UK Society for Biomaterials

UKSB

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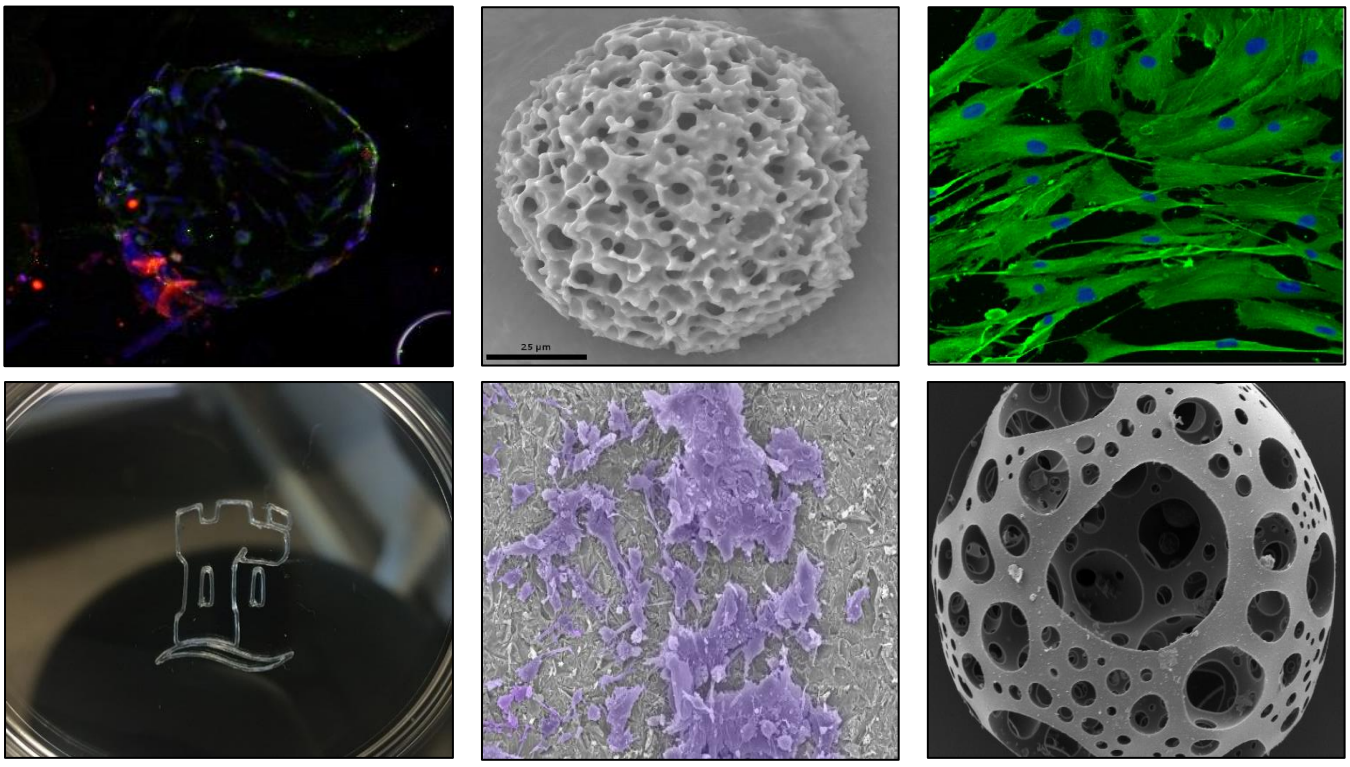
UK Society for Biomaterials

18th Annual Conference



11th - 13th June 2019

Abstract Booklet

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UK Society for Biomaterials 18th Annual Conference

11th - 13th June 2019, University of Nottingham

Local Organising Committee:
Lisa White – Chair
Ifty Ahmed
Mahetab Amer
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Hadi Hajiali
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I-Ning Lee
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Kathryn Thomas
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Cover images:
Induced mesenchymal stem cells grown on GelMA microparticles (Francesco Pappalardo).
Acrylate-based polyHIPE microparticle produced using a microfluidics system (Robert Owen).
Human airway smooth muscle cells cultured in GelMA (Jopeth Ramis).
University of Nottingham logo 3D printed in silicon (Paola Sanjuan Alberte).
Human osteoblast-like cells growing on Ti_6Al_4V substrate (Kathryn Thomas).
Porous calcium-phosphate glass microsphere (Ifty Ahmed).

UKSB Official Sponsors

UKSB Presidents Welcome

Dear UKSB 2019 delegates,

On behalf of the UKSB Council and the local organising committee led by Dr Lisa White, I would like to extend to you all a most cordial welcome to the 18th annual UK Society for Biomaterials conference, this year held jointly with the Tissue and Cell Engineering Society and hosted by the University of Nottingham. Since the beginning of the Society in 2000, we have sought to provide an inclusive and democratic environment to share our research in the interdisciplinary area of biomaterials, at the same time supporting the next generation of researchers developing as postgraduate students and postdoctoral research fellows. The strong scientific programme outlined for this year continues this aim, representing the diverse areas underpinning biomaterials and tissue and cell engineering and providing excellent opportunities for both developing new networks and collaborations and renewing old links.

Over the years, the field of biomaterials has evolved, working closely with the tissue engineering, surface chemistry and bio-fabrication groups worldwide. As a Society we aim 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. This joint meeting with the TCES attests to that aim. There are plans for similar link ups with related UK societies in the coming years.

In line with the aims indicated above, I would draw your attention to our recently established awards providing support for involvement in the European Society for Biomaterials, and funding for inter-laboratory working between student members. Further I would like to highlight the activities taking place at this conference to develop an Early Career Researchers Forum within UKSB and encourage those of you who recognise yourselves by this description to get involved.

Our prize winners this year have been awarded based on their commitment to the field and advances within their particular research areas. I am sure their presentations will provide insight and inspiration.

Finally, the World Biomaterials Congress in Glasgow is less than a year away. We will have a dedicated UKSB special symposium at the Congress. I hope that many of you are planning for this event as it approaches and will help in projecting the profile of the UKSB as a national Society.

With kind regards,

Colin Scotchford

UKSB President

Prizes and Awards

Each year there are prizes given out for those showing excellence in their work, dedication and ambition to further biomaterials research. Due to the success of the launch of the lab-2-lab collaborations and travel awards for the UKSB and ESB last year, these are being continued and you are encouraged to visit www.uksb.org to find details of how to apply and nominate for these awards.

UKSB President's Prize

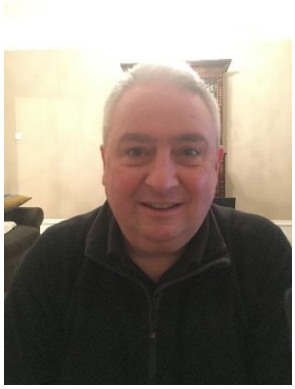
The President's prize was established to recognise the achievement of specific individuals over the course of a career in biomaterials.

The UKSB 2019 President's Prize is awarded to **Professor Kevin Shakesheff**, Pro Vice Chancellor for the Faculty of Science and Director of the UK Regenerative Medicine Platform Hub for Acellular Technologies.

His independent scientific career began at the Massachusetts Institute of Technology under a NATO fellowship following a PhD and his qualification as a registered pharmacist. He returned to the UK as an EPSRC Advanced Fellow and became Professor of Tissue Engineering and Drug delivery in 2001 at the University of Nottingham. In 2013 he became a Royal Society Wolfson Merit Award Holder. He was a Sub-Panel Member for the Research Excellence Framework (REF) for 2014. In 2011 he was made a Fellow of the Royal Pharmaceutical Society and in 2013 a Fellow of the Royal Society of Biologists. In 2014 he was selected as one of the 10 most inspirational scientists in the UK by the Engineering and Physical Sciences Research Council (RISE Leader Award). Kevin's research involves the use of materials to control drug release and cell behaviour in 3D.

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 maxilla-facial repair).



This year the Alan Wilson memorial award is given to **Professor David Wood**. David Wood is the School of Dentistry's Director of Research and Innovation, Professor of Dental Materials (2004-), and the Biomaterials and Tissue Engineering Research Theme Lead (2008-). His first academic appointment was at King's College School of Medicine and Dentistry (1992-1995) before joining Leeds as a junior lecturer in 1995.

He gained BSc Honours (2i) Materials Science from Thames Polytechnic in 1988 and a PhD in Biomaterials Science from the University of Greenwich in 1993, under a CASE Award from the Laboratory of the Government Chemist working on a project taking a glass-ceramic approach to controlling the properties of glass ionomer cements.

Research areas are:

- Natural polymer hydrogels and fibrous materials for medical and dental applications, including wound dressings, guided bone regeneration membranes and 'smart' vehicles for diagnosis and treatment
- Glass-ceramics as bioactive porous scaffolds for hard tissue repair and regeneration.
- Selected areas in Dental Materials, including the characterisation of conventional and translucent zirconia and zirconia implant materials, the development and characterisation of novel dental composites as amalgam alternatives, and characterisation of materials for endodontics.
- Materials and processes in the digital workflow.

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 2019 Larry Hench Young Investigators Prize is awarded to **Dr Asha Patel**. Dr Patel is a Lecturer in Cell & Gene Therapy at the National Heart and Lung Institute, Imperial College London. Her research draws on multidisciplinary approaches including materials science, biochemistry and pharmaceutics to harness the potential of nucleic acid-based therapeutics.

In 2018, she completed her postdoctoral training as an EPSRC E-term fellow in the laboratories of Professors Daniel Anderson and Robert Langer at Massachusetts Institute of Technology. Here, she developed biodegradable vectors for inhaled delivery of mRNA to the lung. Asha graduated with a first class honours degree in Pharmacy from King's College London and remains a member of the General Pharmaceutical Council with extensive professional experience. Dr Patel was awarded her PhD by the University of Nottingham where she investigated the influence of diverse materials chemistry on human pluripotent stem cell and cardiomyocyte behaviour, under the guidance of Professors Chris Denning, Morgan Alexander and Martyn Davies.

Conference Social Events

We are absolutely delighted to welcome you to Nottingham for this year's UKSB conference (TCES-UKSB 2019) and we want to take the opportunity to congratulate all the prize winners and thank all the speakers for sharing their research.

To start off the social events, there will be a Welcome Reception from 6 – 8 pm on Tuesday 11th June after the close of the scientific programme. Following on from this, all Early Career Researchers are invited to join the joint TCES-UKSB Early Career Researchers Social, which will take place in the Student's Union from 8pm (Day 1). Volunteers will lead the way to the Student's Union.

Prior to the conference dinner on Wednesday 12th June, there will be a drinks reception from 7pm which will take place in the Council Room (Room A21) located within the University of Nottingham's iconic Trent Building. This will then be followed by the conference dinner at 8pm in the Senate Chambers, which is also located in the Trent Building. A map has been provided in your bag as part of the conference programme, or please ask any of the volunteers who will be happy to provide directions.

Tuesday 11th June 2019:

Development of New Models, Tools and Technologies

08:00 – 10:00 TCES-UKSB 2019 Registration and Poster Set-up

10:00 – 10:15 Welcome and Introduction to TCES-UKSB 2019

Dr Lisa White, University of Nottingham

10:15 – 11:15 SESSION 1: IMAGING IN TERM

Chair: A/Prof Colin Scotchford, University of Nottingham

10:15 – 10:45 **Keynote - Professor Chris Sammon,** Sheffield Hallam University
Using infrared imaging and spectroscopy to support the development of regenerative therapies

10:45 – 11:00 Mr Dan Merryweather, Loughborough University
Influence of sample preparation methods on the organizational presentation of collagen fibrils in hydrogel electron microscopy

11:00 – 11:15 Prof Elisa Budyn, ENS Paris-Saclay
Reforming the Haversian network in a bone-on-chip

11:15 – 12:15 SESSION 2: Turbo Talks I

1. Ms Lydia Beeken, University of Nottingham (Poster No. 144)
Development of a topical cell therapy for ocular surface disorders

2. Ms Charlotte Lee-Reeves, Imperial College London (Poster No. 264)
Improving stem cell therapies for traumatic brain injury using bioactive ECM scaffolds to attenuate inflammation

3. Mr David Hiram Ramos Rodriguez, University of Sheffield (Poster No. 136)
Development of topographically controlled electrospun scaffolds to deliver proangiogenic agents for wound healing

4. Ms Man Li, University of Liverpool (Poster No. 54)
Nitric oxide releasing electrospun nanofibers for antimicrobial bone tissue engineering

5. Ms Kathryn Thomas, University of Nottingham (Poster No. 240)
Phosphate based glass coatings for rapid Ga3+ release: the challenges of balancing cytocompatibilty with antimicrobial effects

6. Mrs Shirin Hanaei, Nottingham Trent University (Poster No. 109)
Modelling and emulating 3D multi-tissue interactions by microfluidic chip technology

7. Ms Flavia Bonalumi, University of Brighton (Poster No. 104)
Development of a multi-layered cryogel bioreactor with optimised fluid dynamics for bioartificial liver application

8. Mrs Alison Wilson, University of York (Poster No. 73)
Clinical translation of regenerative medicines: a regulatory primer

9. Mr Paul Mardling, Sheffield Hallam University (Poster No. 194)
Auxetic and composite scaffolds show potential for use in tissue engineering

10. Ms Thunyaporn Srisubin, University of Manchester (Poster No. 80)
Phosphonate-modified graphene–laponite composites for bone repair

12:15 – 13:30 Lunch and UKSB Annual General Meeting

13:30 – 15:15 SESSION 3: NEXT GENERATION BIOMATERIALS DISCOVERY

Chair: Prof Morgan Alexander, University of Nottingham

13:30 – 14:00 **Keynote - Professor Jan de Boer,** Eindhoven University of Technology
Life at the cell-material interface

14:00 – 14:15 Dr Mahetab Amer, University of Nottingham
Differentiation by design: Varying surface topographical features of polymeric microparticles influences mesenchymal stem cell fate

14:15 – 14:30 Mr Chinnawich Phamornnak, University of Manchester
Non-woven mats of electroactive composites of silk-PEDOT:PSS for peripheral nerve regeneration

14:30 – 14:45 Dr Jenny Aveyard, University of Liverpool
Nitric oxide releasing contact lens bandages

14:45 – 15:00 Mr David Richards, University of Manchester
Utilising a novel photoresponsive hydrogel with defined surface topography and photoswitchable stiffness to analyse the biophysical regulation of mesenchymal stem cells

15:00 – 15:15 Dr Laura Ruiz, University of Nottingham.
Rapid screening of inks for 3D printing personalised drug delivery implants

15:15 – 16:15 Coffee Break and Posters

16:15 – 18:00 SESSION 4: INNOVATIVE BIOMATERIALS

Chair: Dr Jude Curran, University of Liverpool

16:15 – 16:30 Platinum Exhibitor: **Jellagen**

16:30 – 17:00 **Keynote - Professor Sandra Van Vlierberghe,** University of Ghent
Versatile hydrogel platform for 3D printing applications

17:00 – 17:15 Dr Maxine Chan, Imperial College London
Fabrication and characterisation of endometrial extracellular matrix hydrogel for endometrial regeneration

17:15 – 17:30 Mr George Fleming, University of Liverpool.
Dual-action nitric oxide-releasing micropatterned antibacterial PDMS surfaces

17:30 – 17:45 Dr Richard Balint, University of Manchester.
Graphene-polymer composites for the engineering of cardiac tissue

17:45 – 18:00 Prof Bikramjit Basu, Indian Institute of Science
3D inkjet printing of biomaterials with strength reliability and cytocompatibility: Quantitative process strategy for Ti-6Al-4V

18:00 – 20:00 TCES-UKSB Welcome Reception

20:00 onward TCES-UKSB Early Career Researchers Social, *Mooch, Students Union*

Wednesday 12th June 2019:

Translational Research: Barriers and Breakthroughs

08:00 – 09:00 Meet the Mentor 1

09:00 – 10:45 SESSION 5: BIOMATERIALS AND TERM

Chair: Dr Araida Hidalgo-Bastida, Manchester Metropolitan University

09:00 – 09:15 Platinum Exhibitor: **TA Electroforce**

09:15 – 09:45 **Keynote - Professor Stephen Badylak,** University of Pittsburgh
Clinical Translation of an acellular therapy for Barrett's Oesophagus

09:45 – 10:00 Dr Victoria Roberton, University College London
Local suppression of the T cell response to peripheral nerve allografts using drug-eluting PCL fibres

10:00 – 10:15 Mr James Reid, University of Edinburgh
Biofunctionalizing tailored electrospun scaffolds for influencing vascular cellular performance

10:15 – 10:30 Ms Azadeh Rezaei, University College London
Role of silicate and silicate-bioactive glasses in bone nodule formation

10:30 – 10:45 Ms Roxanna Ramnarine, University of Southampton
Self-assembling structured laponite hydrogels with spontaneous 3D micropatterning of bioactive factors for tissue regeneration

10:45 – 11:15 Coffee break

11:15 – 12:45 SESSION 6: ADVANCES IN TERM

Chair: Dr James Phillips, University College London

11:15 – 11:30 Platinum Exhibitor: **Cell and Gene Therapy Catapult**

11:30 – 12:00 **Keynote - Professor Alicia El Haj,** University of Birmingham
Translation of cell therapy control platforms to the clinic

12:00 – 12:15 Dr Lucy Bosworth, University of Liverpool
Electrospun scaffolds containing decellularised tissue matrix support conjunctival epithelial and goblet cells

12:15 – 12:30 Dr Emily Britchford, University of Nottingham/NuVision Biotherapies
Validation and assessment of an antibiotic decontamination manufacturing protocol for vacuum-dried human amniotic membrane

12:30 – 12:45 Mr Jonathan May, University of Southampton
Study of the effects of microbubbles on bone formation using micro-CT

12:45 – 14:00 Lunch and TCES Annual General Meeting

14:00 – 15:30 SESSION 7: DEVELOPMENT OF TRANSLATIONAL & 3D MODELS

Chair: Dr Paul Roach, Loughborough University

14:00 – 14:15 Platinum Exhibitor: **BioTek**

14:15 – 14:45 **Keynote - Professor Katja Schenke-Layland,** University of Tübingen
Non-invasive high content analysis of 3D in vitro test systems

14:45 – 15:00 Ms Bethany Ollington, University of Sheffield
Generation of an immuno-responsive tissue engineered oral mucosal equivalent containing primary human macrophages

15:00 – 15:15 Dr Laura Macri-Pellizzeri, University of Nottingham
Porous glass microspheres show biocompatibility, tissue ingrowth and osteogenic onset in vivo

15:15 – 15:30 Mr Jeremy Mortimer, University of Edinburgh
Investigations of human tendon width for the anatomical design of an in vitro flexor digitorum profundus enthesis model

15:30 – 16:30 Coffee and Posters

16:30 – 18:00 SESSION 8: ROBERT BROWN EARLY STAGE INVESTIGATOR AND UKSB PRIZES
Chair: Dr Nick Evans, University of Southampton

16:30 – 16:45 Dr Jennifer Ashworth, University of Nottingham
Peptide gels of fully-defined composition and mechanics for modelling cell-matrix interactions in breast cancer

16:45 – 17:00 Dr Nazia Mehrban, University College London
In vivo response to injectable hydrogels: from decellularised ECM to de novo peptides

17:00 – 17:30 **Alan Wilson Memorial Lecture – Professor David Wood,** University of Leeds
The Minimata convention: challenges and opportunities for aesthetic dental materials

17:30 – 18:00 **UKSB President's Prize – Professor Kevin Shakesheff,** University of Nottingham
Minimally invasive delivery of temperature sensitive biomaterials

19:00 – 23:00 TCES-UKSB Conference Gala Dinner *Drinks reception in Council Room (A21); dinner in the Senate Chamber at 20:00, Trent Building.*

Thursday 13th June 2019:

Emerging Research and New Frontiers

08:00 – 09:00 Meet the Mentor 2

09:00 – 09:45 SESSION 9: SENSING & CONTROLLING CELL BEHAVIOUR I

Chair: Prof Sarah Cartmell, University of Manchester

09:00 – 09:30 **Keynote - Dr Roisin Owens,** University of Cambridge
A 3D bioelectronics model of the human gut

09:30 – 09:45 Dr Frankie Rawson, University of Nottingham
Stimulative 3D conducting architectures to modulate cellular phenotype

09:45 – 10:45 SESSION 10: TURBO TALKS II

1. Ms Jazz Stening, University of Nottingham (Poster No. 243)
Optimisation of microparticle formulations for cytokine delivery for macrophage modulation for potential application in spinal cord injury

2. Ms Rina Maruta, Kyoto Institute of Technology (Poster No. 150)
3D culture of epidermis by use of recombinant silk materials containing FGF-7 protein microcrystals

3. Ms Eleanor Porges, University of Southampton (Poster No. 148)
Stable encapsulation of rifampicin and doxycycline in polymersome nanoparticles for delivery to intracellular bacteria

4. Mr George Bullock, University of Sheffield (Poster No. 159)
Bisphosphonate toxicity to the oral mucosa is inhibited in vitro by hydroxyapatite granules

5. Ms Jamie Thompson, University of Nottingham (Poster No. 161)
Investigating glycosaminoglycans in development and disease using fully defined 3D cell culture environments and human pluripotent stem cells

6. Mr Alexander Sturtivant, University of Edinburgh (Poster No. 182)
Use of antifreeze proteins to modify pores in directionally frozen alginate sponges for cartilage tissue engineering

7. Ms Anastasia Polydorou, University of Southampton (Poster No. 122)
Acoustically-stimulated drug carriers for bone fracture repair

8. Dr Valentina Barrera, NHS Blood and Transplant, Tissue and Eye Services R&D (Poster No. 236)
Decellularisation of human femoral nerves in a closed system: towards introducing a new nerve allograft in healthcare in the UK

9.Mr Jordan Roe, Loughborough University (Poster No. 197)
Magnetic hydrogels: tissue engineering constructs with switchable stiffness

10. Ms Michaela Petaroudi, University of Glasgow (Poster No. 149)
Bacterial engineering for the ex-vivo expansion of HSCs

10:45 – 11:45 Coffee and Posters

11:45 – 12:30 SESSION 11: SENSING & CONTROLLING CELL BEHAVIOUR II

Chair: Dr Adam Celiz, Imperial College London

11:45 – 12:00 Dr Nuria Oliva Jorge, Imperial College London
Bioinspired nanomaterials for cell-selective activation of growth factors

12:00 – 12:15 Mr Manohar Prasad Koduri, University of Liverpool
Cytotoxicity and functionality of nano oxy-ph sensors in HMSC environment

12:15 – 12:30 Mrs Eva Barcelona, University of Glasgow
Engineering ligand mobility in the adhesive crosstalk to control stem cell differentiation

12:30 – 13:30 Lunch and Meet the Mentor 3

13:30 – 14:45 SESSION 12: BIOMATERIALS CHEMISTRY AND CHARACTERISATION I

Chair: Dr Cheryl Miller, University of Sheffield

13:30 – 14:00 **Keynote - Professor Elizabeth Cosgriff-Hernandez,** University of Texas
Integrin-targeting materials in regenerative medicine

14:00 – 14:15 Dr Caroline Taylor, University of Sheffield
The potential of pressurised gyration to fabricate polyhydroxyalkanoate aligned fibre scaffolds for peripheral nerve repair

14:15 – 14:30 Mr Morgan Lowther, University of Birmingham
Formulation of an antimicrobial silver-doped magnesium oxychloride cement

14:30 – 14:45 Dr Rachel McCormick, University of Liverpool
Designing a novel bioengineered substrate as a treatment for AMD

14:45 – 15:15 Coffee break

15:15 – 16:00 SESSION 13: BIOMATERIALS CHEMISTRY AND CHARACTERISATION II

Chair: Prof Felicity Rose, University of Nottingham

15:15 – 15:45 **Larry Hench Young Investigators Prize – Dr Asha Patel**, Imperial College London
Chemically diverse materials for cell and gene therapy applications

15:45 – 16:00 Ms Maha Omran, University of Sheffield
Evaluating two powder-based 3d printing techniques for the manufacture of implants for orbital floor repair

16:00 – 17:00 **Prizes and Closing Address**

KEYNOTE LECTURE

USING INFRARED IMAGING AND SPECTROSCOPY TO SUPPORT THE DEVELOPMENT OF REGENERATIVE THERAPIES

Professor Chris Sammon[1]

[1]Sheffield Hallam University, UK

Infrared (IR) spectroscopy is a non-destructive analytical tool commonly used in materials characterisation and forensics, providing a 'chemical fingerprint' based on the vibrations of covalent bonds. These vibrations are sensitive to both the chemistry and environment of the species under investigation. Consequently it is possible to use IR to provide an abundance of information pertinent to TERM applications beyond facile materials identification. In this presentation I will show how IR spectroscopy and imaging can play an important role in biomaterials development and tissue engineering using examples from my lab.
I will present data to show how IR spectroscopy can provide information about the gelation, hydration, swelling and dehydration properties of a family of Laponite® crosslinked pNIPAM hydrogels. These materials have thermal responsive properties close to body temperature and, by careful selection of the hydrogel composition, can drive the differentiation of human mesenchymal stem cells towards predetermined phenotypes for a range of clinical applications.

