorption, washing, and desorption were analyzed by size-exclusion high-performance liquid chromatography (SE-HPLC). Results show varying cellular contact guidance abrogation, cellular spreading, and protein adsorption properties, depending on the quantity and quality of amine groups on the surfaces.

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Keywords: bioactive implant, tissue integration, amino coatings
HYBRID PARTICLES FOR THE PHYSICAL TREATMENT OF THROMBOTIC DISEASES

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Thrombosis is responsible for most strokes and heart attacks, which are the two leading causes of death worldwide (1). Reperfusion is commonly performed with thrombolytics such as recombinant tissue plasminogen activator (rt-PA) but these present numerous limitations (severe adverse effects, few eligible patients, low recanalization rate). With the objective of designing a safer and efficient treatment, we propose here to have a targeted physical action on the thrombus. This non-pharmaceutical thrombolysis would be a combination of thermal and mechanical action, through light and magnetic stimulation, and would be targeted to the occluded vessel by an external permanent magnet (2,3). To achieve this strategy, we designed hybrid organic/inorganic particles that are stable, intravenously injectable, and have great photothermal and magnetic properties. We confirmed the hybrid structure by electronic microscopy and FT-IR spectroscopy. Then, we characterized the size and charge of these new microsystems. Additionally, we studied their stability in saline solution and in time. Magnetization measurements and particle counting were performed to compute the magnetic moment of these hybrid particles. Also, their response to magnetic stimulation was verified. The magnetic evaluation was completed with photothermal tests to estimate their ability to increase temperature when irradiated by a near-infrared laser. Finally, we were able to develop fluorescent hybrid particles and showed that they could efficiently target thrombi in vitro, even under venous or arterial flow, through a magnetic field. In the coming months, we will study the thrombolytic effect of these photo- and magneto-stimulated hybrid particles on ex vivo blood clots. When the therapeutic dose of these microsystems is determined, we will carry out cytotoxicity tests in this range. References:

Keywords: hybrid particles, non pharmaceutical treatment, photothermia, magnetism, mechanical

*Speaker
action, targeting, thrombolysis

**Figure:** Graphical abstract
ATP/COLLAGEN COACERVATES AS NEW PRECURSORS FOR BIOPRINTED DENSE COLLAGEN MATRICES

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Beyond their prevalence in tissues, collagens, and in particular type I collagen’s low immunogenicity and biocompatibility have sparked increasing attention in tissue 3D printing. Despite the potential to play a major role in tissue engineering, research on 3D printing type I collagen has been restricted by its poor mechanical properties and use at sub-physiological concentrations. Attempts to overcome these limitations have proven unsuccessful, because highly concentrated collagen solutions are not compatible with the 3D printing process, due to its increasing viscosity.

Here, we introduce a new strategy for formulating highly concentrated collagen bioinks with drastically reduced viscosity able to bypass current limitations in 3D bioprinting type I collagen. Using adenosine triphosphate (ATP), we can induce a liquid-liquid phase separation, termed simple coacervation, where collagen molecules aggregate and adopt a stacked and aligned rod-like molecular organization in the form of Segment Long Spacing (SLS). This supramolecular arrangement results in a suspension of concentrated collagen coacervates suspended in a solution virtually depleted of collagen, easily adjusted to high concentration by simple centrifugation. To evaluate the potential applicability of the coacervates bioink for in vitro tissue modeling, its mechanical, structural and cell hosting properties were analyzed and compared with conventional collagen gels prepared from acidic solution ink in the same concentration range. Rheological tests were conducted to assess the printability potential and microscopic analysis was used to investigate collagen distribution, organization and orientation from molecular to a supramolecular level in fibrillated constructs printed using the new formulation. Moreover, the biological compatibility of this bioink was correlated to the viability, proliferative status, morphology and migratory ability of normal human dermal fibroblasts (NHDF) in a dense collagen matrix during 14 days of culture.

The results obtained are of great interest in the design of bioprinted dense collagen matrices able to act as hierarchical host materials for biomedical applications. Moreover the use of the new collagen bioink formulation allows us to print the highest collagen concentration reported in literature with spatial control and the ability to preserve the native collagen self-assembly process.

Keywords: 3D bioprinting, Type I collagen, Segment Long Spacing (SLS)

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Utilisation de la technique de la membrane induite de Masquelet en médecine personnalisée : évaluation du silicone et analyse statistique de fémurs de rats.

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La technique de la membrane induite en deux temps opératoires imaginée et popularisée par le Pr AC Masquelet a révolutionné la chirurgie de reconstruction des défauts osseux. Le succès de cette chirurgie repose sur l’implantation transitoire d’un ciment osseux de type polyméthylméthacrylate (PMMA) dans la zone de fracas. La présence du ciment PMMA déclenche une réaction immunitaire de la part de l’hôte conduisant à la formation d’une membrane (appelée “membrane induite de Masquelet”) aux multiples propriétés ostéogéniques favorables à la régénération osseuse. Les besoins cliniques actuels tendent vers de la médecine personnalisée afin d’améliorer la prise en charge des patients et d’adapter les soins en conséquence. Pour ce faire, différents axes de recherches existent. La recherche de nouveaux matériaux inducteurs de membrane est primordiale afin de pallier les inconvénients intrinsèques au PMMA et permettant également leur utilisation en impression 3D. Afin d’utiliser cette dernière, il est nécessaire d’avoir une bonne compréhension de la morphologie des os afin de pouvoir axer le développement de nouveaux outils pour la mise en place de cette stratégie chirurgicale. Les travaux présentés porteront sur l’évaluation du silicone en tant que matériau alternatif au PMMA ainsi que le développement d’outils d’analyses statistiques de la morphologie de fémur de rat.

