UK Society for Biomaterials 16th Annual Conference
20-21st June 2017

Local Organising Committee:
Paul Roach
Claire Lowe
Manisha Mistry
Munya Kamudzandu
Daniel Merryweather
Matthew Dunn
Sandhya Moise

Event photography by Ms Ana Kyoseva, Keele University

Cover images: polymeric gels as osteochondral architecture mimics (Zaid Younus); neural cells differentiated on modified planar surfaces (George Joseph)

UKSB Official Sponsors
Dear UKSB2017 delegates,

On behalf of the UKSB council and local organising committee I would like to extend to you all a most cordial welcome to the 16th annual UK Society for Biomaterials conference, hosted this year at Loughborough University. Since the beginning of the Society back in 1997, we are enthusiastic to provide an inclusive and democratic environment within which we can share our research in the interdisciplinary area of biomaterials, and support the next generation of researchers developing as postgraduate students and postdoctoral fellows. The rich scientific programme outlined for this year will cover the broad areas underpinning biomaterials providing excellent networking opportunities, bringing old friends together whilst fostering new network formation.

Over the years the field of biomaterials has evolved, working closely with the tissue engineering, surface chemical and analysis groups, and now more recently the biofabrication groups worldwide. As a Society we hope to bring together these interconnecting fields, listening to our membership and stakeholders to deliver a modern biomaterials network dedicated to delivering opportunities for the field to grow and tackle the problems at hand and those on the future horizon.

Our prize winners this year have been awarded based on their commitment to the field and advances within their particular research areas. The UKSB has developed a new range of awards this year including support for involvement in the European Society for Biomaterials, and funding for inter-laboratory working between student members. I would like to bring your attention to these schemes and highlight their significance in our Society growing, both in the way we collaboratively work as a network and the strength that shared ideas and resources brings.

We look forward to the World Biomaterials Congress in 2020 where we will have a dedicated UKSB session, and I hope that many of you are planning for this event as it approaches. This will be a great opportunity to present the UKSB as a national Society.

Best Wishes,

Colin Scotchford
UKSB President
Prizes and Awards

Every year there are prizes given out for those showing excellence in their work, dedication and ambition to further biomaterials research. This year we are establishing new awards for lab-2-lab collaborations and travel awards for UKSB and ESB. Please visit www.uksb.org to find details of how to apply and nominate for these awards.

UKSB President's Prize

The President’s prize was established to recognise the achievement of specific individuals over the course of a career in biomaterials.

The UKSB 2017 President’s Prize is awarded to Professor Ruth Cameron, Professor of Materials Science in the Department of Materials Science and Metallurgy and a Director of the Cambridge Centre for Medical Materials. She is Senior Fellow and Director of Studies in Physical Natural Sciences of Lucy Cavendish College, Cambridge.

Her research in regenerative medicine concerns medical materials that will interact therapeutically with the body, which may have bioactive structures that encourage repair and regeneration, provide tailored mechanical support, or release drugs at a controlled rate. Research themes include ice templated scaffolds, biodegradable and bioactive polymers, composites and active surface design.

Alan Wilson Memorial Lecture Award

To celebrate the life and career of the outstanding dental materials scientist Dr Alan Wilson OBE (1928-2011), this award is to recognise the work and contribution of an individual scientist who has contributed significantly to the field of biomaterials science in dentistry (including maxillo-facial repair).

This year the Alan Wilson memorial award is given to Professor David C. Watts. David Watts is Professor of Biomaterials Science in the School of Medical Sciences at the University of Manchester. He is a Fellow of the Institute of Physics, the Royal Society of Chemistry and the Royal Society of Biology. He has personally supervised more than 60 PhD candidates from 20 nations. Since 1998 he has been Editor-in-Chief of Dental Materials (Elsevier). In 2010 he received the research prize of the Alexander von Humboldt foundation. His recreations include classical music and mountain climbing.
Larry Hench Young Investigators Prize

This prize is to highlight the achievements of a promising young research scientist in recognition of outstanding and innovative contributions in a selected field of biomaterials research. Larry Hench (1938-2015) was an Emeritus Professor at the University of Florida and Professor in the Ceramic Materials Department at Imperial College London. He co-founded and co-directed the Tissue Engineering and Regenerative Medicine Centre for 10 years and at the time of his death was the Director of the Technology Centre for Medical Materials and Photonics.

The 2017 Larry Hench Young Investigators Prize is awarded to Dr. Adam D. Celiz.

Adam Celiz was born in London and received a 1st Class Bachelor’s degree in Pharmaceutical and Chemical Sciences from the University of Brighton. He then obtained his PhD in Chemistry under the supervision of Professor Oren A. Scherman in the Melville Laboratory for Polymer Synthesis at the University of Cambridge. His PhD research focused on the preparation of supramolecular polymeric materials. Upon graduating from Cambridge in 2010, Adam pursued postdoctoral research in the Laboratory for Biophysics and Surface Analysis at the University of Nottingham with Professors Morgan Alexander and Martyn Davies. His research focused on high throughput materials screening for novel 2D substrates for human pluripotent stem cell culture.

In 2014, Adam was then awarded a Marie Curie International Outgoing Fellowship (IOF) to build his own research program at the Wyss Institute for Biologically Inspired Engineering at Harvard University with Professor David Mooney. His current research efforts are focused on developing materials for regenerative medicine and tissue engineering applications particularly in Regenerative Dentistry. In August 2017, Adam will become a Lecturer in the School of Bioengineering at Imperial College London and will start his independent research group. He has 13 peer-reviewed publications, 3 patents and was recently awarded an Early Career Researchers Award by the American Vacuum Society’s Biomaterial Interfaces Division. His research has been highlighted by various news agencies and outlets including the BBC, Newsweek, Washington Post and Popular Science.
UKSB 2017 Conference Programme

Tuesday 20 June 2017: Day 1

<table>
<thead>
<tr>
<th>Time</th>
<th>Session 1. Materials Controlling Cells, Chair: Dr P. Roach &amp; Dr Sam Moxon</th>
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<tbody>
<tr>
<td>09.00 – 09.30</td>
<td>Arrival and registration from 8:30am</td>
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<tr>
<td>09.30 – 09.40</td>
<td>Welcome to #UKSB2017</td>
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<td></td>
<td>Paul Roach, Loughborough University</td>
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<tr>
<td>09.40 – 10.20</td>
<td>Keynote: Prof. Morgan Alexander, University of Nottingham</td>
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<td>Bio-instructive Materials Discovery</td>
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<td>10.20 – 10.35</td>
<td>Fabrication of cost effective Proanthocyanidin cross-linked Chitosan-</td>
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<td></td>
<td>Gelatin hydrogel scaffolds with tuneable mechanical properties for tissue engineering</td>
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<td>Ana Encerrado, University of Nottingham</td>
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<tr>
<td>10.35 – 10.50</td>
<td>Fabrication of large-area chemical nanoarrays by polymer pen lithography enable control of mesenchymal stem cell adhesion and differentiation</td>
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<td>Judith Curran, University of Liverpool</td>
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<tr>
<td>10.50 - 11.05</td>
<td>The role of hypoxia and HIF mimetics in bone nodule formation</td>
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<td>Azadeh Rezaei, University College London</td>
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<tr>
<td>11.05 – 11.30</td>
<td>Coffee/Tea Break + Posters</td>
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</tbody>
</table>

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<thead>
<tr>
<th>Time</th>
<th>Session 2. Surface and Cell Interaction, Chair: Dr Siddharth Patwardhan &amp; Tanushree Halder</th>
</tr>
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<tbody>
<tr>
<td>11.30 – 12.00</td>
<td>Larry Hench Young Investigator Award</td>
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<td></td>
<td>Dr. Adam D. Celiz, University of Nottingham</td>
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<td></td>
<td>Synthetic Light Curable Polymers Support Regenerative Behaviour of Dental Pulp Stem Cells</td>
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<tr>
<td>12.00 – 12.15</td>
<td>Design of thermo-responsive polymer-peptide conjugates for human corneal stromal cell phenotypic support</td>
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<td></td>
<td>Floor A A Ruiter, University of Nottingham</td>
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<tr>
<td>12. 15 – 2.30</td>
<td>Nanoscale characterization of cold plasma treated collagen film and its inflammatory response in vitro</td>
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<td>Dr. Rui Chen, University of Liverpool</td>
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<tr>
<td>12.30 – 12.45</td>
<td>Fatty-acid absorption detrimentally changes the physical properties of ultra-high molecular weight polyethylene</td>
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<td>Parnian H Zaribaf, University of Bath</td>
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<td>12.45 – 13.00</td>
<td>Rationally designed and robust vascular-protective growth factors</td>
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<td>Dr. Marlies Fischer, University of Leicester</td>
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<tr>
<td>13.00 – 14.00</td>
<td>Lunch + Posters</td>
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Please note that Loughborough helpers will be on hand to direct you around campus and to the Burleigh Court Hotel, which is a 5 minute walk away from James France Building where the conference is held. There is also a frequent shuttle bus service across campus.

Enjoy the conference dinner and please be prompt for day 2 of the programme.

<table>
<thead>
<tr>
<th>Session 3. New Materials and Neural Engineering, Chair: Dr Alan Smith &amp; Matthew Dunn</th>
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</thead>
<tbody>
<tr>
<td><strong>14.00 – 14.20</strong></td>
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<tr>
<td>Electrically conductive and soft biomaterials for potential tissue engineering scaffold applications</td>
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<tr>
<td><strong>14.20 – 14.35</strong></td>
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<td>Natalija Tatic, University of Nottingham and Université Catholique de Louvain</td>
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<tr>
<td><strong>14.35- 14.50</strong></td>
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<tr>
<td>Dr. Sam Moxon, University of Manchester</td>
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<tr>
<td><strong>14.50- 15.05</strong></td>
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<tr>
<td>Caroline S. Taylor, University of Sheffield</td>
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<tr>
<td><strong>15.05 – 15.30</strong></td>
</tr>
</tbody>
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<thead>
<tr>
<th>Session 4. Antimicrobials/bacterial, Chair: Dr Pamela Walsh &amp; Daniel Merryweather</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>15.30 – 15.50</strong></td>
</tr>
<tr>
<td>Antimicrobial coatings – capabilities and challenges</td>
</tr>
<tr>
<td><strong>15.50 – 16.05</strong></td>
</tr>
<tr>
<td>Dr. Timothy E.L. Douglas, Ghent University and Lancaster University</td>
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<tr>
<td><strong>16.05 – 16.20</strong></td>
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<tr>
<td>Dr. Felicity de Cogan, University of Birmingham</td>
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<td><strong>16.20 – 16.35</strong></td>
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<tr>
<td>George Fleming, University of Liverpool</td>
</tr>
<tr>
<td><strong>16.35 – 16.50</strong></td>
</tr>
<tr>
<td>Katharina Clitherow, University of Sheffield</td>
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<tr>
<td><strong>16.50 – 17.20</strong></td>
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<td><strong>19:30</strong></td>
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<tr>
<td>Burleigh Court Hotel, Loughborough University Campus</td>
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</tbody>
</table>
## Wednesday 21 June 2017: Day 2

### Session 5: 3D Materials, Chair: Dr Colin Scotchford & Ioannis Kouparitsas

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker &amp; Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.00 – 9.15</td>
<td>Stereolithography 3D printing of poly(D,L-lactide)</td>
<td>Alberto Di Bartolo, Heriot-Watt University</td>
</tr>
<tr>
<td>9.15 – 9.30</td>
<td>Suspended Additive Layer Manufacture of Osteochondral Structures</td>
<td>Dr. Alan Smith, University of Huddersfield</td>
</tr>
<tr>
<td>9.30 – 9.45</td>
<td>The manufacture of 3D electrospun scaffolds with complex features</td>
<td>Selina Beal, University of Sheffield</td>
</tr>
<tr>
<td>9.45 – 10.00</td>
<td>Electrospray bioresorbable tissue repair scaffolds: Travelling the pathway from laboratory to clinic</td>
<td>Dr. Peter Iddon, Neotherix Ltd, UK</td>
</tr>
<tr>
<td>10.00 – 10.40</td>
<td>2017 UKSB President’s Prize</td>
<td>Prof. Ruth Cameron, University of Cambridge</td>
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<td></td>
<td>Ice Templated Structures for Regenerative Medicine</td>
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<tr>
<td>10.40 – 11.10</td>
<td>Coffee/Tea Break + Posters</td>
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</tr>
</tbody>
</table>

### Session 6: Clinically facing biomaterials, Chair: Dr Jude Curran & Caleb Nidiya

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker &amp; Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.10 – 11.50</td>
<td>Alan Wilson Memorial Lecture</td>
<td>Prof. David Watts, University of Manchester</td>
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<td></td>
<td>The quest for stable biomimetic repair of teeth</td>
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<tr>
<td>11.50 – 12.05</td>
<td>Development of an osteochondral plug for joint repair</td>
<td>Katherine Pitrolino, University of Nottingham</td>
</tr>
<tr>
<td>12.05 – 12.20</td>
<td>Repair of ovine anterior cruciate ligament using an acellular porcine tendon graft: biomechanical and biological characterisation after 26 weeks in vivo</td>
<td>Dr. Anthony Herbert, University of Leeds</td>
</tr>
<tr>
<td>12.20 – 12.35</td>
<td>An <em>in vitro</em> co-culture model of the retinal vasculature for use in the development of regenerative medicine strategies for diabetic retinopathy</td>
<td>Jessica Eyre, University of Liverpool</td>
</tr>
<tr>
<td>12.35 – 12.50</td>
<td>Delivering the immunomodulatory properties of mesenchymal stem cells</td>
<td>Dr. Sandhya Moise, University of Nottingham</td>
</tr>
<tr>
<td>12.50 – 13.50</td>
<td>Lunch + Posters</td>
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<td>Time</td>
<td>Speaker/Presenter</td>
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<tr>
<td>13.50 – 14.10</td>
<td>Dr. Siddharth V. Patwardhan, University of Sheffield</td>
<td>Bioinspired “Green” nanomaterials for biomedical applications</td>
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<tr>
<td>14.10 – 14.25</td>
<td>Ri Han, Queens University Belfast</td>
<td>The investigation of marine derived biosilica for bone repair strategies</td>
</tr>
<tr>
<td>14.25 – 14.40</td>
<td>Tianhao Zhou, University of Manchester</td>
<td>A novel Mg²⁺-eluting polymer scaffold for bone cell proliferation and mineralization</td>
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<tr>
<td>14.40 – 14.55</td>
<td>Jeffrey Clark, Imperial College London</td>
<td>Hybrid implants for early-intervention osteochondral repair</td>
</tr>
<tr>
<td>14.55 – 15.10</td>
<td>P. Urban, University of Birmingham</td>
<td>Amorphous ti-cu alloy prepared by mechanical alloying for biomedical applications</td>
</tr>
<tr>
<td>15.10 – 15.25</td>
<td>Christopher Gabbott, Loughborough University</td>
<td>A novel scale-down cell culture and imaging design for the machanistic insight of cell colonization within porous substrate</td>
</tr>
<tr>
<td>15:25 – 15.40</td>
<td>Raasti Naseem, Loughborough University</td>
<td>Comparative study on the effect of scaffold geometry on mechanical performance of bioresorbable scaffolds</td>
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<tr>
<td>15.40 – 16.00</td>
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<td>Prize giving and closing remarks</td>
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<td></td>
<td>Finish at 16.00</td>
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Morgan Alexander is Professor of Biomedical Surfaces, the Director of the EPSRC Programme Grant in Next Generation Biomaterials Discovery, a Royal Society-Wolfson Research Merit Award holder and a Wellcome Trust Senior Investigator. He received his BSc in Materials (1988) and his PhD from the same department at The University of Sheffield in 1992. His work involves developing materials for application in human healthcare and characterising relationships between surface structure and biological properties. Understanding these relationships is critical to the development of next generation biomaterials and it is the theme running through his group’s work across a variety of areas spanning control of bacterial adhesion, to engineering cell response for application in medical devices and cell manufacture.

The range of biomaterials found in the clinic today are dominated by materials chosen on the basis of their availability and mechanical properties rather than positive interactions with surrounding cells and tissues. It would be desirable to design our way forward from this situation to new biomaterials. Unfortunately our understanding of the bio-interface is poor, with only isolated cases where a good understanding of cell-material interactions can be cited, and fewer still where material-tissue interactions are well characterised and understood. This paucity of information on the mechanism of biomaterials interactions with the body acts as a roadblock to rational design. Consequently we have taken a high throughput screening approach to discover new bio-instructive materials from large chemical libraries - this approach can be described as engineering serendipitous discovery.[1] These new candidate biomaterials provide a starting point for development of new medical devices and provide opportunities to study their mechanism of action to provide new information to tackle the rational design roadblock.

This screening approach has been used to identify bio-instructive materials in the discovery of polymers with application in expansion of pluripotent human embryonic stem cells and the identification of substrates on which to mature cardiomyocytes.[2,3] Other screening campaigns using macrophage differentiation have identified bio-instructive materials with pro- and anti-inflammatory characteristics with great potential in modulating the human immune system in novel therapies and devices.[4] Materials resisting bacterial attachment and biofilm have also been identified and will be presented, with early data on the investigation of their mechanism of biofilm formation resistance.[5] Work to integrate and expand this range of bio-instructive materials will be previewed, including movement to 3D screening.

4. Rostam et al. in preparation.
Introduction: Extracellular matrix (ECM) stiffness naturally fluctuates within the body, from hard bone to soft brain tissue. Studies have shown that the behavior of anchorage dependent cells is influenced by the substrate stiffness on which they rest. Mechanical signals are as fundamental to gene expression and cell response as chemical signals. Hydrogel properties can be modified to match the requirements of the tissue engineering application. The fabrication of substrates with a tunable stiffness can be a useful technique to control and evaluate the response of a wide cell linages range to their biophysical microenvironment in vitro. However often the final cost of these substrates is high, that restricts its use.

This project was aimed to develop and investigate the biocompatibility and mechanical properties of cost-effective proanthocyanidin (PA) crosslinked gelatin-chitosan hydrogels and to examine the response of cells growth as a function of stiffness of these hydrogels.

Materials and Methods: Chitosan and Gelatin solutions were prepared by solution in acetic acid. These solutions were mixed then neutralized with NaOH. The mixture was then crosslinked by gradual addition of different PA concentrations. Gels were prepared in petri dishes with different thicknesses and cooled overnight at 4°C. Cast hydrogels were washed with distilled water then neutralized before rinsing with PBS. Prepared Gels were sterilized by UV radiation. 24hrs prior to use gels were protein conditioned in fresh media. 3T3 fibroblasts, MG63 osteoblast-like cells were seeded onto the hydrogels. The samples were incubated for 2 hours to allow cell attachment. Media was changed every 48h.

Alamar blue test was performed to estimate the proliferation of both cell lines as a function of metabolic activity. To evaluate the effects of cross-linker concentration on mechanical properties, hydrogels were prepare from a wide range of, cross-linker, substrate concentration and thicknesses. Mechanical testing of the hydrogels was carried out on an Instron machine under compression using a 50 N loading cell. Compression–strain diagrams were obtained and used to calculate compressive modulus. Results suggests that cross-linker concentration is a significant variable to control the gel stiffness.

Result and Discussion: Cell growth on hydrogels showed proliferation comparable to controls on tissue culture plates. By day 7 cells had covered the total sample surface for most formulations, Morphological evaluation of the cell cultures revealed no discernible difference between control and test groups. By changing the ratios of cross-linker, chitosan and gelatin, and thickness it was possible to deliver hybrid hydrogels with suitable mechanical properties, demonstrating the superior biomimetic properties of the cross-linked blend compared with un-crosslinked hydrogels.

Conclusion: The crosslinked hydrogels exhibited very favorable cell proliferation. The cytocompatibility implies that PA cross-linked hydrogels are suitable substrates for tissue engineering of connective tissues. Statistically significant differences in cell growth were found as PA concentration and substrate thicknesses were varied, cues that directly affect substrate stiffness. The results also show that PA is an effective, non-toxic cross-linking agent.
FABRICATION OF LARGE-AREA CHEMICAL NANOARRAYS BY POLYMER PEN LITHOGRAPHY ENABLE CONTROL OF MESENCHYMAL STEM CELL ADHESION AND DIFFERENTIATION

I-Ning Lee¹, Lu Shin Wong², John A Hunt³, Judith M Curran¹
¹Department of Engineering, University of Liverpool, United Kingdom, ²Manchester Institute of Biotechnology, University of Manchester, United Kingdom, ³School of Science and technology, Nottingham Trent University, United Kingdom, Corresponding author: ilee@liverpool.ac.uk – PhD student (2nd year)

Introduction
Current research has proven that cells sense and respond to changes in topography, stiffness, and material surface chemistry.¹ In addition, recent advances have proven that cells sense and respond to stimulus at the sub-micron/nano scale.² Here we report on the fabrication of large-area chemical nano-arrays using polymer pen lithography (PPL) that are designed to control the spatially defined interaction, at the nano-scale, between selected chemical groups (-NH₂ and -CO₂H), and human mesenchymal stem cells (MSC). The data presented defines the ability of selected chemical groups to control initial integrin binding and subsequent cell adhesion, focal contact formation and MSC differentiation. A definitive relationship between spatial orientation of integrins/focal contacts and presenting chemical group is discussed.

Materials and Methods
11-amino-1-undecanethiol (AUT/-NH₂), or 16-mercaptohexadecanoic acid (MHA/-CO₂H) was patterned using PPL to produce square arrays on large-area gold surfaces (2 cm x 2 cm). Each array consisted of multiple circular features size of (modified area) 300 nm ± 5 nm, with variations in the spatial distribution of adjacent features ranging from 1-3 μm. Arrays were passivated with (11-mercaptoundecyl)hexa(ethylene glycol) (m-PEG) enabling controlled interactions with the chemical groups of interest at the point of contact. MSCs (Lonza, UK) were cultured in contact with selected surfaces in basal medium for up to 28 days. Levels of adhesion, integrin expression, phenotype expression and differentiation were defined using a combination of reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry.

Results and Discussion
Results have demonstrated that changes in the nano-scale resolution of presenting –NH₂ and –CO₂H arrays is a powerful tool in controlling initial MSC adhesion and subsequent differentiation. Selected MHA chemical arrays induced hypertrophic cartilage phenotype, clustered cell morphology which was positive for Collagen X and osteocalcin, in contrast, AUT patterned surfaces with identical topography design showed little to no cell adhesion. However, AUT chemical nanoarrays with reduced spatial distribution and increased amount of features led to clustered cell morphology and potential adipogenic differentiation. With further reduced spatial distribution, AUT nanoarrays led to strong but non-clustered cell morphology and osteogenic differentiation which has proven combinatorial effect of surface chemistry and spatial resolution of chemical groups can be used to effectively control MSC responses. In addition, it was proven that different chemical nanoarrays influenced protein adsorption and confirmation, which had a role in dictating the alignment of cells on the surface. The data proved that both sub-micron depositions of chemical groups has definitive effect of MSC behaviour via control of initial cell attachment mechanisms and potential control of alignment, which is associated with mechanotransduction via the organisation of actin fibres within the cell.

Conclusions
We demonstrated defined selected chemical nanoarrays with various presenting end groups combined with feature size and spatial distribution have successfully directly controlled initial integrin binding of MSCs and subsequent differentiation towards desired cell phenotypes. Research shows that both chemistry and spatial distribution of cell adhesive areas are essential design criteria in controlling MSC differentiation.