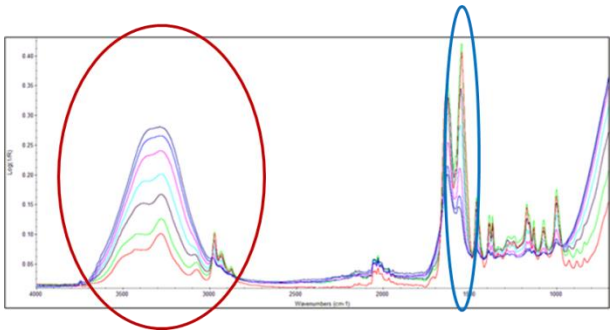


Fig 1: Typical IR data showing the dehydration of a L-pNIPAM hydrogel with time

I will also provide examples of how we use IR imaging to generate pseudo immunohistological stains from tissue sections based on changes in the chemistry. This has some advantages over more traditional IHC approaches such as obtaining multiple 'stains' from the same section and the ability to use the same protocols across species. This approach has been used to characterise the diseased state in both human and goat IVDs and we are currently applying this to assess the efficacy of regeneration of samples using an organ culture system.

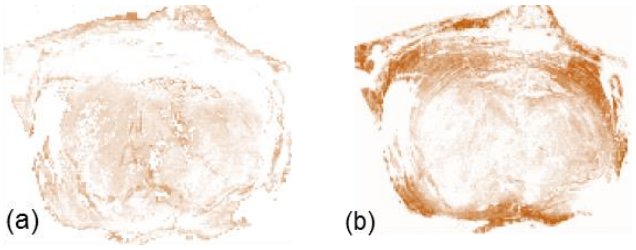


Fig 2: Typical IR images showing the distribution of (a) Col I and (b) Col II from a single goat IVD section

INVESTIGATION OF THE IMPACT OF SAMPLE PREPARATION METHODS AND IMAGING TECHNIQUE ON THE ORGANIZATIONAL PRESENTATION OF COLLAGEN FIBRILS IN HYDROGEL ELECTRON MICROSCOPY

Daniel Merryweather*,[1] Nicola Weston,[2] Chris Parmenter,[2] Mark Lewis,[3] Paul Roach[1]

[1]Department of Chemistry, Loughborough University,
[2]Nano- and Microscale Research Center, Nottingham University,
[3] School of Sports, Exercise, and Health Sciences, Loughborough University
Corresponding author: d.merryweather@lboro.ac.uk

Introduction

Type-I collagen is the most abundant of the collagens and one of the most common biopolymers found in nature, increasingly employed as the basis of 3D cell scaffolds. The collagen-I molecule forms large fibrillar structures comprised of □-helical collagen molecules arranged into larger microfibrils, the interaction and organization of which determine the mechanical properties of the material. Electron microscopy remains a gold-standard method of analysing the nanostructures of a material, however, preparation of samples for electron microscopy may itself induce widespread conformational changes in the polymeric constituent of a gel. Here we present a systematic comparison of various electron microscopy preparative techniques and their effects on the conformational structure of collagen fibrils formed within hydrogels.

Methods

Collagen hydrogels were fabricated from an acid-dissociated solution at 2.0 mg/mL collagen content. The acidic collagen solution was mixed 9:1 with MEM to serve as a pH indicator. This solution was then neutralized by dropwise addition of sodium hydroxide with regular mixing. Once neutralized the solution was held at 37 °C for 30 minutes in order to induce gelation and run to completion. Collagen gel samples were loaded onto a pre-cooled -15 °C sample stand for ESEM and low-vacuum electron microscopy. Dehydrated gel samples were sputter-coated with platinum and imaged via a field-emission gun. Finally, a hydrated gel-state was imaged by slam-freezing collagen gel on a cryogenically-cooled gold-coated copper plate. This sample was transferred to a pre-cooled sample preparation airlock in liquid nitrogen to maintain the frozen state of the gel. This was sputter-coated with platinum within the airlock and transferred to the microscopy stage under vacuum at -190 °C. Further platinum was added via gas-injection of methylcyclopentadienyl platinum trimethyl onto the frozen sample surface. A 15 □m deep trench was milled into the gel bulk using a focused ion beam. The sample was then warmed to -90 °C for 30 minutes to remove gel water content. The sample was returned to -190 °C and the observable fibril matrix imaged.

Results

Collagen fibrils were readily visible within dehydrated gel samples. Low-vacuum SEM of uncoated gels revealed an increasingly dense network of fibrillar structures as the sample-chamber pressure was dropped and gel water content reduced via sublimation. Dehydrated gel samples presented a densely matted net of collagen fibrils between 70 to 150 nm in diameter, with visible structural organizational features such as triplicate braiding of collagen fibrils readily observable under ESEM and FEG-SEM conditions. Under low-vacuum conditions initial fibrils observed presented as isolated structures >200 nm in diameter. As pressure was reduced, an increased density of fibrils could be observed. FIB-milling of platinum-coated slam-frozen collagen gels and subsequent sublimation of the gel water content revealed a highly organized network of fibrillar structures in the range of 70-100 nm in diameter connecting to form 3D porous structures.

Discussion & Conclusions

The collagen molecule is around 300 nm in length and 1.6 nm in diameter according to crystal studies.[1] In biological systems these molecules form a helical microfibril formed from three collagen molecules. These microfibrils in turn may form much larger structures, with size dependent on tissue and cellular population. Water is key in stabilizing the structure of the collagen fibril, forming stabilizing bridges between hydroxyproline residues that interconnect adjacent collagen molecules and microfibrils to form the larger observed structures.[2] As the water content is increasingly restricted to more tightly-bound water directly interacting with the collagen surface, individual collagen fibrils are brought into increasing contact in order to maintain the stable structure imparted by this hydration shell. Increasing density results in increased collagen-collagen interaction with fewer intermediary layers of bound and free water.

References

[1] – Gautieri, A., *et al*., Nano Letters 11.2, 757-766, 2011; [2] – Bella, J., *et al*., Structure 3.9, 893-906, 1995.

Acknowledgements

We thank EPSRC funding in support of the CDT in Regenerative Medicine.

KEYNOTE LECTURE

LIFE AT THE CELL-MATERIAL INTERFACE

Professor Jan de Boer[1]

[1]Laboratory of BioInterface Science, Institute for Complex Molecular Systems and Dept. of Biomedical Engineering, Eindhoven University of Technology, The Netherlands.
www.jandeboerlab.com

In my seminar I will present our latest work on controlling the interaction of cells with biomaterials through design of surface topography. For instance, we are interested in the bone-inducing properties of a subset of porous calcium phosphate ceramics and show how through reverse engineering, we are uncovering an interesting and complex response of cells to materials. Inspired by this, we have started to design high throughput screening strategies of biomaterials libraries, and in particular libraries of surface topographies. Using a design algorithm, we have generated numerous different patterns, which can first be reproduced on a silicon mold and then imprinted onto polymers using microfabrication. After cell seeding, we use quantitative high content imaging and machine learning algorithms to characterize the response of the cells to the thousands of different surfaces and learn more about the relation between surface topography and cell response. For instance, we have identified surfaces which stimulate osteogenic differentiation of mesenchymal stem cells and we are currently testing whether these surfaces can be applied in orthopedic surgery. The focus of my seminar will be on our effort to digitize life at the interface of biomaterials and cells through parameterization of biomaterial properties, –omics based approaches to analyse cell response and computational science to understand and design bio-active biomaterials.

NITRIC OXIDE RELEASING CONTACT LENS BANDAGES

J. Aveyard[1], R. C. Deller[1], R. Lace[2], R. L Williams[2], R. A. D'Sa[1*]

[1] University of Liverpool, Department of Mechanical, Materials and Aerospace Engineering, Liverpool.
[2.] University of Liverpool, Institute of Ageing and Chronic Disease, Department of Eye and Vision Science, Liverpool.
Corresponding author: rdsa@liverpool.ac.uk

Introduction

Blindness due to corneal ulcers represent 5% of all cases worldwide. [1,2] These ulcers are caused by a range of conditions from autoimmune diseases to infections such as fungal and bacterial keratitis. [3] Bacterial keratitis is often contracted through the improper use of contact lenses and treatment regimens often include broad spectrum antibiotic drops, and sometimes the application of a bandage lens to protect the wound. [4] This method of delivery of the drug however is not efficacious as less than 7% of the active agent reaches the site of injury due to the method of administration. Moreover, in recent years, there has been a drive to reduce the use of antibiotics owing to the growing epidemic of antimicrobial resistance.

Nitric oxide (NO) acts as an antimicrobial agent by interacting with simultaneously produced reactive oxygen species such as hydrogen peroxide (H_2O_2) and superoxide (O_2^-) to generate reactive nitrogen species such as peroxynitrite ($OONO^-$), S-nitrosothiols (RSNO), nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3), and dinitrogen tetroxide (N_2O_4).[5,6] It has been shown that these reactive intermediates target DNA, causing deamination, oxidative damage, strand breaks, and other DNA alterations.

Compounds containing the diazeniumdiolate $[N(O)=NO]^-$ functional group have shown great potential in a variety of medical applications requiring the controlled and sustained release of NO [7] Described herein are environmentally friendly methods to develop NO donor contact lenses capable of releasing a controlled and sustained dose of NO to target biofilms on infected wounds.

Materials and Methods

In this work poly-ε-lysine (pεK) is cross-linked with bis-carboxy fatty acids and functionalised with diazeniumdiolate to produce nitric oxide releasing contact lens gels with a high water content and excellent transparency. The chemical properties of the gels were determined using X-ray photoelectron spectroscopy, Fourier transform infrared spectroscopy and UV vis spectroscopy. The NO released was determined using a chemiluminescent NO detector. The antimicrobial efficacy of the gels against S. *aureus* and *P. aeruginosa* was determined after 4 and 24 hr incubation and an indirect cytotoxicity assay was carried out to determine if released NO negatively affected a human corneal epithelial cell line (HCE-T cells).

Results and discussion

NO release from the functionalised contact lens bandages was evaluated at varying pHs in three different solutions; buffer, cell culture media and nutrient broth. The gels demonstrated a burst release at pH 4, and a lower and more sustained release profile at physiological pH 's (pH 7). The antimicrobial efficacy of the contact lenses was observed as reduction colony forming units of *S. aureus* and *P. aeruginosa* using a bactericidal assay. A 3-4 log reduction in *S. aureus* and up to 1 log reduction with *P. aeruginosa* was observed after incubation with the NO releasing gels. The indirect cytotoxicity assay demonstrated that released NO did not negatively affect a human corneal epithelial cell line (HCE-T cells).

Conclusion

The fabrication and functionalisation of nitric oxide (NO) releasing contact lens gel bandages is reported. The contact lens gels exhibit excellent optical and mechanical properties and can release NO under physiological conditions. The gels displayed excellent antimicrobial activity against two of the most common pathogens associated with bacterial keratitis- *S. aureus* and P. *aeruginosa* and did not exhibit significant cytotoxicity against a human corneal epithelial cell line. These contact lens gels could be a promising alternative to current antibiotic eyedrop treatments, that are often inefficient and laborious. The use of NO would not contribute to the growing epidemic of antimicrobial resistance and as the delivery of the treatment is direct to the site of infection, less treatments would be required which would ultimately improve patient compliance.

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Acknowledgements

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RAPID SCREENING OF INKS FOR 3D PRINTING PERSONALISED DRUG DELIVERY IMPLANTS

Laura Ruiz,[1] Vincenzo Taresco,[2] Zuoxin Zhou[1], Richard Hague[1], Clive Roberts[3], Christopher Tuck[1], Morgan Alexander[3], Derek Irvine[1] and Ricky Wildman[1*]

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[3]The Advance Materials and Healthcare Technologies Division/ University of Nottingham
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Introduction

The technology of 3D printing (3DP) is rapidly gaining traction in multiple industrial sectors with some of the most exciting applications being within the pharmaceutical industry. However, the lack of materials able to be successfully 3D printed is constraining the wider adoption of 3DP into industrial processes and consequently stifling the proliferation of these new technologies. Future development pipelines would benefit immensely from a systematic, robust and rapid system for determining the utility of a material for 3DP pharmaceuticals and to establish that information into a materials library.
The aim of this study was to identify, assess and characterise in a rapid way candidate materials for inkjet 3D printing using high throughput (HT) methodologies. The primary goal is to create a library of bioerodible, biocompatible and photocurable materials for the pharmaceutical industry that could be used to 3D print personalised drug eluting implants for heart disease.

Materials and Methods

Polycaprolactone (PCL), poly (lactic acid) (PLA) and poly trimethylene carbonate (PTMC) macromers were synthesised with different methacrylate and acrylate end functionalities and then mixed with two different reactive solvents resulting in a total of 134 different formulations. Materials synthesis was evaluated with NMR. A base library of 18 formulations and a total of 134 combinations were screened using HT methods. The materials were screened for printability, phase separation, mechanical properties, in vitro weight loss, cytotoxicity and drug release. Phase separation and surface mechanical properties were tested in micro array format using ToF SIMS and AFM as described by Louzao el at 2018. The printability of the materials was tested using an automated liquid handler. The 'hit' formulations were used to inkjet 3D print a dual subdermal implant containing the drugs trandolapril and pitavastatin. Drug release was determined with HPLC.

Results and Discussion

The printability results showed that all the base materials combined with NVP and PEGDA were printable at temperatures ranging from 40 °C – 70 °C with the exception of two of them mixed with PEGDA. The phase separation studies showed that PCL and TMC based materials present more phase separation than PLA based materials when mixed with the solvents. This behaviour could help to incorporate hydrophilic and hydrophobic drugs in the same implant avoiding interaction between them. These studies also demonstrated the different surface mechanical properties presented when mixing a non-functionalised material with a di-end functional material. The drug release studies showed that the trandolapril loaded implants were able to continuously deliver the drug for a period of four months.

Conclusions

A HT method for testing printability of materials in a rapid and fully automated way using small amounts of material was developed for the first time in this project. Additionally, by using this rapid screening method we created a library printable biodegradable materials with different degradation times, mechanical properties and drug release behaviour. Formulations were successfully printed and continuous drug release from dual drug implants has been demonstrated.

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KEYNOTE LECTURE

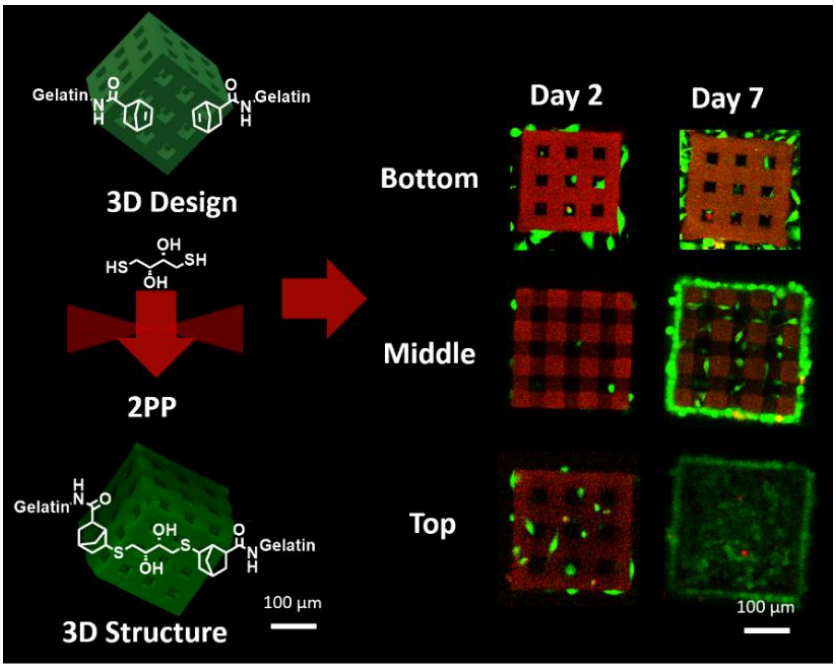
VERSATILE HYDROGEL PLATFORM FOR 3D PRINTING APPLICATIONS

Professor Sandra Van Vlierberghe[1]

[1]University of Ghent

Biofabrication is a specific area within the field of tissue engineering which takes advantage of rapid manufacturing (RM) techniques to generate 3D structures which mimic the natural extracellular matrix (ECM). A popular material in this respect is gelatin, as it is a cost-effective collagen derivative, which is the major constituent of the natural ECM. The material is characterized by an upper critical solution temperature making the material soluble at physiological conditions. To tackle this problem, the present work focusses on different gelatin functionalization strategies which enable covalent stabilization of 3D gelatin structures [1, 2].

In a second part, synthetic acrylate-endcapped, urethane-based precursors will be discussed with exceptional solid state crosslinking behaviour compared to conventional hydrogels [3].
Several polymer processing techniques will be covered including conventional 3D printing using the Bioscaffolder 3.1, two-photon polymerization (see Fig.) and electrospinning starting from crosslinkable hydrogels. A number of biomedical applications will be tackled including adipose tissue engineering [4], vascularization [5], ocular applications [6], etc. The results show that chemistry is a valuable tool to tailor the properties of hydrogels towards processing while preserving the material biocompatibility.



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DUAL-ACTION NITRIC OXIDE-RELEASING MICROPATTERNED ANTIBACTERIAL PDMS SURFACES

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Introduction

As medicine and technology develops so does life expectancy and the prevalence of age-related diseases. Due to this, implantable medical devices, such as: pacemakers, catheters and orthopaedic prostheses have become paramount in modern healthcare and are necessary for prolonging and improving the life of critically ill patients. The increased use of such devices is not without significant problems; one being their susceptibility to bacterial adhesion and subsequent biofilm formation. In this work dual-action micropatterned PDMS surfaces that release nitric oxide (NO) have been fabricated with the aim of controlling bacterial response with both physical and chemical surface modifications, for the potential use in medical implant applications.

Materials & Methods

PDMS replicas were moulded over micropatterned silicon wafers to give PDMS with defined microtopographical features (rectangles, rectangles, inverted rectangles). Aminosilanisation of these surfaces was then carried out using N-(3-trimethoxysilylpropyl)diethylenetriamine (DET3). The amine groups in DET3 facilitated the formation of the NO donor groups, *N*-diazeniumdiolates, when in the presence of high pressures of NO. Planktonic and adhered cell colony forming unit (CFU) assays were carried out against a lab strain of *Pseudomonas aeruginosa* (PA14), to assess the bactericidal and anti-adhesion abilities of the surfaces, respectively. Scanning electron microscopy (SEM) was used to image surface adhered bacterial cells.

Results and Discussion

XPS confirmed the presence of the *N*-diazeniumdiolate groups and AFM analysis showed the well-defined microtopographical features on the PDMS surface. NO release was monitored by chemiluminescence detection and surfaces released up to 981 µmol over 20 hrs at pH 7.4. Planktonic and adhered cell colony forming unit (CFU) assays were carried out against a lab strain of *Pseudomonas aeruginosa* (PA14), to assess the bactericidal and anti-adhesion abilities of the surfaces, respectively. In the presence of non-structured NO-releasing PDMS a bactericidal effect resulted in the complete eradication of bacteria by 4 hrs, due to large NO payloads. Structured NO-releasing PDMS was bactericidal due to NO (62 % reduction) and anti-adhesive due to microtopography (52 % reduction). The results are in agreement with findings reported by Lu *et al*,[1] that microtopography controls bacterial response, through alterations in the cell-surface contact area; when the diameter of surface features are smaller than the diameter of the bacterial cell, cell-surface contact area is minimised and a reduction in adhesion is observed.

Conclusion

Novel dual-action surfaces have been engineered to control bacterial response through multiple mechanisms. Micropatterned PDMS surfaces that release bactericidal concentrations of NO over 20 hrs were successfully fabricated. At 24 hrs, dual-action PDMS surfaces were bactericidal due to NO release and anti-adhesive due to their distinct microtopography.

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KEYNOTE LECTURE

CLINICAL TRANSLATION OF AN ACELLULAR THERAPY FOR BARRETT'S ESOPHAGUS

Professor Stephen Badylak, DVM, PhD, MD[1]

[1]McGowan Institute for Regenerative Medicine, University of Pittsburgh

The clinical translation of any medical technology, including regenerative medicine technology, is dependent upon the ability to overcome recognized barriers including: a genuine clinical need with a sound solution, intellectual property protection, convincing preclinical studies, and regulatory hurdles, among others. Esophageal disease represents a major societal problem which begins with gastric reflux and, in a significant number of cases, progress to esophageal cancer and esophagectomy. This presentation will review the preclinical and clinical studies that resulted in the development of an ECM-based hydrogel for the treatment of esophageal disease. A review of initial human studies and next steps in clinical translation will be discussed.