Mots-Clés: Chirurgie orthopédique, Technique de la membrane induite de Masquelet, silicone, PCA
Thursday, January 18th, 2024

MatSAN Session 2

Chairs:
Yohann WITTRANT, Marjorie DUFAUD

MatSAN Session 2-bis

Chairs:
Benoit TER OVANESSIAN, Sacha BARBERAT
Investigating the influence of bioactive glass 92S6 P123 on 3D-Printed scaffold fabrication

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The use of additive manufacturing techniques for scaffold fabrication has shown remarkable potential in tissue engineering and regenerative medicine. In this study, a novel approach involving a composite material of bioactive glass 92S6 P123 with polylactic acid (PLA) was explored to create intricate three-dimensional (3D) scaffolds. The main objective was to analyze the impact of incorporating bioactive glass 92S6 P123 on the structural properties of 3D-printed scaffolds, subsequently optimizing the architectural design (grid versus gyroid), pore size, and porosity in order to obtain the best compromise between mechanical properties and porosity for sufficient and efficient cell colonization. The selected scaffold architecture, the gyroid, was carefully tailored to accommodate optimal mechanical support and cell proliferation. The outcomes of this study shed light on the significance of incorporating bioactive glass 92S6 P123 within the 3D-printed scaffolds. The findings highlight the enhanced potential for osteogenesis and osseointegration owing to the bioactivity of the glass component. Moreover, the tailored scaffold architecture exhibited promising results in terms of mechanical stability and cellular response. This research contributes to the evolving field of scaffold design for tissue engineering applications, offering insights into the interplay between scaffold composition, architecture, and in vivo performance.

Keywords: bioactive glass, sol, gel, additive fabrication

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Design of a biodegradable bone cement for the treatment of bone fractures induced by breast cancer metastases

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Keywords: Cement, nanofibers, bone metastasis.

ABSTRACT

In 20 to 30% of breast cancer, bone metastases weaken bone tissue and elevate the risk of fractures. In the event of a fracture, bone substitute materials are utilized to repair the damaged tissue and provide mechanical support.

In this work, our main objective was to develop a new biodegradable calcium phosphate cement with enhanced mechanical and drug delivering properties through the incorporation of functionalized polymer-based nanofibers containing an anticancer agent (ACA) to inhibit the growth of metastatic breast cancer cells and promote bone formation. Biopolymer nanofibers, fabricated through electropinning, offer several key advantages, including affordability, ease of use, and high production yields. Moreover, fibers are characterized by a high specific area leading to a high assimilation of drugs presenting poor-solubility.

Figure 1.A displays the morphology of the electrospun nanofibers. Scanning electron microscopy (SEM) images revealed the presence of bead-free, well-defined, and uniformly structured fibrous nanofibers. After crushing at a microscopic scale, the electrospun fibers, loaded or not with ACA, were uniformly integrated into the solid phase (Figure 1.A). No significant changes on the crystallinity of the cement were observed by X-Ray diffraction (XRD) analysis. As demonstrated in Figure 1.B, the inclusion of fibers enhanced the toughness of the cement, evaluated using a standard three-point flexural test after immersion in phosphate buffered saline (PBS) for 72h. Other rheological and physicochemical properties (such as cohesiveness, injectability, setting time, etc.) were not significantly affected by fibers loading. In vitro cell viability tests were realised to evaluate the cytocompatibility of the biomaterial using hFOB 1.19 human fetal osteoblasts. Figure 1.C (top panel) demonstrates the absence of any cytotoxic effects of the biomaterials on human bone cells. In parallel, we validated the effectiveness of the ACA (after its elution from the cement) to inhibit the proliferation of estrogen-responsive MCF-7 human breast cancer cells (Figure 1.C bottom panel).

*Speaker
This work shows the great potential of functionalized fibers-reinforced cement to overcome their brittleness and to reduce the growth of breast cancer cells without cytotoxicity on bone cells. *In vivo* studies using a rat femur model are currently underway to evaluate the behaviour of the biomaterial after implantation in bone and its effect on bone formation.

REFERENCES


Keywords: Cement, nanofibers, bone metastasis

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**Figure 1** (A) Incorporation of the fibers into the cement and SEM images of electrospun nanofibers (B) Toughness of the cements (reference and loaded with ACA fibers or not) (C) *In vitro* biocompatibility and bioefficacy of the biomaterials on hFOB and MCF7 cells
APPLICATIVE POTENTIAL OF BIOPOLYMER PRODUCED BY MICROALGAE IN BONE REGENERATION

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Bone is able to self-healing throw a sequence of inflammation, ossification and remodelling events in which immune and bone cells communicate and interact to restore pre-traumatic anatomy. Despite these remarkable capacities, certain traumatisms or pathologies caused critical size bone defects that cannot be resolved it-self.