References

Acknowledgements
The authors would like to thank the Leverhulme Trust for providing financial support to this project.
THE ROLE OF HYPOXIA AND HIF MIMETICS IN BONE NODULE FORMATION
Azadeh Rezaei¹, Yutong Li¹ and Gavin Jell¹
¹UCL Division of Surgery and Interventional Science
Corresponding author: azadeh.rezaei.14@ucl.ac.uk – PhD student (3rd year)

Introduction
Bone fracture repair is a multistep process involving angiogenesis, inflammation, osteoclast activation, recruitment of mesenchymal stem cells and mineralisation. A signal for healthy bone repair is HIF-1α stabilisation, caused by damage to the local microvasculature network in bone following fracture and the consequent low oxygen (hypoxia) conditions. HIF-1α stabilisation has been shown to increase VEGF production in osteoblasts and enhance fracture healing¹. Furthermore, diabetic and elderly patients have an increased risk on non-union fracture and have a reduced ability to respond to hypoxia²,³. This research investigates the role of HIF mimetics (cobalt and cobalt bioactive glasses⁴) and hypoxia on bone nodule formation, with the larger goal of creating HIF stabilising materials for bone repair.

Materials and methods
Calvarial osteoblastic cells were isolated from 3-day-old Sprague-Dawley rats. Cells were seeded (60,000cells/12 well plate) in MEM-α containing 2 mM β-glycerophosphate, 10 nM dexamethasone, and 50 μg/ml ascorbate. When confluent, cells were treated with HIF-1α stabilising factors, CoCl₂ at a concentration of 12.5, 25 or 50 μM and the dissolution products (2 hours incubation at 37°C at a concentration of 75mg BG in 50ml MEMα on a roller shaker) of 0, 1 and 4mol% cobalt 45S5 bioactive glasses (CoBG) and maintained in normoxia (20%O₂) or exposed to hypoxia (1%O₂). VEGF expression and Alkaline phosphatase (ALP) activity were measured. After 21 days of culture, bone nodules were analysed using FTIR, Alizarin red, TEM and SEM.

Results and discussion
Bone nodule formation was observed in normoxia but not under hypoxic condition, a dystrophic mineral deposition was, however present in hypoxia (Fig.1A). HIF-1α mimetics cobalt (Co12.5µM and Co25µM) did not, however, inhibit bone nodule formation, thereby indicating a difference in cellular action between lack of oxygen (hypoxia) and artificial HIF stabilisation with Cobalt. A reduced ALP production in hypoxia may explain the decreased differentiation ability of osteoblasts into mature mineralising osteoblasts capable of nodule formation (Fig1.B). Bioactive glasses with and without cobalt did not form any bone nodules, in contrast with the previous studies⁵,⁶. VEGF expression was increased in hypoxia, Co12.5 and Co25µM (Fig1.C).

Fig.1- (A) Alizarin red stained bone nodules after 21 days. Normoxia and HIF-1α mimetics (co12.5µM and Co25µM) formed bone nodules, however, hypoxia inhibited nodule formation. Scale bar =200 μm. (B) ALP activity after 21 days normalised with total DNA. Reduced ALP activity under hypoxic condition may explain the decreased differentiation ability of osteoblasts into mature mineralising osteoblasts capable of nodule formation (Fig1.B). Bioactive glasses with and without cobalt did not form any bone nodules, in contrast with the previous studies⁵,⁶. VEGF expression was increased in hypoxia, Co12.5 and Co25µM (Fig1.C).

Conclusions
For the first time this study has demonstrated that the use of HIF mimetics (Co12.5 µM and Co50µM) did not inhibit bone nodule formation whilst hypoxia did. Furthermore, the increased ALP activity and VEGF expression by Cobalt suggest that HIF mimicking materials have the potential to enhance bone repair. Further analysis is needed to quantify these effects in diabetic/elderly cell culture models, together with the design of materials to have the controlled release of HIF mimetics.

References
Larry Hench Young Investigator Award
SYNTHETIC LIGHT-CURABLE POLYMERS SUPPORT REGENERATIVE BEHAVIOUR OF DENTAL PULP STEM CELLS

Adam D. Celiz1,2*, Kyle H. Vining1,3, Jacob C. Scherba3, Alaina Bever3, Morgan R. Alexander1 and David J. Mooney1,3*

1. Advanced Material and Healthcare Technologies Division, School of Pharmacy, University of Nottingham, Nottingham NG7 2RD, UK 2. Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA 02115, USA, 3. Harvard John A. Paulson School of Engineering and Applied Sciences, Cambridge, MA 02138, USA,
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Introduction
Dental disease annually affects billions of patients worldwide and is treated with materials containing monomers, such as bisphenol A glycidyl methacrylate (BisGMA), that can be harmful to dental pulp cells (DPSCs). The dental pulp contains blood vessels, nerves, immune cells, and DPSCs that are capable of regenerating dentin, the bone-like structure that supports tooth enamel. Emerging treatments, such as regenerative dentistry aim to biologically heal dental tissue after injury, but existing restorative materials are not suitable for interfacing with biological tissues. There is a need for restorative materials that support native functions of dental pulp cells (DPSCs).

Materials and Methods
We employed a high-throughput screening format known as polymer microarrays to rapidly evaluate hundreds of light-curable biomaterials for DPSC adhesion. Materials that are discovered as supportive of DPSC adhesion can be scaled up into cultureware to assess proliferation and differentiation of these cells (Figure 1a).

Results and Discussion
We have developed triacrylate polymeric materials that support regenerative behavior of DPSCs isolated from human teeth. We employed thiol-ene “click” chemistry to achieve rapid light-curing and minimize residual monomer. Resulting polymers exhibit stiffness and strength similar to existing materials for dentistry. Triacrylate bulk polymers supported DPSC adhesion, proliferation, and differentiation in vitro. DPSCs require integrin-β1 receptor signaling to remain adhered on triacrylate materials (Figure 1b). Conversely, BisGMA and trimethacrylate materials did not support cell adhesion and caused dysregulation of matrix and cell signaling pathways. Furthermore, we demonstrated how triacrylates can be used similarly to existing materials in a dental pulp injury model.

Conclusions
Triacrylate polymers may enable novel regenerative dental therapies in the clinic by both restoring dental tissue and supporting dental pulp stem cells.
DESIGN OF THERMO-RESPONSIVE POLYMER-PEPTIDE CONJUGATES FOR HUMAN CORNEAL STROMAL CELL PHENOTYPIC SUPPORT

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Introduction
Current cell culture relies on enzymatic treatment to detach cells, which can result in damage to cell membrane proteins. In addition, conventional 2D monolayer culture and culture in high serum conditions can result in loss of functional cell phenotype. For example, human corneal stromal cells (hCSC) lose their quiescent (desirable) phenotype and express a more activated (undesirable) myofibroblast-like phenotype when cultured in these conditions. Over the last decade, many studies have been focused on developing cell culture surfaces that allow enzymatic-free detachment using thermo-responsive materials (REF Okano). Much of this work has led to the development of 2D thermo-responsive materials that are now commercially available. We have developed a thermo-responsive polymer/peptide electrospun fibre-based scaffold for 3D enzyme free cell culture. This was achieved by functionalising a thermo-responsive polymer (PDEGMA) with free thiol groups, followed by the utilisation of thiol-ene chemistry for norbornene functionalised peptide attachment. The peptide sequences GGG-IKVAV, GGG-YIGSR, and GGG-IKVAV-YIGSR were chosen as they have been proven to be important adherence peptides for CSC’s which will be used in this study as an exemplar cell type for this technology.1

Materials and Methods
PDEGMA-co-PDEGOH thiol functionalisation was performed.2 Ellman’s assays confirmed the presence of free thiols. PDEGMA-co-PDEGSH and PLA solution were electrospun to fibrous scaffolds (19 kV, 17.5 cm, and 0.5 ml/hr). Flourescein-3-maleimide labeling confirmed free thiol presence on the scaffold. Norbornene functionalised peptides were synthesised by SPPS3 with a norbornene acid group at the N-terminus. Thiolene UV-curing was performed in the ratio of 1:1 (free thiols: peptide), electrospun scaffold thiols were reduced by 10 eq. TCEP. LAP and peptide/DMSO solutions were added and UV-cured at 365 nm, 6 W/cm² for 15 min.

Results and Discussion
Thiol functionalisation on the PDEGMA-co-PDEGOH was successfully performed. Ellman’s assay showed a conversion of around 30%. This is most likely due to the lower boiling point of the thiol starting product (110°C), resulting in evaporation. An initial pure PDEGMA-co-PDEGS-peptide UV-curing step was performed to confirm the click reaction between the free thiols and the double bond of the norbornene group. FT-IR showed the disappearance of the double bond (Fig. 1Bb) and the appearance of the amino-groups of the peptide (Fig. 1Ba) in the case of the reacted peptide-thiol polymer. The presence of free thiol groups was confirmed by flouresceine-5-maleimide labelling, and attached peptide by UV-curing was later confirmed by ATTO NHS ester labelling.

Conclusions
An electrospun scaffold containing free thiols has been developed and UV-curing of the peptide was confirmed, we now move to test the efficacy of this scaffold in support of serum and enzyme free culture of hCSCs.

NANOSCALE CHARACTERIZATION OF COLD PLASMA TREATED COLLAGEN FILM AND ITS INFLAMMATORY RESPONSE IN VITRO

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Introduction
Cold gas plasma treatments have been widely used to clean, sterilize and/or improve biocompatibility for biomedical devices [1]. The implantation of biomedical devices including collagen-based implants evokes an inflammatory response [2]. Despite inflammation playing an important role in the early stages of wound healing, excessive and non-resolving inflammation is one of major causes which may lead to the poor performance of biomaterial implants in some patients. In this study, effects of cold plasma treatment on the surface hydrophobicity, morphology and elastic modulus of modified collagen films were investigated through contact angle, scanning electron microscopy (SEM) and PeakForce Quantitative Nanoscale Mechanical Characterization (PeakForce QNM); And the effects of cold plasma treatment on the non-specific inflammatory response were also investigated in vitro by the measurement of protein expression and cytokine production after one and four days of U937 cell line cultured on the collagen films.

Materials and Methods
Collagen coverslips were prepared by spin coating and drying for 24 hours and then, placed in 24-well plates within a plasma machine (Emitech K1050X, Quorum Technologies, UK) and treated with either nitrogen or oxygen plasma at a pressure of 0.6 mbar with a flow rate of 15ml/min at 20W or 80W for 2 minutes. AFM analysis was carried out using a commercial AFM (NanoScope VIII MultiMode AFM, Bruker Co., Santa Barbara, CA, USA). PeakForce QNM was conducted in ambient conditions with a silicon nitride probe (Bruker RTESPA-150A, nominal frequency of 150 kHz, nominal spring constant of 5N/m) with a scan resolution of 256 pixels/line. The data were fitted with the Derjaguin–Muller–Toporov (DMT) model to extract the elastic modulus by fitting the contact region of the retract curve close to the contact point. All post-image processing was carried out using IMAGESXM v1.99 and Nanoscope Analysis 1.7 (Bruker, USA).

U937 cells were induced to differentiate by exposing the cells (5 × 10^5 cells/ml) to 5 ng/ml of phorbol 12-myristate 13-acetate (PMA) for 24 hr. Differentiated U937 were seeded at 3 × 10^5 cells/ml on control and plasma treated collagen films for 1 and 3 days. Quantification of human IL-1β, TNF-α and IL-10 in supernatant were achieved using commercially available kits (Invitrogen™). A total of 4 separate repeats were carried out per single culture system.

Results and Discussion
Table 1 showed the surface roughness (Rq) and elastic modulus of collagen films before and after plasma treatment. The results showed that plasma treatment changed the morphology and elastic modulus of collagen film at nanoscale.

<table>
<thead>
<tr>
<th></th>
<th>Rq (nm)</th>
<th>DMT modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.73 ± 1.47</td>
<td>0.75 ± 0.07</td>
</tr>
<tr>
<td>N2 plasma treated 20w</td>
<td>0.62 ± 0.11</td>
<td>0.96 ± 0.53</td>
</tr>
<tr>
<td>80w</td>
<td>0.59 ± 0.15</td>
<td>28.44 ± 2.08</td>
</tr>
<tr>
<td>O2 plasma treated 20w</td>
<td>1.56 ± 0.79</td>
<td>171.58 ± 15.67</td>
</tr>
<tr>
<td>80w</td>
<td>1.83 ± 0.03</td>
<td>105.57 ± 30.17</td>
</tr>
</tbody>
</table>

The results of in vitro U937 cell culture showed that nitrogen plasma treatment may impart an anti-inflammatory effect on collagen film by reducing initial activation of monocytes and macrophages, which led to a lower production pro-inflammatory cytokines IL-1β, TNF-α and higher production of anti-inflammatory cytokine IL-10.

Conclusions
Oxygen and nitrogen cold plasma treatment on collagen films, modified material surfaces not only their chemical composition and surface energy, but also their surface morphology and elastic modulus at nanoscale. Oxygen plasma treatment decomposed the 3D network structure and made the surface rougher and stiffer; nitrogen plasma treatment maintained the 3D network structure of collagen films and made the surface smoother at the nanoscale. The results indicated that compared to oxygen plasma, nitrogen plasma treatment may have produced an anti-inflammatory effect. This could have been due to the combination of the amino chemical group and the smoother of the collagen film, introduced by nitrogen plasma treatment.

References

Acknowledgements
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FATTY-ACID ABSORPTION DETRIMENTALLY CHANGES THE PHYSICAL PROPERTIES OF ULTRA-HIGH MOLECULAR WEIGHT POLYETHYLENE

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Introduction
Retrieval studies have demonstrated that lipids and fatty acids in synovial fluids are adsorbed by the surface of ultra-high molecular weight polyethylene (UHMWPE) joint replacements. James et al. also confirmed the presence of esterified fatty acids within the surface of retrieved implants. However, the effect of fatty acids on the physical properties of UHMWPE is poorly understood. Octadecadienoic acid is one of the main components of the synovial fluid representing approximately 80% of the total fatty acid content. The aim of this study is to investigate the effect of octadecadienoic acid on the percentage crystallinity, melting point and tensile properties of UHMWPE.

Materials and Methods
Five tensile test samples were doped in cis-9,cis-12-Octadecadienoic acid (Sigma Aldrich) for 24h at 100°C. The elevated temperature was used to accelerate the diffusion of the octadecadienoic acid into the UHMWPE samples. An additional set of control samples (n=5) were heated to 100°C in air. Before and after immersion, the dimensions and weight of each sample were measured.

The surface of each sample was analysed using an infrared spectrometer (Perkin-Elmer Frontier L1280032 with ATR, 32 scans, from 4000 to 600 cm⁻¹). The presence of octadecadienoic acid was confirmed from the peak located at 935 cm⁻¹. Approximately 5 mg was taken from the outer surface of each sample for differential scanning calorimetry (DSC) (TA Instruments 250). DSC was performed from 20°C to 200°C at a heat flow rate of 5 °C /min. The crystallinity of each sample was measured from the area under the endothermic peak. Tensile tests were performed in accordance with ISO527 using an electromechanical testing machine (Instron 5965) and a contact extensometer, at a rate of 50 mm/min. Statistical analysis was performed using Mann-Whitney U tests to assess differences between the groups.

Results and Discussion
Octadecadienoic acid doped samples showed an absorbance at 935 cm⁻¹ which was absent in the control samples, which confirmed diffusion of octadecadienoic acid into the surface of the UHMWPE. Our results showed both cross-sectional area and weight increased after doping (area increase: 1.3%±0.2, p=0.006, weight increase: 3% ±0.28,p=0.006). DSC results indicated doping significantly decreased the crystallinity (p=0.028 ,n=5) and the melting temperature (p =0.01, n=5). Reduction in the crystallinity may reduce the wear resistance of the material.

A significant reduction was observed in the yield stress of the doped samples (p=0.012, n=5). This could be due to the plasticising effect of fatty acids on UHMWPE or the lower crystallinity. There was a slight decrease in the elongation at failure, modulus and ultimate stress of the Octadecadienoic acid doped samples; however, the decrease was not statistically significant.

Conclusions
The present study investigated the potential changes in the physical properties of UHMWPE caused by synovial fluid fatty acids. The results demonstrated the absorption of octadecadienoic acid, which happens over time in vivo, alters UHMWPE dimensions, reduces crystallinity, melting temperature, yield stress, and elongation at failure. Consequently, it is important that the effect of fatty acid absorption is taken into account when performing in vitro tests of UHMWPE components, such as wear testing. The dimensional change also has implications for some of the current orthopaedic designs which include close fitting components.

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**RATIONALLY DESIGNED AND ROBUST VASCULAR-PROTECTIVE GROWTH FACTORS**

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**Introduction**
Angiopoietin-1 (Ang1) is a secreted vascular-protective ligand that promotes formation of functional vessels, suppresses endothelial apoptosis, vessel regression, leakage, and inflammation. Ang1 has been shown to enhance vascularization in preclinical models of ischaemic disease, and in tissue engineering applications, crucially acting to improve functionality of neo-vessels. In addition, studies have demonstrated significant therapeutic effects of Ang1 in models of transplant vasculopathy, diabetic retinopathy, oedema, stroke, sepsis and wound healing. The primary receptor for Ang1 is Tie2, a receptor tyrosine kinase expressed predominantly on endothelial cells. Tie2 is activated by Ang1-induced oligomerization. In endothelial cells Ang1 must bind and cluster four or more Tie2 receptors in order to activate signalling.

Despite its therapeutic potential, there are a number of issues that restrict use of Ang1 protein. Native Ang1 is large (280kDa in its active form) and recombinant Ang1 is difficult to produce, prone to aggregation and exhibits variable bioactivity. Furthermore, Ang1 is inhibited by its naturally occurring antagonist Ang2. In this study we have sought to rationally engineer synthetic ligands for Tie2 that mimic the signalling and protective effects of Ang1 but that are facile to produce, small, stable and compatible with deployment in regenerative medicine and tissue engineering applications. These ligands comprise of Tie2-binding peptides fused with flexible linkers to coiled-coil oligomerization domains aimed at binding and clustering the target receptor.

**Materials and Methods**
In order to identify peptides that bind Tie2 extracellular domain, a random 7mer peptide phage display library was screened. Four rounds of screening were performed using Tie2-ectodomain-Fc fusion protein as target. Peptides specifically binding Tie2 were recovered and incorporated into an oligomerization scaffold downstream of a flexible linker. Fusion proteins were expressed in E. coli and purified. Ligand oligomerization state was determined by size exclusion chromatography. Binding of synthetic ligands were assessed by ELISA and cell-surface receptor co-immunoprecipitation assays. The ability of synthetic ligands to activate Tie2 in cultured human endothelial cells was determined by immunoblotting. Functional activity of the ligands was determined in transwell extravasation assays, apoptosis and cell survival assays.

**Results and Discussion**
Synthetic ligands were expressed in mg amounts as pentamers with a molecular mass 60kDa. Ligands specifically bound Tie2 ectodomain in ELISA assays and bound Tie2 on the surface of human endothelial cells. Interaction of ligands with endothelial cells activated the Tie2 receptor and downstream signalling pathways. The ligands inhibited endothelial apoptosis and enhanced endothelial survival to a similar level as the natural ligand Ang1. Peak endothelial protection was observed at synthetic ligand concentrations of 50nM. Synthetic ligands also suppressed endothelial permeability. In contrast to the natural ligand, the synthetic ligands were still active following heating to 50°C and above. Synthetic ligands were resistant to inhibition by Ang2.

**Conclusions**
We have engineered synthetic vascular protective ligands for the endothelial Tie2 receptor. These ligands are much smaller than the natural ligand, stable, easy to produce and tolerate additional features, such as immobilization domains. The ligands activate Tie2 signalling, suppress endothelial monolayer permeability and inhibit endothelial apoptosis as effectively as the natural ligand Ang1. Importantly, the naturally occurring Ang1 antagonist Ang2 does not inhibit the synthetic ligands. These robust ligands have potential for promoting the formation of functional vasculature and vascular protection in regenerative medicine and tissue engineering applications.
ELECTRICALLY CONDUCTIVE AND SOFT BIOMATERIALS FOR POTENTIAL TISSUE ENGINEERING SCAFFOLD APPLICATIONS

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Introduction
Electrically conducting biomaterials have garnered new attentions from bioengineers since they can electrically modulate cellular functions and communicate with cells via electrical signals at the material interfaces [1]. In particular, importance of soft materials mimicking the soft nature of many living tissues has been highlighted in designing biomaterials to effectively interact with biological systems for tissue engineering applications. Hence, the fabrication of electrically conductive and soft biomaterials has been desired.

Materials and Methods
Materials were developed from commercially available starting materials using a combination of well-established chemistry and materials preparation protocols. The chemicals and materials were thoroughly characterised. Cell culture was carried out using commercially available cell lines/medium etc., and analysed using well-established assays.

Results and Discussion
In our group, several electrically conductive hydrogels have been produced by in-situ polymerization of conductive polymer (i.e., polypyrrole) within alginate hydrogels [2] or with pyrrole-grafted hyaluronic acid [3]. These gels show enhanced electrical conductivities compared to conductive polymer-free hydrogels and 20~500 kPa Young’s moduli, and well supported the viability and growth of fibroblasts and human mesenchymal stem cells. In addition, conductive graphene hydrogels were recently prepared by mild chemical reduction of graphene oxide/polyacrylamide (GO/PAAm) composite hydrogels [4]. The reduced hydrogel, r(GO/PAAm), exhibited muscle tissue-like stiffness with a Young’s modulus of approximately 50 kPa and more than ten times lower electrical impedance compared to that of PAAm and unreduced GO/PAAm. Significantly enhanced myogenic differentiation on the r(GO/PAAm) was observed; also, electrical stimulation of myoblasts growing on the conductive hydrogels further facilitated myogenic differentiation.

Conclusions
In conclusion, various electrically conductive hydrogels were successfully produced and their potentials for tissue engineering scaffold applications were demonstrated. Further, I envision that the ultimate progression of these studies will facilitate the use of electrically conducting soft materials as bio-interfaces to modulate tissue functions.

References
HYDROGELS FOR LOCAL DELIVERY TO SPINAL CORD INJURY

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Introduction
Inflammation and a predominant proinflammatory macrophage phenotype are characteristics of spinal cord injury and contribute to progressing neuronal damage. Extracellular matrix (ECM) derived hydrogels have been shown to influence inflammation and have also been used as cell delivery vehicles [1]. This study determined the potential of ECM hydrogels from spinal cord and bone for local delivery of stem cells to spinal cord lesions. In future, successful hydrogels will be tested for their capacity to modulate the spinal cord microenvironment after injury.

Materials and Methods
ECM hydrogels were formed as previously described [1]. Viability of stem cells from the dental apical papilla (SCAP) in hydrogels (1x10^6 cells/mL of gel) was determined using a metabolic assay (PrestoBlue) (n=3, N=3). The ability of hydrogels to gel in vivo was tested in a rat brain biopsy model. Rheological properties were determined with a time sweep (1% oscillatory strain, 1 rad/sec angular frequency) and a sequential frequency sweep test (1% strain, 0.01-100 rad/sec angular frequency) (N=3) [2].

Results and Discussion
SCAP viability decreased significantly (p<0.05) in bone ECM 10mg/mL (bECM10) compared to the other conditions tested (Fig. 1A). 10mg/mL spinal cord ECM hydrogels were heterogeneous, and therefore difficult to deliver reproducibly. A robust gel was formed from bone ECM 8mg/mL (bECM8) in vivo, whereas spinal cord ECM 8mg/mL (scECM8) did not gel. High SCAP viability in scECM8 followed by poor gelation, prompted the testing of combinations with bECM10 and with fibrin. Fibrin in the combinations significantly lowered SCAP viability on day 0, compared to scECM8 (p<0.05) (Fig. 1B). Selected hydrogel combinations: scECM8/bECM10 75/25, scECM8/fibrin 75/25 and bECM10/fibrin 75/25 gelled in vivo. Faster formation and higher storage moduli of bECM8 and selected combinations, compared to scECM8 hydrogels, correlated with in vivo observations (Fig. 2).