ROLE OF SILICATE AND SILICATE-BIOACTIVE GLASSES IN BONE NODULE FORMATION

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Introduction

Silicate-based bioactive glasses (SiBGs) have been reported to promote bone regeneration through the controlled release of ions such as calcium, phosphate and silicate[1]. SiBGs have also exhibited improved bone forming capability compared to non-Si bioceramics[2], which is believed to be due to the biological activity of Si. Dissolution products from 45S5BG have been shown to activate bone-related genes *in vitro*[3], whilst Si can promote angiogenesis, proliferation and collagen production[4]. Considering the invention of SiBGs was over 50 years ago and the commercial use of Si containing materials in dental and orthopaedic applications[5], there is still relatively little known about the cellular mechanism of Si on bone formation or the effect of different concentrations of Si on bone formation. To address and investigate the role of Si and SiBGs in bone formation, we developed an in vitro bone nodule model and characterisation approach that included biological, biochemical, ultrastructure and microstructure quantitative techniques.

Materials and Methods

Calvarial osteoblasts were isolated from neonatal rats and seeded in α-MEM with 2 mM β-glycerophosphate, 10 nM dexamethasone, and 50 µg/ml ascorbate. Cells were treated with Si (0.5, 1, 2 mM) or dissolution products from 45S5BG diluted to achieve similar levels as Si (referred as 0.5SiBG, 1SiBG, 2SiBG). Ion release profiles, metabolic activity, proliferation, VEGF expression and ALP activity of the cells were measured. Nodules were characterised using Alizarin Red staining, SEM and TEM. Raman spectroscopy and interferometry were also used for compositional profiling and quantitative dimensional measurements, respectively.

Results and Discussion

Osteoblasts cultured in all concentrations of Si and SiBGs were capable of forming bone, but a Si concentration-dependent response was observed (Fig1). Si 0.5mM increased the maximum nodule height ($P \leq 0.05$) compared to normoxia. Controlled release of ions and bone nodule formation within the biologically relevant range of SiBGs confirm the role of BG as an ion delivery system and the potential of these materials in tissue engineering applications. TEM showed mineralised nodules formed in all conditions (Fig2); however, nodules seemed to cover larger area in Si 0.5mM and 0.5SiBG compared to Si 2mM and 2SiBG (in accordance with interferometry measurements). A Si concentration-dependent effect on ALP production was observed. Si and SiBG exhibited increased VEGF production compared to control (normoxia) on day 1.

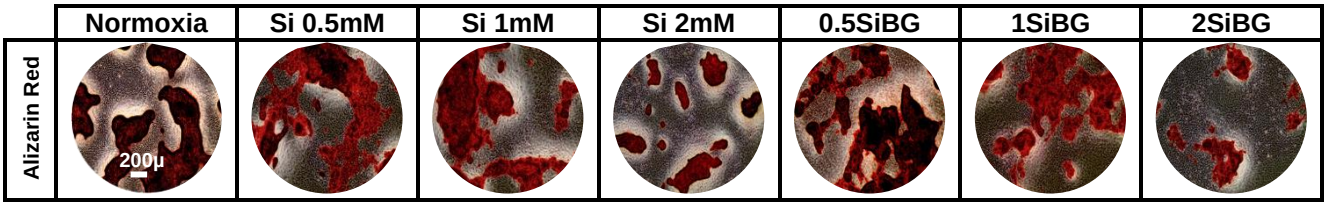

Figure1. Alizarin Red and SEM images of osteoblasts. Neither Si nor SiBGs did not inhibit nodule formation and showed nodules raised from the culture surface; however, a concentration-dependant effect was observed in both Si and SiBGs. 0.5SiBG exhibited denser nodules (dark red) compared to Si 0.5mM which might be due to the presence of other ions.

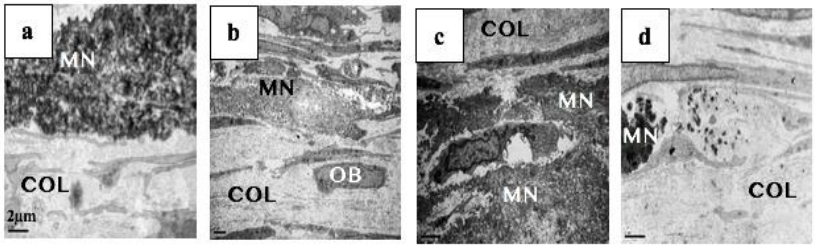

Figure2. TEM micrographs of a) Si 0.5, b) Si 2, c)0.5SiBG, d)2SiBG. Osteoblasts in all conditions were embedded within a fibrous ECM with collagen fibres. Si 0.5 and 0.5SiBG showed denser and larger nodules.

Conclusions

For the first time, we presented quantification and biochemical characterisation of bone nodules using different amounts of Si and Si containing BG conditioned media. This could allow us to use different therapeutic ion concentrations to target different stages of healthy bone regeneration.

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KEYNOTE LECTURE

CELLS IN THEIR DYNAMIC ENVIRONMENT – IMPLICATIONS FOR CELL THERAPY AND REGENERATIVE MEDICINE

Professor Alicia El Haj[1]

[1]Healthcare Technology Institute, University of Birmingham

A variety of cell types respond and adapt to their mechanical environment. These mechanical cues are responsible for maintaining homeostasis, turnover and growth of developmental and adult tissues. Connective tissues are well known for their mechanical functions and a variety of cell types within these tissues have been shown to be mechano-responsive. Our studies on single cell analysis of the biomechanical properties of chondrocytes and the effects of the surrounding matrix in integrating the biomechanical responses demonstrates the challenges for tissue engineers for mimicking natural dynamic cell matrix interactions. Our 3D studies have begun to define the ways we can use bioreactor growth environments to mimic the in vivo environment. Using magnetic nanoparticles approaches, we have begun to target specific mechano-receptors and control cell behaviour remotely. The challenge lies in translating our findings into the clinical setting – can we use biomechanical cues/receptor tagging as therapeutic treatments and if so how? Regenerative medicine gives us a route to explore ways we can define new mechano-active therapies. This presentation will identify the role of physical cues in cell behaviour and stem cell differentiation and the potential for utilising these biomechanical signalling cues ultimately in cell therapy.

KEYNOTE LECTURE

NON-INVASIVE HIGH CONTENT ANALYSIS OF 3D IN VITRO TEST SYSTEMS

Professor Katja Schenke-Layland[1,2,3]

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[2] The Natural and Medical Sciences Institute (NMI) at the University of Tübingen, Reutlingen, Germany
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As the field of regenerative and personalized medicine matures, the need for novel enabling technologies to characterize cells and engineered constructs (i.e. cells/tissue combined with scaffolds and/or growth factors) as well as their individual components in a more insightful, quantitative and preferably non-invasive manner becomes imperative. Raman microspectroscopy is an emerging technique based on light scattering that allows assessing molecular interactions and the biochemical structure of a sample in a non-invasive manner. Specifically for tissue engineering applications, it has been proven to allow determining biochemical information on cells, tissues and/or material-cell tissue constructs without the need for labels.
The aim of this talk is to show the applicability of Raman microspectroscopy for regenerative and personalized medicine applications, and to discuss the added value of the generated data for tissue engineering construct design optimization and preclinical as well as clinical applications.

POROUS GLASS MICROSPHERES SHOW BIOCOMPATIBILITY, TISSUE INGROWTH AND OSTEOGENIC ONSET IN VIVO

Jane McLaren[1‡], Laura Macri-Pellizzeri[2‡], Kazi Zakir Hossain[3], Uresha Patel[3], David Grant[3], Brigitte Scammell[1*], Ifty Ahmed[3*], Virginie Sottile[2,4*]

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[2]Wolfson STEM Centre, Division of Cancer & Stem Cells, School of Medicine, University of Nottingham, UK
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Introduction

Phosphate-based glasses (PBG) are bioactive and fully degradable materials with tailorable degradation rates. PBGs can be produced as porous microspheres through a single-step process, using changes in their formulation and geometry to produce varying pore sizes and interconnectivity for use in a range of applications, including biomedical use. Calcium phosphate PBG have recently been proposed as orthobiologics, based on their *in vitro* cytocompatibility and ion release profile[1,2].

Materials and Methods

In this study, porous microspheres made of two PBG formulations, either containing TiO_2 (P40Ti) or without (P40), were implanted *in vivo* in a sheep model of bone defect. A cylindrical bone defect (8 mm width x 15 mm depth) was created in medial femoral condyles and microspheres were delivered to fill the defect site, with or without bone marrow concentrate. The biocompatibility and osteogenic potential of these porous materials were assessed 13 weeks post-implantation and compared to empty defects and to autologous bone grafts, used as negative and positive controls respectively.

Results and Discussion

Histological analysis showed marked differences between the two formulations, as lower trabeculae-like interconnection and higher fatty bone marrow content were observed in the faster degrading P40-implanted defects, whilst the slower degrading P40Ti material promoted dense interconnected tissue[3]. Autologous bone marrow concentrate (BMC) was also incorporated within the P40 and P40Ti microspheres in some defects, however no significant differences were observed in comparison to microspheres implanted alone. Both formulations induced the formation of a collagen-enriched matrix, from 20 % to 40 % for P40 and P40Ti2.5 groups, suggesting commitment towards the bone lineage. With the faster degrading P40 formulation, mineralisation of the tissue matrix was observed both with and without BMC. Some lymphocyte-like cells and foreign body multinucleated giant cells were observed with P40Ti2.5, suggesting this more durable formulation might be linked to an inflammatory response.

Conclusions

These first *in vivo* results indicate that PBG microspheres could be useful candidates for bone repair and regenerative medicine strategies, and highlight the role of material degradation in the process of tissue formation and maturation.

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Acknowledgements

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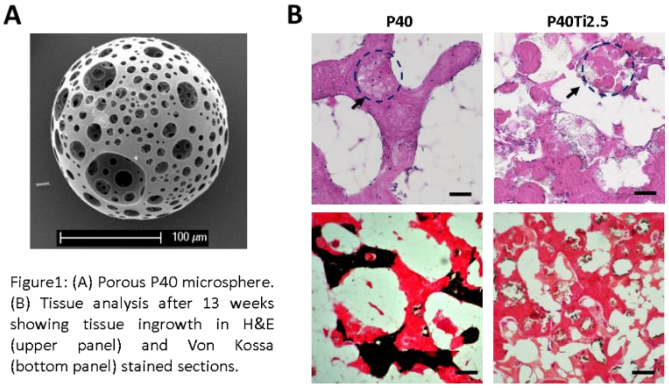

Figure1: (A) Porous P40 microsphere. (B) Tissue analysis after 13 weeks showing tissue ingrowth in H&E (upper panel) and Von Kossa (bottom panel) stained sections.

INVESTIGATIONS OF HUMAN TENDON WIDTH FOR THE ANATOMICAL DESIGN OF AN IN VITRO FLEXOR DIGITORUM PROFUNDUS ENTHESIS MODEL

Jeremy W Mortimer*[1], Mario Macia[1], Nika P Vonk[1], Philippa A Rust[2], Jennifer Z Paxton[1]

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Introduction

Interfacial tissue engineering between soft tissue and bone aims to rapidly generate a matched soft tissue bony insertion (the enthesis) for implantation in an injured area. Our laboratory is designing a model particular to avulsion injuries of the human flexor digitorum profundus (FDP) at the distal phalanx, the most common closed flexor tendon injury in the hand.[1] In repair of flexor tendon injuries, size-matched tendon ends in tenorrhaphy are crucial for smooth tendon glide in the flexor sheath tunnel. This highlights the importance of correct sizing of the tendon analogue for a clinically-applicable *in vitro* model, and we are developing our design from real human FDP morphometric data. Tendon analogue formation is based on an established basic suture to suture fibroblast-seeded fibrin model,[2] and the current study examines how manipulation of suture anchor orientation and size, referencing anatomical data, affects the *in vitro* tendon analogue development over time.

Materials and Methods

64 fresh-frozen human fingers from 8 cadavers donated to The University of Edinburgh Medical School were dissected to expose the FDP tendon insertion onto the distal phalanx. Digital images were taken to measure the tendon width at 12mm and 6mm distances proximal to the bony insertion using ImageJ software. Suture anchor constructs consisted of a fibrin gel seeded with 100,000 rat tendon fibroblasts contracting between 2 suture anchors in 35mm wells. Suture anchors were set up 12mm apart with an intermediate suture size based on the human 12mm proximal tendon width data (5mm) in horizontal vs. vertical or vertical vs. vertical orientations. Larger (10mm) and smaller (2mm) suture sizes were also trialled in vertical positions. Digital photographs of triplicate repeats at multiple time points between days 0-21 were analysed with ImageJ software, measuring gel width at the suture anchor points and midway between them.

Results and Discussion

The mean width (±SEM) of the FDP tendon at 12mm and 6mm distances proximal to its bony insertion was 4.69mm (±0.20mm) and 6.05mm (±0.25mm), respectively, for all fingers and genders combined. Fibroblast-seeded fibrin gel contracted to conform to suture anchor orientation and length, however even by day 21 the gel width was never equal to (or less than) the suture size when measured at the suture anchor point. Standard errors of gel width means were tightest the later the time point and the larger the sutures used in the constructs, suggesting predictability of gel size increases with time and size of anchor. For vertical vs. vertical suture anchor constructs, the gel width midway between 10mm sutures became less than suture size between days 14-17, continuing to decrease with time (**Fig.1**). The equivalent midway measurement between smaller vertical vs. vertical suture anchors had not decreased beyond suture size by day 21, showing that larger anchors may require an intermediate anchoring point to prevent gel/tendon analogue bowing at shorter growth periods.

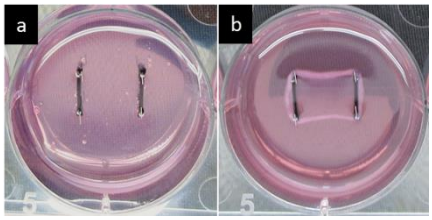

Figure 1. 10mm vs 10mm vertical suture construct; 35mm well. *Gel contraction from day 0 (**a**) (gel covers the entire well) to day 21 (**b**) where gel conforms to anchor length and positioning. Continued gel contraction causes bowing between suture anchors in this design.*

Conclusions

We have quantitatively assessed the tendon morphology at the human FDP insertion to guide tendon analogue design in an anatomically sized *in vitro* FDP enthesis construct. To recreate these tendon sizes we have begun manipulation and assessment of suture anchor constructs to inform contraction pattern predictability and maturation of the *in vitro* tendon analogue over time. This data will form the basis of more finely focused anatomical anchor designs.

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Acknowledgements

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ALAN WILSON MEMORIAL LECTURE

THE MINIMATA CONVENTION: CHALLENGES AND OPPORTUNITIES FOR AESTHETIC DENTAL MATERIALS

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Introduction: Interest in developing new materials to replace amalgam in stress-bearing posterior restorations remains high following the introduction of the Minimata Convention which aims to reduce global environmental mercury levels. The implication for dentistry of the Minimata Convention is a phased reduction in the use of dental amalgam, which for many dentists, particularly in the NHS, is their go-to material for posterior fillings. This opens up the possibility of further developments in the field of direct aesthetic filling materials, including glass-ionomer cements and dental composites. At the other end of the translational pipeline it will be important to understand the barriers to uptake and routine use of these amalgam alternatives.

Materials and Methods: Mechanical properties of commercial and model amalgam replacement materials were assessed with a focus on flexural strength and fracture toughness which are claimed to be key materials properties when trying to project how a material will perform clinically.
The barriers to the uptake of new materials were addressed using a mixed methods approach; the qualitative study involved semi-structured interviews and focus groups with GDPs (private and NHS), dental school teaching leads and NHS dental commissioners.

Results and Discussion: Several of the newly launched amalgam replacement materials and an experimental dental composite exhibited similar/enhanced mechanical properties compared to a highly filled conventional resin composite and significantly higher values compared to a glass hybrid material. Barriers to amalgam phase down included lack of suitable alternatives, costs, time and training in use of alternative materials. Of concern, NHS GDPs considered extraction of teeth as a treatment option in the absence of amalgam and commissioners were concerned that GDP time constraints in the absence of amalgam would lead to reduced access to dental services. Dental schools had reduced teaching of dental amalgam but newly qualified GDPs felt disadvantaged as amalgam was the material of choice for NHS restorations.

Conclusions: The continued development of aesthetic directly placed filling materials suitable for the posterior of the mouth is encouraging. Our data suggest that a complete phase out of amalgam is not currently feasible unless appropriate measures are in place to ensure cheaper, long-lasting and easy to use alternatives are available and can be readily adopted by primary care oral health providers.

UKSB PRESIDENT'S PRIZE

MINIMALLY INVASIVE DELIVERY OF TEMPERATURE SENSITIVE BIOMATERIALS

Professor Kevin Shakesheff[1]

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There are many emerging opportunities in the fields of cell therapy and regenerative medicine to improve efficacy and safety using biomaterials. For example, the use of cell therapies to regenerate tissues is hampered by low retention efficiencies after injection into target tissue sites. Biomaterials can optimize the local environment after administration to protect cells from unwanted physical stresses, provide surfaces for attachment and release molecules to guide cell fate.

For applications in the musculoskeletal system, CNS, liver, eye and other tissues there is a need for minimally invasive delivery of the cell therapy. This places an extra demand on the design of the biomaterials that can be administered through narrow bore needles and acquire their functional structure post-delivery within the body. The Nottingham teams over the last 2 decades have invented new materials and processes to create technologies for cell and drug delivery after minimally-invasive administration. A new class of particulate materials that undergo a process called liquid sintering provide a platform technology for many cell and regenerative medicines. The underlying materials science and potential applications of these materials will be explored in this presentation.

KEYNOTE LECTURE

A 3D BIOELECTRONICS MODEL OF THE HUMAN GUT

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In vitro models of biological systems are essential for our understanding of biological systems. In many cases where animal models have failed to translate to useful data for human diseases, physiologically relevant in vitro models can bridge the gap. Many difficulties exist in interfacing complex, 3D models with technology adapted for monitoring function. Polymeric electroactive materials and devices can bridge the gap between hard inflexible materials used for physical transducers and soft, compliant biological tissues. An additional advantage of these electronic materials is their flexibility for processing and fabrication in a wide range of formats.(*1*) In this presentation, I will discuss our recent progress in adapting conducting polymer devices, including simple electrodes and transistors, to integrate with 3D cell models. We go further, by generating 3D electroactive scaffolds capable of hosting and monitoring cells.(*2*) We are currently working on adapting these scaffolds for a 3D model of the human gut to study microbiome interactions.

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STIMULATIVE 3D CONDUCTING ARCHITECTURES TO MODULATE CELLULAR PHENOTYPE

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Introduction

Biology carefully orchestrates the building of 3 dimensional (3D) architectures and physical-chemical cues which, include electrical, chemical, topographical and mechanical stimuli to carefully modulate cellular function. There is a pressing need for the development of new materials that will allow us to manufacture artificial structures that mimic these biological instructive characteristics. We address this challenge by development of a new conductive composite material compatible with two photon polymerisation (TPP) manufacturing.

Methods

A new biomaterial based on Pentaerythritol triacrylate (PETrA) was polymerised with MWCNTs forming conductive structures. This was printed using TPP and material properties and topography was characterised using impedance spectroscopy, Raman IR, AFM and SEM. Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) were cultured on the new material and structures printed. These were tested for their ability to modulate phenotype by analysing aspect ratio, sarcomere length and contractile frequency via microscopy of the cells.

Results

The developed ink and printed composite structures were shown to be conductive and the MWCNTs were well dispersed. In addition to being biocompatible when 1% photo initiator was used. The hPSC-CMs were cultured on printed structures that had nano-groves printed, and electrical input was applied using AC at 2V at 30 KHz (Figure 1). Cells aligned in the groves. When different structures were tested in the presence and absence of electrical input, the presence and absence of MWCNTs in the composite material we could modulate the sarcomere length up to 2.2 µm. This is similar to mature adult cardiomyocytes.

Discussions and conclusions

The PETrA-MWCNT materials developed was noted to promote hiPSC-CMs maturation in serum free conditions. The biomimetic myofibril-like 3D architecture and the presence of MWCNTs further improved the maturation measured. The application of electromechanical stimulus, superseded the maturation achieved.

BIOINSPIRED NANOMATERIALS FOR CELL-SELECTIVE ACTIVATION OF GROWTH FACTORS

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Introduction

Tissue healing is a highly dynamic process involving multiple cell types and transient biological signalling.[1,2] Efforts in the field of tissue engineering have been focused on developing biomaterials that incorporate dynamic biological information,[3,4] activated by either passive (pH or enzymes) or active (light) triggers. Passive triggers lack spatiotemporal control over release, while active triggers have the challenge of requiring external manipulation. Looking at nature for inspiration, we have developed a novel aptamer-based technology platform that harnesses cellular traction forces to activate growth factors, called Traction Force-Activated Payloads (TrAPs), inspired by the naturally occurring process of activation of TGF-β1 during the healing process (**Fig. 1A-D**).[5]

Materials and Methods

Oligos were synthesised in-house using solid-phase phosphoramidite chemistry, followed by on-column chemical conversions. DBCO-peptides were conjugated to the azide end of aptamers through click chemistry, and the thiol end was conjugated to maleimides on 2D (glass coverslip or polyacrylamide gel) or 3D (collagen sponges) surfaces. Cell proliferation was measured as either metabolic activity (Presto Blue) or viable cell number/field (Cell Tracker Green CMFDA). Established cell culture protocols were used for all cells (human dermal fibroblasts - HDF, human microvascular endothelial cells – HMEC-1 and smooth muscle cells – SMC).

Results and Discussion

This work demonstrates that TrAP technology harnesses cellular traction forces to activate growth factors by unfolding the aptamer. We synthesized aptamers in which one end is attached to a cell-adhesive peptide (e.g., the integrin binding peptide RGD), and the other end has a chemical group that facilitates facile conjugation to any scaffold of interest (e.g., thiol) (**Fig. 1C-D**). We showed that TrAPs platform enables various cells (HFD, HMEC-1 and SMC) to mechanically unfold the aptamers and release active forms of various growth factors (PDGF, VEGF). We demonstrated that the TrAP technology is easy to integrate within a variety of cell culture systems and biomaterials that span both 2D – glass coverslips and polyacrylamide gels – and 3D collagen scaffolds, enabling the addition of cell-activated growth factors to virtually any existing biomaterial of interest. Furthermore, this is the first ever demonstration of cell-selective activation of growth factors – e.g., activation by SMCs but not HDFs after one (**Fig 1E**) and two weeks (**Fig 1F**), by tailoring cell adhesive-peptides to specific cell types (i.e. VAPG to SMCs).