There is growing interest in the use of marine biopolymers as biomaterials, carriers, delivery systems or bioactive compounds for bone regeneration. The aim of this research was to evaluate the potential and safety of biopolymers derived from microalgae in bone healing. The great structural, biological variability and sulphate ratio on macromolecules excreted by microalgae depending on the strains, combined with similarity in composition to certain components of skeletal tissue, leaves the possibility that microagal polymers may have a positive effect on bone regeneration. However, the immune response to an implanted material plays a critical role in all clinical application. An excessive or chronic inflammation can prevent osteogenesis. For this reason, an identification of immunomodulatory properties has been performed by a quantification of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) produced by human monocytes treated with our molecular extracts. This analysis indicated a pro-inflammatory profile of the biopolymer related to lipopolysaccharides (LPS) quantified in this one using an original cell system (HEK-Blue™ LPS Detection Kit 2, Invivogen). Then, LPS were totally removed from the extracts following an alkaline treatment and molecules were characterized by size exclusion and ions exchange chromatography before and after treatment. Results showed that LPS-depleted

*Speaker
molecular extracts lost their pro-inflammatory capacity. However, the alkaline treatment induced a partial denaturation of structure and composition of the macromolecules, with a loss of 2-fold both initial molecular weight and sulphated compounds. Then, we tested osteogenic properties of the different extracts by measuring alkaline phosphatase (ALP) activity during the differentiation of mesenchymal stem cells into osteoblasts secreting bone matrix. Results showed that untreated extracts containing LPS promoted osteoblastic differentiation through a significant increase in ALP activity, 2-fold higher than LPS control. However, LPS-depleted extracts lost osteogenic potential probably due to structural modification induced by alkaline treatment. This work has shown that microalgal biopolymers may have osteogenic potential, but GRAM-negative bacterial contaminants present in microalgal culture, which are potent inflammatory factors, must be absent for any clinical application. Ideally, bacterial contamination should be avoided during microalgal growth, as alkaline treatment used to remove LPS induces macromolecule size and sulphation reduction.

**Keywords:** Biopolymer, Microalgae, Bone regeneration

**Bone Health PhD project**
Antimicrobial and antibiofilm activity of peptide-functionalized hydrogels

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The rising prevalence of chronic wound infections, compounded by the emergence of antibiotic-resistant bacterial strains, presents an urgent and growing global health concern. Conventional antibiotic approaches are impeded by biofilm formation, rendering them ineffective against chronic infections. Consequently, there is a critical need to explore alternative strategies to combat biofilm-related chronic wound infections. Our research addresses this pressing need by focusing on the evaluation of antimicrobial peptides’ efficacy against preformed biofilms and the potential enhancement of treatment outcomes using hyaluronic acid hydrogels loaded with the same antimicrobial peptide. Specifically, we investigated the antimicrobial peptides Poly(arginine)30 (PAR30) and Poly-c-Lysine (c-PLL), both in solution and incorporated within hydrogels, against preformed biofilms of Gram-positive and Gram-negative pathogenic bacteria. Our findings illuminate the potent antibiofilm properties exhibited by hyaluronic acid (HA)-based hydrogels when loaded with these antimicrobial peptides compared to peptides in solution. These hydrogels effectively combat biofilms, demonstrating strong antibiofilm potential. Our study, conducted in both static and dynamic conditions mirroring real-world scenarios, sheds light on their practical use in treating chronic wound infections and combating antibiotic resistance in medical settings.

Keywords: Hydrogels, Bacterial biofilms, Antimicrobial peptides
DEVELOPMENT OF A TOPICAL PLATFORM FOR THE DELIVERY OF NATURAL BIOACTIVE COMPOUNDS AND STAPHYLOCOCCUS AUREUS PHAGES FOR DERMAL APPLICATIONS

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Perturbations of the skin functions are related to many factors, such as high oxidative stress and microbial dysbiosis that, in some cases, may lead to infections and formation of biofilms on the skin surface. Combination therapy based on antioxidants and antibacterials may be a key strategy to consider for the treatment of dermal pathologies.

Curcumin (CUR) is a multi-targeting pharmacologically active compound, known, among others, for its antioxidant properties. However, the hydrophobic nature and the unstable structure of curcumin limit its capacity to penetrate the skin. A strategy to deliver curcumin is to encapsulate it in nanostructured lipid carriers (NLCs), to preserve its antioxidant properties. Lytic phages are viruses that specifically target and infect bacteria, reproducing within the bacterial cells and ultimately causing their destruction. The use of phages is particularly relevant in the context of the increasing prevalence of multidrug-resistant pathogens, where conventional antibiotics may be less effective.

Herein, we propose in this work a bi-functionalized platform for the delivery of curcumin encapsulated in nanostructured lipid carriers (Curcumin-loaded NLCs) and Staphylococcus aureus phages, both entrapped in a hydrogel protective matrix for a topical application. A suspension of NLCs (mainly 85 nm in size) was produced by hot homogenization method and characterized by Dynamic Light Scattering (DLS) and Z-Potential. Entrapment efficiency of CUR in NLCs were assessed by spectrophotometry and more than 50 % of CUR has been encapsulated. A stock of 10°10 UPF/mL of phages GRCS was prepared with the double-layer agar plating method.

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Infectivity studies reveal that the presence of Curcumin-loaded NLCs does not compromise the lytic activity of *Staphylococcus aureus* phages with

First results showed that the combination of Curcumin-loaded NLCs and *Staphylococcus aureus* phages represents a promising strategy for the treatment of skin associated diseases and cutaneous wound.