Conclusions
bECM8 was easy to handle. Potential advantage of scECM8/bECM10 75/25 and scECM8/fibrin 75/25 is that these hydrogels are homologous to the site of injury. In scECM8/fibrin 75/25 and bECM10/fibrin 75/25, lower SCAP viability at the point of application (day 0) was counterbalanced with immediate gelation and adhesiveness of fibrin. bECM8, scECM8/bECM10 75/25, scECM8/fibrin 75/25 and bECM10/fibrin 75/25 are stiff and fast gelling materials, support SCAP viability, overcome the flow of blood in vivo, do not dissolve in brain lesions and have potential for spinal cord application.

References

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DEVELOPING 3D iPSC MODELS OF NEURODEGENERATIVE DISEASE
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Introduction
Neurodegenerative diseases are a global health concern with recent statistics indicating they now cause more deaths per annum than heart disease. Consequently, there is a worldwide effort to determine the underlying disease mechanisms and improve on current therapeutic strategies[1]. Induced pluripotent stem cell (iPSC) models offer a promising approach as they can be produced from somatic cells and subsequently differentiated into neurons harbouring the same mutant phenotypes expressed by patients in vivo[2]. Many current strategies, however, employ 2D culture systems which don’t reflect the mechanical 3D environment presented to cells in the brain. Additionally, there is difficulty in generating mature, functional neurons using 2D systems as the maturation process is lengthy and yields cells that often cannot be cultured for more than 90 days, thus inhibiting expression of disease phenotypes that develop on a more long-term scale[3]. In this study, collagen scaffolds are presented as substrates for enhancing maturation of iPSC-derived neurons with a view to moving towards creating more clinically relevant disease models.

Materials and Methods
iPSCs (provided by the University of Oxford) were cultured to 100% confluence and induced down a neuronal lineage with neural induction media. At 35 days post-induction cells were dissociated and encapsulated in collagen scaffolds (PureCol EZ Gel – Applied Biosystems, Germany) with 2D controls cultured on laminin-coated plates. RNA extraction (RNEasy Kit – Qiagen, UK) and cDNA synthesis (iScript Kit – BioRad, UK) was conducted at days 45, 60 and 90 post-induction. The resulting cDNA was analysed for expression of MAP2, synaptophysin and VGLUT using RT-PCR with GAPDH as a housekeeping gene.

Results and Discussion
Analysis of RNA transcription revealed dramatic differences in expression of neuronal maturation markers between 2D and 3D cultures (Fig. 1). The gene coding for synaptophysin, a synaptic vesicle membrane protein, was first transcribed 60 days post-induction in 2D culture but at 45 days post-induction in 3D culture. Additionally, the gene encoding VGLUT, a glutamate transporter, was first transcribed at 90 days post-induction in 2D culture but at 60 days post-induction in 3D culture. Expression of both markers is often associated with development of neurons with functional synapses. Earlier occurrence of this in 3D culture can likely be attributed to greater cell-cell interaction and signalling due to enhanced neuritogenesis (indicated by increased MAP2 expression) as this is a key mechanism in neural maturation. This could provide a potential platform for development of functionally mature, disease-relevant neurons on a shorter timescale. Consequently, culture in collagen scaffolds may facilitate reconstitution of mature disease phenotypes that cannot be established in 2D due to limits in long term viability of iPSC-derived neurons.

Conclusions
Encapsulation of iPSC neurons in collagen resulted in enhanced neuritogenesis and maturation, yielding cells with functional synapses 30 days earlier than in 2D. Collagen scaffolds seeded with patient-derived iPSCs will now be explored for long term culture (90 days +) and reconstitution of disease phenotypes.

References
THE USE OF MODIFIED AMINOSILANE COATINGS FOR USE IN PERIPHERAL NERVE TISSUE ENGINEERING

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Introduction
Peripheral nerve injuries affect 2.8% of all trauma patients, affecting 300,000 people in Europe alone. Current treatments, determined by the severity of the injury, involve direct end to end suturing or using an autograft, the current ‘gold standard’ treatment to bridge larger gaps.¹ However, there is limited donor nerve available, donor site morbidity and limited nerve function is restored. Synthetic and natural nerve guide conduits are currently available but each has its limitations, such as acidic degradation products and high degradation rates.¹ Coatings have been used to improve the biocompatibility of synthetic nerve guide conduits, as well as providing surface chemistry guidance for the regenerating axon. Natural coatings, such as collagen, can cause an immune response, depending on where they are derived.² However, synthetic coatings, such as aminosilanes, avoid this issue and are much cheaper to produce. They allow the ability to control chemical group deposition, at the sub-micron scale, and any associated topographical profiles.²

Materials and Methods
13mm Glass coverslips were immersed in 3% aminosilanes (Short Chain: 3-Aminopropyl triethoxysilane; Long Chain: 11-Aminoundecyltriethoxysilane) isopropanol solution for 2 hours, washed, and dried overnight. Surfaces were characterised using Water Contact Angle, AFM and XPS. 40,000 NG108-15 neuronal cells were seeded onto long and short chain aminosilane modified coverslips, as well as a plain glass control for 6 days. 60,000 rat primary Schwann cells were cultured on coverslips for 7 days. Dorsal Root ganglion bodies were extracted from rat spinal columns and were dissociated, mechanically and enzymatically, into primary Schwann cells and neurons, which were cultured onto the modified aminosilane coverslips for 7 days. Live/dead analysis was used to confirm the biocompatibility of the aminosilane modified glass coverslips, and immunolabelling for β III tubulin (neuronal cell and neurite marker), S100, GFAP and p75, (Schwann cell markers), was also performed.

Results and Discussion
Cell viability assays found that the highest numbers of live NG108-15 neuronal cells, and primary Schwann cells, were on the long chain aminosilane coverslips, compared to the short chain aminosilane coverslips and plain glass control. Neuronal differentiation studies found that NG108-15 neuronal cells cultured on long chain aminosilane glass coverslips had a higher average number of neurites per neuron and a statistically higher average neurite length compared to the short chain coverslips. The highest average Schwann cell lengths were higher on the aminosilane modified coverslips, compared to the controls. Schwann cells cultured on all conditions stained positively for S100, GFAP and p75 confirming a maintained phenotype. Primary neurons, cultured on the long chain aminosilane modified coverslips, had statistically higher average neurite lengths, compared to the other conditions and there was a statistically higher numbers of neurites expressed per neurons cultured on the aminosilane coverslips, compared to the other conditions.

Conclusions
This study confirmed that long chain aminosilane coatings were the most efficient for NG108-15 neuronal cell, and primary neuron, neurite outgrowth and supported primary Schwann cell attachment, function and proliferation. Differences between the two aminosilane chain lengths were seen in the number of neurites expressed per primary neuron. Both chain lengths increased the numbers of NG108-15 neuronal cell, Schwann cell, and primary neuronal cell attachment, compared to the plain glass control. Future work will involve applying this treatment to synthetic nerve guide conduits for investigations in vitro and in vivo.

References

Acknowledgements
We acknowledge the University of Sheffield for funding.
INTRODUCTION
In the extensive literature on antimicrobial coatings, results at times are surprising or contradictory. For example, when coupling onto a biomaterials surface an antibacterial compound whose mode of action is interference with an intracellular target molecule, how can such a coating be active. Further confusion arises because of differences in media, particularly when assessing cytotoxicity of antimicrobial coatings. Our research groups have focused in a number of studies on questions surrounding covalently anchored graft coatings comprising antimicrobially active molecules (low molecular weight, 300-1200 Da) or polymers. In particular, we have focused on detailed multi-technique surface characterisation, to probe for the presence of bioactive molecules that may not be covalently grafted and can interfere in biological tests, leading to incorrect conclusions that a covalently attached layer of molecules is active.

MATERIALS AND METHODS
We have used a range of antimicrobial molecules, both commercial and experimental, for various designed experiments with various grafted layers. The molecules were immobilised onto plasma polymer interlayers with suitable reactive surface groups. Surface analyses were performed using XPS, ToF-SIMS, and ellipsometry before and after extensive various washing protocols. Antimicrobial activity of samples was evaluated via attachment and biofilm formation assays.

RESULTS AND DISCUSSION
Our results show that physisorption of antimicrobials occurs to substantial extents onto many substrates – even in the presence of reactive surface groups and catalysts, such as carbodiimide. However, much larger amounts of molecules can be absorbed into polymer substrates – again bypassing reactive surface groups. Surface analysis data before and after various washing procedures clearly demonstrate the presence of both covalently grafted and non-covalent molecules. When placed into bioassays, the latter molecules diffuse into solution and interfere with microbes. This can lead to erroneous interpretation that a covalently grafted layer has activity, as we demonstrated using a negative control, levofloxacin, which has an intracellular target and thus no activity when covalently grafted, yet diffusing molecules gave rise to activity.

Our results also demonstrate that detailed surface analytical studies in combination with various washing protocols – including severe conditions such as SDS and high temperature – are needed to ascertain that a putative antimicrobial coating exerts its bioactivity only through covalently grafted molecules rather than through solubilising molecules.

CONCLUSIONS
During covalent grafting, some molecules avoid reacting and physisorb and absorb onto/into the substrate (or spacer hydrogel layers). If not recognised and accounted for, such contributions can confuse interpretations. Some of the surprising and contradictory literature data may have been caused by unrecognised contributions from systems more complex than the intended covalent graft layer.
PECTIN-BIOACTIVE GLASS SELF-GELLING, INJECTABLE COMPOSITES WITH ANTIBACTERIAL ACTIVITY

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Introduction

Hydrogels, i.e. 3D crosslinked polymer networks with entrapped water, are interesting biomaterials owing to their injectability, permitting minimally-invasive implantation, and ease of incorporation of multifunctional microparticles. For applications in bone regeneration, the presence of an inorganic component is considered desirable. One such inorganic component are bioactive glasses. Besides their bioactivity, glasses release several types of ions, including calcium, which can be exploited to crosslink anionic polymers to induce hydrogel formation. One such type of calcium-binding polymers are pectins, which are used as thickeners in foods such as jams. In this study, a calcium-rich bioactive glass produced by a sol-gel method, hereafter named A2, was added in particle form to pectin solutions in order to induce their gelation, resulting in self-gelling, injectable hydrogel-particle composites. Two different A2 particle diameters, namely 2.5 and <45 µm were compared. Two different amidated pectin preparations derived from apple (AA) and citrus (CA) were compared. Composites were characterized physiochemically, microbiologically and cell biologically.

Materials and Methods

A2 (54% CaO, 40% SiO2, 6% P2O5, all molar %) was prepared as described previously (1). Particle size was determined by laser diffraction. A2 particles and pectin solution (1.5% (w/v)) were sterilized by autoclaving. A2 were added to pectin solution to yield a final concentration of 20% (w/v) and shaken vigorously to form composites. Gelation speed of pectin-A2 composites was studied by rheometry. Mineral formation after incubation in Simulated Body Fluid (SBF) was studied by SED/EDX, FTIR and XRD. Distribution of A2 in composites was studied by µCT. Antibacterial activity was tested against methicilin-resistant Staphylococcus aureus (MRSA). The proliferation of MC3T3-E1 osteoblast-like cells on the composites was studied.

Results and Discussion

A2 particles of both sizes, 2.5 and <45 µm, induced hydrogel-particle composite formation, for both AA and CA pectins. Gelation was completed within 20 minutes (Figure). Hydroxyapatite (HA) was formed in all composites after incubation for 28 days in SBF (Figure). µCT revealed inhomogeneous distribution of particles within composites. All composites were highly antibacterial against MRSA, inhibiting bacterial growth almost completely. MC3T3-E1 cells attached and proliferated on all composites, although a certain cytotoxic effect was observed when cells were cultured in the presence of eluates from composites.

Conclusions and Outlook

A2 bioactive glass particles induced the gelation of both AA and CA to form injectable self-gelling hydrogel-particle composites, which induced HA formation upon incubation in SBF, composites exhibit antibacterial activity against MRSA and support MC3T3-E1 osteoblast-like cell adhesion and proliferation. No major differences were seen between composites containing AA or CA, or A2 of particle diameters 2.5 and >45 µm. Further experiments are required to test the composites’ ability to support osteogenic differentiation.


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**ANTIBACTERIAL TITANIUM: PREVENTING INFECTION FOLLOWING CRANIOPLASTY**

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**Introduction,**
The current management of microbial infection in injured tissues is by the systemic administration of antibiotic drugs. These are designed to eliminate pathogens while leaving the patient unharmed. This method is of limited efficacy as systemic antibiotics are not targeted to the site of infection and will often have poor penetration to areas around wounds and implants. The widespread use of prophylactic antibiotics has also led to increasing microbial resistance.

Biomaterial solutions to target implant infections rely heavily on introducing an initial coating such as a polymer, which then allows antibiotic agents to be included onto an implant surface. The antimicrobial agent can then either leach from the polymer or be covalently attached to the polymer. While this methodology is well established there are two significant drawbacks. Firstly, the inclusion of coatings such as polymers onto the surface of metal implants can hinder the osseointegration of the implant. Secondly, the leaching of materials gives sustained release over several months yet only tend to last for months. The majority of joint infections occur at greater than one year, this means the biomaterials don’t address the real unmet clinical need.

In this work, we report a novel attachment method which allows attachment of novel antimicrobial peptides directly to a metal surface. This grafting method also remains on the surface for extended periods of time (>1 year).

**Materials and Methods,**
Titanium was plasma nitrided at 440 °C for 19 hours. The metals were polished and washed using acetone to clean the surface. The surfaces were functionalised in N,N-dimethylformamide (DMF) using antimicrobial peptide (100µg), HBTU (0.5mM in DMF) and DIEA (60% v/v in DMF). The surfaces were washed extensively to remove unreacted material and then tested for efficacy at inhibiting bacterial growth against a range of bacteria and fungi. Lab strains were used initially, and multi-resistant clinical isolates later.

**Results and Discussion,**
TOF SIMS showed the peptides were attached evenly across the surface (Figure 1a) The peptides showed broad spectrum activity against a range of bacteria including lab strains and clinical isolates of both Gram positive and Gram negative bacteria. Peptides were tested for inhibition of growth in solution and their ability to prevent bacterial growth on the metal surfaces. Peptide coated titanium designed for orthopaedic implants showed significant reductions in bacteria on the surface for patient isolates of both S. aureus and S. epidermidis (Figure 1b).

Figure 1: a) Efficacy of antibacterial titanium against S. aureus and S. epidermidis isolates. b) TOF SIMS 3x3 mm map of titanium surface showing even peak coverage.

**Conclusions,**
We have demonstrated the efficacy of our novel coating technology to both reduce bacterial load in solution around an implant and prevent bacteria creating a biofilm on the implant surface. We believe this has great potential to revolutionise implant materials and reduce the rate of implant associated infections.
THE ANTIMICROBIAL EFFICACY OF NO-RELEASING POLYMERIC SUBSTRATES

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Introduction
Given the global epidemic of antimicrobial resistance, coupled with 1 million new cases of healthcare-related infections reported each year in England,1 developing new antimicrobial strategies is an urgent clinical need. Nitric oxide (NO) is an endogenously produced free radical that is involved in various physiological and biological processes,2 and plays a key role in the host defense against microbial agents.3 As NO is a reactive gas, the utility of NO is problematic due to the lack of suitable vehicles for storage and delivery. Compounds containing the diazeniumdiolate functionality [N(O)=NO] are generated by subjecting secondary amines to high pressures of NO and are known to release NO under physiological conditions.4,5 Described herein are environmentally friendly methods to tether diazeniumdiolates onto polymer surfaces that are capable of releasing a controlled and sustained dose of NO for targeting bacterial infections. Functionalised surfaces are comprehensively characterised in terms of chemistry and topography. NO release and the antibacterial efficacy of the surfaces is evaluated against two strains of *Pseudomonas aeruginosa*.

Materials and Methods
Silicone elastomer (SE) and polyethylene terephthalate (PET) were first subject to oxygen plasma treatment. Both polymers were then silanised with aminosilanes, N-[(3-trimethoxysilyl)propyl]diethylenetriamine (DET3) and N-[(3-(trimethoxysilyl)propyl)aniline (PTMSPA). Aminosilane-grafted polymers were then reacted with high pressures of NO (5 bar) to give the desired diazeniumdiolate-grafted materials. The reaction steps were followed with static water contact angle and changes in surface roughness ascertained using atomic force microscopy (AFM). Chemical composition was characterised through XPS analysis. NO release was determined by Griess assay and electrochemical detection. Antimicrobial efficacy was assessed with antibiofilm colony forming unit (CFU) assays on strains of *Pseudomonas aeruginosa* (PA01, PA14).

Results and Discussion
This study was carried out to ascertain the feasibility of tethering antimicrobial NO donor groups (diazeniumdiolates) onto polymer surfaces. Plasma treatment of SE and PET was carried out allowing the formation of reactive oxygen-containing functional groups enabling silanisation to occur. AFM imaging confirmed each step in the reaction scheme with an increase in roughening after plasma treatment followed by a decrease after silanisation, and tethering of the diazeniumdiolate. XPS corroborated these results by showing an increase in oxygenation for the plasma treated substrate and the appearance of an N 1s peak after silanisation. The tethering of the diazeniumdiolate was confirmed with the N 1s curve fitting at 402.7 eV6, representing the (O)N=N(O) group. The diazeiumdiolate PET surfaces released 5000 nM of NO and 3500 nM for SE grafted surfaces. The antimicrobial efficacy of the surfaces was evaluated by assessing the reduction in biofilm adhesion of two strains of *P. aerugionosa*. A significant 1-log reduction was observed on SE grafted surfaces with PA14.

Conclusion
This work has shown a low-cost, environmentally-friendly technique in developing NO-releasing substrates. Initial bacterial tests have shown promising evidence of the antimicrobial efficacy of these surfaces.

References
ANTIFUNGAL AGENTS INCORPORATED IN ELECTROSPUN POLYMER PATCHES TO INHIBIT 
CANDIDA ALBICANS

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Introduction
Oral candidiasis varies in severity from acute stomatitis to chronic oral mucosal conditions. Topical anti-fungal drugs are the main therapy. However, therapy is limited because of rapid drug loss due to saliva washing that occurs for creams, tablets and mouthwashes as well as an increasing number of candida strains being resistant to anti-fungal drugs. Medium-chain saturated fatty acids are known to have anti-fungal properties and may offer an alternative therapy for oral candidiasis. Electrospinning is a versatile technique to manufacture a delivery device with potential muco-adhesive properties. This project aimed to develop an electrospun polymer patch for sustained therapeutic delivery of fatty acids to inhibit Candida albicans, the main causative organism of candidiasis.

Materials and Method
Anti-fungal ability of fatty acids
Agar disc diffusion test: 2 x 10^5 CFU/ml C. albicans (strains BWP17 and SC5314) were spread across YPD agar plates on top of which fatty acid containing (C5:0 to C12:0) (0.2 M) filter-paper discs (6.27 mm ø) were placed. Plates were incubated for 24h at 37°C after which inhibition zones are measured. Amphotericin B and DMSO were used as positive and negative controls respectively.

Electrospinning process:
Fatty acids (C5:0 to C12:0) (0.2 M) were incorporated in a poly (ε-caprolactone) (PCL) (10 w/w%)/ dichloromethane dope and then electrospun. Electrospinning conditions: 15 cm tip-to-collector distance, 3 ml/h flow rate and 17 kV voltage. Electrospun patches (12.7 mm ø) were punched from the spun mats.

Anti-fungal ability of the electrospun patches
Agar disc diffusion test: same technique as before with electrospun fatty acid patches tested on candida strain SC5314 and an azole resistant strain CAR17.

Biofilm viability: 1 x 10^6 CFU/ml C. albicans SC5314 were pipetted into pre-treated 24 well plates and incubated at 37°C overnight to grow biofilm formations. Electrospun patches containing fatty acids (C7:0 to C12:0) are placed on the biofilm along with 400 μl RPMI. After incubation XTT analysis is performed to test biofilm viability.

Results and Discussion
Fatty acid C8:0 and C9:0 showed the greatest inhibition of yeast C. albicans when placed on filter discs and when incorporated in electrospun patches (fig. 1) respectively. On the azole resistant strain CAR17 PCL C9:0 showed the greatest inhibition, even compared to C9:0 on a filter disc. On the biofilm the electrospun PCL patches C8:0 to C12:0 all showed significant inhibition.

Conclusion
Fatty acids (C7:0 to C12:0) successfully inhibited three strains of Candida albicans, including an azole resistant strain, both in its yeast and hyphal (biofilm) states. Furthermore, the electrospun patch was able to act as a delivery device for the release of fatty acid as a therapeutic agent to inhibit C. albicans.

References

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STEREOLITHOGRAPHY 3D PRINTING OF POLY(D,L-LACTIDE)

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Stereolithography 3D printing (STL) shows great potential for the fabrication of bioengineered parts, where design fidelity and high detail resolution are required. In STL a liquid resin is selectively photo-polymerized in a layer by layer fashion in order to fabricate the desired solid object. Research in this area can benefit from the growing number of low-cost desktop printers made commercially available, but it is still hindered by the lack of biocompatible resins. As part of our research on biofabrication, we present the formulation of a poly(D,L-lactide) (PDLLA) based resin and its application on a low-cost STL printer.

We synthesised methacrylated PDLLA macromonomers via ring-opening polymerization of D,L-lactide (Corbion Purac) at 130 °C under inert atmosphere. 1,6-hexanediol and stannous octoate (Sigma-Aldrich) were used respectively as initiator and catalyst. Functionalisation with methacrylic anhydride (Sigma-Aldrich) was performed over 6 hours at 120 °C in the presence of K₂CO₃. ¹H-NMR (Bruker Ultrashield 300) was performed to assess molecular weight and functionalisation of the macromonomers. To formulate an STL resin, diethylene glycol diethyl ether (39.1 wt%) was added to the macromonomer (58.6 wt%) as non-reactive diluent, using diphenyl(2,4,6-trimethylbenzoyl)phosphine-oxide (TPO, 2 wt%) and hydroquinone (0.3 wt%) as photo-initiator and inhibitor respectively. The resin was characterised for its photo-curing behavior on the Ember STL desktop printer (Autodesk, Inc.). The printer is open-source and equipped with a 22.5 mW/cm², 405 nm LED. The same printer was then used for the fabrication of computer designed parts.

A degree of functionalisation of 97% was confirmed by ¹H-NMR spectra analysis. From the same, the number average molecular weight was found to equal 2.8 kg/mol. Figure 1 shows the semi-logarithmic plot of thickness of a cured layer, Cₒ (µm), against the irradiation dose, E (mJ/cm²) for our resin tested on the Ember. The trend-line (working curve) obtained informs on the critical dose, Eₘ (mJ/cm²), at which the gel point is reached and the depth penetration, Dₚ (µm), which defines the penetration of light in the resin and the attainable range of layer thickness. For our application the critical dose was 56.7 mJ/cm² and the depth penetration 335 µm. To test printability, 10 × 5 × 2.5 mm³ slabs with a 5x3 array of square pores (1 mm²) were designed and uploaded to the Ember. The light exposure time was set to 20 seconds for the first layers, in order to achieve good attachment to the printer build head. All other layers were printed at 5 seconds exposure. The printer step height was set to 50 µm which results in a total of 50 layers to print. Figure 2 shows the printed part, with the insert representing a magnified top view. We found that the designed geometry was largely reproduced, whilst pores were partially occluded in deeper layers.