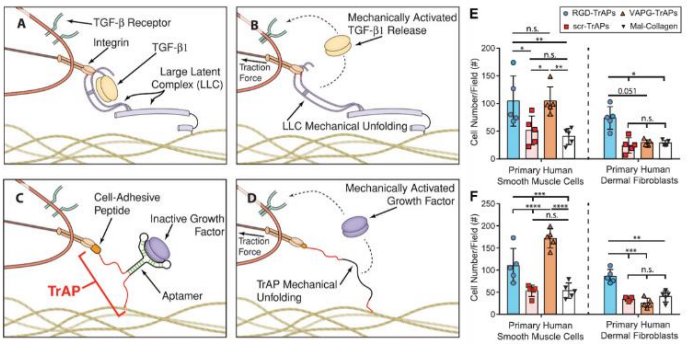

Figure 1. A,B) Natural TGF-β1 activation from LLC. **C,D)** Aptamer-based synthetic mimic of LLC. Selective GF activation by SMC but not HDF using VAPG TrAPs after **E)** one and **F)** two weeks. Adapted from ref. 5 under CC-BY permission.

Conclusions

TrAP technology platform harnesses cellular traction forces as a biophysical trigger to activate and release therapeutics without the need of passive or external triggers, unlocking the potential to develop highly dynamic biomaterials that enable spatiotemporal, cell-selective activation of localized bioactivity for regenerative medicine. Multiplexing different TrAPs (for various growth factors and/or selective for specific cell types) within a scaffold will allow to tune the activation of defined growth factors to the arrival of specific cell types within a local microenvironment, mimicking the complex and dynamic natural healing process.

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This work was supported by the Wellcome Trust [109838/Z/15/Z], Engineering and Physical Science Research Council [EP/R041628/1], and the Department of Bioengineering at Imperial College London. N.O. acknowledges support from a TECNIOspring PLUS fellowship, part of the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement no. 712949.

CYTOTOXICITY AND FUNCTIONALITY OF NANO OXY-PH SENSORS IN HMSC ENVIRONMENT

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Introduction:

Oxygen levels have been identified as an important parameter in stem cell cultivation and differentiation. In addition pH level levels can also be used as an indicator of the physiological conditions associated with the cell environment [1]. Therefore real-time monitoring of these factors, in spatially defined locations within a tissue/cell construct can provide abundant and valuable information directly relating to the optimal physiological conditions required to control cell function and performance within a 3D construct in vitro. For implantable medical devices and in vitro cell modeling this information can be used to optimize/develop novel materials and culture scenarios that can be used to control cell function, eliminating the need for supplementation with exogenous biological factors. In addition, the sensors must not affect cell viability or induce changes in cell function or phenotype whilst optimizing sensitivity, accuracy, resolution, linearity, dynamic range and hysteresis. To validate the use of this technology in Human Mesenchymal Stem Cell culture systems viability and cytotoxic effect of Nano Sensors to monitor oxygen and pH levels in real time in vitro was assessed.

Material and Methods

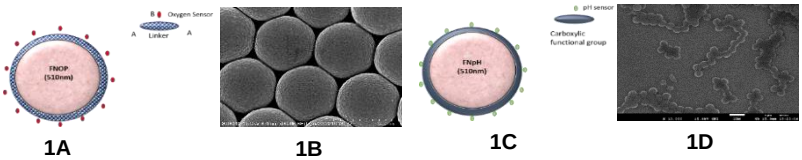
Nano Oxygen and Nano pH sensors were fabricated using Polystyrene Nano beads (PSB) with surface modified by carboxyl groups (Thermo SCIENTIFIC, W050C) s. For Nano Oxygen sensors Pluronic F127 (a triblock copolymer), was employed (all are from Aldrich Sigma) and attached onto the surface of PSB by an esterification process. Oxygen-sensitive red fluorescent molecule Ru (dpp) $_3Cl_2$, ($C_{72}H_{48}Cl_2N_6Ru$) (Fluka, excitation at 470-490 nm and emission at 613 nm), was attached to the structure of a hydrophobic polymer Pluronic F-127 [2] in ethanol. For Nano pH sensors, initially, an intermediate sulpho NHS ester was formed with the help of EDAC/ NHS coupling reaction. The intermediate compound was further mixed with amine conjugated FITC fluorophore resulting in the synthesis of Nano pH sensors as shown in figure 1.

Results and Discussion

Three different concentrations (0.1, 1, and 10 µL/mL) of each sensor was tested and measured using live cell and MTT assays in 96 well plates. Cells cultured in 96 well plates at a seeding density of 5000 cells/ $cm^2$are as shown in figure 2 and 3. Control cells, grown in the absence of any sensors, demonstrated a normal gradient of increase in cell number and the same profile was shown with 10µL/mL of both Oxygen and pH sensors. In contrast w 1µL/mL of both sensors shows an increased growth rate at day 11, indicating a reaction to the presence of the sensors. At a sensor concentration of 0.1µL/mL of both sensors a Gaussian distribution of cell proliferation was observed.

Conclusion

In this study we demonstrate a novel O_2 and pH sensor synthesis and its cytotoxic effect in HMSC cellular environment.



1A 1B 1C 1D

Figure 1 Schematic and SEM image of Nano-Oxygen sensor (A,B) and Nano- pH Sensor (C, D

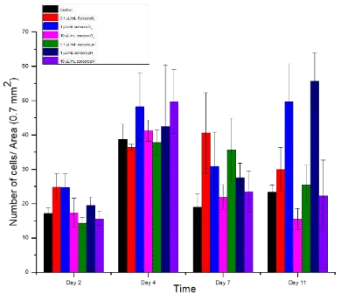


Figure 2: Cell Viability assay (Calcein Green)

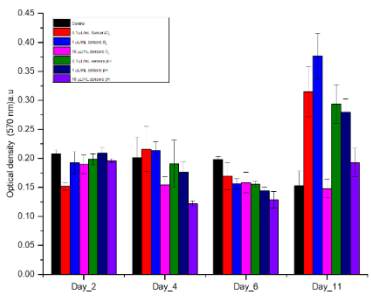


Figure 3 MTT Assay

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ENGINEERING LIGAND MOBILITY IN THE ADHESIVE CROSSTALK TO CONTROL STEM CELL DIFFERENTIATION

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Introduction

The behaviour of mesenchymal stem cell (MSCs) is strongly influenced by their local surroundings, which provide them with biochemical and physical signals. The biochemical cues are mediated by interactions with the extracellular matrix (ECM) through integrins and by interactions between cells via cadherins. On the other hand, cells are equally sensitive to the physical properties of the microenvironment, such as stiffness or viscosity (1). The metabolical pathways involved in the adhesive crosstalk converge to regulate MSCs mechanosensing, provoking changes in their behaviour and eventually determining cell fate (2). Nevertheless, these mechanisms are not fully understood yet.
In this work, we address the role of ligand mobility in cell fate, and how it affects the adhesive crosstalk between integrins (RGD receptors) and cadherins (HAVDI-containing proteins) to ultimately elucidate stem cell mechanosensing of viscosity.

Materials and Methods

We used a model system based on supported lipid bilayers made by following the vesicle fusion method. To determine how ligand mobility and hence viscosity affects MSCs behaviour, two kind of bilayers were used: one of lipids that present a fluid phase (DOPC) and the other one made of lipids with gel phase (DPPC) at cell culture conditions. Glass is used as a non-mobile control surface. All the surfaces are functionalised with varying ratios of RGD and HAVDI which mimic cell-ECM and cell-cell contacts respectively.
Then, human MSCs are cultured on these biointerfaces and parameters such as cell adhesion, protein translocation or expression of transcription factors are investigated. To achieve this, several techniques are being used such us immunostaining, atomic force microscopy or in-cell western.

Results and Discussion

An increase in cell area and cell adhesion, in particular its strength, is observed when the viscosity of the surface and the amount of RGD increases. Nevertheless, when HAVDI is included in the bilayers, cell spreading is reduced and changes in the location of mechanosensitive proteins (i.e. YAP) are observed, revealing an altered sensing of viscosity (Figure 1).

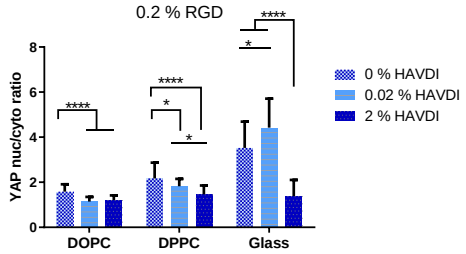

Figure 1: YAP/TAZ ratio of hMSCs cultured on surfaces with different viscosities and different amounts of HAVDI.

The presence of HAVDI provokes not only differences in adhesion and mechanosensing in a viscosity-dependent manner, but also in the expression of transcription factors (e.g. osteogenic), which is an indicator of the influence of viscosity in cell fate.

Conclusions

Our findings reveal not only that there is an influence of viscosity in the adhesive crosstalk between integrins and cadherins, but also that the mobility of the ligands affects stem cell differentiation.
These results show that, when cell-cell interactions are involved, MSCs have a different perception of the mechanical properties of their surroundings compared to when only cell-ECM interactions are present. This altered sensing of the physical cues (viscosity, in this case) provokes changes in cell behaviour and cell fate. Further investigations of these changes in cell behaviour will allow to establish a paradigm to understand and exploit cell response to viscous interactions.

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Acknowledgements

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KEYNOTE LECTURE

INTEGRIN-TARGETING MATERIALS IN REGENERATIVE MEDICINE

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The ability to direct cell behavior has been central to the success of numerous therapeutics to regenerate tissue or facilitate device integration. Collagen often serves as a design basis for bioactive materials due to its putative role in regulating cell adhesion and phenotype, which occurs in part through α1β1 and α2β1 integrin adhesion signals it presents to cells. These integrins are involved in an array of cell activities including angiogenesis, cell migration, adhesion, and proliferation. However, all collagen-containing products on the market today utilize materials from slaughterhouses with the associated disadvantages including no means to optimize the molecular composition of the collagen to guide regeneration. We propose to circumvent these limitations by generating novel bioactive materials using a collagen-mimetic protein engineered to have enhanced therapeutic action and improved scale-up potential. Initial sequence design was based on the collagen-like protein, Scl2 in Streptococcus pyogenes. Whereas native collagen has numerous binding sites for integrins present on a wide range of cells, the Scl2 protein acts as a biological blank slate that only displays the selected receptor-binding sequences programmed in by site-directed mutagenesis. We used site directed mutagenesis to introduce human integrin binding sites into this protein and have provided evidence that human integrin binding sites function within the engineered protein bind and activate α1β1/α2β1. To generate robust materials based on this technology, the collagen-mimetic protein was conjugated into a poly(ethylene glycol) (PEG) based hydrogel to generate bioactive hydrogels. This platform technology is currently being explored in several tissue engineering applications including chronic wound dressings, bone grafts, and vascular grafts. It also provides a unique opportunity to investigate the contribution of collagen binding integrins in a variety of regenerative processes and disease pathogenesis.

FORMULATION OF AN ANTIMICROBIAL SILVER-DOPED MAGNESIUM OXYCHLORIDE CEMENT

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Introduction

There is great interest in developing cement formulations with high strength and easy preparation for bone regeneration. In comparison to existing materials such as calcium phosphates (CaP), magnesium oxychloride cements (MOC) offer greater resorbability and can be tailored by the addition of phosphoric acid. Excess MgO is frequently added to MOC mixes for improved strength. Interestingly, this could lead to inherent antimicrobial and antifungal functionality due to the efficacy of micron scale MgO powders [**1**]. Current prophylaxes are often face difficulties penetrating biofilms. Supplementing with an inorganic antimicrobial such as silver can disrupt biofilm formation, enhancing the efficacy of conventional antibiotic treatments [**2**]. In this work, the potential for antimicrobial MOC formulations has been investigated. Both the mechanism of any inherent antimicrobial behaviour and the addition of a silver phosphate to increase efficacy have been studied. Importantly, correlations between critical cement requirements have been explored, identifying a formulation that balances mechanical properties and efficacy.

Materials and Methods

Cements were manufactured by mixing light MgO powder with MgCl brine [**3**], with a range of formulations with excess MgO produced. Phosphate-modification was by addition of H3PO4 to the brine. When producing silver-modified cements, Ag3PO4 was added to the dry MgO. For a subset of formulations, deliberate porosity was induced by addition of a porogen.

Samples were produced by extrusion into moulds of 6 mm diameter and 12 mm height, curing for 48 hours before demoulding. Compression testing was performed with load rate 1 mm/min. Helium pycnometry and X-ray diffraction (XRD) were performed on ground cements. Semi-quantitative assessment of spectra was made and normalised based on the ratios of reagents used. Perfusion and elution assessments were made, with elutants assessed by inductively coupled plasma spectroscopy (ICP-OES).

Antibacterial efficacy was assessed compared to CaP controls. Zones of inhibition were assessed for S. aureus and E. coli on tryptic soy agar (TSA) after 24 hours incubation. Efficacy against planktonic bacteria in broth was assessed by serial dilutions inoculated on TSA for overnight culture.

Results and Discussion

Dry compressive strength was comparable to CaP ceramic cements, with ultimate compressive strength (UCS) up to 30 MPa. Increasing the powder to liquid ratio had no significant effect on UCS, but increased relative density, suggesting critical flaw size remained consistent. Addition of Ag3PO4 had negligible effect on UCS, however addition of H3PO4 to the brine reduced strength by a factor of a half, and prevented extrusion at powder/liquid ratio of 1.6.

XRD results indicate retardation of 5Mg(OH)2.MgCl2.8H2O (5-phase) formation by orthophosphate modification, with associated brine acidification reducing Mg(OH)2 to MgO. Silver modification also reduced the yield of 5-phase, however this was associated with depletion of chloride ions by formation of AgCl.

Antibacterial efficacy was shown both by the formation of zones of inhibition, and reduced viability of both planktonic and adhered bacteria in broth versus CaP controls. Unmodified cements showed significant reduction in viability, suggesting that MOC shows some inherent antimicrobial efficacy.

Conclusions

Magnesium oxychloride cements have been shown to possess a degree of inherent antimicrobial efficacy associated with MgO. Addition of a porogen and silver phosphate improved efficacy, both against adhered and planktonic bacteria in broth. This formulation shows promise as an antimicrobial cement that exhibits more rapid degradation than calcium phosphate cements.

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LARRY HENCH YOUNG INVESTIGATORS PRIZE

CHEMICALLY DIVERSE MATERIALS FOR CELL AND GENE THERAPY APPLICATIONS

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Introduction

The development of defined biomaterials for cell & gene therapy could overcome issues associated with scalability and reproducibility that hinder biologically derived systems. However, rationale design of biomaterials is difficult without fully understanding cell-material interactions, therefore to enable identification of novel chemical ligands capable of cellular interaction, we employed parallel screening approaches that correlate biological response with materials chemistry [1].

Two key areas that my research has focussed on to date are developing synthetic materials that can support cell culture [2] and non-viral vectors that encourage cell uptake and gene delivery [3,4]. More recently we have demonstrated that physical properties such as polymer topology can influence gene delivery, broadening the toolkit to manipulate material properties for control of cellular interaction.

My current research investigates how chemically diverse messenger RNA constructs can alter pharmacokinetic profile to enable controlled protein expression.

Methods

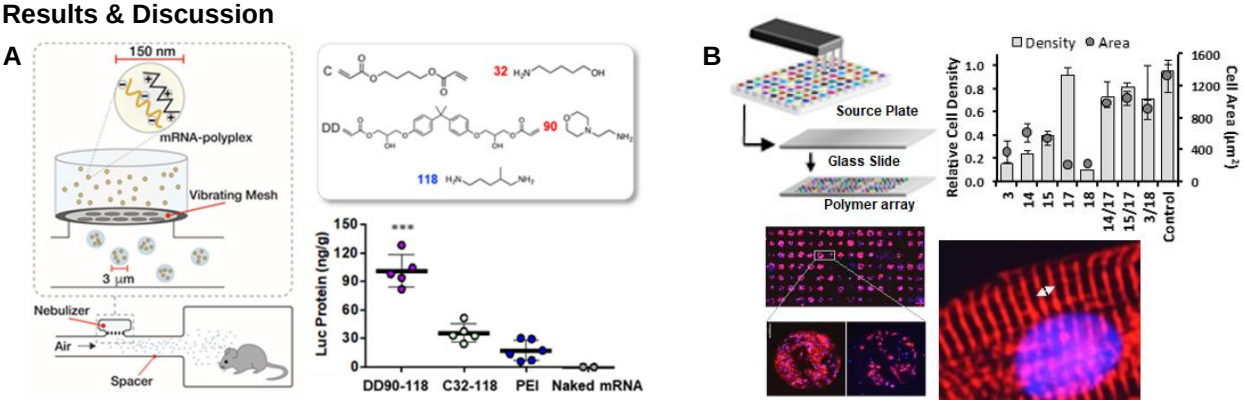
Nanoformulated polyplexes for gene delivery

Chemically diverse acrylate and amine monomers were reacted via Michael Addition to generate linear or hyperbranched cationic polymers. The ability of these materials to complex with and protect nucleic acids such as DNA and mRNA was assessed, as well as transfection efficiency for intracellular gene delivery.

Chemically defined substrates for cell culture

Acrylate monomers with diverse side chain chemistry were pin printed onto glass slides and polymerised in situ via free-radical polymerisation. Cells of interest such as human pluripotent stem cells and cardiomyocytes were cultured on the slides to identify materials capable of supporting cell survival in the absence of undefined components such as Matrigel™ or fetal bovine serum.

Results & Discussion



A) Cationic amine polymers were synthesised for nebulised messenger RNA delivery. Chemical modification of the polymer greatly influenced gene transfection in vivo, with DD90-118 based polymers able to generate greater luciferase protein in the lung compared to C32-118 polymers [3].

B) Chemically diverse microarrays were seeded with human pluripotent stem cell derived cardiomyocytes. Cardiomyocyte attachment density, as well as cell morphology and sarcomere alignment were influenced by surface chemistry [2].

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EVALUATING TWO POWDER-BASED 3D PRINTING TECHNIQUES FOR THE MANUFACTURE OF IMPLANTS FOR ORBITAL FLOOR REPAIR

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Introduction

The delicate structure of the orbital floor makes it prone to fractures. Titanium mesh is often used for repair as it can be tailored to the estimated size and shape of the defect by the surgeon, however, sharp edges are common which can cause pain and discomfort to the patient [1]. Incorrectly shaping or positioning the implant are other drawbacks which can lead to additional surgeries. 3D printing offers customisable implants based on patient scan data, aiming to reduce inaccuracies in implant shaping in-situ. Two possible techniques for manufacturing such an implant are laser sintering (LS) and high speed sintering (HSS). LS sinters powders using a laser while HSS utilises an ink and an infrared lamp to sinter powders layer by layer [2]. One problem with these techniques is that the polymer commonly used, polyamide 12 (PA12), is not osteoconductive, and can limit bone regeneration. Hydroxyapatite (HA) is a widely used osteoconductive material, similar in composition to natural bone [3]. Therefore, this project aims to assess the feasibility LS and HSS for the manufacture of implants with osteoconductive properties for orbital floor regeneration.

Materials and methods

HA was added to PA12 in wt% of 0, 5, 10, 20, 30 and 40 wt% and mixed for 45 min. Discs and bars were manufactured by LS and HSS. Tensile testing and 3-point bending was performed on all printed compositions to determine their mechanical properties. Biocompatibility was evaluated by seeding MG63 cells on discs which were incubated for up to 7 days. PrestoBlue™, PicoGreen™ and alkaline phosphatase assays were used to determine *in vitro* cell viability, quantify DNA and assess osteogenic activity, respectively. Scanning electron microscopy (SEM) was used to study the surface topography of the samples.

Results and discussion

For higher wt% of HA, parts were printed more reliably with HSS than LS. SEM images showed that HSS samples were more porous than LS samples, which is likely to have influenced the mechanical properties as shown by Figure 1. T-tests showed that LS samples had a significantly higher ultimate tensile strength (UTS) than HSS samples, therefore, LS implants can potentially withstand more strain before failure. Additionally, laser sintered samples with HA equal to or greater than 20 wt%, had a significantly lower UTS than the control. This trend was not seen for HSS samples. Initial cell viability studies showed that the samples were biocompatible.

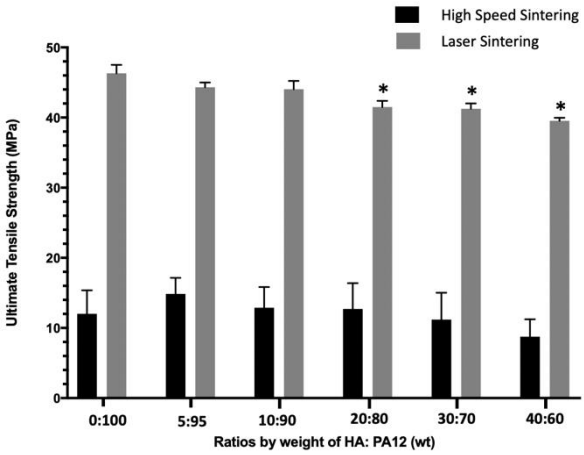

Figure 1: UTS of LS and HSS samples composed of different ratios of HA: PA12 from tensile testing. One-way ANOVA was used to statistically analyse the samples at different ratios manufactured using the same fabrication technique. Statistical significance ($p<0.05$) compared to 100 wt% PA12 is indicated by *.

Conclusions

LS samples had superior mechanical properties to HSS samples, however, as the orbital floor is a non-load bearing bone, a high mechanical strength is not critical, thus, both techniques have the potential for the fabrication of patient specific implant for orbital floor repair. Initial biocompatibility results suggest that both techniques produce biocompatible materials, however further work is required to eliminate the use of potentially carcinogenic inks from HSS.

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Acknowledgements

We are grateful to Wendy Birtwisle and Kurt Bonser for their assistance in the 3D printing processes. Thank you to University of Sheffield for financial support as part of institutional commitment to the EPSRC Centre for Innovative Manufacturing in Medical Devices (MeDe Innovation) grant: EP/K029592/1.

Turbo Talk Presentations

NITRIC OXIDE RELEASING ELECTROSPUN NANOFIBERS FOR ANTIMICROBIAL BONE TISSUE ENGINEERING

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Introduction

Bacterial adhesion and biofilm formation leading to infections is a major reason for failure of guided bone regeneration.[1] Compared to traditional bactericidal agents such as antibiotics, antiseptics and silver, which can lead to drug resistance or high cytotoxicity, nitric oxide (NO) is an attractive antimicrobial as it highly effective without leading to antimicrobial resistance.[2, 3] However as NO is a reactive gas, with a relatively short half-life, delivery of this antimicrobial is challenging. In this study we have synthesized NO releasing coatings on poly(ε-caprolactone) (PCL) and gelatin blended nanofibers. The NO donor used is an *N*-diazeniumdiolate which is formed using amino functionalities in the nanofibers. The biofilm inhibition to *Staphylococcus aureus* (*S. aureus*) was employed in evaluating the biological response of NO release nanofibers.

Materials and Methods

Five blends of PCL:gelatin nanofibers were prepared in mass ratios of 100:0, 75:25, 50:50, 25:75 and 0:100. Then samples were placed in a NO reactor to synthesis diazeniumdiolates. Diazeniumdiolates modified samples were analysed using contact angle and XPS. NO release was monitored using a chemiluminescent NO detector. Biofilm CFU assays were performed to determine the inhibition of biofilm formation on NO releasing surfaces after 6h and 24h incubation. In addition, SEM was used to determine *S. aureus* morphology after 6h incubation on NO releasing surfaces.