Acknowledgements: This work was funded by CY Initiative of Excellence (grant "Investissements d'Avenir" ANR-16-IDEX-0008") and by MCIN/AEI/10.13039/501100011033 and by ERDF A way of making Europe (Grant PID2021-128213OB-I00).

**Keywords:** Staphylococcus aureus phage, Nanostructured lipid carrier, Hydrogel
SITE-SPECIFIC DRUG DELIVERY: INNOVATIVE DEVICE FOR MITIGATING PELVIC RADIOTHERAPY SIDE EFFECTS IN THE COLON

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Pelvic Radiation Disease (PRD), an inflammatory condition affecting the bowel, commonly arises as a side effect of radiotherapy for pelvic cancers, including prostate cancer, bladder cancer, gynecologic cancers, rectal cancer, and lymphoma. Five to 15% of patients undergoing pelvic radiotherapy will exhibit clinical manifestations of radiation colitis. The current treatment primarily focuses on providing symptomatic relief, pausing radiotherapy, and, in severe cases, resorting to surgical intervention. With the growing population of cancer survivors, there’s an increasing need to mitigate colorectal complications resulting from pelvic radiation treatment. In response, this study introduces an innovative approach by developing a biodegradable, three-layered, self-rolling biomedical device specifically designed for drug encapsulation and site-specific release (Fig.1A).

The initial steps involved the preparation of photo-crosslinkable hydrophilic and hydrophobic poly-(ethylene glycol)-poly(lactide) (PEG-PLA) star-shaped copolymers to create cytompatible and degradable bilayered films that self-roll in water within 30 seconds (1). Subsequently, these films unrolled in the ulcerated region within the colon during colonoscopy deposit (Fig.1B). They were then loaded with an anti-inflammatory agent, specifically prednisolone. Optimal concentrations for film loading, as well as the time, speed, and direction of anti-inflammatory release, were determined (Fig.1C). In vitro tests evaluated the efficacy of the released anti-inflammatory, inhibiting pro-inflammatory cytokines in macrophages stimulated by E. coli (2). Additionally,
a third catechol bioadhesive layer was incorporated (3), preserving self-rolling properties.

Subsequently, we designed a non-surgical colonoscopy-based treatment to test the self-rolled films in an *in vivo* setting. A rat model underwent fractionated pelvic irradiation of 3x10 Gy, simulating clinical radiotherapy. Our primary focus was to understand the kinetics of the inflammatory phases in the colon within this model and strategically determine the optimal timing for introducing the self-rolled, anti-inflammatory loaded patches (Fig.2A).

Our findings indicate the peak of the colon’s inflammatory process occurring on the fifth day following the final irradiation fraction (Fig.2B-C). The study successfully demonstrated the feasibility of patch implantation via colonoscopy in non-irradiated rats, affirming the devices’ non-toxic nature and easy local deposition within the colon (Fig.2D). Future study goals include evaluating the impact of localized patch implantation in resolving the inflammatory process in the irradiated rat model.

By focusing on an innovative approach of implanting bioresorbable patches, we aim to ameliorate colorectal damage, contributing to enhanced patient welfare during and after pelvic radiotherapy.

REFERENCES


**Keywords:** Colorectal Inflammation, Medical Device, Colonic Implantation
Oral presentations MatSAN Session 4 - Friday, January 19th, 2024

Fig. 1 - (A) Experimental design of ANR-OPENN: CONception of bioresorbable self-rolled Patches for the local trEatment of inflammatioN induced in the coloN after irradiation. (B) Degradable bilayered self-rolling films. (C) Prednisolone release.

Fig. 2 - (A, B) Epithelial irradiation lesions. (C) Inflammatory phases kinetics. (D) Patch insertion in the colon.
AN INNOVATIVE PROCESS TO REVEAL WHARTON’S JELLY POTENTIAL

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Wharton’s jelly is a gelatinous connective tissue surrounding umbilical vessels in the umbilical cord of mammals. It is rich in glycosaminoglycans, proteoglycans, collagen fibers, and growth factors. Thanks to all its components, Wharton’s jelly is known to improve the biological processes of healing and reducing inflammation. At TBF, umbilical cords are sampled according to directive 2006/17/CE. The vein and the two arteries are removed from the umbilical cord and the umbilical cord lining containing Wharton’s jelly is treated using a viral-inactivating chemical treatment to ensure its safety. This treatment does not alter the proteins constituting Wharton’s jelly. Then, Wharton’s jelly is ground and can be molded into different forms thanks to its viscous properties. The molded forms are freeze-dried. This freeze-drying step allows to remove water bound to proteoglycans and to the collagen and elastin fibers of Wharton’s jelly to rearrange. The moisturizing properties of proteoglycans are preserved through freeze-drying. Thus, a molded flexible form with a foamy appearance presenting a cohesive texture is obtained, and this form can recover in volume when exposed to water or other solutions. This last property gives absorption and trapping properties to the product, allowing to use it as a long-term releasing matrix. After freeze-drying, Wharton’s jelly can be further ground to form a powder (1). Thus, using this process with or without grinding after freeze-drying, Wharton’s jelly may be used in a large variety of forms: as a gel, in a molded form, bound to umbilical cord lining, or as a release matrix for active substances. Therefore, we developed products for various applications: a plug to treat anal fistulas (SygeLIX-F) made of a molded form of Wharton’s jelly with a guide of umbilical cord lining (Figure 1a), a gel to treat complex anal fistulas (SygeLIX-G) made of a powder of Wharton’s jelly which can be reconstituted to form a gel at the time of surgery (Figure 1b), and eye drops to treat corneal ulcers or ulcerative keratitis (SygeLIX-Coll-T) made of Wharton’s jelly mixed with trehalose (Figure 1c). This last product can be loaded with active substances to gradually release them. All these products are currently in clinical trials.