In conclusion, the printability of a PDLLA based STL resin is here reported. We show that a custom resin can be readily formulated and used on an unmodified, low-cost desktop printer. The printing was achieved with the geometry being closely reproduced. In this work we focused on printability; in order to increase printing resolution we will introduce a suitable UV absorber in the resin formulation to decrease Dₚ and undesired overcuring. Cytotoxicity and bioreabsorbability studies will be conducted on the parts after extraction of soluble fraction.
**SUSPENDED ADDITIVE LAYER MANUFACTURE OF OSTEOCHONDRAL STRUCTURES**

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**Introduction**

Additive Layer Manufacturing (ALM) is a technique that has showed promise in the field of tissue engineering, with the potential to create detailed biological constructs of defined shape. This could be applied to fabricate implants for patient-specific defects or replicating interfaces between different tissues such as bone and cartilage. Limitations in ALM however, are caused by the small range of materials that are compatible with the technique and that have mechanical properties that resemble native extra cellular matrix. Here we use a novel method of extrusion-based ALM whereby the printed material is extruded into a suspending, self-healing gel media rather than onto solid surface. To demonstrate the complexity achievable with this technique, scaffolds that mimic the structuring and cellular organization of an osteochondral defect were manufactured using composite hydrogel structures with anisotropic mechanical properties that mimic the native osteochondral environment.

**Materials and Methods**

Osteochondral plugs were produced by the addition of chondrocytes and osteoblasts to 1.5% w/v gellan and 1.5% w/v gellan-5% nano-hydroxyapatite respectively. The consecutive extrusion of these two solutions into the suspending fluid gel bed followed by ionic crosslinking produced a bi-layered construct that was implanted into a femoral condyle defect in vitro and cultured for 4 weeks. Following culture qRT-PCR, fluorescent IHC and H&E staining were performed.

**Results and Discussion**

Layered scaffolds were fabricated from biopolymer hydrogels using a suspended ALM method producing an osteochondral analogue that was implanted into a full thickness osteochondral defect (Fig 1A). Scaffolds retained their structure throughout the culture process. PCR for types I and II collagen revealed a gradient in ratio of expression after 30 days culture in the simulated defect. Expression of COL2A, was highest in cells located in the cartilage region of scaffolds. Conversely, COL1A showed the lowest levels of expression in cells within this region (Fig 1B).

**Conclusions**

It was shown that suspended manufacture could recapitulate the structure of the osteochondral region as defined by micro CT. The bioprinted composite structure maintained its shape and mechanical robustness over a period of 30 days of culture during which the encapsulated cells retained their phenotype. This novel method of manufacturing 3D tissue-like structures has significant promise for the study of interfaces and regeneration of complex tissue.

**References**


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THE MANUFACTURE OF 3D ELECTROSPUN SCAFFOLDS WITH COMPLEX FEATURES

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Introduction
Electrospinning may be used to manufacture 3D scaffolds for use in in vitro models or regenerative medicine applications. Researchers at Sheffield have previously developed a medical device based on an electrospun scaffold that contained regions tailored for the delivery of limbal stem cells in ophthalmology.1 Recent research suggests that surface morphology, including fibre alignment, may influence the behavior of stem cells on scaffolds2. The aim of this research was to develop and evaluate a templating method for the preparation of complex heterogenous electrospun meshes that could be used to investigate the alteration of cell behaviour without biochemical or similar modification.

Materials and Methods
Polycaprolactone (PCL) scaffolds were manufactured by electrospinning. PCL (Sigma or Corbion) was dissolved in solvent (DCM/DMF, HFIP, Chloroform) at 10% or 15% (w/v) then stirred until a uniform solution was produced. The charged polymer was collected on an earthed flat collector or a cylindrical collector using either a slow rotation (100-200 rpm) or high speed (2000-2500 rpm). Electrospinning onto a custom stainless steel template prepared using metal additive manufacturing was used to introduce heterogeneous 3D microfeatures. Scanning electron microscopy (SEM) was used to image scaffolds, and Fibremetric software (PhenomWorld) was utilised to analyse fibre characteristics.

Results and Discussion
The results demonstrated that electrospun fibres could be spun using both polymer sources with the three solvent systems. Due to small molecular weight differences, the Sigma PCL was spun effectively at 10 % and 15% (w/v), while the Corbion PCL generated a more consistent product at 15 % (w/v). Collection onto a flat collector produced random fibres. Drum rotation speed was used successfully to produce either random fibres at slow speeds or aligned fibres at the higher speeds. Comparative studies of polymer source and solvent showed that the Corbion polymer with chloroform was the best system for electrospun scaffold manufacture. This method produced the more reliable scaffolds for both random and aligned fibre orientations. These scaffolds were characterised in terms of the fibre diameter and level of alignment present. Therefore, parameters have been defined to manufacture random and aligned scaffolds. The incorporation of a range of 3D microfeatures into the electrospun mats was further demonstrated using the custom template (Figure 1).

Conclusions
Scaffolds containing random and aligned fibre orientations were reproducibly manufactured and characterised. Polymer source, solvent system and the collection method (eg random or aligned) were all shown to be determinants of the properties of the mesh produced. Further work is ongoing to investigate how these scaffolds can be utilised as a research tool to analyse the effect of fibre orientation on the behaviour of stem cells, ultimately using electrospinning as a manufacturing technology for the manufacture of enhanced devices for regenerative medicine.

References

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ELECTROSPUN BIORESORBABLE TISSUE REPAIR SCAFFOLDS: TRAVELLING THE PATHWAY FROM LABORATORY TO CLINIC

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Introduction
Over the past two decades there has been much research into the use of electrospun biomaterials for applications in regenerative medicine, drug delivery, and in vitro tissue modelling. Although a promising technique for the manufacture of a wide variety of functional biomaterials, complex technical challenges and regulatory requirements must be satisfied in order to develop clinically-acceptable products using an electrospinning manufacturing process. Electrospinning uses a large electric potential difference to cause an electrically charged jet of polymer solution to be ejected from conducting needles towards a collector. Electrostatic repulsion, a rapid whipping motion of the jet, and solvent evaporation all contribute towards significant narrowing of the jet as it travels towards the collector. Very fine (10 nm to 10 µm diameter) fibres are formed as the jet dries, and these accumulate on the collector resulting in highly porous materials with very large surface areas. Since 2007 Neotherix has been developing novel electrospun biomaterials, and has encountered a number of technical challenges in the establishment of a reliable, scalable and commercially viable electrospinning process. The technical solutions generated have allowed the completion of, to the best of our knowledge, the first clinical trial of an electrospun regenerative device [1].

Materials and Methods
Neotherix uses a custom-built electrospinning rig, designed to have a highly variable configuration to satisfy the requirements of biomaterial research, while having the capacity to act as an early stage manufacturing rig following release of a product onto the market. It can be set up in six different high voltage configurations: needles and target can be at positive, negative or zero potential (independently). Process settings can be independently varied including: number of needles, potential difference, electric field polarity, collector diameter and rotation speed, needle to collector distance, and needle traversing rate. Electrospun scaffold biomaterials were prepared using this rig according to methods described in several patent applications [2].

Results and Discussion
The challenges encountered in the development of electrospun biomaterial scaffolds, and the resulting technical and design solutions will be discussed. Design challenges include optimisation of the 3D architecture for the cell types of interest, material selection, and selection of suitable product sterilisation processes. Process related challenges include determining how to solubilise a difficult to dissolve polymer, the best means for encapsulation of active compounds, methods for stabilising problematic formulations, and how to manufacture bilayer scaffolds. Manufacturing challenges include how to maintain process reliability and material quality while scaling up production volume, and how to control the manufacturing process to allow production of a sterile medical grade scaffold to international regulatory standards. The successful translation of a novel biomaterial from laboratory to the clinic will be used as a case study to explore how electrospinning can be developed into a reliable, scalable and commercially viable process. Additional data will be presented showing how Neotherix’ scaffolds can be used for in vitro 3D tissue modelling applications, for example the modelling of bovine endometrium [3].

Conclusions
Neotherix has developed a reliable and scalable electrospinning process for the manufacture of safe and clinically-relevant implantable biomaterials. A resorbable tissue repair scaffold was successfully translated from laboratory to clinic using our ISO 13485 certified process, culminating in the first clinical trial of an electrospun regenerative device despite an increasingly challenging regulatory environment. Lessons learned in travelling this pathway can be applied to the development of other earlier-stage pipeline technologies, potentially leading to the translation of even more sophisticated electrospun biomaterials.

References
1. See study details at: https://clinicaltrials.gov/show/NCT02409628

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2017 UKSB President’s Prize

Professor Ruth Cameron, University of Cambridge

ICE TEMPLATED STRUCTURES FOR REGENERATIVE MEDICINE

The talk will describe how ice templating technologies can be used to create novel, complex and biomimetic 3D environments for the control of tissue growth adaptable to a wide range of medical applications.

Ruth Cameron is Professor of Materials Science in the Department of Materials Science and Metallurgy and a Director of the Cambridge Centre for Medical Materials. She is Senior Fellow and Director of Studies in Physical Natural Sciences of Lucy Cavendish College, Cambridge.

Prof. Cameron’s research in regenerative medicine concerns medical materials that will interact therapeutically with the body, which may have bioactive structures that encourage repair and regeneration, provide tailored mechanical support, or release drugs at a controlled rate. Research themes include ice templated scaffolds, biodegradable and bioactive polymers, composites and active surface design.
Direct restoration of damaged teeth requires biomaterial formulations than can undergo rapid in situ transformation to strong solids. Dr Alan Wilson (1928-2011) developed aesthetic and clinically accepted Glass Ionomer cements (GIC) for this purpose, based upon the reaction between polycarboxylic acids and ion-leachable glasses. His lasting contribution was in the chemistry of these reactive glasses that produce abundant Ca2+, Al3+ and F- ions in response to an acidic challenge. This creative initiative stimulated the exploration of alternative co-poly-acids, incorporating phosphoric acid moieties as well as –COOH functionality. Some of these developments have led, decades later, to novel designs of electro-spun scaffolds for bone regeneration.

Meanwhile, in parallel to GIC, an alternative strategy emerged based around the in situ polymerization of viscous di-methacrylate monomers (or ‘resins’) containing high volume-fractions [up to ca. 70 % v/v] of silanated, but otherwise inert glass powders. These are known as resin-composites or resin-based-composites (RBC) and may be considered – along with GICs – as generically biomimetic, where the glass powders are analogous to hydroxyapatite (HAP) and the polymer matrix to collagen. During recent decades progressively more sophisticated glass-powders have been developed, especially with nano-hybrid size ranges.

In the early 1980s, photo-polymerization of RBC pastes was widely introduced using QTH and more recently LED blue light sources. This technology has been successful clinically, although not always deployed optimally. However, some intrinsic problems have been identified – notably polymerization shrinkage stresses that may compromise the integrity of adhesive interfaces. R&D has been directed towards mitigating such effects inter-alia by alternative monomer systems and irradiation protocols. Since about 2010, attempts have been made to incorporate bioactive glasses in RBC formulations. We will survey the current status, combining personal and global insights.

David Watts is Professor of Biomaterials Science in the School of Medical Sciences at the University of Manchester. He is a Fellow of the Institute of Physics, the Royal Society of Chemistry and the Royal Society of Biology. He has personally supervised more than 60 PhD candidates from 20 nations. Since 1998 he has been Editor-in Chief of Dental Materials (Elsevier). In 2010 he received the research prize of the Alexander von Humboldt foundation.
Introduction

Disease and injury to the joints are increasingly common in an ageing population. Current tissue engineering approaches are limited in their capacity to regenerate the bone/cartilage interface, which is important to provide enough strength for the repaired tissue to bear sufficient loads to be fully functional. A multilayer non-delaminating functional chitosan scaffold is being developed to promote dual differentiation of mesenchymal stem cells and specifically to replicate the in vivo bone/cartilage environment.

The aim of this project is to optimise the current scaffold and its production process to facilitate successful translation to the clinic.

Materials and Methods

Process mapping techniques, which help identify the key process stages and highlight areas of variability, have been used to produce a more robust and repeatable output.

The main functional criteria of the scaffold have been investigated and tests performed to determine how well the current product meets the criteria. This work includes details of the scaffold’s compressive strength and modulus, delamination properties under tensile stress, pore size and porosity, degradation profile and the scaffold’s ability to support cell attachment, proliferation and differentiation into the bone and cartilage lineages. An in vivo model of degradation has been trialled in vitro, to mimic the effect of enzymes present at the elevated levels compatible with sites of inflammation and injury such as osteoarthritis.

Results and Discussion

The scaffolds compressive strength and degradation profile have been improved by optimising the concentration of a natural cross-linker. The use of this cross-linker has enhanced the cyto-compatibility of the scaffold and produced a porous scaffold with graded pore size to mimic the natural architecture of the cartilage and subchondral bone regions. The incorporation of Nano-Hydroxyapatite into the structure has increased the osteoconductive properties of the bone phase and the scaffold’s osteogenic potential has been shown by tracking levels of alkaline phosphatase activity and presence of mineralisation during culture with mesenchymal stem cells.

Conclusions

A biomimetic and resorbable scaffold has been produced using a scalable manufacturing route that produces a uniform output with graded pore size. The production process has been optimised to provide a non-delaminating biphasic plug with an improved compressive modulus and defined degradation profile that is able to support attachment, proliferation and differentiation of mesenchymal stem cells to support cartilage and subchondral bone repair.

Acknowledgements

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REPAIR OF OVINE ANTERIOR CRUCIATE LIGAMENT USING AN ACELLULAR PORCINE TENDON GRAFT: BIOMECHANICAL AND BIOLOGICAL CHARACTERISATION AFTER 26 WEEKS IN-VIVO

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Introduction: The decellularisation of xenogenic tissues offers a promising solution to the repair of damaged musculoskeletal structures such as the ACL by delivering immunologically safe reconstructive biomaterials in plentiful supply. This study aimed to investigate the consequences of in-vivo regeneration and integration with the host environment on the biomechanical properties of acellular porcine super flexor tendon (pSFT) grafts following 26 weeks implantation in an ovine ACL model.

Materials and Methods: pSFT’s were harvested from 4-6 month old large white pigs and trimmed to dimensions of 6.5-7mm diameter and 100mm length. Decellularisation was carried out using a previously established procedure [1], including antibiotic washes, low concentration detergent (sodium dodecyl sulphate (SDS), 0.1%) washes and nuclease treatments. Allograft ovine flexor tendon (oFT) grafts were harvested aseptically from 1 year old Texel sheep to serve as a control. Both graft types were tested for sterility prior to packaging and all grafts were gamma irradiated with a minimum dose of 25kGy.

pSFT and oFT grafts were implanted for biological assessment (n=4) or for biomechanical testing (n=6) in 2-4 year old skeletally mature sheep. For biological assessment, samples of graft and surrounding soft tissues were fixed and embedded in paraffin wax using standard techniques. Bone tunnels were embedded in poly (methyl methacrylate) (PMMA) resin and sections stained with Modified Paragon stain. For biomechanical testing, the extremities of the tibia and femur were potted in PMMA cement allowing for secure mounting to an Instron 3365 (Instron, UK) (figure 1). Ten loading cycles between 0 and 20N at a rate of 100mm/min were used to precondition each specimen prior to a ramp to failure at 200mm/min until rupture was achieved. In addition, acellular pSFT’s were implanted into ovine knees (n=6) immediately prior to testing to serve as a t=0 control. Load-extension data was fitted to a bi-linear model [2] to determine values for toe and linear region stiffness in addition to load at failure and extension at failure. Statistical variances between specimen groups were determined by 1-way analysis of variance (ANOVA). Tukey’s honestly significant difference test was used for post hoc evaluation and a p-value of <0.05 was considered to be statistically significant.

Results and Discussion: After 26 weeks in-vivo, no significant differences were found between acellular pSFT and oFT allograft groups for any of the biomechanical parameters investigated. The control acellular pSFT at t=0 group had substantially reduced biomechanical parameters when compared to either implanted article groups (t=26 weeks). Histopathological analysis points to good integration at 26 weeks, including signs of ossification, formation of Sharpey’s fibres, cellular infiltration and ligamentisation of the graft (figure 2). The results of the biomechanical testing indicate that acellular pSFT grafts perform equally as well as oFT allograft controls. In both cases bone tunnels were found to have healed around the implanted grafts.

Conclusions: The improvements found in the biomechanical parameters between t=0 and 26 weeks in-vivo are evidence of the successful and continuing regeneration and integration of the acellular pSFT within ovine knees.

References:
AN IN VITRO CO-CULTURE MODEL OF THE RETINAL VASCULATURE FOR USE IN THE DEVELOPMENT OF REGENERATIVE MEDICINE STRATEGIES FOR DIABETIC RETINOPATHY

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Introduction
Diabetic retinopathy (DR) is characterised by early stage breakdown of the blood retinal barrier and loss of capillary viability. Greater understanding of early cellular changes, including cell loss and progenitor cell migration, will promote development of earlier intervention strategies to prevent vision loss. An optimised 3D co-culture model of the human retinal microvasculature will provide a useful tool to study the effect of potential cellular therapies on the diabetic retinal microvasculature. The aim of this study was to develop a co-culture model using primary human retinal cells to explore their response to diabetes mimicking stresses such as high glucose and low oxygen.

Materials and Methods
Human retinal microvascular endothelial cells (hREC) and human retinal pericytes (hRP) (both CellSystems) were cultured in various concentrations of serum, physiological glucose, high glucose, 20% oxygen and 5% oxygen. Cells were characterised using immunofluorescence staining for change in expression of proteins involved in angiogenesis and oxidative stress. Cells were grown in monoculture or co-culture in 24-well plates to explore the cell-cell contacts in monocultures or endothelial-pericyte interactions in co-cultures, mimicking the retinal microvasculature. A 3D co-culture model was optimised, growing hRP and hREC on either side of polyethylene terephthalate (PET) transwell membranes (MilliCell®), in healthy vs. diabetic conditions for up to 3 weeks. hRP and hREC were CellTracker tagged to confirm successful attachment to PET transwells. Cells on the PET membranes were imaged using confocal microscopy. Barrier properties were measured using trans-endothelial electrical resistance (TEER) and metabolic activity was monitored using resazurin sodium salt.

Results and Discussion
hRECs and hRP were characterised by immunocytochemistry (ICC) and maintained their morphology over time in vitro. Metabolic activity (resazurin assay) of both cell types was monitored over time, with little change in healthy vs. diabetic glucose over 7 days, suggesting longer timescale of conditioning is required to affect cell number and metabolism. The cells responded differently in mono vs. co-culture. Expression of proteins and cell surface receptors was visualised using fluorescently tagged antibodies in hypoxic (5% oxygen) and high glucose (33mM [D+] glucose) conditions either as mono- or co-cultures in static conditions and changes were seen in superoxide dismutase-1 (SOD-1) and angiopoetin-2 (Ang-2) expression, which are involved in the oxidative stress and angiogenic responses respectively. Further investigation of secreted proteins will be carried out using multiplex ELISA. Both cell types can survive on the PET membrane for up to 21 days. Serum was essential for hRP attachment to the underside of the transwell insert but 5% FCS allowed sufficient hRP cell growth and metabolic activity. There are examples of in vitro 3D co-culture blood-brain and blood-retinal barrier models, but all use either animal, immortalised cell lines or cells isolated from larger vessels [1,2]. This model is the only example of human primary retinal microvascular cells we are aware of, making it more translatable to diseases of the human retina. This model can then be used to aid in understanding early changes in the retinal microvasculature in conditions such as diabetic retinopathy, an increasing problem worldwide.

Conclusions
Both hRP and hRECs have been maintained successfully for up to 3 weeks in 3D co-culture. Changes in expression of oxidative stress and angiogenic response molecules have been observed in both cell types in static co-culture conditions. The next step is to add flow to the model to determine any changes to cell behaviour in static versus dynamic conditions.

References

Acknowledgements
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DELIVERING THE IMMUNOMODULATORY PROPERTIES OF MESENCHYMAL STEM CELLS

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Introduction

Mesenchymal stem cells have been extensively investigated for exploiting their trilineage potential in regenerative medicine therapies. In addition, they also exhibit immunomodulatory behaviour that aids in wound healing and regeneration. Understanding the mechanisms through which mesenchymal stem cells regulate inflammation will help identify the best delivery routes of these cells for treating inflammatory conditions and immune diseases. In this study we investigate the different mechanisms by which mesenchymal stem cells communicate and respond to an inflammatory niche. We have developed an in vitro model to assess the immunosuppressive behavior of MSC when loaded within different biomaterials.

Materials and Methods

Our initial studies look at the effect of Balb/c mouse MSCs on the proliferation of concanavalin A-activated Balb/c mouse splenocytes in vitro. In the bioassay, MSCs either as monolayers or within different biomaterials are plated onto the well bottom while the activated splenocytes stained with cell tracker violet dye are added to a transwell insert. Proliferation of the splenocytes following exposure to the MSC is measured using flow cytometry at various timepoints. MSCs were loaded into alginate and prepared as macro-beads or loaded into Fibrin clots.

Results and Discussion

A ~50% reduction in the proliferation was observed for each batch of activated splenocytes exposed to same batch of MSCs monolayer confirming the immunosuppressive nature of MSCs in vitro. While alginate (without MSCs) in itself did not affect splenocyte proliferation, MSC-encapsulated alginate showed immunosuppressive effect although only after repeated exposures to fresh batches of activated splenocytes. Initial results on Fibrin show that the biomaterial alone could have immunosuppressive properties while MSC-loaded Fibrin a higher potency than MSCs encapsulated in alginate.

Conclusions

A bioassay has been established to assess the immunosuppressive potential of MSCs. MSC encapsulation within different biomaterials has an effect on this property and by comparing the effect the biomaterials have on the immunomodulatory behavior of the MSCs the best material and design can be identified for further in vivo delivery of the cells.
BIOINSPIRED “GREEN” NANOMATERIALS FOR BIOMEDICAL APPLICATIONS

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Abstract:
Nanostructured materials are widely studied for their use in biomedical applications. These materials vary in their compositions (e.g. inorganic or organic), their sizes, shapes, surfaces and efficacies. Although good progress is being made in evaluating the safety of using such novel materials, there has been very little attention given to the safety or sustainability of their synthesis/preparation, let alone their manufacturing.

This issue is illustrated with the case of silica as an example. Except a gold-coated silica product (Auroshell, which is in the first stage of development as an anticancer agent), there are currently no silica-based drug delivery systems on the market, despite the fact that extensive studies on mesoporous silicas have shown them to be very promising drug delivery systems, nearly 15 years ago. This delay is due to the use of hazardous chemicals and harsh conditions. Similar issues are common with most other materials under investigation for biomedical applications.

In recent years, biologically inspired green methods have been developed to produce a range of nanomaterials. This presentation will explore such new methods, analyse their “green-ness” and compare efficacy in biomedical applications (including toxicity) of nanomaterials produced using these new methods. The presentation will end by drawing design principles for green nanomaterials and tuning their properties for a range of applications.
THE INVESTIGATION OF MARINE DERIVED BIOSILICA FOR BONE REPAIR STRATEGIES

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Introduction
Silica incorporation into biomaterials, such as Bioglass and Si-substituted calcium phosphate ceramics has received significant attention in bone repair. It has been suggested that Si ions stimulate the differentiation and mineralisation of osteoblast precursor cells1, and that silicate-substituted hydroxyapatite can promote fast bone growth and apposition in vivo2. Bioglass® 45S5 (trade name PerioGlas®) developed by Larry Hench has now been implanted into one million patients with maxillofacial defects3. In spite of the success of silica application in this area, its specific role in bone healing remains unclear.

Diatoms are unicellular microalgae, that have a unique silica cell wall, known as frustules. There are over 25,000 described species, with approximately 184 new species being identified each year (3). Each species has its own unique topography and shape offering a good opportunity to study the influence of the physiochemical properties of silica on bone repair. The aim of this project is to improve the osteogenic potential of biopolymer scaffolds, and enhance the mechanical properties of bioresorbable polymer composites via incorporation of diatom biosilica. In the process, we hope to gain a better understanding of the specific role of silica in bone repair.