Results and Discussion

Results have demonstrated that the formation of diazeniumdiolates on nanofibers. The binding energies of N 1s peak at ~401 eV for N^+ and ~402 eV for N-O were observed from XPS analysis, representing the $-(O^-)N^+=N(O^-)$ group. The kinetics of NO release were dependent on pH, with acidic conditions (pH 4) resulting in the release of higher NO concentrations than pH 7.4 and pH 8.5. The surface releasing the highest concentration of NO showed more than 1 log reduction in *S. aureus* biofilm formation.

Conclusions

Diazeniumdiolates were successfully PCL:gelatin nanofibers. These electrospun membranes have the potential to be use as anti-infectives for guided bone regeneration.

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PHOSPHATE BASED GLASS COATINGS FOR RAPID GA^{3+} RELEASE: THE CHALLENGES OF BALANCING CYTOCOMPATIBILTY WITH ANTIMICROBIAL EFFECTS

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Introduction

Biofilm infections affect 1-4% of orthopaedic implants, representing cyclical chronic pain for patients and at a cost of ≈ £2 billion annually [1]. A successful antibacterial coating must release a dose of a bactericidal agent at the wound site, preventing bacterial adhesion on the implant surface. Human bone cells must later colonise the surface to ensure implant fixation. Ga^{3+} has been reported to be osteogenic at <100 µM and antimicrobial against a wide range of pathogens at <9 mM [2][3]. Radio Frequency Magnetron Sputtering can deposit degradable Phosphate based glass (PBG) coatings with excellent substrate conformity and adhesion strengths exceeding the FDA guidelines and Ga_2O_3 can be incorporated into the glass structure.
This project's objectives were to produce Ga_2O_3 PBG content coatings for rapid Ga^{3+} release and to determine whether rapid ion release could be tolerated by human osteoblasts. For the first time this was compared to a Ga_2O_3 coating.

Materials and Methods

RF magnetron sputtering of suitable targets deposited coatings on to Ti6Al4V substrates. Ga_2O_3 was incorporated into the coatings in two ways: sputtering from a 10 mol% Ga_2O_3 PBG target and blending from a PBG and Ga_2O_3 target. Coatings compositions and structure were investigated by EDX, XPS, FTIR and RHEED. Degradation and ion release was performed in DMEM and compositional changes were investigated by EDX and XPS. Cytocompatibility testing used MG63 human osteoblast-like cells for Neutral Red and Alamar Blue assays. Bacterial attachment of *Staphylococcus aureus* to the coatings was visualised using confocal microscopy and quantified using COMSTAT 2 ($\mu m^3/\mu m^2$).

Results and Discussion

Blending Ga_2O_3 with PBG resulted in a 71.9 mol% Ga_2O_3 coating (355nm thick). The glass target gave a 22.4 mol% Ga_2O_3 coating (156 nm thick). XPS confirms that Ga is present as an oxide in all coatings. These coatings are the highest Ga_2O_3 content glass coatings manufactured to date.

Table 1- Key characterisation data from the Ga containing PBG coatings. Ga_2O_3 content calculated from EDX analysis

Targets used	Coating Ga_2O_3 content (mol%)	Ga^{3+} release at 8 h (ppm)	Ga^{3+} concentration at 8 h (µM)	Ga content 0 h (atomic %)	Ga content 168 h (atomic %)
Gallium doped PBG	22.4	1.9	27	2.6	2.0
PBG and Ga_2O_3	71.9	16.5	237	12.2	8.1
Ga_2O_3	100.0	62.5	897	18.0	0.6

Key findings from the degradation of the coatings in DMEM are summarised in the table above. All coatings were present after 168 h, whereas full degradation was recorded previously in H_2O. We suggest that Ga^{3+} release (which occurs mainly in the first 8 h) from the PBG containing coatings was limited due to Ga being bound into the glass structure, while Ga present in nano-crystallites (as detected by RHEED) was released into DMEM. No coating released enough Ga^{3+} to give antimicrobial mM concentrations, resulting in no difference in biofilm growth across any of the samples. Human cell growth on the coating surfaces is limited in all cases including the PBG control, and significantly so in the 71.9% and 100% Ga_2O_3 coatings. The higher cell growth on the 22.4 mol% samples compared to the higher Ga_2O_3 content coatings suggests that some Ga^{3+} exposure can be tolerated by the cells, as micrographs show healthy cell morphology on the 100% Ga_2O_3 coating after 168 h.

Conclusions

RF Magnetron offers the ability to coat high Ga_2O_3 PBG coatings, which will release Ga^{3+} into DMEM. The degradation of PBG structures in DMEM has not been widely reported and therefore different glass compositions must be investigated to ensure full degradation and Ga^{3+} release. Our results suggest that cells can recover after initial Ga^{3+} exposure and therefore cell response to a Ga^{3+} ion burst must be investigated further to determine if a short lived PBG coating releasing Ga^{3+} could prevent initial bacterial attachment without damage to surrounding tissue.

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Acknowledgements

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DEVELOPMENT OF A MULTI-LAYERED CRYOGEL BIOREACTOR WITH OPTIMISED FLUID DYNAMICS FOR BIOARTIFICIAL LIVER APPLICATION

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Introduction: Liver disease is a condition that currently affects 29 million European[1] people and there are still no effective treatments other than liver transplantation [2]. Bioartificial liver devices aim to replace the detoxification and metabolic functions of the liver in people with liver failure. Cryogels are supermacroporous hydrogels which hold potential as cell scaffolds with open porosity, interconnectivity of pores and suitable mechanical properties. Cryogel monolith columns have been used by other groups to support HepG2 cells and fill the bioreactor chamber of a BAL prototype [3, 4]. However, device performance was not maintained for more than a few hours. Shear stress and local velocities created by flow inside porous scaffolds need to be optimised to improve cell viability and avoid inflammation but there are no relevant studies to date. We have previously carried out fluid dynamic measurements using a purpose-built micro-particle image velocimetry (μPIV) setup with video post-processing in order to extrapolate the effect of flow inside the cryogel matrix to improve cell viability and avoid blood cell activation. Starting from PIV results, a multi-layered bioreactor composed of spaced cryogel discs was developed to maximise blood/hepatocyte mass-exchange. Acrylate-based cryogels were previously used by our group for blood detoxification purpose [5] and were here modified to enhance the non-fouling and cell adhesive properties of the surface. This study aimed to investigate whether these cryogel formulations are suitable for use as cell scaffolds in a perfusion device and to assess whether the multi-layered design possess improved bioreactor performance compared to the column version.

Materials and Methods: p(HEMA-co-MBA) cryogels were synthesised by cryogelation technique. Cryogels were then functionalised with alginate by post-synthesis functionalisation. RGD peptide was synthesised by solid phase method and covalently bounded to the surface through activation of hydroxyl groups with CNBr. Porous structure was analysed with SEM, confocal imaging and micro-computed tomography (μCT). Porosity and permeability was derived from the μCT scan. Non-fouling properties were investigated by protein absorption studies. Cell viability was assessed by MTT/ATP activity and live/dead imaging. Hepatocyte functionality in bioreactors was investigated by quantification of albumin and urea production using ELISA and a colorimetric assay, respectively.

Results and Discussion: Synthesised cryogels possessed an open porosity, with pore sizes of up to 100μm, and an interconnected network of pores, making them suitable to be used in a perfused system. Alginate limited protein absorption from plasma, which is an important precursor to avoiding blood cell activation and inflammation. RGD peptide enhanced hepatocyte functionality in terms of albumin and urea production. Cryogel-perfused bioreactors maintained hepatocyte viability and functionality for up to 1 week. The multi-layered bioreactor design allowed a significantly higher hepatocyte production of albumin and urea compared to the column version. Also, cell colonization and proliferation through the device were significantly increased.

Conclusions: In this study, RGD-alginate-p(HEMA)-based cryogels with engineered surface and optimised fluid dynamics were successfully synthesised and were able to maintain a high fraction of metabolising hepatocytes over time compared to HEMA alone. Flow was previously characterised using an ad-hoc built μPIV setup which allows flow visualization inside the cryogel porous structure and extrapolation of local velocities. These measurements led to the development of a new multi-layered design scaffold with improved hepatocyte adhesion and functionality, compared to the column version. The multi-layered scaffold design shows promise for use in a BAL application.

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MAGNETIC HYDROGELS: TISSUE ENGINEERING CONSTRUCTS WITH SWITCHABLE STIFFNESS

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Introduction

Multiple factors dictate the design process of potential biomaterials. Materials with tuneable and reversible properties offer a desirable advantage over conventional biomaterials, which can be hard to manipulate in a clinical setting. Here we explore the integration of hybridised magnetic nanoparticles into synthetic hydrogels, with the intention to utilise these scaffolds as a cell-seeded regenerative medicine strategy, that has switchable mechanical properties and is non-invasive.

Methods

Hybridised magnetic nanoparticles (HNPs) were synthesised according to previous work. [1,2] Briefly, gold coated iron oxide nanoparticles containing a poly(ethylenimine) (PEI) intermediate layer (2 mL) were stirred with allyl methyl sulphide (0.5 mL) and sonicated with a pre-polymer solution of poly (ethylene glycol) methyl ether methacrylate (PEGMA) or poly(N-isopropylacrylamide) (PNIPAM). The resultant mixture was degassed using nitrogen and injected into moulds and cured using UV light for 35 minutes. Subsequent hydrogels were swollen in dH_2O, inoculated with 50,000 SH-SY5Y neuroblastoma cells and cultured for 7 days. Samples were processed for live/dead staining and cell viability measurements using Alamar Blue at 3 and 7 days. Samples were analysed using plate-plate rheometry and multi-axial tension testing.

Results

Rheological and strain-tracking tension tests of loaded and unloaded hydrogels reveals tuneable storage moduli dependant upon the attraction of HNP's to a magnetic source and concentration of crosslinker in the pre-polymer solution. Preliminary cell culture experiments indicate the presence of live cells on the surface of the hydrogels.

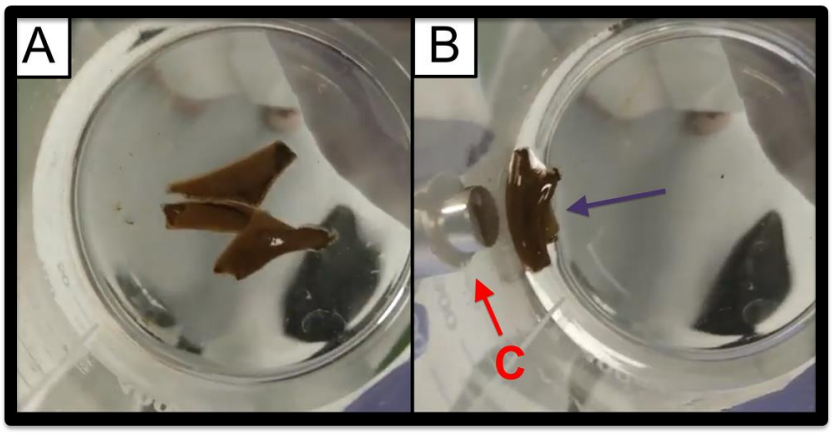

Fig. 1: Swollen PEGMA hydrogels encapsulating HNP's (A) quickly respond to the influence of a neodymium magnet (C).

Discussion & Conclusions

This work presents the possibility of creating new biocompatible smart materials that respond to the influence of magnetic fields. The mechanical properties of the hydrogels themselves may be tailored to suit the target tissue and have been shown to be biocompatible over a 7-day period. Future work will focus on exploring the rheology of such gels under the influence of magnet, as well as further investigations into the cell compatibility of a hydrogel under the influence of a magnet for prolonged periods.

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BACTERIAL ENGINEERING FOR THE *EX-VIVO* EXPANSION OF HSCS

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Introduction

Hematopoietic stem cells (HSCs) constitute a rare cellular population residing in the bone marrow (BM) and have recently gained traction in research due to their significant clinical potential. These multipotent, self-renewing cells have the unique capacity to regenerate the whole hematopoietic system in the event of haematological disorders. This particular property has placed HSCs in the spotlight of experimental haematology, making *ex-vivo* HSC expansion a significant challenge for the research community. This unmet challenge would produce clinically relevant numbers from a small sample of cells that could be used for BM transplantation, somatic cell gene therapy as well as differentiation into mature blood cell types.

The complexity of the BM and the variety of signals that support HSC stemness and self-renewal have proven a limiting factor in current approaches to HSC expansion methods. We propose a system focused on engineering a dynamic microenvironment that will provide the signals that HSCs experience in their natural niche, combined with the structural characteristics of the BM. Our approach includes the use of non-pathogenic, genetically-engineered bacterial biofilms (*Lactococcus lactis*), producing key factors (CXCL12, TPO, VCAM-1, FN) that contribute to HSC phenotype maintenance and proliferation. Additionally, the use of hydrogels will mimic the BM architecture. Finally, we aim to incorporate mesenchymal stem cells (MSCs) into the system, to provide a closer representation of the BM.

Materials and Methods

Recombinant proteins (CXCL12, TPO, VCAM1, FN) were cloned in the pT2NX plasmid and the constructs were transformed in *L. lactis* using electroporation. For the CD34+ cell experiments, HSCs were seeded on top of *L. lactis* biofilms developed on Sigmacote-coated coverslips at a density of 50,000 cells/ml. After 5 days of incubation at 37°C, 5% CO_2, the HSCs were collected and phenotyped in a flow cytometer. In parallel, the effect of the biofilms on BM-MSCs were tested. Briefly, cells were seeded at 10,000 cells/cm^2 and incubated at 37°C, 5% CO_2. MSC spreading and adhesion was evaluated using immunofluorescence and their phenotype was assessed using In Cell Western analysis.

Results and Discussion

We have characterised both protein production and the effect of the cytokines to MSCs and HSCs. Neither cell type seems affected by the presence of bacteria. Furthermore, our results suggest that our system supports HSC phenotype maintenance and can achieve up to 19-fold HSC expansion in 2D experiments. Analysis of the interaction between MSCs and the biofilms has also shown that the MSCs exhibit a niche-like phenotype and could potentially produce more HSC self-renewal factors.

Conclusion

Our data suggests that our system has a promising potential to closely mimic the native BM microenvironment and induce *ex-vivo* HSC expansion.

Poster Presentations

Tuesday 11th June

Poster Number	*Submission Number*	*Author*	*Title*
P001	54	Man Li	Nitric oxide releasing electrospun nanofibers for antimicrobial bone tissue engineering
P004	104	Flavia Bonalumi	Development of a multi-layered cryogel bioreactor with optimised fluid dynamics for bioartificial liver application
P009	240	Kathryn Thomas	Phosphate based glass coatings for rapid GA3+ release: The challenges of balancing cytocompatibilty with antimicrobial effects
P012	31	Georgia Duffy	Porous poly-ε-lysine for an artificial cornea application
P013	33	Jake Edmans	Towards transmucosal peptide delivery: Incorporation of an active model protein into a mucodahesive nanofibre patch using uniaxial electrospinning
P014	38	Robert Deller	Antibacterial efficacy of nitric oxide releasing hydrogels on 2D and 3D human skin models
P016	50	Kiran Mann	Gelatin microparticles as carriers for the delivery of antimicrobial peptides
P017	56	Sophie Louth	A novel weight-bearing antibiotic eluting temporary hip spacer manufactured by selective laser melting
P018	58	Kayla Kret	Characterization of detachable gelatin/chitosan hydrogels for tissue engineering applications
P020	62	Steven Gibney	Towards the development of an implantable biodegradable sensor for the electrochemical monitoring of therapeutics
P024	70	Nathalie Sallstrom	Nanocomposite hydrogel system for biomedical applications
P026	78	Chidimma Mbadugha	Identification of scalable polymers capable of modulating macrophage polarisation
P027	82	Soher Jayash	A novel organic-inorganic hybrid hydrogel for cell encapsulation and drug delivery
P031	88	Matthew Wadge	Investigations into novel titanate conversion of dc magnetron sputtered titanium thin films for biomedical applications
P038	117	Sophie Mountcastle	A new method to quantify biofilm formation on biomaterials surfaces

Wednesday 12th June

Poster Number	*Submission Number*	*Author*	*Title*
P005	138	Mohamed Elsawy	Ultra-short constrained βeta-sheet forming peptides for the fabrication of versatile soft biomaterials
P009	143	Tochukwu Ozulumba	Nanoporous enterosorbent Yaq001 as an oral treatment for liver disease through endotoxin adsorption and moderation of immune dysregulation
P011	146	Laurissa Havins	Bioengineering Dual Gradient Platforms for the Control of Cell Behaviour and Differentiation
P015	166	Iek Man Lei	Embedded bioprinting an in vitro cochlea model for studying cochlear implants
P018	176	Jessica Senior	Suspended layer additive manufacture of a tri-layer skin model
P021	181	James Kinsella	Understanding neural networks: the development of single neuron-neuron brain-on-a-chip models
P026	196	Ting Yan Ng	Synthesis of various calcium phosphate nanoparticles from a magnesium-free simulated body fluid.
P032	208	Ignacio Medina-Fernandez	Acelullar gelatine-alginate scaffolds for dentine-pulp regeneration
P034	218	Aidan Meenagh	3D printed scaffolds for functional ex vivo cardiac tissue models
P035	219	Michael Moore	Development of a hydroxyapatite-based ink for the 3D printing of bone tissue-like scaffolds
P040	235	Fritz de la Raga	Development of a bioprocess for the expansion and differentiation of IPSCS
P042	242	Jeremy Mortimer	Designing an angled interface for an in vitro flexor digitorum profundus enthesis model through human histological investigation
P043	245	Sofia Perea Ruiz	Bioengineering 3D microenvironments to study mechanotransduction and vascularization in bone regeneration

Thursday 13th June

Poster Number	*Submission Number*	*Author*	*Title*
P003	149	Michaela Petaroudi	Bacterial engineering for the ex-vivo expansion of HSCs
P008	197	Jordan Roe	Magnetic hydrogels: Tissue engineering constructs with switchable stiffness
P018	256	Zaribaf Fedra	Examination of the suitability of lipiodol as a contrast agent for polyethylene biomaterials
P019	258	Ian Richards	Novel porous structures for enhanced osteointegration in orthopaedic devices
P021	260	Joel Turner	Understanding cellular uptake of silicate species in bone cells
P029	275	Reece Oosterbeek	Structural and mechanical changes in PLLA-based polymer blends during hydrolytic degradation
P030	276	Adriana-Monica Radu	Regulation of the HIF pathway for controlled bone remodelling in patients with impaired fracture repair
P034	283	Ya Hua Chim	Unique patterns of elastin degradation in ascending aortic aneurysms in bicuspid aortic valve patients
P035	284	Laura Bowker	The application of cold atmospheric plasma gas to direct wound healing in equines

POROUS POLY-Ɛ-LYSINE FOR AN ARTIFICIAL CORNEA APPLICATION

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Introduction

The cornea is an integral part to the functioning of the eye, as it is the most important refractive layer and acts to focus light onto the retina[1]. It is made up of three main layers: a stratified epithelium, the stroma, and a single layered endothelium. These three layers maintain the transparency of the cornea via various mechanisms. If the cornea becomes damaged, these mechanisms will be compromised and the cornea will become opaque. Corneal opacities account for 5 % of blindness worldwide[2], with the leading treatment being the replacement with a cadaveric donor cornea. This treatment has several limitations, such as low availability, tissue rejection and a high cost[3], which introduces the need for a more suitable alternative. This research aims to produce an artificial cornea made of a porous poly-ε-lysine hydrogel. A preliminary gel has been produced and tested for its porosity, transparency and cytotoxicity *in vitro*.

Materials and Methods

Gel chemistry: Poly-ε-lysine (PεK) hydrogels were manufactured using 1-ethyl-3-(3-dimethylaminopropyl) (EDCI) and N-hydroxysuccinimide (NHS) as activators to cross-link PεK with octanedioc acid (Su). These gels were made with varying percentage cross-linking.

Gel preparation: The four reagents were dissolved in water and added to a beaker contained within a stirrer device (Fig. 1), which fragmented the gels during polymerisation. After 20 minutes, the fragments were removed from the stirrer device. They were left to set between two glass plates, pressed with a spacer of 0.5 mm. The fragments then set together as a thin porous sheet.

Percentage light transmittance: The gels were cut and placed into a 96-well plate with 100µl of water, which was inserted into a spectrophotometer and read at 486 nm with an emission filter of 520 nm. The resulting absorbance values were converted into percentage light transmittance using the equation: Transmittance (%) = $(10^{(-1*Abs)})*100$.

Cell viability: Human corneal epithelial cells (HCE-Ts) were seeded onto the gel. A solution containing reagents for a Live-Dead assay, calcein AM and ethidium homodimer (EthD-1), was added to each well containing cells. The wells were imaged on a fluorescence microscope and a percentage cell viability was calculated.

SEM: Prior to imaging, the samples were freeze-dried and loaded onto a carbon strip on the SEM mount with both the top surface and the cross section visible. All SEM images were taken on a Tabletop SEM TM3030 according to the manufacturer's protocol.

Figure 2: Photo of stirrer device

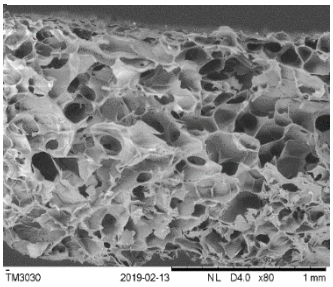

Figure 2: SEM of cross section of Su 30% 0.0714 g/ml fragmented gel

Results and Discussion

Percentage light transmission: The percentage light transmission was measured for gels with two different cross-linking amounts, 30 % and 45 %. Su 30 % 0.0714 g/ml had the highest light transmittance of 75 % compared to 15 % for Su 45% 0.0714 g/ml. This is approaching the value of 90 % observed by the human cornea.

Cell viability: A percentage viability was calculated for the cells on the Su 30 % 0.0714 g/ml fragments after 24 hours then again after 7 days. The hydrogel had a cell viability of 91 % after 24 hours and 80 % after 7 days, compared with a tissue culture plastic (TCP) control that had a cell viability of 98 % after 24 hours and 93 % after 7 days. Despite being lower than TCP on both time points it is more than 75 % cell viability, which deems the Su 30 14 fragments non-cytotoxic to HCETs.

SEM: The images taken show that the gel has a porosity both throughout its cross-section and on its surface. The pores range from 100-200 µm in size and appear to be interconnected throughout the gel (Fig. 2)

Conclusions

In conclusion, a porous hydrogel has been produced with 30 % cross-linking and 0.0714 g/ml polymer density, which demonstrates a modest transparency and was not cytotoxic to HCET cells. Future work will include conducting mechanical testing on the gels to establish their tensile and compressive strength and seeding stromal fibroblast cells onto the gels to mimic a stromal graft.