Figure1. Different products made from Wharton’s jelly. a) SygeLIX-F. b) SygeLIX-G. c) SygeLIX-Coll-T.

*Speaker
References


Keywords: Wharton’s jelly, allograft, extracellular matrix

Figure1. Different products made from Wharton’s jelly. a) SygeLIX-F. b) SygeLIX-G. c) SygeLIX-Coll-T.
Friday, January 19th, 2024

MatSAN Session 3

Chairs:
Christophe DROUET, Chloé DUJARDIN
PROCESSED INVERTED HUMAN UMBILICAL VESSEL AS NERVE REGENERATION CONDUIT IN THE TREATMENT OF HAND NERVE SECTION

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Treatment of peripheral nerve injury, particularly those with a gap, remains a challenge. Recovery of finger sensitivity is often incomplete and can interfere with personal and occupational activities. The need for better regeneration outcome has given rise to the development of alternative treatments such as nerve conduits (1). A multicenter, open, and prospective clinical trial was conducted to evaluate the safety and efficacy of a conduit of freeze-dried inverted human umbilical vessel in the regeneration of hand nerve sections (Figure 1) (2).

Patients between 18 and 65 years old with hand nerve section of 2 mm to 20 mm with static 2-point discrimination (s2PD) > 15 mm had to be included. The primary objective was the recovery of the sensibility of the hand 12 months after conduit implantation defined by s2DP ≤ 8 mm. Secondary objectives were the evaluation of the clinical tolerance and of the functionality of the hand. Evaluations were performed at 1, 3, 6, and 12 months.

Twenty-four lesions of 23 patients with mean nerve gap of 6.22 mm (2; 30) were included. At 12 months, primary objective, s2PD ≤ 8 mm, was achieved by all but 2 patients (one had chronic alcoholism with peripheral neuropathy, the other had nerve gap of 30 mm) (p-value < 0.001). Complete innervation was recovered by 88% of patients. Pressure sensation and quality of life related to the hand significantly increased while symptoms due to nerve section (pain, cold sensation, hypoesthesia, hyperesthesia) decreased to almost zero. No safety issue related to the nerve conduit were reported.

Thus, this prospective clinical trial showed that processed inverted human umbilical vessel is a safe and effective option as conduit for hand nerve regeneration.

References

*Speaker
Oral presentations MatSAN Session 4 - Friday, January 19th, 2024


Keywords: Nerve, allograft, umbilical vessels

Figure 1. Macroscopical structure of the inverted vessel product
POLY(BETA-AMINO)ESTER ELECTROSPUN WOUND DRESSING WITH MODULATED DEGRADATION KINETICS FOR CHRONIC WOUNDS TREATMENT

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Chronic wounds show no healing tendency after a period of up to 4 to 6 weeks despite any local treatment. This wound presents a persistence of the inflammatory stimulus and a high sensitivity to bacterial infection. Engineering innovative wound dressing is a key to facilitate the wound treatment, reduce healing time and prevent recurrent infections. In this context, this project focused on the design of electrospun wound dressing based on Poly(Beta-amino)esters polymers (PBAE) and loaded with an antibiotic (ciprofloxacin, CFX). The release kinetics of CFX was studied by varying the structure of PBAE polymers and therefore their biodegradability rates. PBAE macromers were synthesized through bulk Michael addition of Poly(ethylene glycol) diacrylate (Mn= 575 and Mn= 250 ) and Isobutylamine at 90°C for 72H and characterized by NMR and SEC2. The electrospinning of PBAE macromer, Poly(ethylene oxide) (900 kDa, PEO), 2-dimethoxy-2- phenylacetophenone (DMPA) (mass ratio of 73:27 PBAE:PEO) and CFX solutions in N,N- Dimethylformamide (DMF) was optimized and followed by UV-crosslinking for 120 min. Fiber diameters were quantified using ImageJ (v1.42q, NIH) on SEM micrograph (FLEXSEM1000). Degradation (n=6) of fibrous samples was performed in phosphate-buffered saline (PBS) at 37°C for up to 96H. The CFX release from fibrous mats was studied under dynamic conditions using a USP apparatus 4 (Sotax®). The in vitro cytotoxicity of fibers was assessed with a NIH3T3 cell line, according to ISO 10993-5 standard, with extraction method by AlamarBlue® Assay.

We successfully synthesized viscous liquid PBAE macromer with two different PEGDA leading to two different molecular weights (av. 1900 and 2300 Da) and hydrophilicities. SEM images of electrospun PBAE membranes confirmed the formation of homogeneous and defect-free fibers with micron scale diameters (Figure 1). UV post-treatment was successfully monitored using FTIR analysis and led to reduced degradation rates in PBS (~70% mass loss after 48h). The cytocompatibility of membranes was proved by cell viability (> 70% with regard to control) of NIH3T3 cells. Release assays confirmed the different kinetics relative to the PEGDA used.