Materials and Methods
C. meneghiniana a marine diatom, collected from the Mississippi River, USA was used in this study. Preparation involved a nitric acid digest and washing in dH2O until a neutral pH was obtained. After washing, heat treatment will remove the organic matter (30 to 35 wt.%) from diatom. To quantify diatom dissolution rate, 100mg of dry weight diatom was added to 10mls of PBS. Mesoporous silica (MCM-41) was used as a control. Scanning Electron Microscope (SEM) and Brunauer-Emmett-Teller (BET) were studied to measure the morphological profile and pore size of diatom. Samples were immersed in PBS solution (pH 7.4) at 37°C under dynamic mixing conditions for 1, 3, 5 and 7 days. At each time point, the pH was recorded and the supernatant was analysed for Si ions using Inductively coupled plasma mass spectrometry (ICP-MS). The diatom was also added to Poly(DL-Lactide-Co-Glycolide) (PDLGA) at 1 and 5 wt.%. Diatom and PDLGA granules were compounded via twin-screw melt-extrusion, to form in a filament, which was either compression moulded or cut into dumbbells for tensile testing or utilised for (3D FDM) printing of scaffolds.

Results and Discussion
Fig. 1 shows the typical morphology of a marine diatom, which was not significantly changed by seven days’ dissolution test in PBS media. Concurrently the ICP results demonstrated near-linear Si release kinetics up to 58 ppm at 7 days. The diatom medium demonstrated a slight drop in pH from 7.4 to 7.1 after 24 hrs in media thereafter no change was observed. Fig. 2 shows Young’s moduli of various PDLGA composites after tensile testing. The Young’s modulus of PDLGA with diatom (5 wt.%) was significantly enhanced (3319±67 MPa) compared to the pure polymer. An enhancement was also noted for tensile strength (56.9±1.7 to 64.4±0.9 MPa).

Conclusion
This project is investigating the potential use of diatoms as a component of bioresorbable polymer scaffolds. As an additive in PDLGA, diatoms can provide a significant benefit in terms of Young’s modulus and strength. Concurrently they can provide a valuable source of low-release Si with near-linear dissolution kinetics. Future work will investigate the influence of FDM scaffold production on the performance of these promising composite biomaterials.

Reference
A NOVEL Mg2+-ELUTING POLYMER SCAFFOLD FOR BONE CELL PROLIFERATION AND MINERALIZATION

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Introduction
Mg2+ is a co-factor for many different enzymes and is important for cell proliferation, protein synthesis and apoptosis¹. Increased intracellular Mg2+ is able to serve as a regulator of cell cycle by enhancing the protein synthesis². In this paper, we introduce novel magnesium ion-eluting polymer materials, synthesized by a novel in-situ ring-opening polymerization technique, which can be potentially used for the enhancement of osteogenesis. It is the first time a biodegradable and magnesium-containing polymer has been developed with a 3D structure suitable bone for tissue engineering.

Materials and Methods
Lactone based monomers (L,D-Lactide, ε-caprolactone and δ-valerolactone) were pre-mixed at various ratios and heated at 150 °C for 24 h, with the addition of layered double hydroxide (LDH) as the polymerization initiator. The ring structures of the monomers were opened by the LDH, and the magnesium and aluminium ions from the initiator cross-linked the opened linear polymer chain in the reaction. Consequently, a divalent cross-linked ionomer materials were formed with a fibrous network, creating a porous 3D structure.

The resulting ionomers were ground and filtered, then coated using Pt for SEM imaging. ICP was used to measure the ion concentration leaching out from the ionomer at different time points within 2 weeks.

Saos-2, an osteosarcoma cell line, was selected as the model to investigate osteogenesis potential. Cells were cultured indirectly with ionomer materials installed in the bio-inserts. Pico Green and ALP were applied to indicate proliferation and differentiation of cells, respectively.

Results and Discussion
The fibrous microstructure of the ionomer materials is demonstrated by scanning electron microscopy (SEM) (Figure 1). This material provides a suitable architecture for direct cell attachment and migration. Magnesium ions are detectable in the rinsed culture medium at 3.125 mg/ml for 3 monomer compositions, respectively (figure(2)). The copolymer-based ionomers had higher Mg2+ ion concentrations compared to that of the homo-polymer.

For proliferation, the samples with lower concentrations of ionomer appeared to show only marginal differences compared with the TCP control in the culture medium, without osteogenic induction factors (Figure (3)). On the other hand, samples at 3.125 and 6.25 mg/ml concentrations exhibited higher ALP levels which indicated enhanced osteogenic effects relative to the control. By moderating the Mg²⁺ ion release from the materials, the osteogenesis of Saos-2 cells can be enhanced and optimised (Figure (4)).

Conclusions
Magnesium cross-linked ionomers have a slightly increased proliferation and better mineralization effect when used to culture Saos-2 bone cells at specific concentrations. Further experiments will explore the effect of osteogenic factors role on the proliferation and differentiation. Also, PCR will be performed to confirm the genetic effect on osteogenic marker genes.

References
HYBRID IMPLANTS FOR EARLY-INTERVENTION OSTEOCHONDRAL REPAIR

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Introduction

Osteochondral damage can affect a wide variety of the population: from young adults, caused by sporting injuries and trauma, through to older generations facing degenerative diseases such as osteoarthritis, which alone affects one quarter of the population. The best case scenario is to spot cartilage damage early and if this is the case, micro-fracture is a commonly implemented surgical technique. This involves releasing stem cells from the subchondral bone marrow in the hope that they differentiate into cells that produce the required hyaline (articular) cartilage. In reality, the technique results in fibrocartilage and after 2-5 years revision surgery is often required. Rarely does microfracture result in a good long term solution and there remains a demand for new solutions1.

An appropriately designed degradable implant, exposing cells to suitable mechanobiological stimuli in-situ, provides a potential solution to regenerate articular cartilage and restore joint health and mobility. Sol-gel hybrid materials provide an excellent platform for creating suitable implants. Such hybrids are covalently bonded co-networks of bioactive silica and degradable polymer produced through bottom-up synthesis2. Mechanical properties can be tailored to match that of the local tissues and the material degrades congruently.

The proposed implant combines an additively manufactured scaffold, with an optimised pore architecture to promote cell regeneration, and a monolithic low-friction articular surface to maintain joint mobility and enclose the cells in the scaffold. Hybrid scaffolds produced by 3-D printing have been shown to promote collagen type II, indicating articular cartilage growth. Mechanical properties must be matched to that of the surrounding tissues such that an appropriate strain gradient is experienced by the cells in the scaffold. Micro-CT imaging in combination with digital volume correlation (DVC) provides a novel technique to visualise and quantify the strain field in a three dimensional volume encompassing the implant and surrounding tissue3. DVC tracks movement of voxels and relies upon patterns in the imaged materials to infer relative movements.

Materials and Methods

Silica-polycaprolactone (SiO2-PCL) hybrids were prepared by sol-gel synthesis of the SiO2 and, prior to gelation, addition of the polymer with glycidyloxypropyltrimethoxysilane (GTPMS) as the coupling agent. Monoliths were cast in Teflon moulds and scaffolds were 3-D printed by robocasting. After an appropriate gelation time the two components were joined and aged at 40°C. For micro-CT scanning, bovine tissue samples were scavenged from the femoral condyles of a slaughtered animal and an artificial osteochondral defect was created in to which the hybrid device could be implanted. For the purposes of imaging cartilage, samples were stained in a 1% w/v solution of phosphotungstic acid (PTA) and DI H2O. Prior to scanning, samples were washed in a DI H2O bath to remove excess surface PTA. Hybrid samples were submerged in DI H2O and micro-CT scanned both individually and in the tissue defect.

Results and Discussion

Fusion between the two hybrid components was confirmed by SEM imaging. Preliminary micro-CT scans showed good imaging resolution of the 3-D printed architecture and surfaces of the articular component. Staining aided the cartilage imaging but was unable to penetrate the bulk hybrid surface hence introducing straining during the synthesis should be considered. In a single scan it was possible to capture bone, cartilage and the hybrid implant including clear boundaries between the materials.

Conclusions

The highly tunable properties of SiO2-PCL hybrid implants: both in regards to material composition and 3-D printing architecture, provides an encouraging route to match the required mechanobiological properties to regenerate articular cartilage.

References (where applicable)

Acknowledgements (where appropriate)
This project has been supported through EPSRC funding (EP/N025059/1) and the student holds the Imperial College Class of 1964 Scholarship.
AMORPHOUS TI-CU ALLOY PREPARED BY MECHANICAL ALLOYING FOR BIOMEDICAL APPLICATIONS

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Introduction
Recent studies reported that copper inhibited microbial, fungal and viral infections [1] and have been widely used as antibacterial agent for clinical applications because of its excellent antimicrobial properties, low toxicity and high cytocompatibility [2,3]. Zhang et al. successfully prepared antibacterial Ti-Cu crystalline alloy with >99% antibacterial rate (in comparison with cp-Ti) by a high pressure sintering process [4]. However, crystalline metals can be replaced by amorphous/nanocrystalline ones to improve the strength, hardness, wear resistance, and corrosion resistance. Mechanical alloying (MA) is one of the most important routes to obtain amorphous or nanocrystalline alloys.

Materials and Methods
Pure titanium powder Se-Jong (97.5wt% Ti) and copper powder CH-L10 (98.2wt% Cu) were used as elemental powders. The powder mixtures with a composition of Ti80Cu20 were mechanically alloyed in a high energy attritor ball mill using stainless steel vial and balls. The ball to powder ratio of 20:1 was used. The milling times used were 1, 5, 10, 15, 20, 30, 40, 50 and 60 hours, and the products after milling were analyzed by SEM (Philips XL 30), TEM (CM-200) and X-ray diffraction (Siemens D500) using CuKα radiation.

Results and Discussion
Ti80Cu20 powder mixture, after milling for the first 40 hours, reduce its particle size down to about 8.4 μm (from the initial size of 25.6 μm for Ti and 33.7 μm for Cu) and acquire an equiaxed particles shape (Figure 1a). From 40 to 70 h particles continue being equiaxial and their size slightly decreases up to approximately 5.5 μm. Figure 1b shows the X-ray diffraction patterns. The intensity of the diffraction peaks of Ti and Cu diminishes with milling time, and, after 40 h, a broad peak with the maximum at about 2θ = 39°, typical of an amorphous or nanocrystalline phase, appears. Formation of amorphous/nanocrystalline phase after 40 h of milling has been confirmed with TEM (Figure 1c).

Conclusions
Based on TEM and XRD analysis, the crystalline to amorphous/nanocrystalline phase transition induced by ball-milling during 40 h was verified for the Ti80Cu20 alloy. The amorphous/nanocrystalline phase is maintained until 70 hours of milling.

References

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A NOVEL SCALE-DOWN CELL CULTURE AND IMAGING DESIGN FOR THE MACHANISTIC INSIGHT OF CELL COLONIZATION WITHIN POROUS SUBSTRATE

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Introduction
Understanding the complex cell colonisations within porous 3D biomaterials, is still a fundamental challenge in tissue engineering. It is especially difficult to assess cell-cell and cell-biomaterial interactions at the single- or multiple-cell level during tissue culture using common optical microscopes, due to their limited focus depth, the relative ‘large’ size and complex structures of 3D biomaterials. To combat this we deconstructed the complex 3D environment into thin sections using TEM grids, which were situated inside novel miniaturised bioreactors. Thus the cells cultured on the thin modular substrate can be imaged at the single cell level using conventional microscopic techniques. Human dermal fibroblasts cultured on these modular substrates were imaged through different optical microscopes during cell culture. Complicated dynamic processes were observed to be utilised by both individual and coordinated cells to bridge and segment porous structures. Further in-situ analysis via SEM and TEM provided high quality micrographs of cell-cell and cell-biomaterial interactions at the micro-scale, and also illustrated the relation between different mechanical states of the cells and the internal cytoskeletal structures at nano-scale. Thus this novel scale-down design was able to improve the mechanistic understanding of cell colonisation within porous biomaterials, which could be difficult to obtain using other cell or tissue culture systems.

Materials and Methods
Neonatal foreskin human dermal fibroblasts (HDFs, Intercytex) were used in this study. The 3D cell culture imaging system (3D CCIS) was fabricated from Nylon 12 (PA2200, EOS), and Selective laser sintering (SLS, Formiga P100, EOS) was utilised to print two discs and a stopper for the fabrication of each set of 3D CCISs. Several imaging techniques were used in this study: phase contrast microscopy (PCM, Nikon Ti), fluorescent microscopy (Nikon Ti), confocal microscopy (Nikon Ci, Japan), scanning electron microscopy (SEM, JSM 7800F, JEOL), and transmission microscopy (TEM, JSM 2000FX, JEOL).

Results and Discussion
This study improved understanding of the mechanistic behaviours found during cell colonization within porous substrates using TEM specimen supporters inside the 3D CCIS. Micrographs of the exemplary HDFs cultured on the thin modular substrate were captured at multiple- and single-cell levels using optical microscopes during cell culture; in-situ analysis was also conducted at micro- and nano-scales after cell culture via SEM and TEM. Our research validated that the scale-down study design is an effective approach to systematically evaluate individual elementary structural components of complex 3D matrices. Thanks to both the thin substrate and the miniaturized 3D CCISs, it was feasible to noninvasively monitor cellular behaviours on the porous substrate using PCM during cell culture.

Conclusions
Use of the 3D CCIS in this study, demonstrated that this cell culture and imaging system was easy to fabricate and use, and the cells were analysed from multiple- and single-cell levels to micro- and nano-scales using optical and electron microscopes. Apart from systematic evaluation of the elementary structural features, this novel platform also established the potential to investigate other biochemical, biomechanical properties and bioactive molecules usually engineered in 3D matrices, which will provide the necessary mechanistic understandings to address the translational challenges in Tissue Engineering.

References

Acknowledgements
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COMPARATIVE STUDY ON THE EFFECT OF SCAFFOLD GEOMETRY ON MECHANICAL PERFORMANCE OF BIORESORBABLE SCAFFOLDS

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Introduction
Percutaneous coronary intervention is a standard procedure to resolve blockages within artery, which involves the implantation of stents to maintain vessel patency. Currently, bioresorbable scaffolds (BRSs) are in the process of replacing the metallic permanent predecessor (drug eluting stents) commonly used in stenting. BRSs are made of poly (L) Lactide (PLLA), an aliphatic polyester which is biodegradable and biocompatible with a wide range of medical applications. The pre-degradation performance of these scaffolds is not well defined. It is not understood how mechanical properties of the scaffolds change with processing and implementation of scaffold geometry out of the extruded polymer tubing. The aim of this project is to assess the mechanical performance of PLLA scaffolds with a direct comparison to the extruded tubing from which scaffold geometry is cut.

Materials and Methods
Scaffold segments and tubing samples will be subjected to indentation testing in order to assess the performance of each. Load versus depth analysis will be carried out on each sample in order to assess the properties throughout the depth. Tension testing for the ring samples and tubing will be carried out in order to characterize the pre-degradation states for each sample.

Results and Discussion
The preliminary data obtained suggests that the laser cutting of the scaffold design has minimal effect on the scaffold integrity and mechanical properties. However, it is noted that these properties are seen on a local scale and do not define the overall global mechanical performance.

Conclusions
The results obtained here will help gain a better understanding of mechanical properties of scaffolds pre- and post-incorporation of stent design. This will enable development of materials in order to be able to withstand processing conditions which may alter the mechanical properties.

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Poster Presentations
Chronic diabetic foot ulcers significantly impact societies around the world. These devastating wounds have an enormous negative impact on an individual's quality of life, leading to loss of mobility and sleep deprivation and contributing to increased risk of amputation, anxiety, and depression. Diabetic have a 23-fold increase in the rate of amputation following ulceration compared with non-diabetics, with up to 85% of amputations preceded by DFUs. Moreover, the 5-year mortality rates associated with DFUs or DFU-related amputations have been found to be as high as or higher than those of breast and prostate cancer. With an increasing frequency of diabetes, these values are only expected to rise without any further innovations for treatment.

Here, I will discuss our efforts to develop new materials-based methods for treating diabetic foot ulcers. Specifically, we are interested in how to manipulate cell signaling networks within diabetic foot ulcers to reprogram them to heal. We aim to do this through a combination of nanotechnology-based approaches, which we use to study patient-derived cells from diabetic foot ulcers and to modulate healing in a mouse model of diabetic ulcers. These methods enable us to modulate cell signaling via both localised growth factor stimulation and RNAi-based knockdown. These approaches have demonstrated the ability to modulate multiple aspects of wound repair, leading to significant increases in vascularisation and the rate of wound healing when used in vivo.

POSTER 2: INVESTIGATION OF RESORBABLE PYROPHOSPHATE GLASS FOR ORTHOPAEDIC APPLICATIONS

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Introduction
An estimated one in two women and one in five men in the UK will suffer from osteoporosis in their lifetime, costing the NHS nearly £1.73 billion per annum [1]. Pyrophosphates and their by-products are of interest as they are a close analogue to bisphosphates as used in drug therapy for osteoporosis. This work specifically focused on interpreting structural, thermal and degradation profiles for a series of Phosphate Based Glasses (PBGs).

Materials and Methods
Series 1 quaternary glasses were produced in the system (P2O5)40-x - (MgO)24 - (CaO)(16+x) - (Na2O)20, where x = 0, 2.5, 5, 7.5 and 10 respectively, using the melt quench method with the following precursors; NaH2PO4, CaHPO4, MgHPO4, CaCO3, MgCO3, P2O5 (Sigma Aldrich, UK). Each formulation was analysed using XRD and EDX to confirm amorphous state and composition. 31P MAS NMR obtained at the EPSRC UK National Solid-state NMR Service at Durham outlined Qn structure and DTA showcased thermal properties. Dissolution tests were conducted by submerging glass discs (9 x 2 mm, annealed at 10°C above Tg for 60 mins) in 30 ml ultra-pure water. Mass, dimensions and pH measurements were taken at timed intervals.

Results and Discussion
31P MAS NMR analysis presented a chemical shift relating to a decrease in Qn species from Q2 → Q1 → Q0 as P2O5 content was reduced, indicating an increase in the number of chain terminators in the phosphate network [2]. Furthermore, these results coincide with a decrease in rate of ions released from glass discs with decreasing P2O5 content. For example, Ca2+, exhibited a rate of 0.6592 ppm/day for P40, compared to 0.3985 ppm/day for P30. In addition, thermal analysis identified a decrease in Tc and the processing window (Tg – Tc) with decrease in P2O5 content, suggesting a lower cross-linking density and shorter chain length in the phosphate network [3]. P30 glass did not follow this trend, showing an increase in Tc and processing window. Additionally there is an increase in all four ions released from P30 (For eg, Ca2+: 0.3985 ppm/day) when compared to P32.5 (Ca2+: 0.3792 ppm/day). Further investigation under SEM has found precipitate material on the disc surface, that of which XPS has identified an increase in Calcium to Phosphate ratio relative to an un-treated sample, suggesting the formation of a precursor to hydroxyapatite, the mineral component of bone.

Conclusions
Material characterisation confirms composition and amorphous state of the glass series. Dissolution studies and thermal analysis indicate a decrease in ion release rate, Tc and processing window with lower phosphate content from P40 to P32.5. The next stage of investigation will aim to understand how bone marrow derived human Mesenchymal Stem cells interact with Series 1 glass formulations.

References

Acknowledgements
The authors would like to take this opportunity to thank Orthopaedic Research UK for funding this project.
POSTER 3: ASSESSMENT AND OPTIMIZATION OF HYDROGELS FOR 3D NEURONAL CELL CULTURE

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Introduction
Hydrogels used in cell culture can be defined as permissive or promoting according to their ability to support cellular adhesion to the three-dimensional polymer matrix. Gene expression in 3D culture has been demonstrated to more accurately reflect conditions found in vivo. The use of promoting hydrogels to guide cell migration and neurite extension has potential to allow for the formation of guided 3D neural constructs which may serve as new fundamental tools for neuropharmaceutical drug discovery as well as assessment of neural circuits in a controlled in vitro environment.

Materials and Methods
Dilutions of a commercial nanofibrillated cellulose (NFC) hydrogel and rat-tail collagen I were seeded with 1.5x10^6 cells/ml of the SH-SY5Y human neuroblastoma cell line. Cultures were maintained for 7 days using DMEM-Glutamax with 1% pen-strep and 10% FBS before treatment with differentiation media containing 2% horse serum, 25ng/ml BDNF and 2 μM retinoic acid for a further seven days. Images were taken daily and half the total media volume replaced. After seven days of differentiation cultures were stained with a live/dead stain and imaged using fluorescent microscopy. Gels were fixed using a 1:1 solution of acetone:methanol and stained with an anti-β-tubulin antibody and DAPI.

Results
NFC hydrogels were determined to be permissive as cells rapidly clustered into aggregates that were observed to expand over time. 0.5% NFC gels were determined to produce relatively larger diameter aggregates however the observed increase was not statistically significant. In contrast neurite outgrowth was determined to be significantly greater in 1% gels compared to 0.5%. Nanofibrillated cellulose was found to degrade after 12 days in vitro resulting in the loss of floating aggregates. Collagen gels of varying concentration were found to be promoting and supported cellular adhesion and elongation in three dimensions. Viability of SH-SY5Y cells was found to increase as the concentration of collagen in solution was decreased.

Discussion & Conclusions
Due to the lack of recognizable motifs cell adhesion was not supported in cellulose hydrogels. Whilst proliferation and neurite outgrowth was supported in cellulose the lack of guidance results in random outgrowth of neurites and dense clustering of cells that may result in internal necrosis due to poor mass-exchange. In contrast collagen I gels are suitable for the guidance of neurite outgrowth. The observed correlation between neural cell viability and collagen I concentration supports the biological nature of collagen I as a component of glial scar tissue that hinders neuronal infiltration into damaged tissue. The short-lasting nature of nanofibrillated cellulose hydrogels limits their application in neuronal culture due to the need to maintain cellular connections and the risk of damaging extended processes whilst replenishing the gel.

References
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Acknowledgements to the EPSRC for funding through the Centre for Doctoral Training in Regenerative Medicine.
POSTER 4: DESIGNER NEURAL CIRCUITS: A MICROFLUIDIC MODEL FOR PARKINSON’S DISEASE

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Introduction: Parkinson’s disease (PD) is a debilitating and progressive neurological disease that still features many unknowns. With a lack of clinical progress towards PD treatments we turn to pre-clinical methods. Tissue slice cultures and animal models both have numerous shortcomings, whereas cell models involving neural cells cultured in an isolated single population isn’t sufficient to recreate brain architecture. As a solution, we have developed a five-port microfluidic device to serve as an in vitro pre-clinical neural model, mimicking the basal ganglia, the causative area of PD in the brain. By growing primary cells from the cortex, striatum, globus pallidus and substantia nigra within the ports of the device, allowing them to connect via micro-scale channels, and recording the spontaneous electrophysiological activity via extracellular electrodes, a functional neural circuit is formed. This device mimics the in vivo basal ganglia and allows the device to be a powerful platform for study and testing of potential PD treatments.

Materials and Methods: The device itself was fabricated via two-stage soft lithography, resulting in a high resolution pattern: two input ports, one centre port, and two output ports, with tapered micro-channels between each set of ports to direct axonal growth in a single direction. Primary cells were dissected from rat embryos ranging from E12-16 depending on the experiment type, and were seeded in each separate port. Cell viability, morphology and connectivity were assessed via fluorescent microscopy, while the growth surface featured a multi-electrode array (MEA) in order to assess cell function via extracellular electrophysiological recordings.

Figure 6: Cells cultured within a five-port device. The left image shows axons extending through the channels (green: β-tubulin, red: MAP2, blue: DAPI) and the right brightfield image shows cells and an electrode (the black square).