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TOWARDS TRANSMUCOSAL PEPTIDE DELIVERY: INCORPORATION OF AN ACTIVE MODEL PROTEIN INTO A MUCODAHESIVE NANOFIBRE PATCH USING UNIAXIAL ELECTROSPINNING

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Introduction

The oral delivery of peptides is challenging because of degradation in the gastrointestinal tract. Transmucosal drug delivery is an attractive alternative due to avoidance of the gastrointestinal tract and hepatic first-pass metabolism, and favourable ease of administration and patient compliance in comparison to subcutaneous parenteral delivery. However, significant obstacles remain for the development of effective formulations including permeation through the epithelial barrier and loss of biological activity. We have developed a biodegradable, mucoadhesive oral patch[1] that demonstrates long residence times in vivo[2] and is currently involved in a stage 2 clinical trial. The patches are comprised of a two-layer electrospun polymer system composed of a highly bio-adhesive inner layer and an outer saliva-resistant, durable but flexible protective layer. This research aims to further develop the patch for transmucosal delivery of therapeutic peptides.

Materials and Methods

Lysozyme, an antimicrobial enzyme, was incorporated into poly(vinylpyrrolidone)/Eudragit RS100 polymer nanofiber patches as a model protein using a variety of ethanol/water mixtures as solvents and uniaxial electrospinning. Loading rates, bioactivity, and release profile were investigated by soaking the patches in PBS to release the enzyme and then analysing the supernatant using enzyme kinetics and protein assays. The nanofiber morphology was analysed using scanning electron microscopy. The hydrophobic backing layer was produced by electrospinning an additional poly(caprolactone) layer and melting at 65 °C to produce a continuous film. Residence times were evaluated using a simple *in vitro* test and agar disc diffusion assays were used to assess any antimicrobial effect against oral bacteria strains.

Results and Discussion

For solvent mixtures in the range of 97 - 40 wt% ethanol, the bioactivity of the released enzyme was above 90 % and there was no significant difference between solvents. The loading efficiencies ranged from 70 – 100 % with no significant difference between solvents. The average fibre diameter is significantly decreased at 60 and 40 wt% ethanol due to higher solution conductivity and lower viscosity. Samples were taken from different parts of the patches, showing that the distribution of lysozyme is homogenous. The release profile showed that 87 % was released within 1 hour, which is desirable given that the existing patches show residence times of around 2 hours. There was no significant decrease in bioactivity after melting the backing layer at 65 °C.

Conclusions

The resulting protein-loaded patches displayed high bioactivity and clinically relevant release rates making them a promising proof of concept for the delivery of bioactive peptides to the oral mucosa. Additionally, lysozymes' antimicrobial properties may give the patches a potential application as antiseptic dressings for oral wounds.

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ANTIBACTERIAL EFFICACY OF NITRIC OXIDE RELEASING HYDROGELS ON 2D AND 3D HUMAN SKIN MODELS

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Introduction

The healing of burn wounds are hindered by bacterial infections which can lead to increases in morbidity and mortality rates.[1, 2] This problem is exacerbated by the rise in multidrug resistant bacterial strains that limit the efficacy of antibiotics.[3] Alternative approaches such as nitric oxide have shown promise as potent and broad-spectrum antimicrobial agents which can interact with DNA, lipids and proteins thereby killing the bacteria.[4] Nitric oxide also serves as a signalling molecule that can stimulate the immune response and wound healing processes.[5] Here we investigate the antimicrobial efficacy of nitric oxide releasing materials against *Staphylococcus aureus* and a secreted extracellular protease (V8 protease) capable of disrupting epithelial barrier function.[6, 7]

Materials and Methods

A variety of standardised biophysical techniques and commercially available *in vitro* biochemical assays have been utilised to characterise the physical and chemical properties of our nitric oxide releasing materials (e.g. chemiluminescence) and their efficacy against *S. aureus* (NCTC 13811) and V8 protease. Subsequent impact on cell viability (e.g. alamar blue) and cell functionality (e.g. transepithelial electrical resistance) utilised HaCaT (skin keratinocyte) and WS1 (skin fibroblast) human cell lines co-cultured on 1 µm Polyethylene Terephthalate (PET) membranes.

Results and Discussion

Here we demonstrate a pH mediated burst release of nitric oxide from diazeniumdiolate functionalised hydrogels over a 24 hour period. Diazeniumdiolate functionality was confirmed by FT-IR and pH mediated nitric oxide release via chemiluminescence. The bactericidal efficacy of our nitric oxide releasing materials was assessed directly against *S. aureus* at several initial seeding densities and time points. Activity against the *S. aureus* secreted V8 protease was determined by changes in the rate of proteolytic cleavage of a fluorescently labelled tripeptide and changes in the transepithelial electrical resistance of co-cultured HaCaT and WS1 cells on 1 µm PET membranes. Subsequent viability (e.g. Live/Dead) and barrier functionality (e.g. zonula occuldens-1 immunostaining) assessments against co-cultured skin keratinocytes (HaCaT) and skin fibroblasts (WS1) highlight the biocompatibility of our nitric oxide releasing materials. Preliminary work has also explored the utilisation of our nitric oxide releasing materials against a wounded and subsequently *S. aureus* infected 3D human skin equivalent (Labskin[1.1]). Current work is focussing on the administration of *S. aureus* to our co-cultured systems and subsequent treatment with our nitric oxide releasing materials to assess concurrent antimicrobial and functional activities.

Conclusions

pH responsive nitric oxide releasing materials are capable of acting as a potent antimicrobial agents against *S. aureus* without detrimental impact to the viability and functionality of multiple human skin cell types.

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GELATIN MICROPARTICLES AS CARRIERS FOR THE DELIVERY OF ANTIMICROBIAL PEPTIDES

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Introduction

Antimicrobial peptides (AMPs) are naturally occurring macromolecules that demonstrate a potent antimicrobial activity against a broad range of microbes, including viruses, bacteria, and fungi. AMPs are part of every organism's innate immune response and act as a first line of defence against infection. The mechanism of action of AMPs is dissimilar to that of current clinically used antimicrobial agent and therefore, they are not susceptible to developing resistance and hence there is interest in the development of AMPs for as therapeutics for multidrug-resistant infections. While the use of AMPs for infection control shows significant promise, efficacy can be hampered owing to proteolytic degradation and low bioavailability. Entrapment of AMPs into drug delivery vehicles can significantly increase the therapeutic index of AMPS by stabilising the peptide, increasing the residence time and potentially targeting it to the site of action.

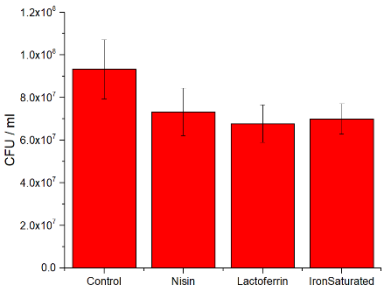
This study investigates two potent and FDA approved AMPs (nisin and lactoferrin (LF)) encapsulated in gelatin microparticles in a facile one-pot synthesis for the treatment of infections. Nisin is a versatile AMP due to its diverse applications in many fields from medicine to the food industry. It is a cationic amphiphilic peptide consisting of 34 amino acids with a cluster of hydrophobic residues at the N-terminus and hydrophilic residues at the C-terminus[1]. Lactoferrin (LF) is a glycoprotein produced in various mammalian species, which is found in bodily secretions, including tears, saliva, bile, gastrointestinal fluids, urine, milk and colostrum. LF displays strong antimicrobial activity against a broad spectrum of bacteria, fungi, yeasts and viruses and in addition displays anti-inflammatory and anti-carcinogenic properties[1]. The influence of the choice of AMP, particle size, zeta potential value, drug loading and in vitro drug release was studied.

Materials and Methods

Gelatin microparticles were prepared using an oil in water (O/W) emulsion technique. An aqueous solution of gelatin was added to corn oil to form the O/W emulsion, which was subsequently precipitated using acetone. A solution of nisin or LF was then left to incubate with the gelatin microparticles for 24 hrs to entrap the AMPs onto the microparticles. The microparticles were characterized with Fourier-transform infrared spectroscopy (FTIR), Zeta potential, particle size analysis and Scanning electron microscopy (SEM). The antimicrobial efficacy were tested against *Staphylococcus aureus S.aureus* using the spread plate technique and the colony forming units (CFU) were measured at 4 and 24 hr time points.

Results and Discussion

The influence of a number of experimental variables of microparticle synthesis and AMP used was investigated. The control particles sizes varied from 148.7 d.nm to 4893 d.nm, with a zeta potential of 3.34. After entrapment of the AMPs the particles sizes there was an increase in size and zeta potential. The antimicrobial efficacy of the AMP-loaded microparticles was tested against *S. aureus* and shown in figure 1. There was a 1-log reduction in the nisin and lactoferrin loaded microparticles compared with the control gelatin.



Conclusion

In this study we have developed a procedure to entrap antimicrobial peptides gelatin microcapsules using an oil-in-water emulsion method. Characterisation of the particles was performed using Zeta potential, particle size analysis FTIR and scanning electron microscopy. The AMP-loaded microparticles showed promise as a drug delivery vehicle to improve the delivery of the active agent for the treatment of infections.

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A NOVEL WEIGHT-BEARING ANTIBIOTIC ELUTING TEMPORARY HIP SPACER MANUFACTURED BY SELECTIVE LASER MELTING

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Introduction

Hip implant failure due to infection is a major problem with over 8000 UK patients receiving a revision due to microbial colonisation in 2017 [1]. The gold standard for revision of an infected prosthetic is a two stage procedure, including thorough debridement of the soft tissue and the use of a temporary spacer that elutes antibiotics [2]. These devices are often made of bone cement and are not fully load bearing leading to extensive periods of bed rest [2]. The focus of this work is to develop a novel hip spacer, to enable patients to load bear during this 6 – 8 week period. Conventional implant manufacturing techniques, such as casting, are unable to create the complex structures required to house an antibiotic eluting biomaterial. Selective laser melting (SLM) is an additive manufacturing technique that enhances design freedom through layer-by-layer manufacture. The novel porous lattice design explored is built from Ti-6Al-4V using SLM. In order to assess the feasibility of the design, four key research areas require exploration. The lattice, which provides the main mechanical support for the spacer as well as controlling drug release. The biomaterial embedded in the lattice that incorporates the antibiotic, in initial experiments this has taken the form of a brushite cement. The channels through which the antibiotic is released, these have been investigated in terms of filling the internal volume and release of the antibiotic. Finally the surface properties can be manipulated to minimise adhesion of both bone cells, as this is a temporary device that needs to be easily removed, and of bacteria to reduce the risk of additional infections.

Materials and Methods

In order to find the optimal lattice design, cylindrical lattices (12 mm diameter, 15 mm height) were generated in Element (nTopology, USA), and built on a Ren AM500M (Renishaw PLC, UK) from gas atomised Ti-6Al-4V powder using optimised in-house parameters, and compression tested in accordance with ISO 13314:2011. The initial biomaterial investigated was dicalcium phosphate dihydrate known as brushite. β-tricalcium phosphate (β-TCP) and monocalcium phosphate monohydrate (MCPM) powders were mixed with deionised water in a powder-to-liquid ratios (PLR) of 2:1 for 30 s. To investigate the best strategy for placement of the channels, three model implants were designed with a 2 mm diameter hole in the top and four 1 mm diameter holes in either the sides horizontally, inclined at 45 degrees, or vertically at the bottom. These were filled using the brushite cements loaded into a 5 mL syringe attached to a 15G needle. Micro-CT was then used to visualise the cement inside the model. To investigate the elution characteristics of a gentamicin loaded brushite cement, the antibiotic was dissolved in deionised water at 100 mg/mL, and then mixed at a PLR of 2:1, resulting in a final concentration of 50 mg per 1 g of cement. Cement cylinders and implant models containing cement were immersed in 10 mL of phosphate buffered saline incubated at 37°C. 10mL samples were withdrawn at intervals over 6 hours. These were tested for gentamicin using a CE 7500 UV–Vis spectrophotometer (Cecil Instruments, UK) and compared to cement without gentamicin.

Results and Discussion

The compression testing found that the BCCZ lattice at 60 % volume fraction had the highest compressive yield strength at 444.3±6.9 MPa, which is around double the strength of bone, while leaving 40 % of the volume for the biomaterial. Micro-CT visualisation of the cement filled model implants found all designs were filled both in the reservoir and channels. However, defects were seen in the cement of all designs, particularly for the vertical channel model. The cumulative release of gentamicin from the cement after 6 hours was greatest from bare cement, with only 28 % release from the vertical channels, 10 % from horizontal, and 5% from inclined, the quantity of defects may have had an influence on this. The minimum inhibitory concentration of gentamicin against S. epidermis and S. aureus was found to be 1 and 16 µg/mL. All the model implant designs were shown to elute sufficient concentrations of gentamicin to inhibit the growth of both S. epidermis and S. aureus.

Conclusions

This work has demonstrated the possibility to exploit additive manufacturing technologies to enhance the value of medical devices. More specifically, the design freedoms of this technique have been exploited to generate load bearing structures for use in two-stage revision of infected hip arthroplasty. Work on the mechanical and chemical interactions between the biomaterials and the lattice structure is on-going. Future work will look at whether the lattice structure can be used to tailor antibiotic release from the device in order to treat the infection quickly using a minimum quantity of the drug and without creating antibiotic resistance.

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CHARACTERIZATION OF DETACHABLE GELATIN/CHITOSAN HYDROGELS FOR TISSUE ENGINEERING APPLICATIONS

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Introduction

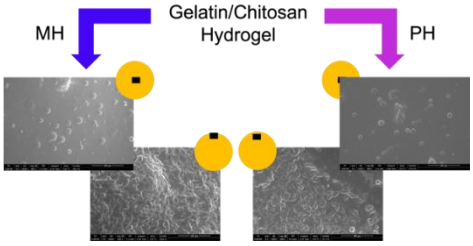
Hydrogels are becoming an increasingly popular biomaterial for scaffolds within tissue engineering. In this research, gelatin and chitosan are used as the core materials due to their abundance, low cost, and cytocompatibility. These materials interact strongly with the substrate material, which then results in the inability to produce isolated hydrogels. In order to form freestanding hydrogels using these materials, an intermediate layer was utilized that enabled complete detachment of the hydrogels after gelation. In this research, the hydrogel discs will be characterized to determine their practicality for tissue engineering applications. In future research, these freestanding, detached hydrogels will be further developed and adapted to create a hybrid tissue engineered ligament.

Methods

A mixture of 3% chitosan and gelatin was combined at 37°C with a 2% solution of proanthocyanidin as a binding agent to form the hydrogel. Three hydrogel samples were created: hydrogel cast into a well plate as a control (NH), hydrogel cast onto an intermediate layer of cell culture medium (MH), and hydrogel cast onto an intermediate layer of PBS (PH). Cytotoxicity assays were performed on the hydrogel samples using NIH-3T3 cell line, including an elution assay from the intermediate layer. ESEM images were taken of the samples before cell culture and after 5 days of cell culture at a low seeding density. The surface chemistry of the samples was analysed using Near Ambient Pressure XPS. The hydrogel was formed into rectangles to allow for MicroTensile testing to determine their applicability in a tissue engineered ligament application.

Results and Discussion

By casting the hydrogel onto an intermediate layer, the hydrogel samples, MH and PH, become fully detached after gelation and removal of the liquid. The freestanding discs maintained their integrity throughout cell culturing, imaging, and tensile testing. The hydrogel was successfully cast into discs for cell culture and a variety of rectangular shapes for tensile testing. The cytotoxicity assay showed the high cytocompatibility of the hydrogel composition. At day 3 of cell culture, the assay showed significant difference between both MH and PH to NH. Although by day 7, there was no significant difference between MH and NH. The ESEM images after cell seeding show the cell morphology on the NH samples is typical of fibroblasts on a relatively stiff surface, spread and elongated with very few rounded cells. The cells on the MH and PH samples showed a rounded morphology near the center of the hydrogel disc. Nearer the edge, the cells began to elongate and are more numerous. The surface chemistry revealed that there were no deposits of Na and Cl, concluding that the intermediate layer does not have a unfavourable effect on the surface of the hydrogel. The MicroTensile testing showed that MH was significantly stiffer than PH.



It is well documented that fibroblasts will attach and elongate on a perceived stiffer surface (1,2), the NH sample in this case. Both the MH and PH samples are lacking the substrate tension that fibroblasts prefer to become elongated and proliferate. This can be attributed to the lack of anchorage of the hydrogel sample to a substrate. This may be the cause of the lower proliferation at early time points on MH and PH. At the longer timepoints, it is believed that the cells have adapted to the surface and have begun to sense the other cells on the surface, seen in the monolayer near the edge. The cell growth can be optimised through the anchoring of the detached hydrogel in application. The hydrogel composition shows high cytocompatibility, a necessary characteristic of scaffolds for tissue engineering. The durability of the scaffolds, ease of creation, and manipulation of size and shape shows promise for this method of creating isolated hydrogels to be used for a variety of different applications.

Conclusions

The hydrogel composition shows high cytocompatibility. The cytocompatibility is maintained after casting the hydrogel onto the intermediate layers. The freestanding hydrogel discs are durable in handling, a positive sign for tissue engineering applications. The intermediate layer did not leave any residue on the surface of the hydrogel that diminishes cellular activity. Although, the PH elutant treatment had significantly lower cell viability than that of the MH treatment and control treatment. The freestanding hydrogel was successfully cast into a rectangular shape for tensile testing, a similar shape when used for tissue engineered ligaments. Overall, the MH samples had higher cellular viability and was stiffer in tension than PH.

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DEVELOPMENT OF A FULLY IMPLANTABLE BIODEGRADABLE SENSOR FOR THE ELECTROCHEMICAL MONITORING OF THERAPEUTICS

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Introduction

Recent advances in biomaterial development have made it possible to explore a number of novel avenues concerning the emerging field of bioelectronics. Specifically, in relation to the field of biosensors the identification of new materials has made the possibility of developing a sensor platform capable of functioning *in vivo* is now a realistic possibility. The ability to measure an analyte of interest *in vivo* is seen as a major leap forward in therapeutic monitoring; current therapeutic monitoring involves recurring and difficult sampling procedures making data acquisition inconvenient, expensive and potentially inaccurate [1]. The successful development of a functioning *in vivo* sensor would overcome these limitations, however a number of challenges remain before this goal can be achieved. The largest of such challenges include poor biocompatibility, loss of functionality over time, and loss of sensitivity when placed in a complex environment [2]. To overcome these challenges a multidiscipline approach must be undertaken. Based on this, a combination of electrochemistry, polymer chemistry and biomaterial development will be used to develop a novel nano/micro-sensor capable of detecting and accurately monitor the systemic concentration of antidepressants.

Materials and Methods

A range of surface characterization techniques have been used to characterise blended-polymer films constructed from the conductive polymer poly(3,4-ethylenedioxythiophene): polystyrene sulfonate (PEDOT:PSS) and a range of commercial biodegradable polymers. Films were formed using either drop or spin coating techniques. The techniques used to characterise these films include Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) to determine the distribution of polymers at the surface of the film, as well as Atomic Force Microscopy (AFM) to determine the topographic structure of the films. Finally, cyclic voltammetry and electrochemical impedance spectroscopy (EIS) have been used in order to understand the films conductive properties. Similarly, investigation has been carried out in to the redox behavior of potential therapeutic analytes. This primarily relied on electrochemical techniques, such as cyclic voltammetry, to determine the specific signal produced by therapeutic compounds when interrogated at various electrode surfaces in both simple and complex media. Furthermore, the effect of potential interferents, such as ascorbic acid, uric acid and glucose, was assessed to determine the feasibility of detecting tricyclic antidepressants (TCA's) in a complex environment.

Results and Discussion

Thus far, surface characterization of PEDOT films has shown that while PEDOT films can be easily manufactured the properties of the resulting films are influenced by the composition and technique used to create the film. Furthermore, electrochemical study has demonstrated that while the films are electroactive they require further optimization to achieve the resolution needed to be used as a sensor platform.

Secondly, electrochemical characterization of the redox behavior of certain TCAs indicates that specific signals are detectable using both glassy carbon electrodes (GCE) and gold electrodes. However, the results differ from those published previously and further work is required to determine how the electrode surface and electrolyte solution influence the analytes redox behavior [3].

Conclusions

Overall, results indicate that the detection of TCAs is possible however further optimization of electrode surfaces and study of the reaction kinetics is required. Likewise, while PEDOT is a promising material further work is necessary to understand how PEDOT interacts with both other polymers and electrode surfaces. If successful this project would act as a proof-of-concept for a novel sensor platform which would provide a step forward for translational research and contribute towards the growing fields of bioelectronics, therapeutic monitoring and personalised medicine.

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NANOCOMPOSITE HYDROGEL SYSTEM FOR BIOMEDICAL APPLICATIONS

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Introduction

Hydrogels are insoluble polymeric networks which can contain large quantities of water. Since hydrogels contains a lot of water, they mimic the soft natural tissues and thus are of interest in tissue engineering. One of the main issues of hydrogels are their poor mechanical properties which limit their practical use. There are various methods to improve mechanical properties, and in this work nano-clay is used as a crosslinker which can attach to several polymer chains and thus reinforcing the hydrogel. The monomer used is a zwitterionic sulfobetaine monomer which was selected due to its non-adhesive potential as well as its potential non-cytotoxic nature.

Materials and Methods

Hydrogel preparation: Laponite XLG (4-10wt%) was dispersed in deoxygenated deionised water, then the monomer N-(3-Sulfopropyl)-N-methacroyloxyethyl- N,N-dimethylammonium betaine (SPE) (10-50wt%) and a photoinitiator Irgracure 2959 (0.5wt%) were added and the suspension was placed in moulds and UV-cured for 1hr.

Mechanical testing: Uniaxial tensile and compressive properties of the hydrogels were evaluated using universal testing machine (Instron 5944) fitted with a 2kN load cell. Hysteresis tests were also performed.

Additive manufacturing: Syringe based extrusion printing was used to create structures using the Bioplotter (envisionTEC) by using a printing then curing approach.

Cell culture: SH-SY5Y neuroblastoma cell line was used to evaluate cellular response to the hydrogels. Three different conditions were tested; control, indirect and direct. Alamar blue assay was performed to evaluate cell viability and fluorescent staining used to evaluate cell count and neurite length of the different conditions.

Result and discussion

A soft hydrogel system with very high elongation abilities (~1200%) was developed. The hydrogels could also recover well from compression forces and did not break after 90% compression. The hydrogels also displayed self-healing abilities which is caused by the ionic interactions between the polymer and the clay platelets. Furthermore, due to the shear thinning properties of the pre-hydrogel solution, the material could be extruded successfully and since the material quickly recovered its original properties, a structure could be fully printed before curing was required.

The cell culture work showed that hydrogels did not cause cytotoxic responses to the neuroblastoma cell line used, there was no difference in cell viability between the different conditions. Cells were also shown to grow on top of the hydrogel surface which could potentially be caused by the clay providing anchoring points for the cells to attach to.