*Speaker
A promising biocompatible fibrous membrane with suitable degradation and release kinetics was developed. This PBAE/PEO membrane could be used as bilayered wound dressings for the release of two different drugs with distinct time scale release kinetics.


Keywords: Electrospinning, Wound dressing, Wound Healing, Chronic Wound, Poly(beta amino)ester, PBAE

Figure 1- SEM Image of electospun PBAE fibers
Development of a Biphasic Osteochondral Model for Joint Tissue Repair using Extrusion-based 3D Bioprinting of a Natural Composite Hydrogel

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Extrusion-based 3D bioprinting is a promising technique to produce complex, three-dimensional (3D) structures that mimic the native extracellular matrix (ECM), which is pivotal for developing advanced tissue engineering solutions. Composite hydrogels, which are combinations of two or more distinct materials, can be used as a printing medium that offers several advantages over a single biomaterial due to their tunability, including improved mechanical properties and biocompatibility. These materials can be engineered to have specific physical, chemical, or biological properties, making them useful for a variety of applications. Extrusion-based 3D bioprinting of these hydrogels has generated high expectancy for joint tissue engineering, in particular for cartilage and bone repair. Here, we report the development of a 3D bioprinting process for the generation of biphasic 3D constructs for osteochondral repair of full-thickness joint lesions.

The bioink used in this study is based on a natural composite hydrogel developed at 3d.FAB Platform (Villeurbanne, France) comprising of gelatin (5%), alginate (2%), and fibrin (2%) loaded with murine mesenchymal stromal cells (mMSCs) expressing the differentiation factor BMP-2 under inducible conditions – a factor critically involved in both chondrogenesis and osteogenesis. Following printing using the BIO X 3D printer (CELLINK, Sweden) and crosslinking with a solution of transglutaminase/CaCl2/thrombin, the 3D bioprinted constructs were cultured individually under proliferation, chondrogenic or osteogenic conditions for a period of 28 days in order to optimize the culture conditions for enhanced cell differentiation.

Our findings showed: (i) high cell viability (> 90%) of mMSCs across all conditions using the Live/Dead assay; (ii) sustained cell proliferation using the PrestoBlue Proliferation Assay; and (iii) cell differentiation towards either the chondrogenic or osteogenic lineages as shown by the up-regulation of specific differentiation markers by RT-qPCR. Cell differentiation was further analyzed at the protein level using immunofluorescence labelling: type II collagen and aggrecan for cartilage; osteocalcin for subchondral bone. Finally, a biphasic construct comprising a cartilage compartment and a subchondral bone compartment was printed in an “all-in-one”

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strategy and analyzed by immunofluorescence following different culture conditions. It showed the feasibility of printing complex cell-laden 3D constructs with different composition along the depth.

Overall, although some challenges remain to be overcome, our approach of 3D bioprinting holds great promise for the development of an improved osteochondral repair strategy for joint tissue injuries.

**Keywords:** Bioprinting, Osteochondral repair, MSCs
CHITOSAN-GRAFTED-FIBRONECTIN FOR BIOACTIVE THERMOSENSITIVE HYDROGELS

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Chitosan (CS) solutions buffered with sodium β-glycerophosphate (βGP) are thermosensitive liquids having a gelation point near 37°C, a property interesting for cell encapsulation and delivery using 3D scaffolds. Recently, a formulation platform using βGP and ammonium hydrogenophosphate (AHP) has been developed in our lab, providing cyto-compatible macroporous hydrogels with both physiological pH and osmolarity. However, these hydrogels exhibit a poor bioactivity, attributed to the lack of interactions between the cells and the chitosan scaffold. This issue could be overcome by decorating the surface of the macropores with adhesion proteins, such as fibronectin (Fn). In this work, we graft CS (Mw 250 kDa) and Fn (Mw 550 kDa) using carbodiimide chemistry, leading to CS-g-Fn. The grafting was also conducted using CS and Fn labelled with different fluorescent probes. Time-sweep measurements demonstrate that both CS-g-Fn and unmodified CS have similar gelation kinetics and mechanical properties. Using confocal microscopy, we compared the morphologies of CS hydrogels containing CS-g-Fn, and CS hydrogels simply mixed with Fn. By quantifying the colocalization of fluorescently labelled CS and Fn, we show that Fn is immobilized in the CS network containing CS-g-Fn, whereas it is not in the mixture of CS and Fn. Image analysis using the morphological sieving technique shows a wider pore distribution for the CS-g-Fn hydrogel compared to the unmodified CS hydrogel. Bioactivity was assessed by encapsulating Tomato (+)-MPNST cells in CS or CS-g-Fn hydrogels. After 48h culture already, a significantly larger number of cells present cytoplasmic extensions in the CS-g-Fn hydrogel as compared to the CS hydrogel (Figure a and b). This study demonstrates that Fn grafting provides an efficient way to improve the bioactivity of chitosan hydrogels while preserving their thermosensitivity and macroporous structure.