Results: Primary cultures (of both neural and glial cells) successfully extended processes through the micro-channels unidirectionally, creating a neural circuit similar to that found in the basal ganglia in vivo. Fluorescent imaging confirmed the connectivity, observing synapse formation between separate cell populations. These connections were further determined to be functional via MEA, with extracellular electrophysiological activity altering between isolated and connected cell populations, especially those connected to a population of dopaminergic neurons.

Discussion and Conclusions: We demonstrate functional activity between specific neural cell populations, by creating interconnections within a fabricated neural circuit. This device can currently be used as a physiologically relevant model of the basal ganglia and used for study towards both Parkinson’s and Huntington’s disease. By selectively removing dopaminergic neurons from the substantia nigra portion of the device, it can be used as a Parkinsonian pathological disease model, with its accessibility allowing for testing of neuroprotective factors, cell replacement therapies and different biochemical/physical treatments, all on an in vitro model representing a hard-to-access in vivo neural area. This device represents a powerful platform for experimentation and study.

Acknowledgements: We acknowledge funding support from the EPSRC Doctoral Training Centre in Regenerative Medicine.
POSTER 5: THE EFFECT OF NANOFIBRES AND NANOTUBES ON THE STRENGTH OF GLASS IONOMER CEMENTS

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Introduction
Glass ionomer cements (GICs) are one of many restorative materials available to dental clinicians. These are widely used due to their fluoride release, biocompatibility, ease of use and ability to adhere to dentine/enamel without bonding agents. However, the poor strength compared to materials such as composites and amalgams, limit their applications. Hydroxyapatite Nanofibers (HANF) and Halloysite Nanotubes (HNT) were substituted into a GIC and effect on diametral and compressive strength assessed.

Materials and Methods
HANF were created using a reflux condensation described modified from Chen1 and analysed using scanning electron microscopy (SEM), transmission electron microscopy (TEM), fourier transform infrared spectroscopy (FTIR), and x-ray diffraction (XRD). HANFs were substituted into a GIC (Diamond Carve, Kemdent, Swindon) in 1, 2, 3, 5, 10 and 15% and tested for compressive strength (CS) (6 mm height, 4 mm diameter, n = 40) and diametral tensile strength (DTS) (4 mm height, 6 mm diameter, n = 40). After 24 hours samples were loaded to failure using a Zwick/Roell Z020 Testing Machine (Zwick, Leominster) with a crosshead speed of 0.5 mm/min and force at fracture used to calculate CS or DTS. HANF acid lability was assessed by fixing fibers onto an adhesive and immersing into a pH 2 solution for 10 minutes to mimic the GIC neutralisation reaction. HNT (Durtec GmbH, Germany) were also investigated for acid stability using the same method. HNTs were imaged using SEM and TEM and substituted into GICs at 1, 2 and 3% by two approaches: adding HNTs to the GIC powder and mixing on a tube roller (Denley Instruments Ltd, Cambridge) for 15 minutes or milling the powders using a tube mill (IKA, Oxford) for 120s and CS tested. Samples taken every 30 seconds assessed dispersion by dying the HNT with acridine orange before mixing into the GIC and imaging the cement using optical/fluorescent microscopy.

Results and Discussion
HANF SEM/TEM showed rod morphologies 10–50 nm diameters and lengths of 5–10 µm. FTIR showed a peak around 1000 cm⁻¹ for PO₄³⁻ and the XRD spectrum matched library data for HA. HNT SEM/TEM images showed irregular, hollow, tubular structures with a diameter of 10–30 nm and lengths of 1–5 µm.

<table>
<thead>
<tr>
<th>% substitution</th>
<th>HANF CS (MPa)</th>
<th>DTS (MPa)</th>
<th>HNT CS (MPa) Mixed (15 mins)</th>
<th>Milled (120 s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>156.62 (+25.86)</td>
<td>6.70 (+1.64)</td>
<td>140.25 (+26.14)</td>
<td>149.78 (+21.21)</td>
</tr>
<tr>
<td>1</td>
<td>129.84 (+21.26)</td>
<td>6.11 (+1.33)</td>
<td>128.96 (+35.59)</td>
<td>122.97 (+22.12) *</td>
</tr>
<tr>
<td>2</td>
<td>130.92 (+16.83)</td>
<td>6.26 (+1.84)</td>
<td>116.89 (+14.16) *</td>
<td>120.29 (+13.30) *</td>
</tr>
<tr>
<td>3</td>
<td>150.44 (+23.18)</td>
<td>6.84 (+2.00)</td>
<td>119.79 (+16.36) *</td>
<td>119.90 (+15.81) *</td>
</tr>
<tr>
<td>5</td>
<td>126.01 (+18.96) *</td>
<td>6.85 (+1.87)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>117.53 (+14.83) *</td>
<td>6.25 (+1.54)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>107.77 (+14.95) *</td>
<td>8.39 (+2.10) *</td>
<td></td>
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</tr>
</tbody>
</table>

Table 1 - CS and DTS of HANF and HNT substituted into GIC powder (*significant difference)

HANF dissolved when immersed into pH 2 solution and likely the reason of reduced CS as the HANF left voids or reaction inhibiting calcium phosphate upon dissolution. Acid dipping HNTs showed no dissolution or change in morphology. Milling visibly dispersed the dyed HNTs through the GIC matrix.

Conclusions
At 1, 2, 3, 5, 10 and 15% HANF show no significant increase in CS and are unlikely to survived the acidic GIC conditions. HNTs appear more acid resistant but still show no significant increase in CS.

References

Acknowledgements
The authors acknowledge Associated Dental Products Ltd. for funding the project.
**POSTER 6: CHELATION OF SODIUM ALGINATE WITH SELECTED IONIC SPECIES FOR BONE SCAFFOLDS**

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**Introduction**

Tissue engineering approaches aim to address bone loss due to disease or trauma with scaffolds serving as a temporary skeleton to accommodate and stimulate bone growth. Alginites are attractive in tissue regeneration due to their capacity to replicate characteristics inherent to the extracellular matrix, biocompatibility, biodegradability, non-antigenicity, and chelating ability hence composites of hydrogels with hydroxyapatite, bioglass and others have been reported to promote attachment and proliferation of bone forming osteoblasts yielding calcified matrices. The presence of ions such as strontium and zinc are also known to accelerate bone healing¹ whilst zinc ions additionally possess antibacterial properties², thus the incorporation of these ionic species within scaffolds for bone regeneration is of significance. Bone is a complex and hierarchical tissue, which predominantly is a biocomposite of nano-hydroxyapatite and collagen³. The aim of the study is to develop multifunctional hydrogel complex biocomposites with different ionic species incorporated in the ECM mimicking matrix hence as a first step the current work examines the properties of the ionic alginate gels on chelation with strontium, zinc and calcium ions.

**Materials and Methods**

Sodium alginate solution 4% (M₆₀~2.3x10⁶ g/mol, M/G ratio: 40/60; w/v) were air-dried to make thin films (<1mm), which were subsequently immersed in 10% (w/v) solution of Ca(NO₃)₂, ZnSO₄, or Sr(NO₃)₂ for 3 hours. The crosslinked alginate films were washed twice with H₂O and air-dried. FTIR spectroscopy, tensile tests (ISO527-3), water uptake, and MTT cytotoxicity assay with primary human osteoblasts (HOB) were conducted. Statistical analysis (one-way ANOVA and Bonferroni) was done with GraphPad Prism 7.

**Results and Discussion**

The results are summarised in Table 2. One-way ANOVA showed significant differences (p<0.0001) in tensile strength, Young’s modulus, and equilibrium water content (EWC) for both high-G and high-M alginites, whilst Zn²⁺ ions had significantly lower tensile properties for the high-G alginites. In contrast, the high-M alginites showed improved tensile strength with higher elongation to break. Alginites consist of guluronic (G) and mannuronic acid (M) units, which form regions of M-blocks, G-blocks, and alternating MG-blocks⁴. Divalent cations are believed to bind to alginate G residues, explained by the ‘eggbox’ model⁴. The ions selectivity caused Ca²⁺ to form more extensive ‘egg box’ conformations, hence providing tougher gels; whilst Sr²⁺ form less crosslinking junctions leading lower tensile properties and EWC. The lack of binding specificity in case of zinc ions led to random networks, leading to higher water retention and softer gels. Finally, the ionically bound Sr²⁺ & Zn²⁺ species control the release of these ions as indicated by the MTT results that showed no evidence of any cytotoxicity.

**Conclusions**

Calcium, zinc and strontium are ions of interest due to their osteogenic properties and this study shows that they can be successfully used to tailor the properties of alginites to modify the matrix of composites for the development of scaffolds for bone tissue.

**References**


**ACKNOWLEDGMENTS**

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POSTER 7: THE EFFECT OF MGO/TIO2 ON CRYSTALLISATION BEHAVIOUR OF PHOSPHATE BASED GLASSES (PBG)

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Introduction: This work aimed to investigate the role of Mg/Ti content on thermal, crystallisation behaviour and structural properties of PBG in a glass series 40P2O5-(24-x)MgO-(16+x)CaO-(20-y)Na2O-yTiO2 (where 0≤x≤22 and 0≤y≤1).

Materials and methods: Six different types of glass formulations in the above mentioned glass series were prepared by melt-quenching using the following precursors sodium dihydrogen phosphate (NaH2PO4), calcium hydrogen phosphate (CaHPO4), magnesium hydrogen phosphate trihydrate (MgHPO4.3H2O), titanium dioxide (TiO2) and phosphorous pentoxide (P2O5) (Sigma Aldrich, UK). The melt temperatures were in between 1150-1250 °C for 1.5-2 hours, depending on the titanium content in glass composition.

Results and discussion: The compositions and amorphous state of produced glasses were confirmed by energy-dispersive X-ray spectroscopy (EDX) and X-ray Diffraction (XRD) analysis, respectively. Thermal analysis was conducted using differential thermal analysis (SDT) and it was seen that all glasses exhibited single crystallisation peak which shifted towards higher temperature with increasing particle size. The glass transition (Tg) and crystallisation (Tc) temperature of the glass particles (45-90 µm) at 10 °C/min heating rate, decreased from 449 °C to 431 °C and 571 °C to 539 °C, respectively with decreasing MgO content from 24 mol% to 8 mol%. However, below 8 mol% MgO containing glasses showed strong tendency towards crystallisation. 8 mol% MgO with 1 mol% TiO2 containing glasses showed around 2 % higher in Tg and 6 % higher in Tc than for 8 mol% MgO containing glasses. Activation energy for crystallisation (Ec) was calculated using Kissinger equation. Ec decreased from 115 KJ/mol to 96 KJ/mol with decreasing MgO content from 24 to 8 mol%. 1 mol% TiO2 containing glasses (≤ 8 mol% MgO) showed higher Ec than for 8 mol% MgO without TiO2 containing glasses. Additionally, density of all glasses have been investigated by Archimedes’ principle. Fourier transform infra-red spectroscopy (FTIR) and nuclear magnetic resonance (NMR) were also carried out for all glass formulations.

Conclusions: Thermal properties and activation energy of crystallisation (Ec) decreased with the substitution of MgO for CaO. 1 mol% TiO2 inhibited the crystallisation for below 8 mol% MgO containing glasses in the above mentioned glass series.


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POSTER 8: AN ALVEOLAR STRUCTURAL MIMIC: A TISSUE ENGINEERING SOLUTION FOR EMPHYSEA

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Introduction

Respiratory diseases are the second highest cause of death in the UK and lead to a huge economic burden to the NHS. The lung has a complex three-dimensional structure. The basic unit of the lung is the alveoli, the site of gas exchange with the blood. Tissue engineering approaches represent an attractive potential to treat lung disease. Our aim is to create an alveolar-like structure to replace damaged lung areas caused by respiratory diseases such as emphysema. Surgispon® is a gelatine sponge and widely used in clinics with high porous structure and good elasticity. In this study, Surgispon® has been modified to increase its stability and cellular biocompatibility as a scaffold, to generate alveolar-like structures.

Methods

Surgispon® scaffolds were crosslinked with 25% glutaraldehyde (GTA) vapour for 24 hours. Scaffold mechanical stability was compared to untreated scaffolds. The crosslinked sponges (scaffolds) were vapour cleared by exposure to air and sterilized by UV before any cell culture work.

A549 human lung carcinoma epithelial cells were used for cell culture experiments following the protocol. Cells were seeded onto six-well plates and cultured for two days to investigate cytotoxicity of residual GTA on the scaffolds via Alamar Blue cell viability assay. After cytotoxicity analysis, scaffolds were coated with either fibronectin, collagen type I (rat tail), collagen type IV (rat tail) or collagen solution (human fibroblast) and uncoated scaffolds were used as a control group. A549 cells were cultured on the scaffolds and Alamar blue, live-dead and DAPI stains were performed at day 7, day 14 and day 21.

Results

Only crosslinked scaffolds survived incubation in media for longer than 21 days. Cytotoxicity testing showed that crosslinked scaffolds support cell growth.

Alamar blue, live-dead and brightfield microscopy indicated that there was less cell attachment on uncoated scaffolds than collagen coated scaffolds and only a slight improvement via fibronectin coating. However, all three types of collagen coated scaffolds showed higher cell metabolic activity compared to the tissue culture plate itself.

![Fig. 1: Images of A549 cells on treated scaffolds after 21 days culture. A and B: collagen solution coated scaffolds. C and D: uncoated scaffolds. A, C: brightfield and B, D: DAPI.](image)

Discussion and Conclusions

Surgispon requires cross linkage to maintain structural stability and biomolecule coating for better cell attachment and proliferation. The interconnected porous structure of Surgispon® has great potential to create an alveolar-like structure.

Acknowledgements

We acknowledge funding support from EPSRC Centre for Doctoral Training in Regenerative Medicine and Keele University.
POSTER 9: STRONTIUM AND ZINC CO-SUBSTITUTED NANOPHASE HYDROXYAPATITE

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Introduction
It is well documented that biological hydroxyapatite (HA) differs from pure and synthetically produced HA, and contains a mixture of calcium phosphate (CaP) phases in addition to a range of impurity trace ions, such as strontium (Sr²⁺), zinc (Zn²⁺), magnesium (Mg²⁺), fluoride (F⁻) and carbonate (CO₃²⁻), but to name a few. Further to this, biological apatite is generally in the form of rod (or needle-like) crystals in the nanometre (nm) size range, typically 60 nm in length by 5-20 nm wide. The in vitro and in vivo performance of HA can be modified by the use of nanoscale HA and/or the addition of different trace ions, such as Sr²⁺ or Zn²⁺, to more closely mirror the complex chemistry of human bone. To date, most of the work in the literature has considered single ion-substituted materials and coatings, with limited reports on co-substituted calcium phosphate systems [1,2]. Therefore, the aim of this study was to produce a range of Sr²⁺ or Zn²⁺ co-substituted nanoscale apatites and to undertake a detailed characterization of the attendant materials' properties.

Materials and Methods
In this study, a range of nano-hydroxyapatite (nHA) and co-substituted nHA (based on Sr²⁺ and Zn²⁺) were manufactured using an aqueous precipitation method. Sr²⁺ and Zn²⁺ were chosen due to the significant performance enhancements that these substitutions can deliver. Sr²⁺ and Zn²⁺ co-substituted nHA were produced with 2.5%, 5% and 10% total (and equal) Wt% substitutions. They were referred to as Sr/Zn-2.5%-nHA, Sr/Zn-5%-nHA and Sr/Zn-19%-nHA, respectively. The materials were then characterised using Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), X-Ray Photoelectron Spectroscopy (XPS) and Transmission Electron Microscopy (TEM) techniques.

Results and Discussion
The FTIR and XRD results confirmed that in the case of the nHA, no impurities were detected and the Ca/P ratio was 1.61±0.04, close to that for stoichiometric HA. TEM analyses showed rod-like nanoparticles of 99 nm x 34 nm in size. In the case of the different Sr²⁺ and Zn²⁺ co-substituted materials, the FTIR spectra all showed significant dehydroxylation, peak broadening and increased carbonate content. The XRD results also exhibited peak broadening with increasing Sr²⁺ and Zn²⁺ co-substitution, along with peak shifting to higher 2θ values, indicating that Sr²⁺ had substituted for Ca²⁺ in the lattice. The XPS data revealed that the Sr/Zn/Sr+Zn+Ca ratio increased as the levels of co-substitution increased. The TEM results revealed that both the nHA and Sr/Zn-2.5%-nHA materials were rod shaped with an aspect ratio of over 3. However, the aspect ratio of the higher concentration co-substituted materials were closer to 2, indicating a more rounded morphology. The nanoparticle dimensions were also observed to decrease with increasing levels of co-substitution.

Conclusions
None of the materials produced contained any other impurity CaP phases, and were all on the nanoscale, as supported by the XRD, FTIR, XPS and TEM measurements recorded here. Small amounts of CO₃²⁻ were found within each of the materials (which was not unexpected). However, its presence, along with that of both Sr²⁺ and Zn²⁺ ions are highlighted in the literature as having the potential to have a positive influence on the osteoblast response. The next step in this work is to undertake detailed dissolution and in vitro testing of these materials to probe their potential bioactivity.

References
Introduction
Magnesium alloys offer significant benefits as biomaterials for the fabrication of orthopaedic implants[1]. In addition to having ultra-high ductility, high strength, and toughness they can undergo complete resorption in vivo after bone healing. This offers advantages over current devices (e.g. pins, rods, intramedullary nails, meshes, etc.) that use permanent metals/metal alloys for the treatment of bone fractures. Importantly, once implanted, devices employing magnesium alloys do not need a second surgery for their subsequent removal. Key to creating a suitable magnesium based alloy implant is the control of the alloy’s dissolution rate. This can be achieved by varying the alloy composition and/or using advanced bioactive coating methods, based on the physical deposition of hydroxyapatite (HA), to control key properties of the magnesium alloy-biological interface for orthopaedic clinical application. HA biocompatibility has been thoroughly investigated, and is known to show improved adhesion, proliferation, differentiation and increased alkaline phosphatase activity of primary human osteoblast cells. [2]

Materials and Methods
Commercial magnesium aluminium alloy AZ31 (96Mg-3Al-1Zn)(Goodfellow Ire.) was abraded using SiC papers beginning at 800 grade rising to 1200 grade. The HA coating was then applied using radio frequency (RF) magnetron sputtering (150W, 5 hrs, 3.3W/cm², 0.00005 Pa). The target used was pressed using commercial HA, 11g (Plasma Biotal Ltd). The material characterisation included use of X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared (FTIR).

Results and Discussion
XPS, FTIR and XRD data was initially collected using the base alloy material with no coating present, AZ31. The same characterisation techniques where then utilised following the 5 hour RF sputtering using a HA target. It can be seen from the XPS spectrum that in the sputtered sample (Fig 2) there is the presence of strong Ca 2p (346-352 eV) and P 2p (133 eV) peaks which are much less prevalent and not present in the raw AZ31 spectrum (Fig 1) respectively, giving a strong indication that deposit ion of hydroxyapatite (calcium phosphates) has occurred. This is also assumed by the dampening of the Mg 2p high resolution peak in the sputtered samples. Both FTIR and XRD data confirm the presence of HA after sputtering.

Conclusions
Coating hydroxyapatite onto magnesium alloys is achievable via RF magnetron sputtering method. This is confirmed by characterisation data above. These results are however preliminary and just indicate that a HA coating will adhere to the alloy. Future work would be to create coatings evenly dispersed of a desired thickness, and to investigate the stability of the alloy when exposed to simulated body fluid (SBF), in particular, using nanoindentation and AFM techniques to follow the evolution of mechanical properties as a function of depth. The aim to achieve a controlled resorption of the HA coating and Mg alloy, whilst improving bioactivity and maintaining structural integrity.

Future Work
Future work would be to create coatings evenly dispersed of a desired thickness, and to investigate the stability of the alloy when exposed to simulated body fluid (SBF), in particular, using nanoindentation and AFM techniques to follow the evolution of mechanical properties as a function of depth.

References
POSTER 11: EFFECT OF FOAMING AGENT ON NOVEL PORTLAND CEMENT FOR VERTEBROPLASTY

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Introduction
Portland cement (PC) is a hydraulic calcium silicate based cement which has been successfully used in Dentistry as an endodontic cement known as mineral trioxide aggregate (MTA) [1]. In 2012, an injectable novel PC containing 5wt% CaCl2 as a liquefier and accelerant was proposed for load-bearing application e.g. vertebroplasty; that is stabilisation of vertebral bodies [1]. PC has the potential to substitute polymethylmethacrylate (PMMA) due to no exothermic polymerisation and monomer toxicity issues, yet it lacks macroporosity (>100µm) for better cellular reactions [1]. Recent studies on calcium phosphate cements (CPCs) reported that, foamed gelatine could act as a template for macropores, which would provide a higher surface area for bone colonisation and angiogenesis [2]. To date, no studies have evaluated the effect of foamed gelatine on PC for vertebroplasty application at a high powder-to-liquid ratio (PLR).

Materials and Methods
The PC model system consisted of 75wt% PC, 20wt% Bi2O3 and 5wt% CaCl2. Type B gelatine was dissolved in distilled water (DW) at a concentration of 10% (w/v) to produce foam. Foamed gelatine (FG) was added to the powder phase at 1, 5 and 10wt%. Cement slurries were hand mixed with DW for 2 min at a PLR of 4.0g/ml and set for 6 h at 37°C. The setting and injectability were measured using Gilmore needle tests and universal testing machine respectively. The wet compressive strengths (CS) were measured at a crosshead speed of 1mm/min after 7 days of immersion in DW. The relative porosity (RP) was measured using helium pycnometry and weight loss experiments. To observe surface porosity, samples were analysed for by SEM at 5kV and magnification of x40. All data were analysed for p<0.05 using Sigmaplot (V13.0).

Results
Percentage of extruded cement was significantly improved up to 96% (p<0.05) by adding 10wt% FG (Fig.1). CS and RP were inversely proportional, the increase in the FG content, reduced CS significantly while increased porosity (p<0.05) as shown in Fig.2. SEM images indicated presence of pores greater than 100µm for 10wt% FG group compared with the control which had a relatively smooth surface (Fig.3).

Discussion and Conclusions
Improvement in injectability of PC was attributed to the role of gelatine which has been stated as cohesion promoter in literature as well as addition of foam increases the liquid content of this novel cement [2]. The foaming process created bubbles and macropores (>100µm) which were maintained during the setting of cement, and interestingly some pores were interconnected even at this high PLR. Despite the reduction of CS which was attributed to the increasing in the size and number of pores, the CS of 10wt% FG group was still higher than the CS of cancellous bone (2-10MPa). Therefore, for the first time, an injectable novel PC with macroporosity and sufficient strength was introduced for load-bearing applications, which could suggest further investigation in terms of cellular reactions.

References
POSTER 12: THE MANUFACTURE OF ELECTROSPUN MEMBRANES WITH OSTEOGENIC AND ANTIMICROBIAL PROPERTIES FOR ORTHOPAEDIC AND DENTAL SURGERY

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Introduction

After surgery, the prevention of deep bone infections and the enhancement of bone regeneration reduces the time required for patient recovery and the costs associated with long hospital stays. The regeneration of bone injuries could be significantly improved by a ready source of mesenchymal stromal cells (MSC) supplied by an artificial reservoir. If a synthetic niche environment could be prepared that maintained a MSC population, this could enhance local bone tissue regeneration in medical devices. To facilitate bone healing surgeons currently employ nano-hydroxyapatite (nHA) to stimulate bone regrowth, however this has no innate antimicrobial properties. Deep bone infections are very challenging to treat due to the difficulty of achieving a suitable antibiotic concentration in the affected area. Silver has long been used to successfully treat bacterial and fungal infections. We investigated the substitution of silver into nHA and subsequent incorporation into electrospun membranes patterned with artificial stem cell niches and its impact on bacterial and mammalian cells.