Conclusion

SPE successfully formed mechanically robust hydrogels with clay as a crosslinker. The material could be extruded with a printed then curing approach due to its shear thinning behaviours. The hydrogels did not have any negative effects on the cell viability and cells were shown to grow on the hydrogel surface.

Acknowledgements

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IDENTIFICATION OF SCALABLE POLYMERS CAPABLE OF MODULATING MACROPHAGE POLARISATION

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Introduction

Host immune responses to biomaterials play a critical role in clinical success or failure of medical devices[1]. Adverse immune responses elicited by macrophages against biomedical implants has been a long standing issue in the field of biomedical engineering. A potential approach to limit these reactions is developing biomaterials with immunomodulatory abilities. These materials could direct macrophage polarisation away from a pro-inflammatory phenotype and towards pro-healing phenotype, thereby accelerating healing while reducing tissue damage and fibrosis[2]. Biomaterials surface chemistry has been shown to influence functions of different cell types, including macrophages[3]. In this study, using a high throughput screening strategy, we sought to identify immune-instructive polymers with the ability to induce macrophage polarisation towards pro or anti-inflammatory phenotypes.

Materials and Methods

Using a high-throughput micro-array screening approach, we investigated the immunomodulatory effects of 283 acrylate or acrylamide polymers, on the polarisation of primary human monocyte-derived macrophages. Screening was carried out by quantifying expression of pro-inflammatory (M1) and anti-inflammatory (M2) markers, calprotectin and mannose receptor respectively, expressed by cells on different polymers. Polymers observed to impact macrophage polarisation were scaled up. Subsequently, the impact of these scaled-up polymers on cell viability, cytokine production, gene expression and phagocytic ability was assessed.

Results and Discussion

The development of biomaterial design principles that will enhance the generation of favourable cell-material interaction has been the focus of various studies. Polymer micro-array strategy employed in this study presents a time and cost efficient approach, for screening a wide range of materials for desired responses. From the initial screen, polymers which influenced macrophage polarisation were identified. Following polymer scale-up, we observed a differential expression of M1 and M2 associated cytokines and transcription factors by macrophages grown on different polymer materials, with some polymers inducing a highly polarised M1 or M2 phenotype. Furthermore, polymers were seen to have varying effects on macrophage phagocytic ability.

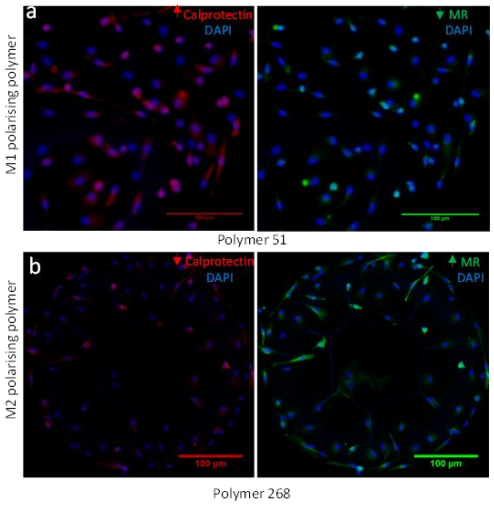

Figure 1: Immunofluorescence images of macrophages on different polymers spots. Images show differential expression of calprotectin and mannose receptor by cells on M1 and M2 polarising polymers. A) polymer 51 B) polymer 268. Representative images are shown from n=3 biological replicates.

Conclusion

Our findings show that polymer choice influence macrophage behaviour. Future work will focus on investigating the molecular basis of material-induced macrophage polarisation.

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A NOVEL ORGANIC-INORGANIC HYBRID HYDROGEL FOR CELL ENCAPSULATION AND DRUG DELIVERY

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Introduction

Chitosan is a natural polysaccharide copolymer which is widely used in drug delivery and bone tissue engineering. Thiolated chitosan (TC) has several advantages compared with unmodified chitosan, including significantly improved permeation and mucoadhesive properties arising from thiol groups on side chains. Moreover, soluble TC displays gelling properties that facilitate controlled drug release, cell encapsulation and bioprinting (1). Recent studies in polymer and bioengineering have resulted in developments in hydrogels for therapeutic delivery. In the present study, a novel hydrogel was prepared based on thiolated chitosan (TC) and silica to be used for bone regeneration in future applications.

Materials/methods

A range of novel organic-inorganic hybrid hydrogel composed of a range of ratio TC and silica were prepared. A low molecular weight chitosan (LMWC)/silica hydrogel was prepared for comparison. The chemical structures of the hybrid hydrogels were confirmed using Fourier-transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectroscopy. The fundamental rheological properties of gels were determined using an oscillating rheometer. Hydrogel degradation was examined in phosphate buffer saline (PBS) or PBS containing 1.5 mg/mL lysozyme. The sample solution was extracted at 30 minute, 1 hour, 7 hours and 24 hours and up to 504 hours and replaced by fresh solution. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to obtain the soluble silica release profiles in the degradation solution and high-performance liquid chromatography (HPLC) was used for the quantification of chitosan and glycerol. Moreover, the cytotoxicity of the hybrid hydrogels on osteoblasts (SAOS-2) was evaluated by both direct and indirect contact methods. For the indirect contact method, hydrogels were incubated in McCoys media for 24 hours. The extraction ratio was 0.2 g/mL, according to ISO 10993-12. After seeding cells, cultures were fed with extraction media. For the direct contact method, cells were cultured on the surface of the hydrogel samples in a culture plate. Cell cultures were then incubated for 24, 48 and 72 hours. The viability of cells was determined by using the alamarBlue assay. Cell viability of SAOS-2 cells encapsulated in hydrogels was evaluated using the live/dead assay and confocal microscopy image analysis.

Results

The hybrid spectra of hydrogels synthesised here shown to exhibit characteristic absorption bands which included: Amide II (1570 cm^{-1}), Si-O (924 cm^{-1}) and Si-O-Si (854 cm^{-1}). Also, NMR techniques showed a reaction between the epoxide ring of silica and chitosan. Strain and frequency sweep tests demonstrated a solid-like response of the hydrogel, which increased for the TC/silica hydrogel compared with the LMW/silica hydrogel. Silicon release was more rapid during the first 24 hours of the experiment and subsequently silicon release remained at a relatively slow rate over 21 days. All hydrogels exhibited limited cytotoxicity as viability of osteoblasts seeded on hydrogels remained at >60% over 72 hours culture. Notably the growth of osteoblasts seeded on hydrogels increased gradually as exposure time increased. For the indirect contact method, viability of osteoblasts was greater than 80% over the 72 hour period.

Conclusion

The newly developed TC/silica hydrogel exhibited specific degradation and mechanical properties which showed no significant cytotoxic effects during material-cell contact. Thus, the hybrid hydrogels have potential to be used for cell encapsulation, tissue engineering and intelligent drug delivery.

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INVESTIGATIONS INTO NOVEL TITANATE CONVERSION OF DC MAGNETRON SPUTTERED TITANIUM THIN FILMS FOR BIOMEDICAL APPLICATIONS

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Introduction

The current process for improving implant surfaces to be bioactive, therefore, providing a more natural fixation, is reliant on high temperature (>1500 K[1]) plasma spraying of hydroxyapatite (HA). However, these surfaces have been shown to spall due to their brittle nature, high internal stresses, and weak mechanical adhesion[2]. Titanate surfaces have been developed as an alternative since the mid-1990s by Kokubo *et al.*[3], however, their applicability have been limited to titanium (Ti) and its alloys only *via* chemical conversion routes. The authors propose a novel method for generating nanoporous titanate surfaces on non-Ti biomedical materials through conversion of DC magnetron sputtered Ti films.

Materials and Methods

Commercially pure polished 10 mm 316L S.S. discs were subjected to DC magnetron sputtering using a cp-Ti (Miba Coatings; 99.5% purity) target (1.56 kW/cm^2 power density; optional 0 to -100 V substrate bias and up to 300 ^{o}C substrate heating). The produced *ca.* 4 µm films were then treated in NaOH (5 M; 60 ^{o}C; 24 h) to assess the effect of sputtering parameters on titanate conversion. The samples have been labelled according to the following convention: negative substrate bias (V)/applied substrate temperature (^{o}C), e.g. 100V/150^{o}C for -100 V bias and 150 ^{o}C applied temperature. Characterisation using SEM, EDX, XPS, Raman, FTIR, XRD, and texture coefficient analysis was conducted.

Results and Discussion

SEM micrographs *(Figure 3A-D*) demonstrated 3.89 ± 0.04, 3.90 ± 0.03, 3.71 ± 0.04 and 3.68 ± 0.02 µm thick Ti coatings for the 0V, 100V, 100V/150^{o}C and 100V/300^{o}C samples, respectively. Through application of a substrate bias, and bias in conjunction with substrate heating, the density of the films increased (reduction in coating thickness and surface voids). Furthermore, texture coefficient analysis of measured XRD spectra (*Figure 3E-F)* using the Harris equation[4], exhibited a shift from columnar (preferred orientation in the Ti HCP (002) plane (PDF 00-044-1294); Texture coefficient ($T_{c(002)}$ = 3.39) in the 0V sample, to more equiaxed ($T_{c(002)}$ = 1.54; for pure equiaxed, T_c = 1) in the 100V/300^{o}C sample. Subsequent titanate conversion of the above samples produced 1.12 ± 0.04, 1.20 ± 0.02, 1.20 ± 0.03, and 1.63 ± 0.06 µm thick titanate layers for the 0V, 100V, 100V/150^{o}C, and 100V/300^{o}C samples, respectively. Despite the proposed hypothesis that increased porosity would allow better NaOH penetration, therefore, increasing titanate conversion, this was not observed in the samples tested. EDX, XPS and Raman analysis, showed incorporation of 7.6 ± 0.1, 8.9 ± 0.1, 11.5 ± 1.8, and 7.6 ± 0.1 at.% of Na in the 0V, 100V, 100V/150^{o}C, and 100V/300^{o}C samples, respectively.

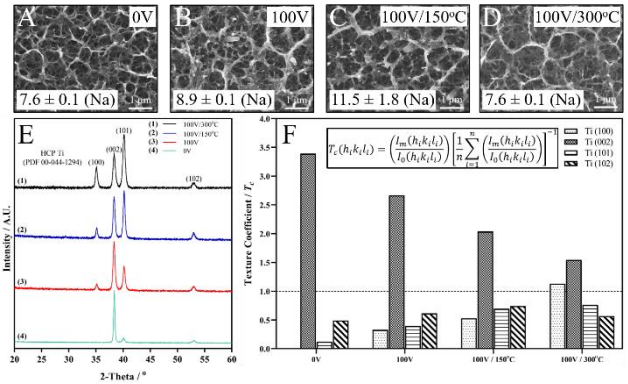

Figure 3. A-D) SEM micrographs and Na EDX inclusion (Insert) in titanate converted samples. E) XRD spectra and F) Texture coefficient calculations (Harris equation; Insert) of pre-converted samples.

Conclusions

Overall, the data presented demonstrates the successful conversion of DC magnetron sputtered Ti coatings into titanate structures, with increased Na inclusion for the unbiased (7.6 at.%), biased (8.9 at.%), and biased/heater runs (11.5 at.% & 7.6 at.%), respectively. The hypothesis that increased penetration in the more porous unbiased film was not evidenced, however, clear structural differences in the film produced have an effect on Na inclusion and titanate morphology of the conversion layer.

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A NEW METHOD TO QUANTIFY BIOFILM FORMATION ON BIOMATERIALS SURFACES

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Introduction

There are numerous studies focusing on improving biomaterials to generate antimicrobial properties and reduce rates of implant failure due to infection[1,2]. Biofilms account for up to 80% of infections, including those related to implantable devices[3], and therefore are vital to consider when evaluating antimicrobial activity. However, quantifying biofilm formation on surfaces is challenging as microbiological methods often rely on manual counting, and are laborious and resource-intensive. Confocal imaging is a useful technique as it enables visualisation of biofilm formation in 3D. However, there is little consensus on analysing the resulting images. This work aims to develop a robust image analysis method to enable automated quantification of biofilm formation from confocal micrographs. Ultimately, this will support the development of much needed approaches to prevent and treat costly infections.

Materials and Methods

Streptococcus sanguinis was seeded on coverslips (n=5). Traditional cell counting methods were carried out for comparison with computational analysis. The number of live bacteria at 0, 1, 2, 5 and 7 days was established using CFU-plating. Total bacteria at each time-point were counted using a haemocytometer. Live-dead staining of biofilms at each time-point was imaged using confocal laser scanning microscopy (LSM 700, Zeiss, Germany) using a x40 oil immersion objective. Five random locations were scanned on each biofilm sample. Z-stacks were taken for each time point for 3D visualization. Image analysis was carried out using two software packages, Fiji (ImageJ, v1.52h) and Matlab (v2017b). The percentage of viable and dead bacteria in each image was determined by calculating the number of pixels corresponding to the numbers of dead (red) and live (green) bacteria (Figure 1). A similar methodology was applied to determine cell numbers.

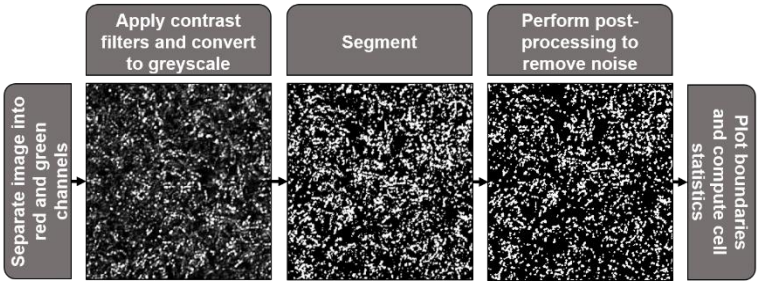

Figure 4: Schematic outlining image processing methods utilised to determine percentage of live cells for single-species biofilms. Images of key stages are presented.

Results and Discussion

Computational image analysis methods were compared with each other, and with standard cell counting techniques. The overall trend in live cell percentage varied between methods. The Matlab script demonstrated a similar trend to the manual counting methods but overestimated the percentage of live cells at all time-points. Calculating cell numbers resulted in a lower estimation of live cells at early time-points, but was more closely aligned to the biological cell count at 5 and 7 days. The reasons for these differences will be explored and further development of the computational analysis techniques will be discussed. Finally, results from the 3D image analysis will also be presented and compared with 2D data.

Conclusions

Computational analysis has advantages by automating the quantification process, whereas traditional methods rely heavily on manual counting. Improving and standardising analysis techniques will enable better comparison of antimicrobial surfaces. The technique developed here can be applied more broadly to the medical implant and biofilm fields.

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ULTRA-SHORT CONSTRAINED BETA-SHEET FORMING PEPTIDES FOR THE FABRICATION OF VERSATILE SOFT BIOMATERIALS

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Introduction

Nature has exploited molecular self-assembly to develop the complex higher macromolecular structures of both the genome and proteome. Inspired by nature, we have recently developed *de novo* ultra-short constrained amphiphilic peptides that self-assemble into bioinspired β-sheet nanofibers.[1,2] The amphiphilic nanofibers were used for the fabrication of various soft biomaterials, such as hydrogels in aqueous medium, emulgels in biphasic media as well as nanofibrillised microcages/microspheres (Fig. 1). The developed materials were used as scaffolds for dental pulp stem cells (DPSCs) and for the controlled delivery of 5-fluorouracil (5-FU).

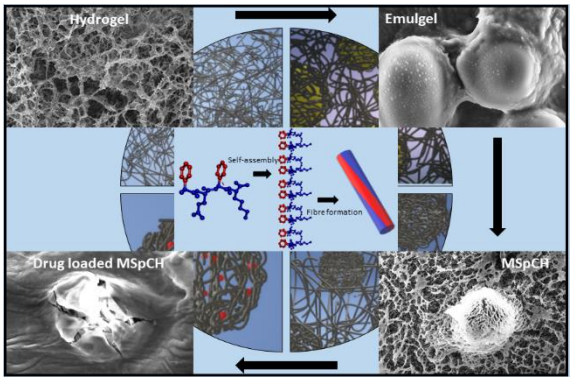

Figure 1: Self-assembly of the amphiphilic ultra-short peptide and the versatile systems fabricated from the nanofibers

Materials and Methods

ATR-FTIR: molecular characterisation of self-assembly.
Oscillatory rheology: characterisation of the viscoelasticity of the fabricated materials.
AFM, SEM, TEM and SAXS: characterisation of nanofibrillar systems topology, morphology and structure.
Proliferation assay: alamar blue assay used to test the proliferation of DPSCs cultured on the hydrogel scaffolds.

Results and Discussion

FTIR showed that the ultra-short peptides self-assemble to form anti-parallel β-sheet structure in response to pH change of the aqueous solution, with prominent peaks at 1689, 1624 and 1524 cm^{-1} corresponding to an extended β-sheet conformation. AFM showed the formation of nanofibers with diameter size revealed to be of ~9nm from SAXS Guinier analysis. Both SEM and TEM showed the formation of entangled nanofibre networks forming hydrogels in aqueous medium with critical gelation concentration of 3% W/V as revealed from the inverted vial test and oscillatory rheology (shear modulus ~4500). In biphasic media, the amphiphilic nanofibers formed stable O/W emulsions (Melissa oil phase) compared to commercial emulsifiers such as poloxomer, cetrimide, SDS and Tween 80 used at same molar concentrations under various environmental conditions (phosphate, chloride and thiocyanate salts; 60°C for 3 hrs; 3 weeks storage…etc.). AFM, TEM and SEM micrographs showed the formation of nanofibrillised microspheres at the O/W interface confirming that the emulsion stabilisation was mediated by the amphiphilic nanofibers (Fig. 1). Oscillatory rheology data showed the viscoelasticity and injectability of the formulated emulgels. The nanofibrillised microspheres were formulated from emulgels (chloroform oil phase) by vacuum evaporation and were loaded with 5-FU, which showed sustained release profiles compared to hydrogels, following Korsmeyer-Peppas (KP) release model. DPSC 2D culture on peptide hydrogel showed cell viability between 3 and 4 %W/V that was not significantly different from controls, suggesting that DPSC growth at these concentrations was comparable to growth on tissue culture treated control wells. However, at day 7, cells cultured on 5 %W/V scaffold showed a significant increase in viability ($p=0.0090$) compared to the control groups. Therefore 5 %W/V is an ideal concentration for further studies.

Conclusions

Ultra-short amphiphilic peptides were designed to self-assemble into bioinspired β-sheet nanofibers that formed the bases for the fabrication of various soft materials with great potential for biomedical (ECM scaffolds for tissue regeneration, anti-infective materials…etc.) and pharmaceutical (emulsifiers, drug delivery vehicles…etc.) applications.

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Diamond for beam time award (SM17102) and all the staff on beamline I22 at Diamond for their support with the SAXS experiments. Staff in the EM facility of the University of Manchester, for their assistance, and the Wellcome Trust for equipment grant support to the EM facility.

NANOPOROUS ENTEROSORBENT YAQ001 AS AN ORAL TREATMENT FOR LIVER DISEASE THROUGH ENDOTOXIN ADSORPTION AND MODERATION OF IMMUNE DYSREGULATION

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Introduction

Disruption of gut barrier function and passage of bacterial endotoxin and other inflammatory products to extra intestinal sites exacerbates immune dysregulation to instigate potentially lethal sepsis in liver cirrhosis. No treatments beyond antibiotics exist to suppress the mechanisms by which bacterial translocation occurs and antibiotics raise issues of resistance and increased gut microbiome disruption. Enteric adsorption may be used to remove bacterial endotoxin, cytokines and other inflammatory products in order to limit translocation into the mesenteric lymph nodes, portal vein and systemic circulation. However, significant adsorption is challenging to achieve because of the large size of key inflammatory molecules which must be removed and the complex nature of the enteric environment. We have investigated the repression of endotoxin mediated inflammatory stimulus by a nanoporous adsorbent, Yaq001, using a THP-1 monocyte cell model. The aim was to investigate adsorptive capacity and mechanism of effect in order to potentially modulate immune dysregulation through physical adsorption of gut derived inflammatory products in the treatment of liver cirrhosis.

Materials and Methods

Phenolic resin derived activated carbon beads (Yaqrit Ltd) were characterised by mercury and gas nitrogen porosimetry, scanning electron microscopy and energy dispersive X-ray analysis. Bead adsorption of molecular weight marker molecules, albumin, myoglobin and caffeine were measured by UV/Vis spectroscopy. Endotoxin adsorption was measured using a chromogenic LAL assay. Impact on *S. aureus* and *E. coli* bacterial growth kinetics was measured by optical density analysis. Production of cytokines IL-8, IL-6 and TNF by THP-1 monocytes stimulated with endotoxin and impact of incubation with Yaq001 was measured using ELISA.

Results and Discussion

Physical characterisation of the beads indicated a nanoporous structure with a high surface area and bimodal pore size distribution in the microporous (<2 nm) and macroporous (50-150 nm) range. The beads removed small, middle and high molecular weight marker molecules in comparison to micropore only controls which were unable to remove significant amounts of high molecular weight albumin. Significant endotoxin adsorption was observed by Yaq001 from simulated intestinal fluid but not by the microporous control. No disruption to bacterial growth kinetics was observed. Significant adsorption of cytokines IL-8, IL-6 and TNF occurred in contrast to microporous controls where TNF adsorption was half that of the test samples. This was mirrored in the THP-1 results where repression of LPS stimulated TNF production by the cells at the 4-hour time point was almost complete for Yaq001 but not for the microporous control. Endotoxin stimulated THP-1 cytokine production was significantly reduced by bead incubation suggesting a disruptive mechanism which removes both bacterial toxin stimulus and excessive cytokine response.

Conclusions

Enterosorbent Yaq001 provides adsorptive surface area and internal nanoporosity enabling bound bacterial endotoxin removal and subsequent repression of primed inflammatory THP-1 cytokine production. In the gut, removal of key bacterial and inflammatory products could moderate the negative impact of disrupted gut barrier function and impaired immunity in liver disease.

Bioengineering Dual Gradient Platforms for the Control of Cell Behaviour and Differentiation

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Introduction

In an aging population, diseases targeting neuron subsets in the brain are becoming more prevalent. In the UK, approximately 270,000 people in the UK suffer with Parkinson's disease which is poorly treated and understood. Treatments available currently depend on delaying progression rather than prevention or cure and therefore it is crucial that new methods of treatment become available. Generation of mature and functional neurons as a clinical therapy for a therapeutic benefit is one possible avenue.

Neuron development *in vivo* is reliant on the surrounding environment for signalling cues, morphogen gradients and extracellular matrix (ECM) support (1). These factors play an important role in deciding neural progenitor fate. In a tightly controlled, specific environment, biological proteins provide a concentration gradient, driving progenitors through differentiation (2). The ability to harness these distinct characteristics for *in vitro* work may enable the differentiation of neuron subtypes, which could ultimately be used within the clinic.