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**Keywords:** Thermosensitivity, Biofunctionalization, Hydrogel

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**a)** Tomato (+)-MPNST cells (red fluorescence) encapsulated in CS or CS-g-Fn hydrogels after 96h of culture (fluorescently-labelled CS* appears with green fluorescence). Arrows indicate cytoplasmic extensions. **b)** Percentage of cells with cytoplasmic extensions in both gels after 2 and 4 days of culture.
A degradable nanofibrous scaffold of poly(ε-caprolactone-co-lactide) for intervertebral disc regeneration

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The intervertebral disc (IVD) consists of a gelatinous core (nucleus pulposus, NP) surrounded by a fibrous ring (annulus fibrosus, AF). IVD herniation is a major cause of low back pain, affecting 90% of people in their lifetime. Discectomy, which involves surgically removing the herniated NP, effectively relieves pain. However, it does not address the underlying injury to AF, which can lead to re-herniation and accelerated disc degeneration. Here, we designed a dual-repair approach targeting the NP with the injection of a commercially available hyaluronic acid gel, and the AF by implanting a bio-inspired multi-lamellar scaffold with oriented microfibres, a self-anchoring system, and controlled biodegradability to promote the regeneration of a spatially organized AF tissue. To this aim, we synthesized a novel poly(ε-caprolactone-co-lactide) (PCLA) with various molar percentages of lactide (10, 20, and 30 %) and structures (copolymer or blend) that were electrospun into sheets of aligned nanofibers. Thermal, mechanical, and structural properties were analyzed, showing a Young’s Modulus ranging from 20 MPa to 37 MPa, close to that of the targeted AF tissue. In vitro degradation of gamma-sterilized PCLA showed a molar mass loss of 70 ± 0.4% after 6 months, associated with visible fiber breakage, which could facilitate the deposition of newly formed AF tissue over time. Furthermore, we demonstrated that PCLA sheets guided the in vitro alignment and proliferation of ovine AF cells and maintained the expression of AF markers for 4 weeks (collagen type I, type II and aggrecan). Finally, we produced a multi-lamellar 3D implant by stacking multiple layers of PCLA sheets and incorporating a self-anchoring system. We assessed the feasibility of maintaining this multi-lamellar scaffold within an annular defect (4mm biopsy punch) of a sheep IVD during 4 weeks of culture and analyzed cell infiltration and matrix deposition.

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Keywords: Intervertebral disc, poly(ε, caprolactone, co, lactide), Electrospining
Régénération diaphragmatique par bioimpression de microparticules pré-cellularisés

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La bioimpression 3D est un technique de fabrication additive innovante d’ingénierie tissulaire. Cependant cette technique rencontre certaines limitations notamment concernant la survie cellulaire post-impression. Nous avons mis au point une technique utilisant des microparticules poreuses servant de support de prolifération pour les cellules et permettant de les protéger des stress mécanique lors de l’impression. Dans le cadre d’un projet portant sur la hernie diaphragmatique congénitale, nous avons choisi d’imprimer des cellules musculaires et des fibroblastes en coculture avec ces microparticules sur un matériau biocompatible à base d’albumine. La combinaison de ces deux techniques, l’utilisation de microscaffold et l’impression sur ces biomatériaux, a permis une meilleure prolifération des cellules ainsi qu’une meilleure interaction entre les deux types cellulaires offrant la perspective d’une nouvelle solution d’ingénierie tissulaire de diaphragme.

Keywords: Diaphragme, Bioimpression, microscaffolds, coculture

*Speaker
Thursday, January 19th, 2024

MatSAN Session 4

Chairs:
Ana SFARGHIU, Mansoor CHAABAN
SURFACE MODIFICATION OF A 3D-PRINTED POLYURETHANE STENT-GRAFT FOR ANTI-THROMBOTIC PROPERTIES

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INTRODUCTION

In the context of patient-specific treatment of complex abdominal aortic aneurysms, a custom-made stent-graft (SG) was fabricated by 3D printing with medical-grade thermoplastic polyurethane (TPU) (1). A common complication after SG implantation is thrombosis in 10% of cases (2). The strategy for preventing blood coagulation and thrombosis was to immobilize heparin on the TPU SG. A preliminary treatment with polydopamine (PDA), a biocompatible and bio-inspired adhesive polymer, and polyethyleneimine (PEI) was used as a versatile platform for the immobilization of biomolecules like heparin. PDA will allow the coating’s adherence, thanks to catechol functions while PEI will provide surface amine groups to graft the heparin (3,4).

EXPERIMENTAL METHODS

TPU was immersed in a dopamine/polyethyleneimine solution (tris buffer 50 mM, pH = 8.5) to form a PDA/PEI coating (TPU-PDA/PEI). An immersion in heparin solution (1 g/L, PBS 1X, pH = 5.7, 400rpm, 24 hours) was performed to create an electrostatic bond between the carboxylic acid of heparin and amine groups on the TPU-PDA/PEI surface (TPU-PDA/PEI-Hep) (Fig 1). The functionalization process was evaluated at each step by colorimetric assay (Toluidine Blue and acid orange (5,6)), Fourier-Transform InfraRed spectroscopy (FTIR, Perkin Elmer), Scanning Electron Microscope (SEM, Hitachi) and water contact angle (KRÜSS).