Materials and Methods

Silver substituted nHA was produced using a modified rapid mixing wet precipitation method at 2, 5, 10 mol % silver. The nHA was dried and ground before addition at 20 wt % to a solution for electrospinning consisting of polycaprolactone, dichloromethane and dimethylformamide. Transmission electron microscopy, scanning electron microscopy, energy-dispersive X-ray spectroscopy and X-Ray Diffraction Analysis were used to analyse the nHA, as a powder and within the electrospun membrane. Unsubstituted nHA/PCL and PCL only scaffolds were used as controls. Fibres were electrospun onto metallic templates formed by additive manufacturing (SLM). Clinically relevant isolations of E. coli and S. aureus were collected from the China centre of Industrial Culture Collection (CICC) and tested against the electrospun scaffolds using both diffusion and contact methodologies. MSCs, collected from Wister Rat femurs, were cultured in non-contact (toxicity study) and in direct contact (osteogenicity study) with the membranes. PrestoBlue® and ALP measurements were used to analyse the cells in culture.

Results and Discussion

This investigation found that silver nHA membranes significantly reduced E. coli and S. aureus bacterial populations while maintaining cytocompatibility with mammalian cells and inducing the differentiation of MSCs into osteoblasts. SEM, TEM and EDX identified silver nanoparticles within the HA and confirmed the presence of the HA within the fibres. SEM imaging of the membranes demonstrated the presence of artificial stem cell niches which contained regions of aligned and non-aligned fibres. Both diffusion and contact bacteria studies demonstrated reduced bacterial presence, with E. coli and S. aureus undetectable after 48 hours of contact exposure. An increase in MSC activity was observed over the culture period with the cells cultured on samples containing nHA observed producing alkaline phosphatase, a key marker for osteogenic differentiation. The inclusion of additive manufacturing as part of the fabrication process allows the creation of intricate structures that can be designed to mimic certain aspects of the native stem cell niche. MSCs were identified in the niches, cell proliferation was observed and morphological differences in cell structures were recorded. Silver nHA containing membranes have the potential to act as an antimicrobial membrane while stimulating bone recovery through the action of artificial stem cell niches.

Conclusions

Silver substituted nHA was successfully produced, characterised and electrospun into membranes. These membranes were fabricated to include artificial stem cell niches. These membranes showed bactericidal activity against E. coli and S. aureus within 48 hours and supported the proliferation of rat MSCs, which were observed within the artificial stem cell niches.

Acknowledgements

We would like to acknowledge the UK-China bridges and Newton foundation for providing funding for a researcher exchange programme. In addition, we acknowledge the funding from MeDe and the Women Academic Returners’ Programme (WARP) at UoS for making this work possible.
Introduction
Many of the medications currently in use for the treatment of oral mucosal diseases are administered in the form of creams or mouthwashes, leading to irregular dosage, systemic administration, and undesired side-effects. Additionally, movements inside the oral cavity and the production of saliva may facilitate the removal of the medication from the delivery site, decreasing contact time and impairing treatment efficiency. Thus, there is a clear clinical need to develop systems capable of delivering controlled amounts of drug locally, whilst remaining attached to the oral mucosa for prolonged periods of time. The aim of this project was to develop mucoadhesive polymeric patches for mucosal or transmucosal drug delivery using polymers approved for pharmaceutical applications and the electrospinning manufacturing technique.

Materials and Methods
Solutions of polyvinylpyrrolidone (PVP) and Eudragit® RS100 (RS100) were prepared in ethanol at room temperature and under continuous stirring. Particulate dextran (Dex) and poly(ethylene oxide) (PEO), insoluble in ethanol, were added to the solutions to enhance the mucoadhesive properties of the electrospun membranes. All polymeric solutions were electrospun using optimised process parameters. A hydrophobic backing layer was fabricated using poly(caprolactone) (PCL) to produce dual layer systems capable of unidirectional drug delivery. Solution properties were studied through rheometry. Patch morphology was examined using scanning electron microscopy, and patch wetting properties were investigated in vitro using optical tensiometry and solubility studies. Adhesion properties were studied in vitro on plastic and porcine mucosa.

Results and Discussion
The viscosity of the polymeric solutions varied greatly depending on composition and concentration, affecting the formation of electrospun fibres visibly. The addition of RS100 to PVP resulted in increased surface hydrophobicity and reduced patch solubility. The particles of Dex and PEO, insoluble in ethanol, retained their original morphology and were located on the surface of the electrospun fibres. A hydrophobic backing layer was successfully produced using electrospun PCL, with enhanced attachment between both layers achieved through the application of a thermal treatment that melted PCL but did not affect the mucoadhesive layer. PVP only patches did not adhere to plastic or porcine mucosa, whilst PVP and RS100 patches with and without Dex or PEO were tightly adherent, demonstrating the enhanced adhesive properties of the system.

Conclusions
The combination of PVP and RS100 showed to be a versatile polymeric system, allowing for the manufacture of patches with varying degrees of solubility. This may be used to control the rate of drug release and mucoadhesive properties of the patch by selecting specific compositions. Furthermore, electrospinning allowed for the easy addition of various substances to the patches (drugs, bioadhesive particles), and the large surface area of electrospun materials may enhance adhesion and drug release. In conclusion, these compositions showed great potential for the fabrication of mucoadhesive patches for efficient treatment of oral mucosal diseases.

References

Acknowledgments
The authors would like to thank Patrick Rider for providing essential assistance with the sessile drop technique assays, Dr. Robert Moorehead with the rheometry analysis, and BASF and Evonik Industries AG for kindly providing PVP and Eudragit RS100, respectively. The manufacturing elements of this research, and Santocildes-Romero and Hatton are linked to the EPSRC Center for Innovative Manufacturing in Medical Devices (MeDe Innovation).
**POSTER 14: OPTIMISATION OF MICROCRYSTALLINE CELLULOSE SOL-GEL REACTION FOR THE DEVELOPMENT OF NEXT GENERATION BIOMATERIALS**

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**INTRODUCTION**

Microcrystalline cellulose (MCC) offers a natural alternative to synthetic, non-renewable materials and its use in composites is hindered by its poor solubility due to aggregation (1). Aqueous sodium hydroxide (NaOH) is a well-known solvent that causes MCC to swell and disrupt hydrogen bonding (2). Dissolution of the network is essential to gain control over particle size and remove impurities. The aim of this study was to optimise MCC sol-gel reaction in aqueous NaOH for post-modification of the MCC network to develop next generation biomaterials such as hydrogels or 3D matrices.

**MATERIALS AND METHODS**

Stock solutions of MCC and NaOH was prepared by mixing 0.6M MCC and 3.8M NaOH separately in 50 ml deionised water (dH\(_2\)O). The solutions were cooled to 3 oC and stored in a refrigerator before use. Stock solutions were mixed at a stirring rate of 1000 rpm until a transparent solution was obtained. The 3 oC solution was degassed (A) and gradually heated to 20°C (B) and to 50°C (C) on a magnetic hotplate. Samples from each solution were sputter coated using a gold-target (Quorum Technologies, Q150 RS) and imaged using a Zeiss Sigma vpFESEM at an accelerating voltage of 2 kV. Transparency of each solution was measured using a JASCO UV-vis spectrophotometer at 600 nm. Viscosity was recorded for 10 min at 20°C using a Bohlin CVO 100 rheometer. Samples were evaluated for cytotoxicity using L929 fibroblasts in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin.

**RESULTS AND DISCUSSION**

![Fig 1. SEM images of MCC dissolution at 3°C (A) and after heating at 50°C (C).](image)

It is known that NaOH cleaves the intermolecular hydrogen bonds within the MCC network to form agglomerates (2). At 3°C, the agglomerates self-assembled into 1.4 nm x 0.4µm nanofibrils (Fig 1A). As temperature increased, the nanofibrils broke into agglomerated microparticles with diameters of 0.4 ± 0.2 µm (Fig 1C), which were closely packed with less hydrodynamic volume (3). MCC solution (A) at 3°C showed high viscosity, which decreased at 20°C forming a liquid phase (B). At 50°C, a viscous hydrogel was obtained, which dried it to a solid film (C). The change in viscosity can be explained by the structural transition from nanofibrils to agglomerates. Solution (A) turned opaque when heated to 50°C and an increase in viscosity occurred between 20°C and 50°C to indicate phase separation and beginning of gelation (2).

**CONCLUSION**

The behavior of MCC network in aqueous NaOH was subjected to change with temperature. A structural transition was observed from nanofibrils to agglomerated microparticles as the temperature increased. Changes in the structure of the network, transparency and viscosity was observed with temperature resulting in a viscous solution (A) with a clear liquid phase (B), a viscous hydrogel that dried as a solid film (C). Such MCC structures can be further optimised and serve as templates to develop novel hydrogels or 3D matrices for nanocomposites.

**REFERENCES**


**ACKNOWLEDGEMENTS**

The authors would like to acknowledge the support from technical staff at the Royal Free Hospital.
POSTER 15: SUSPENDED ADDITIVE LAYER MANUFACTURING: AN ALTERNATIVE APPROACH TO 3D BIOPRINTING USING LOW VISCOSITY BIOPOLYMERS

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Introduction

Biopolymer hydrogels are frequently used as tissue engineering scaffolds as they can have physicochemical similarities to extracellular matrix (ECM). The anisotropic nature of ECM, in both material and cellular composition, is particularly challenging to replicate. Therefore to facilitate the creation of a more native environment, techniques to integrate multiple materials with different chemical and material behaviour into a single construct are of great interest. 3D printing using Additive Layer Manufacturing (ALM) is one approach that has showed promise to create detailed constructs of defined shapes. Creating hydrogel structures from biopolymers using ALM however is particularly difficult due to the low viscosity of the gelling solutions causing the printed structure to collapse and lose its shape before solidification. Another problem when 3D printing biopolymer hydrogels is the inability to integrate multiple layers of material once gelled preventing the production of integrated gels with regional variations in mechanical behaviour. Recently in our laboratories we have developed a technique to create cell-laden structures from low viscosity biopolymers with the potential to design distinctly anisotropic structures from multiple materials [1]. This approach uses a particulate fluid gel bed in which the cell laden biopolymer materials are extruded into a bed of micrometre sized gel particles that provide sufficient support to prevent the structure collapsing under its own weight. Here we demonstrate the power of this technique to create a range of composite biopolymer hydrogel structures using a commercially available 3D bioprinter.

Materials and Methods

A particulate fluid gel bed was prepared by cooling a solution of 0.5% w/w agarose from 85 to 20 °C under constant shear using a magnetic stirrer rotating at 700 rpm. A range of biopolymers were selected and used at various concentrations that included gellan gum, alginate, gelatin and collagen. These biopolymer solutions were used to create integrated composite structures using an Inkredible® extrusion based bioprinter. Briefly, the solutions were added to extrusion cartridges in the bioprinter and then extruded into the agarose fluid gel base to create a range of 3D geometrical shapes from G-code. A second biopolymer was then loaded into the extrusion cartridge of the bioprinter and then layered on the surface of the previously printed shape. Once the final composite construct was printed the polymers were crosslinked by addition of 200 mM calcium chloride solution to the fluid gel bed. Following gelation the supporting phase was removed and the printed structures washed prior to inspection.

Results and Discussion

This study has demonstrated the potential for ALM using fluid gels as a supporting media integrated with a 3D bioprinter as a method for manufacturing layered tissue culture scaffolds from low viscosity biopolymers. Figure 1 illustrates the process and shows examples of final printed constructs of distinct biopolymer materials that were precisely printed and integrated creating a single structure with specific regions of different mechanical and chemical properties.

![Fig 1](image-url) A schematic diagram showing the manufacturing process for 3D biopolymer structures using a commercially available bioprinter adapted to facilitate the suspended additive layer manufacturing method. A) Integrated (10 mm³) cubes of gellan and gellan containing a red dye and B) integrated collagen (6 mm) gellan (4 mm) cylinders

Conclusions

We have shown the potential of using suspended additive layer manufacturing for 3D bioprinting constructs of defined shape. Anisotropic biopolymer hydrogels with integrated layers were also produced using this technique. This method is currently being investigated to create multi-layered and multicellular constructs for tissue regeneration.

References

POSTER 16: EFFECT OF BORON OXIDE ADDITION ON THE CYTOCOMPATIBILITY AND FIBRE DRAWING ABILITY OF NOVEL PHOSPHATE BASED GLASSES WITH 40 MOL% P₂O₅ CONTENT

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Introduction: Various formulations of Phosphate based glasses (PBGs) have been studied extensively for their biocompatibility. Different studies have shown that the biocompatibility of these glasses is affected strongly by their solution degradation rate and associated ion release. Therefore, the biocompatibility of PBGs is expected to be affected by the addition of different metal oxides, which are known to alter the chemical durability of the glasses. It has been reported that it is difficult to draw fibre from glass formulations with P₂O₅ content lower than 50 mol%. The aim of this study was to investigate the effect of B₂O₃ addition on the cytocompatibility of P₂O₅-CaO-MgO-Na₂O-B₂O₃ with phosphate contents fixed at 40 mol%. The effect of boron incorporation on continuous fibre drawing via the melt drawn process was also investigated.

Materials and Methods: In this study PBGs in the system (P₂O₅)₄₅-(CaO)₁₆-(Na₂O)₁₅₋ₓ-(MgO)₂₄-(B₂O₃)ₓ where x = 0, 5 and 10 were produced via melting the appropriate amount of precursors at 1200°C for 2 hours. The cell culture was conducted using MG63 cells (human osteosarcoma). Fibres were produced from the glasses via melt-spinning using a dedicated in-house fibre manufacturing facility. The mechanical properties of the fibres were measured via single fibre filament test method using LEX810 tensile tester.

Results and Discussion: The morphology of the cells cultured on phosphate glass discs was visualised using SEM (see Figure). A representative image of MG63 osteosarcoma cells cultured on phosphate glass specimens for 7 days is presented. In general, cells cultured on all glass surfaces showed a confluent layer at 7 days of culture. Due to the inherent (cancerous) nature of MG63 cells large nodules of cells were found at initial time points which resulted in the formation of a dense cell layer at later time points. This observation was consistent for all the glass samples. Continuous fibres of 18-20 microns were successfully drawn from glasses containing 5 and 10 mol% B₂O₃ with tensile strengths of 990 and 1100 MPa respectively, while fibre a non-continuous fibre drawing process occurred for the glass composition without B₂O₃. The NMR studies conducted on the glasses revealed that the addition of B₂O₃ increased the chain length by becoming a part of the phosphate main structure which eventually made the fibre drawing process continuous.

Figure: SEM images of the MG63 cells cultured on the surface of different glass compositions after 7 days of culture.

Conclusions: A dense cell layers was observed on all the glass samples investigated after 7 days of cell culture. The increased chain length as confirmed by NMR studies were suggested to be due to B₂O₃ incorporation within the glass backbone which also facilitated the ease of fibre fabrication from these formulations as compared to the non-boron containing glasses.

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POSTER 17: APPROACHES TO ANTI-MICROBIAL SURFACES FOR ORTHOPAEDIC APPLICATIONS

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Introduction
Biofilm formation on medical devices, such as total hip replacement devices, poses a great threat to the health of patients and a great expense to the NHS. In the EU 0.9% of total hip replacements and 3% of partial hip replacements result in a surgical site infection [1], and biofilm formation plays a major role in these cases. Despite the sterility of the medical devices themselves, often the biofilm may be formed by the bacteria from the patient’s own flora, and therefore materials which prevent microbe adherence as opposed to relying on sterilisation alone are required.

A collaborative project with Zimmer Biomet will explore new technologies and existing structures based on degradable and non-degradable materials, multilayers and surfaces. This will also explore fundamental understanding of the mechanisms through detailed microbiology, and more in depth in-vitro cytocompatibility with detailed temporal materials characterisation to elucidate the behaviour of microbes in response to the different surface and media conditions at different time points.

By approaching both degradable and non-degradable surfaces as potential antimicrobial structures this work aims to develop a comprehensive microbiological testing set of protocols that can be used in future assessments of materials with a wide variety of antimicrobial mechanisms. Surface topography from the nano- to the macro-scale will be investigated, as well as optimising surface chemistry in order to prevent microbial adhesion or biofilm formation.

A broad range of material characterisation, imaging, human cell culture and microbiological studies will be used in order to ascertain the antimicrobial mechanisms for these structures. Optimisation of these structures will then be pursued once the mechanisms can be fully understood and exploited, allowing for the combination of multiple approaches for greatest effect.

Both polymeric and metal scaffolds have been produced and obtained to assess a wide range of different antimicrobial materials and additive molecules. An approach using quorum-sensing molecules, which bacteria use to communicate, will also be explored.

Materials and Methods
Due to the unique challenges of analysing biofilm formation inside 3D scaffolds, made from both very high- and very low density materials, an analysis protocol comprising of several techniques will be utilise to provide a standardised method of analysing such structures regardless of their density. Scanning with micro-CT has been attempted, with an Agarose hydrogel acting as a synthetic biofilm for means of proof of concept due to its similar density to a bacterial biofilm, as well as a combination of freeze-drying and confocal microscope imaging in order to provide an insight into how the biofilm is distributed inside the scaffold; ergo the ability of bacteria to penetrate through the structure. 3D scaffolds will be tested against flat samples for comparison and any effects of the geometry itself on bacterial adherence can also be assessed.

Results and Discussion
Bacterial colonies have been successfully cultured inside the 3D structures in vitro, confirmed by microscopy. The method of freeze-drying and confocal microscope imaging provided insight into the amount of biofilm formed, with quantifiable results, and the penetration of bacteria through the scaffold through cross-sectional analysis. Micro-CT scanning, however, has been unsuccessful in yielding meaningful data, due to the large difference in density of materials.

References
POSTER 18: A STUDY OF STRONTIUM SUBSTITUTED HYDROXYAPATITE

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Introduction:
It is well documented that hydroxyapatite (HA) is chemically and structurally alike to that of the main components that make up bone tissue. However, unlike synthetic HA, biological HA contains several calcium phosphate (CaP) phases and other ions, such as zinc (Zn), magnesium (Mg) and strontium (Sr). Sr has been the focus of several studies as it has been found to not only promote bone formation, but also slow bone resorption. Previous studies have shown that the addition of Sr into the HA lattice has improved signalling pathways of pre-osteoblastic cells, encouraging them to differentiate into an osteogenic lineage1. The key aim of this work was to prepare a range of strontium substituted HA materials and to study their in vitro behaviour with primary human osteoblasts and human Mesenchymal Stem Cells.

Materials and Methods:
HA powders were synthesised through aqueous precipitation at 4 different Sr %wt doped concentrations. This included pure HA (HA), HA with 2%wt Sr (2SrHA), HA with 5%wt Sr (5SrHA) and HA with 10%wt Sr (10SrHA). These powders were pressed into disks using 0.3g of powder per disk (13mm x 1 mm) and were then sintered at 600˚C. The materials were analysed using X-ray Photon Spectroscopy (XPS), Fourier Transform Infrared Spectroscopy (FTIR), Transmission Electron Microscopy (TEM) and X-Ray Diffraction (XRD).

Results and Discussion:
As observed for the XRD, FTIR and XPS results, the crystallinity, purity and stoichiometry of the HA powders were as expected. The XRD results show broad peaks, indicative of small grain size, low crystallinity and the substitution of Sr2+ for Ca2+ in the HA lattice. Further evidence for the substitution of Sr2+ for Ca2+ was indicated by the shift in peak positions to lower 2θ in the XRD results and the loss of hydroxylation in the FTIR spectra. XPS data generated showed the presence of Sr3p peak at ~269 eV for each of the Sr substituted samples, as expected, whilst there was no Sr detected in the pure HA sample.

Conclusions:
The XPS, FTIR and XRD results show that each of the powders have definite similarities but change as expected upon the rising concentrations of Sr. It was also clearly shown that Sr2+ was substituted for Ca2+ in the HA lattice and that no significant impurities were detected in the materials. The next step in this work would be to understand how increasing strontium concentration in these samples affects their in vitro performance.

References:
POSTER 19: HYDROGEL SCAFFOLD ENGINEERING FOR OSTEOCHONDRAL DEFECTS

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Introduction

Osteochondral defects have been set to be one of the world’s most common problems affecting variable groups of people due to different pathological or accidental damage problems [1]. Tissue engineering has emerged to provide a better solution for osteochondral defects using combinations of cells with biomaterials fabricated in a 3D scaffold construct in order to mimic the natural tissue environment [1-4]. The present work focuses on testing selected biomaterial hydrogels which are N-isopropylacrylamide (NIPAM) and N-tert-butylacrylamide (NTBAM) to identify the effects of specific features of the gels and their architecture on bone cell line behavior, activity, and osteogenic potential after specific time of culturing.

Methods

Gels have been characterized to identify the specific IR spectral pattern and functional groups, the water contact angle have been measured as well to verify the hydrophilic/hydrophobic behavior. Scanning electron microscopy images of the gels were used to clarify internal gel architecture and pore size. Cell viability/cytotoxicity staining test has been performed to identify the percentage of viable MG63 cells after specific time of culturing. Cytoskeletal fibrin and cellular nucleus have also been examined using phalloidin and Dapi staining to identify specific MG63 cells behavior and morphology during interaction with these scaffolds. Tests, including alkaline phosphatase (ALP) and Alizarin red staining, support the findings for osteogenic activity and verification of calcium deposits produced by the cells.

Results:

The IR spectral results together with the water contact angle, showed an obvious hydrophobic behaviour for pNTBAM compared to pNIPAM hydrogel. SEM imaging revealed a different internal architecture with a larger pore size measured for pNIPAM compared to pNTBAM. MG63 showed a good percentage of viable cells after 21 days of culturing. A proof of calcium deposition has been noticed on both of the gels with a higher deposition upon pNTBAM.

Discussion & Conclusions:

Results demonstrate the impact of wettability, surface stiffness and internal architecture of pNIPAM and pNTBAM on MG63 bone like cells behaviour. The variable aspects of viability and mineralization profile, may suggest the validity of these hydrogels as a tissue replacement matrix to support variable tissue regeneration including bone and cartilage as in osteochondral tissue damage.

References:

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Acknowledgements

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POSTER 20: COMPOSITE MEMBRANES FOR GUIDED PERIODONTAL TISSUE REGENERATION
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Introduction
The development of bioactive membranes for the guided tissue regeneration (GTR) of periodontal structures is an area of increasing interest in the treatment of periodontitis, an infectious disease that destroys the tooth-attachment apparatus [1]. The GTR approach involves the placement of a barrier to exclude the epithelial and gingival tissues from the exposed root surface in order to enable the more slow-growing periodontal ligament and hard tissues to regenerate [1]. Chitosan is a biodegradable carbohydrate polymer that has been evaluated as a scaffold material for in vitro and in situ bone and periodontal tissue engineering [2]. The present study investigates the bioactivity and antibacterial properties of solvent-cast chitosan-bioactive glass composite films for potential use as GTR membranes to repair damaged periodontal structures.

Materials and Methods
Bioactive glass (BG) and 5 wt% gallium-doped glass (BG-Ga) were prepared by the sol-gel method [3], and both glasses were ion-exchanged with silver ions (BG-Ag and BG-Ga-Ag). Chitosan and bioactive glass were blended in 1% aqueous acetic acid solution at a mass ratio of 100:35. The solutions were cast on to polycarbonate surfaces and dried in air at 60 °C. The in vitro bioactivity of the composite membranes was evaluated by monitoring hydroxyapatite (HA) formation on their surfaces in simulated body fluid (SBF) at 1, 3, 7 and 14 days [4]. HA was confirmed by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) with energy dispersive X-ray analysis (EDX). Twelve nutrient agar plates were spread with 0.2 cm³ of Escherichia coli (1.7 × 10⁸ CFUcm⁻³). Three 8 mm discs of each composite were immediately placed in the centre of each plate and the samples were incubated at 37 ºC for 24 hours, after which time the zones of inhibition were measured. Each test was carried out on triplicate plates for every composite material.