RESULTS AND DISCUSSION

The deposition of the PDA/PEI coating is highlighted by surface analysis with a new peak at 3400-3500 cm⁻¹ on the FTIR spectra, corresponding to dopamine phenol. SEM also corroborates the presence of a homogeneous coating on the TPU’s surface, with polydopamine nanoparticles

*Speaker
trapped inside the coating. Colorimetric assay confirmed a minor change in the amount of catechol surface function (with respectively 0.66±0.18 nmol/cm² for TPU and 2.33±0.20 nmol/cm² for TPU-PDA/PEI), the increase of amine groups after the first step of functionalization (from 13.78±3.66 nmol/cm² for the TPU to 29.02±1.68 nmol/cm² for the TPU-PDA/PEI sample) and the presence of carboxylic acid group after the addition of the heparin layer.

CONCLUSION

This study confirmed the functionalization of the 3D-printed TPU prototype with heparin. Therefore, this prototype is ready for anticoagulant tests and biological evaluation to confirm its anti-thrombotic properties and cytocompatibility.

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ACKNOWLEDGEMENTS

This research was funded by the Métropole Européenne de Lille (MEL).

Keywords: 3D, printing, vascular stent, graft, functionalization
Fig 1. Functionalization process of a 3D printed stent-graft
Reactive CO2 laser sintering of powders to produce bioactive glassy scaffolds

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The use of additive manufacturing techniques for scaffold fabrication has shown remarkable potential in tissue engineering and regenerative medicine (1). This work describes a new method to produce bioactive glass using a CO2 laser as the unique heat source, from soda-lime silicate glass beads and/or oxide precursors explored to create intricate three-dimensional (3D) scaffolds. The formulations chosen for this study are all in the bioactive area of the Hench triangle, including the 45S5® composition (2).

Figure 1. Scaffolds made by powder bed fusion with CO2 laser

The scaffolds produced are characterised using conventional solid-state chemistry methods (XRD, DTA-TGA, SEM, Raman), as well as the quality of the produced objects will be studied as a function of the laser machine parameters (Power, scan velocity, hatch spacing) and the raw materials batch composition. The biocompatibility of CO2 laser sintered samples is evaluated by cytotoxicity tests and their behaviour in Simulated Body Fluid (SBF) medium.

(2) L.L Hench, Journal of Materials Science: Materials in Medicine, 17, 2006, 967-978

Keywords: Bioactive glass, additive fabrication, Selective laser sintering

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Figure 1. Scaffolds made by powder bed fusion with CO₂ laser
FORMULATION OF BIO-INKS DEDICATED TO SPECIFIC CARTILAGE LAYERS USING COLLAGEN AND HYALURONIC ACID ADDITIVES

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Hyaline articular cartilage is a tissue with limited repair capacity. Thanks to 3D printing, a specific layer-by-layer reconstruction of cartilage is possible by formulating bio-inks dedicated to the different specific layers of cartilage. We therefore assessed the effect of type I collagen and hyaluronic acid supplementation on the chondrogenic properties of human bone marrow-derived mesenchymal stem cells (MSCs). Collagen is known for its chondrogenic properties, and combined with alginate, it improves cell adhesion to the support and the expression of chondrogenic markers. Hyaluronic acid is a component of cartilage ECM. It also has interesting chondroinductive properties.

For this purpose, after expansion, MSCs were suspended in an alginate-based bio-ink containing different amounts of soluble collagen I (0, 0.5, 1 and 5 mg/mL of bio-ink) or hyaluronic acid (0 and 16 mg/mL). The substitutes were produced using a 3D printing by bio-extrusion and then polymerized. They were then cultured in control or TFG-β1-enriched medium to induce chondrogenesis for 28 and 56 days, at the end of which cell viability, gene expression and matrix synthesis were assessed.

No toxicity was observed, whatever the ink or culture conditions. At D28, gene expressions show chondrogenic differentiation of MSCs under the effect of TFG-β1. Low level of added collagen (0.5 mg) promotes the expression of chondrogenic markers, while the high level of 5 mg induces a more osteogenic profile. In substitutes containing hyaluronic acid, we also noted a significant increase in the expression of chondrogenic markers at D56.

Histological and immunohistochemical analyses confirmed the chondrogenic effect of TFG-β1, with type II collagen synthesis potentiated by the lowest collagen quantity (0.5 mg). In contrast, the 5 mg amount of collagen induced more calcifications within the substitutes. However, we

*Speaker
found no significant difference in matrix synthesis between bio-inks with and without hyaluronic acid, even though early whitening was observed, associated with superior substitute strength in the presence of hyaluronic acid.

In conclusion, the Col0.5 bio-ink is a promising candidate to reproduce the superficial layers of hyaline cartilage. In contrast, the Col5 bio-ink can mimic the calcified layer and the hyaluronic acid bio-ink to reproduce the deep layer of hyaline cartilage.

Acknowledgments: This work was supported by "la Direction Générale des Armées (ANR-16-ASTR-0021)", "l’Université de Lorraine et le Grand Est 2016" (AAP-002-037), "la Fondation de l’Avenir" (AP-RM-16-042) and "la Fondation pour la Recherche Médicale" (No ECO201906008942), CPER IT2MP and FEDER.

**Keywords:** 3D bioprinting, chondrogenesis, MSCs
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(Only participants who agreed to appear)
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**List of participants - Journées BIOMAT & MatSAN 2024 - Super-Besse, January 14-19, 2024**
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**Polymer and Hydrogel; Biomaterials Science; Drug Delivery; In vitro and In vivo Evaluation; Medical Devices**
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