Results and Discussion
The characteristic sharp doublet of crystalline hydroxyapatite at 570 – 605 cm⁻¹ was present in the FTIR spectra of all of the composite membranes following a residence time of 3 days in SBF. The presence of hydroxyapatite was additionally confirmed by SEM and EDX.

Zone of inhibition data are listed in Table 1. These data indicate that the composites blended with the bioactive glasses, BG and BG-Ga, failed to demonstrate any antimicrobial activity against E coli. Conversely, composites containing the silver-bearing glasses, BG-Ag and BG-Ga-Ag, showed clear zones of inhibition which were not significantly different (p = 0.445).

Table 1. Zone of inhibition data for composite membranes (standard deviations in brackets)

<table>
<thead>
<tr>
<th>Sample</th>
<th>BG</th>
<th>BG-Ga</th>
<th>BG-Ag</th>
<th>BG-Ga-Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean zone (mm)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.27 ± 0.18</td>
<td>0.28 ± 0.15</td>
</tr>
</tbody>
</table>

Conclusions
Antibacterial composite films can be prepared by solvent-casting mixtures of chitosan and Ag⁺-exchanged bioactive glass. The incorporation of 5 wt% gallium into the sol-gel-derived glass influenced neither in vitro bioactivity nor antimicrobial activity.

References
**POSTER 21: 3D PRINTING LIVER ON DEMAND**

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**Introduction**
Generation of hepatocytes, the primary liver cell, from embryonic stem cells (ES cells) is of interest to many fields, from pharmacology to tissue-engineering. As ES cells can divide indefinitely and can give rise to any cell type in the body, they are in theory an unlimited source of hepatocytes. However, poor stability and function of stem-cell derived hepatocytes in current culture conditions prohibit their use for research.

Bioprinting would allow multiple cell types and biomaterials to produce a 3D structure mimicking liver tissue. The goal of this project is to use a bioprinter to produce a 3D culture platform for stem cell-derived liver cells, which will improve the stability, viability and function of these cells.

My research involves encapsulating embryonic stem cells in an alginate hydrogel, before maturing them into hepatocytes using a defined protocol. Viability, function and stability of these cells are the key parameters to determine if the encapsulation and maturation are successful.

So far my research has shown that ES cells can be successfully encapsulated in alginate hydrogel, maintain their viability, and begin the differentiation process to hepatocyte-like cells.

**Materials and Methods**
ES cell spheroids are generated using either suspension culture or micro-wells and then encapsulated in alginate. Analysis of cell viability after encapsulation has been performed extensively using fluorescent imaging and chemical assays. The Hay protocol (Hay et al., 2008) for hepatocyte differentiation was employed to generate hepatocyte-like cells from the spheroids. Characterization of the cells was undertaken at keys points of differentiation relative to development: endoderm formation; hepatoblast formation; and the mature hepatocyte-like stage. Immunofluorescence and qPCR were the primary means to characterize the cells.

**Results and Discussion**
Initial encapsulation tests showed low cell viability, so ES cells were first made to form spheroids before encapsulation. This greatly improved their viability. Various biomaterials were mixed with alginate to form composite hydrogels and were trialled to assess their effects on viability and maturation, but none were seen to be significantly better than the base alginate condition. Optimising the ES spheroid differentiation was then the focus, as while the Hay protocol is well defined and efficient for 2D culture, it appears insufficient to generate hepatocyte-like cells in 3D. Tweaking the differentiation protocol has so far greatly improved the generation of definitive endoderm (Sox17+) cells in the spheroids.

**Conclusions**
High viability of encapsulated ES cell spheroids has been achieved in alginate, and while addition of bioactive materials did not prove to be beneficial, optimisation of the differentiation protocol has already greatly improved endoderm formation in the spheroids. The next steps will be to optimise the later stages of the differentiation protocol and conduct full characterisation of the spheroids and cells throughout the process. When the protocol has been well optimised, the resulting hepatocyte-like spheroids can be utilised for *in vitro* drug trials, toxicity studies, or for *in vivo* transplantation work.

**References**
**POSTER 22: ANTIBACTERIAL BIOMATERIAL DEVELOPMENT USING Azadirachta indica EXTRACT**

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**Introduction**

Polyhydroxyalkanoates (PHAs), bacterial polyesters, are highly biocompatible, biodegradable and have varied mechanical properties. These properties make PHAs suitable candidates to develop medical devices and tissue engineering scaffolds (Nigmatullin et al., 2015). There are two types of PHAs: short chain length (SCL) PHAs and medium chain length (MCL) PHAs (Khanna and Srivastava, 2005). In this study, we have focused on SCL PHAs, Poly(3-hydroxybutyrate), P(3HB). P(3HB) is one of the most well studied SCL-PHA and has high value of stiffness, tensile strength and is also known to exhibit piezoelectric property (Basnett et al., 2014). Within the biomedical field, one of the main concerns are the emergence of multidrug resistant (MDR) bacteria that are capable of biofilm development. These adhere on medical devices, implants and spread by creating wounds and infections in the human body (Percival et al., 2015). Hence, it is essential to develop medical devices and implants with inherent antibacterial properties. Incorporation of organic or inorganic antibacterial agents into PHAs is one possible route for the development of antibacterial PHAs. It has been proven that extracts of the Asian medicinal plant, *Azadirachta indica* (neem), has antibacterial properties (Sharma et al., 2009). Hence, in this study, antibacterial PHAs were developed using *Azadirachta indica* (neem) extract.

**Materials and Methods**

*Bacillus subtilis* OK2, was used for the production of P(3HB). *Azadirachta indica* extract (JIVA Ayurveda, India) was used as the active agent. Batch fermentation was conducted in 14 L Applikon fermenters for the production of P(3HB). To purify and extract the polymer, soxhlet extraction was used (Rai et al., 2011). To evaluate the antibacterial properties of the selected agent, the agar-well diffusion method and agar-disc diffusion method were carried out against the Gram positive bacteria, *Staphylococcus aureus* ATCC 6538 and the Gram negative bacteria, *E. coli* ATCC 8739. The antibacterial tests were carried out in triplicate.

**Results and Discussion**

P(3HB) was successfully produced using *Bacillus subtilis* OK2 with a total yield of 35% dry cell weight. The antibacterial tests, confirmed that the *Azadirachta indica* extract had the potential to inhibit/prevent growth of both Gram positive and Gram negative bacteria. These results are presented in Figure 1. It was observed that the *Azadirachta indica* extract was more effective against *S. aureus* than on *E. coli*. The diameter of the inhibition zones obtained using the extract were mostly higher than that obtained using the positive control (streptomycin), further confirming the highly effective antibacterial activity of the *Azadirachta indica* extract.

**Conclusion**

Antibacterial tests confirmed the antibacterial activity of the *Azadirachta indica* extract against both Gram positive and Gram negative bacteria. Further studies, will involve the production of antibacterial P(3HB) using this active agent and its use in the production of medical prototypes.

**References**

POSTER 23: HOW DOES MECHANICAL LOADING OF 3D BIOMIMETIC SCAFFOLDS EFFECT COLLAGEN PRODUCTION?

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Introduction: Collagen is the major structural protein in the body, occurring as 28 different isoforms [1]. The different types of collagen are found in specific tissues, imbuing the extracellular matrix with appropriate properties. Examples include, type I in bone, type II in cartilage [2] and type X [3] in endochondral mineralisation during development. The continuous mechanical loading applied by bone growth during both embryogenesis and neonatal development influences the production of the extracellular matrix of cartilage, tendon and ligament. In adulthood production is largely regulated by the dynamic mechanical stimuli that results from exercise, generating tissue that is optimised to specific loading conditions [4].

Methods: Utilising the EBERS TC-3 bioreactor and 3D printing technology, physiological mechanical stimuli will be recreated under controlled conditions in the lab and applied to stem cells cultured in biomimetic 3D scaffolds. The aim is to determine how specific mechanical forces result in functional collagenous extracellular matrix by guiding differentiation and synthesis of particular collagen isoforms. This research presents the opportunity to selectively grow different tissues from mesenchymal stem cells by controlling their differentiation using specific loading forces.

Results: In vitro fibrin acts as a 3D biomimetic scaffold. Cells are cultured and then cast within the Fibrin gel. By forming the fibrin gel around two anchor points it will contract forming a rod like 3D biomimetic structure with the cells encased. The anchors have been designed to act as the loading points for the fragile fibrin gels. Once contraction is complete loading will be applied as required. Figure 1 shows the anchor points used to load the cells.

Discussion and Conclusions: It is currently unknown how stem cells respond to specific types of stretch or compression in their environment by differentiating and producing appropriate extracellular matrix [5]. This investigation will focus on using the different tissue specific collagen isoforms as markers of functional differentiation in mesenchymal stem cells.

References:
Introduction
Polyesters are described as sustainable and bio-active/bio-inert; such qualities make them attractive for biomedical applications. More specifically, Poly (lactide) (PLA) and Poly (ε-caprolactone) (PCL) are two thermoplastic polyesters that are well known for their bio-compatible and environmentally sustainable nature. PLA shows high tensile strength and a brittle nature at room temperature. On the contrary, PCL exhibits a more ductile character. The reciprocal traits of these two polymers could be advantageous if combined, however their very poor miscibility, restricts their use as a single compound when mechanically mixed (e.g. with an extrusion process). Rheological and microstructural studies on the use of a newly synthesized di-block co-polymer PCL-PLA has been explored to achieve blending of these two otherwise immiscible polymers. 1

Materials and Methods
Commercial grades of PCL and PLA were used in the project. The resulting PCL-PLA di-block copolymer was synthesised according to Teyssie 2 with minor modifications according to Ronca 3. Rheology has been performed using a TA instruments ARES G2 in 25mm Plate-Plate geometry. SEM imaging performed with a Carl Zeiss (Leo) scanning electron microscope.

Results and Discussion
Rheology proved an improvement in miscibility of the two starting polymers that is dependent on the composition of the ternary mixture and requires concentrations of copolymer as low as 5% to achieve such results. Further investigations are in progress to establish a correlation between block lengths and further reduction of copolymer content; however this is limited by the activity of the chosen catalyst. Hence, reviewing available catalysts as well the production of novel ones are also in progress.

Conclusion
It was shown that improvements can be achieved through the use of a di-block co-polymer as a compatibiliser. However the microstructure of the co-polymer seems to play a role into the blending process and the resulting homogeneity of the blends. Work is currently in progress on fine-tuning the microstructure of the copolymer via different ligands and metals.

References
**POSTER 25: TAILORING THE STEM CELL ENVIRONMENT THROUGH CONTROLLED ION RELEASE FROM DEGRADABLE CALCIUM PHOSPHATE-BASED GLASS MICROSPHERES**

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**Introduction:** The use of tailored materials is largely investigated as an alternative strategy to support and potentiate the regenerative ability of stem cells. In the field of bone repair, phosphate-based glasses (PBG) have gained great interest since they are fully bioresorbable and their formulation can be manufactured to resemble the chemical composition of the inorganic part of bone (1). PBG have been produced in different geometries including microspheres (MS), which are particularly advantageous for biomedical applications as they are suitable for delivery via minimally invasive routes into the target area (2). The main advantage of PBGs is that their degradation can easily be controlled releasing a mix of ions into the local microenvironment to influence the cell response (3). Mesenchymal stem cells (MSCs) are multipotent stem cells characterized by the ability to differentiate towards mesodermal lineages including bone tissue, and have already been used in the clinic (4). The aim of this study was to characterize the ion release rate, cytocompatibility and pro-osteogenic potential of various PBG-MS formulations manufactured as both bulk and porous microspheres via in vitro studies, using human bone marrow derived mesenchymal stem cells.

**Methods:** For the study of the PBG-MS ion release rate, different formulations of PBG-MS were incubated in DMEM at 37°C for 14 days. Samples of DMEM containing the PBG-MS degradation products were collected over 14 days and analysed by ion chromatography. For the biological characterization, medium conditioned with PBG-MS was used to culture the human bone marrow-derived MSCs for 14 days. Cell metabolic activity was monitored during the experiment and assayed at day 2 and day 12. The alkaline phosphatase (ALP) activity was assayed at day 12.

**Results:** The results of ion chromatography revealed that the PBG-MS manufactured as bulk and porous products released respectively 0-18 and 0-27ppm of Magnesium, 7-23 and 0-255ppm of Calcium, 25-89 and 33-127 ppm of Phosphate, and 0-230ppm and 0-243ppm of Sodium ions in 48h in DMEM. The results of the cell metabolic assay indicated that PBG-MS supported cell growth over a period of 14 days similarly to the standard medium. The formulations tested also promoted the hMSCs osteogenic response by inducing ALP activity.

**Conclusions:** Our results suggest that the degradation products from the PBG-MS formulations tested are cytocompatible and suitable for long-term cell exposure. Moreover, they seemed to promote osteogenic commitment in hMSCs, therefore suggesting that these products could be promising candidates for bone regeneration strategies.

**References:**

**Acknowledgements**
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POSTER 26: A METHOD FOR QUANTIFYING BACTERIAL COVERAGE ON COATED METALLIC SUBSTRATES

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Introduction
My research aims to develop a surface treatment or coating suitable for metallic implants that prevents biofilm infections occurring by disrupting the processes by which the bacteria initially adhere to the substrate surface. It is suggested that the first 6 hours post-surgery are critical to the successful formation of a biofilm that is developed enough to protect the bacteria inside it. 1 Phosphate based glass coatings, which degrade fully in aqueous environments2, offer an opportunity to create degradable coatings that may prevent biofilm formation by preventing bacteria from adhering to the implant surface within the 6 hour window.

In order to develop effective coatings, it is necessary for me to develop a testing protocol to determine the effects of my materials on bacterial growth. While established methods exist for testing biofilm growth on different substrates, very little work has been done on metallic substrates. Although microscopy can be used, a higher through-put method is desirable for the initial screening of candidate materials to show a marked reduction in bacterial adhesion and therefore warrants further investigation.

Materials and Methods
3 different substrates were prepared. Medical grade titanium (Ti6Al4V) was wire eroded to give circular (10mm diameter) disks and then sandblasted with 120 p Alumina Grit (distance from substrate approx. 50 mm, pressure at 7x10^5 Pa) to give the Ti samples. Solid Copper disks of the same size were sandblasted as well to give Cu samples. These samples were sterilised (UVC radiation for 30 mins per side) and 3 samples of one type were placed into sterile petri dishes.

Staphylococcus Aureus Newman strain with an eGFP tag was cultured in Tryptone Soy Broth (TSB) and Chloramphenicol (Cm) to a concentration of 10 µg/ml to an Optical Density (OD600) of 1. The culture was washed twice in TSB. The sterile petri dishes containing the samples were filled with 15ml pre-warmed (37°C) TSB, and then inoculated to 0.01 OD600 from the washed culture. The dishes were incubated (37°C at 60 RPM) for 6 hours. After incubation the samples were washed in PBS and placed face-up into a glass bottomed 24 well plate (Grenier Bio-one) and read by the TECAN Infinite® M200 PRO (Excitation = 488 nm, Emission = 540 nm).

Results and Discussion
The TECAN data shows a clear difference between the signal reported from the Ti disks and the Cu disks, indicating a difference in bacterial adhesion between the two substrates. Presenting the data as a heatmap also allows for low-resolution indication of the distribution of cells across the surface of the disk.

Conclusions
The TECAN data shows that high through-put screening of candidate materials on metallic substrates is possible. By comparing candidate samples to both the Ti samples (a positive control) and the Cu samples (a negative control) it will be possible to determine their relative efficacy in preventing bacterial adhesion. The candidate materials that show promising results in this test can then be subject to further assays to further characterise these materials and their antimicrobial effects.

References
POSTER 27: THE EFFECT OF GELATIN BEADS IN THE PSEUDOISLETS ON THE SECRETION OF INSULIN

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Introduction
Diabetes mellitus affects a large percentage of the world community. It has two major forms: Type I and Type II. Type I diabetes mellitus is characterised by complete loss of β-cells in the pancreatic islets of Langerhans, these cells regulate insulin secretion and blood glucose homeostasis. The detrimental effects of uncontrolled blood sugar call for the development of therapeutic strategies that help in restoring insulin level as close as possible to the normal secretion of insulin endogenously. Islet transplantation has been a promising and progressing strategy to fight diabetes; however, the limit in obtaining donors and the low yields of native islets for transplantation in addition to the long-term use of immunosuppressive drugs are the main challenging obstacles that prevent the widespread use of islet transplantation. The aim of this study is to develop a new technique, which can improve the functionality of Pseudoislets through using a β-cell line in order to explore effective approaches in the constitution of Pseudoislets.

Materials and methods
BRIN-BD11 rat pancreatic β-cell line was used to generate Pseudoislets. The cells were cultured on ultra-low attachment 96-well plate. The cell seeding density was 32,000 per well. Gelatin beads were produced following the water-in-oil emulsion method. Gelatin A in a 15 % solution was preheated and dropped into vegetable oil under constant stirring. Gelatin beads (GBs) with 40µm and 30µm were crosslinked with 5% glutaraldehyde. Then the gelatin beads of both sizes were incorporated into the PIs. The GBs incorporated PIs were stimulated with 16.7 mM glucose for 20 minutes. The releasing of insulin was determined using an ELISA kit.

Results and Discussion
Figure 1 showed BGs incorporated PIs morphology and a significant increase of insulin secretion in BGs incorporated PIs, supporting the hypothesis that Pseudoislet survival could be improved with the incorporation of GBs. The use of GBs could make porous scaffold in the PIs which increased the viability due to a better for the diffusion for small molecules like oxygen and nutrients. The higher cell viability improved the insulin secretion.

Conclusion
Our results showed that incorporation of GBs in the PIs lead to improve viability, especially on day 7, hence supporting the hypothesis that Pseudoislets survival could be improved with incorporation of GBs for a better insulin secretion when they stimulated with glucose. These experiments have demonstrated the important features of incorporation of gelatin beads allowing applications in medicine and Bioengineering as encapsulation devices of drug delivery vehicles as well as a tool to improve the cell viability

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Fig.1 (I) Optical microscopy images of PIs with incorporated GBs (40 µm) at day 3 (A), day 5 (B) and day 7 (C) compared to unincorporated PIs at day 3 (D), day 5 (E) day 7 (F). (II) Insulin released from Incorporated PIs at day7
POSTER 28: CHEMICALLY FUNCTIONALISED AND TOPOGRAPHICALLY TEXTURED MICROPARTICLES TO SCREEN STEM CELL-PARTICLE INTERACTIONS

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Introduction
Stem cell technologies require suitable biomaterials capable of supporting cell proliferation and modulating cell response. Biomaterial design therefore becomes crucial for a successful regenerative medicine therapy. It is well-known that material properties such as chemistry1, topography2 and elasticity3 are capable of inducing particular cell behaviours and individually control stem cell fate. The aim of this work is to study this concept on large libraries of 3D materials which combine all these properties by fabricating a series of chemically modified textured microparticulate architectures.

Materials and Methods
Microparticles of biodegradable poly(lactic acid) (PLA) (74 kDa) were prepared by homogenisation of PLA solution in dichloromethane in a 1% w/v polyvinyl-alcohol (98% hydrolysed, 13-23 kDa) aqueous solution. Agitation speed and polymer concentration were varied to target different particle sizes. Subsequently, microparticles were treated with ethylenediamine solution (0.5M in isopropanol) at room temperature and the polymerisation initiator α-bromoisobutyryl bromide was directly coupled to the generated amino groups. The extent of aminolysis and initiator immobilisation were assessed by the 2,4,6- trinitrobenzene sulfonic acid assay. Textured particles were obtained by incorporating fusidic acid (FA) in the organic phase at different polymer/FA ratios. Emulsification was performed as described and dimpled particles were obtained after FA release in PBS. Immortalised human mesenchymal stem cells (iMSCs) were seeded onto the microparticles either statically or dynamically, and their attachment, viability, and aggregation were assessed using Live/Dead staining and scanning electron microscopy (SEM).

Results and Discussion
By changing emulsion conditions, smooth microparticles in a size range from 50 to 300 µm were obtained (Fig. A). Preliminary cell experiments showed that seeding iMSCs on the microparticles resulted in the formation of cell-particle aggregates (Fig B) independent of seeding conditions employed (static vs dynamic). However, particle size had an influence on aggregate formation, with larger particles resulting in smaller aggregate sizes. Next, particles were aminolysed in order to enhance surface functionality and reactivity. Control of the particle exposure time to aminolytic regents is essential not only in order to achieve greater functionalisation, but also in order to avoid particle damage (time periods above 1 hour were detrimental for particle morphology). Amino groups now available on particle surface can further react with chemistries which have demonstrated significant capabilities in modulating cell response. Here, the ATRP initiator α-bromoisobutyryl bromide was directly linked onto the particles allowing to graft polymers from these 3D structures. Monomers of interest will be subsequently polymerised from the activated surfaces.

In addition, topographical features can be induced by incorporation of FA into the sample. The phase separation of the drug from the polymer during solvent evaporation, generate particles with a characteristic ‘golf ball-like pattern on the surface’. (Fig C) Moreover, dimple size can be tuned by varying the amount of FA added to the initial polymer/FA mixture. Future work will combine these topographies and chemistries and the impact of these combinations on cardiomyocyte maturation and MSC differentiation in 3D will be evaluated.

Conclusions
In this work, chemically and topographically modified microparticles have been presented. The methodologies selected allowed us to incorporate functionalities of a very dissimilar nature by following a common approach from a single core material. In addition, these approaches retain bulk material properties and the topographical features whilst changing the outermost surface characteristics. In conclusion, these materials present a platform for new biomaterials discovery in 3D for potential regenerative medicine applications.

References

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POSTER 29: LOW-VOLTAGE CONTINUOUS ELECTROSPINNING PATTERNING

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Introduction
Electrospinning is a versatile technique for the construction of microfibrous and nanofibrous structures with considerable potential in applications ranging from textile manufacturing to tissue engineering scaffolds. In the simplest form, electrospinning uses a high voltage of tens of thousands volts to draw out ultrafine polymer fibers over a large distance. However, the high voltage limits the flexible combination of material selection, deposition substrate, and control of patterns. Prior studies show that by performing electrospinning with a well-defined “near-field” condition, the operation voltage can be decreased to the kilovolt range, and further enable more precise patterning of fibril structures on a planar surface.

Main section
In this work, by using solution dependent “initiators”, we demonstrate an ultralow voltage continuous electrospinning patterning (LEP) technique, which reduces the applied voltage threshold to as low as 50 V, simultaneously permitting direct fiber patterning. The versatility of LEP is shown using a wide range of combination of polymer and solvent (aqueous and organic) systems for thermoplastics and biopolymers. LEP offers advantages over traditional electrospinning protocols in the range of usable materials and substrates, providing continuous deposition mitigating electric sparks. In order to guide the onset of fiber formation at voltages at around 100 V, different types of initiators should be used depending on the polymer conductivity; to this end, the electrospinnable range of the polymeric solution concentrations is also widened. We demonstrated fibril patterns of various polymers, directly deposited onto substrates such as hydrated agarose gel and PDMS with millimeter thicknesses. Novel functionalities are also incorporated when a low voltage mode is used in place of a high voltage mode, such as direct printing of living bacteria; the construction of suspended single fibers and membrane networks. LEP may bring a potential route to the deposition of fibrous structures of a variety of materials for future organ-on-chip applications, as well as precision construction of fibrous scaffolds for tissue engineering.

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