The aim of this work is to drive work towards recapitulating a controlled biochemical gradient environment. Cell attachment is tightly regulated through the use of a cell-phobic poly(potassium 3-sulfopropyl methacrylate) (PKSPMA) polymer brush layer. The polymer brush surface is designed as a dual gradient, whereby the a density gradient is presented orthogonally to a polymer chain-length gradient, thus allowing fine control over biochemical attachment and exhibition. Biological proteins are attached to the ends of the polymer brush, creating a change in protein concentration across the gradient surface. Our hypothesis is that this surface environment will affect attachment and subsequent behaviour of cells.

Materials and Methods

Borosilicate glass coverslips are chemically modified by a polymer brush using a surface initiated atom transfer radical polymerisation methodology. After immersion in 2-bromoisobutyryl bromide- acetonitrile (BIBB-ACN) solution for 1hr, initiator prepared coverslips are then immersed in pKSPMA solution and polymer is grown for 1hr. NHS-ester functionalisation is carried out in two stages, first using tris(2-aminoethyl)amine in DMF for 48 hrs under nitrogen at 65 °C. This is followed by immersion in DCM with DSMO and DMAP for 18 hrs under nitrogen. FITC staining was carried out and imaged using fluorescence microscopy. Cell culture was carried out using SH-SY5Y neuroblastoma cells seeded at 200,000 per well in a six well plate. Cells were fixed with formaldehyde and stained with DAPI and F-Actin after two days. Plates were imaged using fluorescence microscopy. Analysis was carried out on chemistry treated slides using x-ray photoelectron spectroscopy (XPS), fourier- transform infrared spectroscopy (FTIR) and drop shape analysis (DSA).

Results and Discussion

A BIBB-ACN initiator gradient was analysed and confirmed through DSA, with a clear contact angle gradient. The pKSPMA polymer brush treated coverslips were analysed using XPS, FTIR and DSA to discern whether the chemistry had been deposited correctly. Results confirmed chemical modification of the surface. SH-SY5Y seeding on half-treated polymer surfaces showed a preference of attachment on the clean glass rather than the polymer. FITC binding after the NHS-ester functionalisation step showed fluorescence on the polymer half of the coverslips only, confirming again that the functionalisation chemistry had worked. The results show clearly that polymer brush chemistry has been deposited on the surface, and acts as a 'cell-phobic' zone for cells seeded onto the surface. This allows us to manipulate cell attachment on the surface in a highly controlled way, especially when a gradient is employed.

Conclusions

With this biomaterial interface, the ability to control and manipulate cell growth is optimised. The dual gradient system allows for two variables to be introduced in a controlled manner, without the need for complex or messy experiment set ups. The addition of a biological gradient reflects the signalling system seen within the brain during development and neurogenesis, which is an important aspect when attempting to derive mature neurons. Whilst in its early stages, this work lays the foundation for designing a high-throughput system of generating neurons in a controlled manner, in an environment reflecting *in vivo* ECM like support.

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We acknowledge support from EPSRC CDT in Regenerative Medicine and Lboro School of Science for funding.

EMBEDDED BIOPRINTING AN *IN VITRO* COCHLEA MODEL FOR STUDYING COCHLEAR IMPLANTS

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Introduction

Since the mid-1980s, cochlear implants have been used to treat severe hearing loss, remarkably improved patients' quality of life[1]. Though its successful clinical outcome, several issues of the current cochlear implants, such as the distortion problem caused by the uncontrolled current spread within cochleae, enormous individual differences in outcomes and the lack of pre-implant predictor of outcomes, have yet to be addressed[2,3]. In addition, the absence of predictive models for cochlear implant studies is an obstacle to improving the current implants. Animal models have been extensively used in the preclinical hearing research, however these models fail to represent the anatomical features and variability of human cochleae. In an effort to reduce *in vivo* approaches and to develop a personalised approach for cochlear implant testing, this work aims to develop an *in vitro* cochlea model as a tool for cochlear implant research. Here, we demonstrate a novel technique to fabricate a cochlea model by embedded printing a fugitive ink inside a bone-mimetic matrix.

Materials and Methods

All experiments in this work were performed with a custom-built 3D bioprinting system. The 3D fugitive template of human anatomical cochlea structure was printed inside a bath of polysaccharide-hydroxyapatite composite matrix. After printing, the matrix was crosslinked and subsequently the fugitive ink was removed, leaving a hollow structure of cochlea inside a bone-mimetic matrix. To optimise the matrix formulations, rheological and swelling properties of the matrix material were assessed. Electric field imaging (EFI) profiles measured in patients and the bioprinted models were compared to evaluate the potential of the model as a tool for cochlear implant studies.

Results and Discussion

We show a novel embedded bioprinting approach to fabricate a freestanding hollow structure of cochlea inside a bone-mimetic matrix. Our bioprinted model closely mimics the anatomical feature of human cochlea, and the matrix formulation has been tailored to mimic the biochemical composition of cortical bones. The EFI measurements show similarities between patients and the bioprinted model.

Conclusions

In this work, we have fabricated an *in vitro* cochlea model by embedded printing a fugitive cochlea structure inside a bone-mimetic matrix. The geometry of the model and the matrix composition closely mimic native cochleae. We anticipate that our biomimetic model can accelerate the advancement of cochlear implants and possibly advance the development of a personalised model for testing cochlear implants.

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SUSPENDED LAYER ADDITIVE MANUFACTURE OF A TRI-LAYER SKIN MODEL

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Introduction

The treatment of skin defects caused by illness or trauma remains a major healthcare problem - particularly in chronic non-healing wounds. The current gold standard in chronic wound management is the split-thickness autograft. Split-thickness autografting, however, is not suitable for the treatment of wounds which compromise the subcutaneous layer and can lead to contraction and scarring at the wound site following transplantation. Due to the shortcomings of current wound care treatments along with limited donor availability, there is a clinical need for an alternative wound therapy. The use of biologic scaffolds loaded with spatially allocated cells offers a potential solution to these problems by preventing tissue contraction and scar formation. Using suspended layer additive manufacture, whereby 3D scaffolds are printed layer upon layer within a supporting agarose fluid gel [1,2], it is possible to fabricate full thickness tri-layer skin models *in vitro*. These scaffolds feature physiochemically and mechanically defined layers which replicate the epidermis, dermis and hypodermis *in vivo* and have the potential to overcome the issues that we currently face in the treatment of chronic wounds.

Materials and Methods

Agarose fluid gels were prepared by shear cooling agarose solutions (0.5% w/v) and loading into deep petri dishes. Primary human epidermal keratinocytes (HEK), human dermal fibroblasts (HDF) and stromal vascular fractions (SVF) were isolated from an abdominoplasty approximately four hours after excision and cultured to confluency prior to use. The heterogenous SVF population was cultured in order to isolate a homogenous population of adipose derived mesenchymal stem cells (ADSC). SVF heterogeneity and ADSC homogeneity was quantified by flow cytometry. ADSCs were then cultured for a further three weeks for the differentiation of adipocytes as indicated by staining with Oil red O solution. Using a 3D bioprinter (INKREDIBLE®), ADSC loaded pectin / collagen blends (ratio 2:1) were printed to form the hypodermis layer. Subsequent layers of HDF loaded pectin / collagen blends (ratio 1:1) to form the dermal skin regions were printed atop the subcutaneous layer and surface seeded HEKs formed the epidermal region (**Fig 1A**). Once printed, the constructs were crosslinked (thermally for collagen and ionically for pectin) prior to removal from the supporting bed. Cell viability and morphology was assessed using confocal microscopy and the microstructure evaluated using scanning electron microscopy.

Results and Discussion

Cells were successfully isolated from the human abdominoplasty and SVF populations were homogenised giving rise to ADSCs (**Fig 1B**). 3D printed skin models exhibited structural integrity throughout the complete part of neighbouring skin regions (**Fig 1C**) and the successful cultivation of spatially allocated cells was achieved. Scanning electron microscopy showed the material microstructure that was comparable to that of native skin (**Fig 1D**).

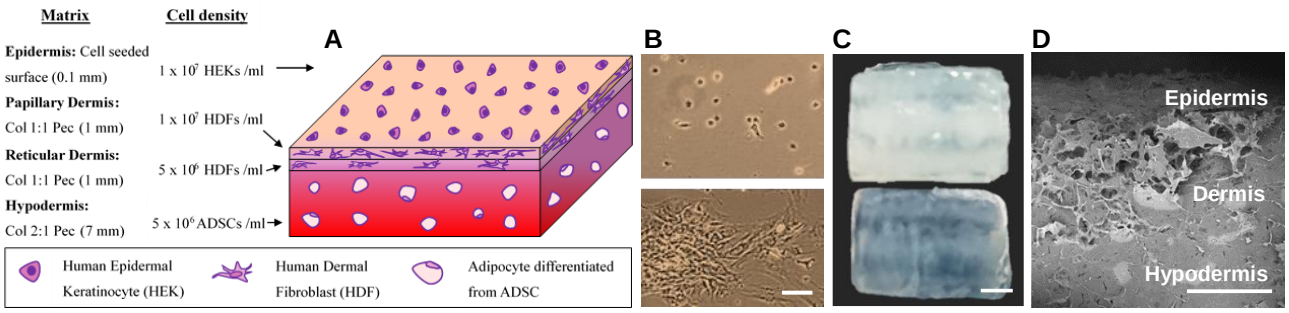

Figure 1 | Design and fabrication of a tri-layer skin model using suspended manufacturing methods. **A)** Design of a tri-layer skin model (15.0 x 15.0 x 9.1 mm) featuring a hypodermis, dual compartment dermis and epidermis. **B)** Culture of cells isolated from the stromal vascular fraction (SVF) at day 1 (upper) and homogenized into ADSCs at day 7 (lower) (scale bar = 100 µm). **C)** 3D printed tri-layer scaffold (upper) and cross section (lower) displaying a gradient in material matrix (scale bar = 3 mm). **D)** Scanning electron micrograph of a 3D printed tri-layer scaffold displaying a gradient in material matrix at the micron scale (scale bar = 1 mm).

Conclusions

These results demonstrate that tri-layer skin models can be intelligently designed and fabricated using low viscosity collagen and pectin blends in order to manufacture a final construct that contains physiochemical gradients similar to the gradients present within skin tissue.

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UNDERSTANDING NEURAL NETWORKS: THE DEVELOPMENT OF SINGLE NEURON-NEURON BRAIN-ON-A-CHIP MODELS

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Introduction

Understanding the structure of complex neural networks and how these interactions result in such a wide array of functions has been of wide interest across the sciences. Owing to its complexity, understanding the brain has yielded multiple approaches. Largely due to an increase in life expectancy with higher risk of age-related neurological disorders, there is a growing need to better understand the brain, how it functions and dysfunctions and how we can aim to treat such illness. To treat such disorders, advanced *in vitro* models need to be developed in order that pharmaceuticals and cell therapies can be assessed at a higher level before clinical trials. However, owing to neural complexity and physical structure, studying and understanding large neural networks is difficult. Developing *in vitro* models that focus on analysing small neural networks via multi-electrode arrays (MEAs) could help improve our understanding and therefore improve treatment development. The work presented here is aimed towards this goal, being focused on chemical patterning to position and control the development of engineered neural architecture.

Materials and Methods

Oxygen plasma-cleaned coverslips were prepared with a cell-phobic polymer brush structure presented in patterns to control the quantity, location and direction of neuron growth. Model MEA surfaces were also prepared by spin coating a thin layer of SU8-10 onto coverslips; this is an epoxy-based negative photoresist that is used to insulate the top surface of MEAs. S1813 (positive photoresist) was used as a sacrificial template around which chemical brushes were generated via a surface-bound ATRP reaction.
SU8-10 was applied via the use of a pre-coater (GLYMO) spin coater rotating first at 500 rpm then 3000 rpm. Coverslips were baked at 95 ^{o}C for 60 seconds, exposed to i-line UV, baked again at 65 ^{o}C (60 seconds), 95 ^{o}C (60 seconds), 130 ^{o}C (120 seconds) and then left to cool slowly. S1813 was spun on at 500 rpm (30 seconds) 3000 rpm (60 seconds), prebaking at 110 ^{o}C for 75 seconds and exposed to i-line UV before developing. Patterns were post-baked at 110 ^{o}C for 10 minutes.
Polymer brushes were generated via three stages: 3-aminopropyl triethoxysilane (ATPES) was first applied, onto which α-bromoisobutyryl bromide (BIBB) was then bound, followed by the 3-sulfopropyl methacrylate potassium salt (pKSPMA) being used as a monomer to grow the polymer brush. Chemical patterning was adapted from Pardo-Figuerez, et al.[1] Surface chemistry and patterning were analysed by x-ray photoelectron spectroscopy (XPS), drop shape analysis (DSA) and fluorescent staining (conjugation of FITC). A neuroblastoma cell line, SH-SY5Y, were cultured onto treated coverslips and analysed via brightfield and fluorescent microscopy.

Results/ Discussion

Chemical patterning onto glass and SU8-coated coverslips was confirmed by multiple methods, with cells demonstrating preferential attachment and direction growth control following these patterns down to a few microns in feature size. Cell seeding densities showed some control over pattern conformity, with organization of neural networks being demonstrated towards the single cell-cell level.

Conclusion

Chemical patterning can be used to control the localization and controlled connectivity of neuronal cells, towards the production of specifically designed neural network architectures. This ability to control cell growth combined with reduced cell seeding density will enable the development of single neuronal networks onto multiple platforms starting with SU8-10/S1813 combination and then eventually leading to chemical patterning of MEAs.

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SYNTHESIS OF VARIOUS CALCIUM PHOSPHATE NANOPARTICLES FROM A MAGNESIUM-FREE SIMULATED BODY FLUID.

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Introduction

Hydroxyapatite (HAp) [idealized formula $Ca_{10}(PO_4)_6(OH)_2$] is chemically and structurally similar to the main inorganic component in bone and teeth. Although it has been the focus of much research as a bone replacement material, the ability to synthesise it as nanoparticles has led to applications such as a drug delivery system and for use in cell transfection as an alternative to viral systems [1]. Here, the aim of the study was to synthesise nanoparticles of calcium phosphate (CaP) using a biomimetic inspired approach. Thus, a simulated body fluid (SBF), a solution containing similar inorganic ion concentrations to those of human plasma, was used for HAp synthesis. Appropriate cues, such as small increases in calcium and phosphate ion concentration or the presence of nucleation sites can facilitate spontaneous nucleation and HAp crystal growth from SBF at physiological pH (7.4) and body temperature (37°C) [2]. In this study a modified SBF composition, through the removal of magnesium ions, was used to prepare various CaP nanoparticles by adjusting the pH.

Materials and Methods

Mg-free SBF solution was prepared by dissolving reactants in deionised water, adapting the method described by Kokubo *et al*. [2]. The pH value of the solution was measured throughout the experiments. Aliquots of Mg-free SBF were then warmed to 37°C and the pH was adjusted from 7.40 to values between 7.75 and 9.15 by the addition of small volumes of 1M NaOH (aq). After an hour, precipitates were collected from the SBF, dried, then analysed using X-ray powder diffraction (XRD), Fourier transform infrared spectroscopy (FTIR). Dispersed nanoparticles were imaged using transmission electron microscopy (TEM).

Results and Discussion

The XRD patterns of precipitates obtained from Mg-free SBF by adjusting pH to values from 7.75 up to 8.75 resulted in a HAp phase, with broad diffraction peaks consistent with nano-scale crystallites of apatite. In contrast, precipitates formed from Mg-free SBF at pH values of 8.90 up to 9.15 resulted in the formation of an amorphous calcium phosphate (ACP) phase, with no resolved diffraction peaks appearing and a characteristic 'amorphous' pattern observed. The formation of a crystalline apatite or an amorphous calcium phosphate phase was also confirmed by FTIR analysis; the former produced a doublet in the ~550-600 cm^{-1} region of the IR spectrum, while for the latter a single broad peak was observed. The morphologies of the apatite and ACP nanoparticles were also studied using TEM analysis. Needle and ribbon like crystals (Fig 1a) were observed with the apatite phase and low aspect ratio nanoparticles (Fig 1b) were observed for the ACP phase.

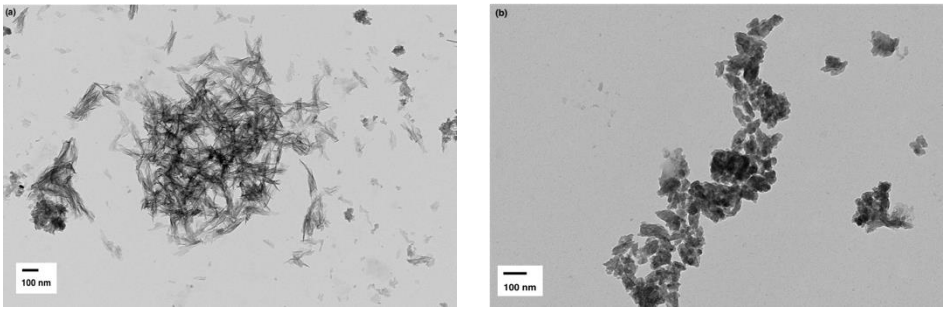

Figure 1. Images of (a) the needle and ribbon shaped crystals precipitated from a Mg-free SBF solution at pH 8 (20000x); (b) low aspect ratio nanoparticles precipitated from a Mg-free SBF solution at pH 9 (30000x).

Conclusion

This study shows that by careful control of synthesis conditions, different calcium phosphate phases can be produced: HAp nanoparticles can be synthesised by using Mg-free SBF solution and different calcium phosphate phases can be synthesised under different pH values of the Mg-free SBF solution. These may behave differently in terms of how they may be internalised by cells and therefore behave as gene delivery systems.

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ACELULLAR GELATINE-ALGINATE SCAFFOLDS FOR DENTINE-PULP REGENERATION

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Introduction

Dental decay and poor long-term outcomes of traditional endodontic treatments have led the search for new dental tissue regeneration strategies. There is a lack of biomaterial approaches that harness the native dental pulp stem cells (DPSCs), which constitute one of the main agents responsible for the intrinsic regenerative capabilities of the pulp. To achieve this, a hybrid gelatine-alginate scaffold crosslinked via tetrazine-norbornene click chemistry incorporating bioceramic particles as an odontogenic moiety is proposed.

Materials & Methods

Sodium alginate (KIMICA, Japan) was modified with 2-norbornene (Sigma-Aldrich, UK) and gelatine (Nitta Gelatin Inc. Japan) will be functionalised with 5-(4-(1,2,4,5-tetrazin-3-yl)benzylamino)-5-oxopentanoic acid, which is being synthesised using nickel triflate as catalyst as described by Alge et al.[1] Proton Nuclear magnetic resonance (^{1}H NMR) will be used to characterise the tetrazine and confirm the functionalisation of alginate and gelatine with norbornene and tetrazine, respectively. Biphasic calcium phosphate (BCP), a mixture of hydroxyapatite (HA) and tricalcium phosphate (TCP) was prepared via wet precipitation and calcination at 1000 ºC. Bredigite ($Ca_7Mg(SiO_4)_4$) and ß-dicalcium silicate (ß-DCS; Ca_2SiO_4) were synthesised via a sol-gel process and calcination at 1150 ºC and 800 ºC, respectively. X-ray diffraction (XRD) was used to assess the crystallinity and formation of the bioceramics. Extracts of these were prepared via incubation at 37 ºC and 5 % CO_2 in DMEM for three days at 200 g/L. DPSCs were cultured in dilutions of the extracts (from 0 to 100 g/L) and MTT assays were performed after 1, 3 and 5 days to assess DPSC proliferation.

Results & Discussion

XRD spectra confirmed the formation of BCP with a high ß-TCP/HA ratio (approximately 80/20), pure bredigite and ß-DCS after calcination. A more prevalent ß-TCP phase is considered desirable as BCPs with higher ß-TCP/HA ratios have been described to be more odontogenic, presumably due to the higher bioresorbability of ß-TCP.[2] Ceramic extracts displayed no significant DPSC cytotoxicity against control (media without extract), except for BCP extracts for which DPSC viability greatly decreased at the highest concentrations, (2x and 4x dilutions) but remained high at the lowest concentrations (16x and 64x dilutions). Polymer modification with tetrazine and norbornene moieties allows for bio-orthogonal crosslinking which will enable encapsulation of bioactive molecules such as growth factors and cytokines to engineer endogenous DPSCs.

Conclusion

In this work, preparation and characterisation of the main components of an acellular scaffold for dentine-pulp regeneration is presented. The hybrid scaffold combines the tuneable mechanical properties of alginate with the pro-attachment moieties and biodegradability of gelatine. Future work includes optimisation of hybrid scaffold parameters such as porosity and Young's modulus to support DPSCs in-vitro and incorporation of bioceramics and other odontogenic elements for differentiation of endogenous DPSCs.

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Acknowledgements

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3D PRINTED SCAFFOLDS FOR FUNCTIONAL EX VIVO CARDIAC TISSUE MODELS

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Introduction

The development of new procedures, interventions and medications for the treatment of cardiovascular disease is dependent on rigorous preclinical and regulatory testing to ensure safety and the efficacy for the patient. A central aspect of this testing is the use of animal models, in particular porcine testing, as an in vivo element of the preclinical assessment regime. The provision of improved human based *in vitro* models that can significantly reduce this dependency on animal studies has clear benefits. Approaches using a blend of the knowledge gained from tissue engineering and lab-on-chip technologies are being developed whereby cardiac cells are combined with a 3D scaffold material and integrated with a suitable measurement platform to generate key elements of functional cardiac tissue. These so-called *ex vivo* systems offer a means to attain optimum physical, mechanical, and electrophysiological characteristics in a system designed to replicate the native myocardium. A key consideration of this approach is the ability to mimic the electrical properties of the myocardium in way that will reflect an appropriate respond to various types of treatments, e.g. drug and/or electrostimulation. In this work, the effects of integration of graphene to enhance the electroactive properties of a poly-e-caprolactone 3D scaffold designed to act as a matrix for functional cardiomyocytes is presented.

Materials and Methods

Poly-e-caprolactone (PCL) powder with a relative molecular mass of 50,000 amu (Polysciences Europe, Germany) with a particle size of <600 µm (98%) was used as the base system for 3D printing. Graphene nanoplatelets (2-10 nm) with a molecular weight of 12.01 g/mole (ACS, Pasadena, USA) were added to the PCL to create 0.25%, 0.50%, 0.75%, 1%, 2% and 5% w/w mixtures. Each powder mixture was placed in a hot extrusion cell of a bioplotter (EnvisonTEC, Germany) and used to print a range of 3D honeycomb structures that have been previously shown to promote cardiomyocyte function. The resulting structures and materials have been characterised by SEM, Conductivity measurements and TGA.

Results and Discussion

Figure 1 shows Scanning Electron Microscopy (SEM) images of honeycomb type 3D structures printed from PCL/Graphene w/w mixtures. These data indicate that it is possible to create the required structures with up to 5% graphene thereby resulting in a conductivity of $1x10^{-3}$ S/m compared to $1x10^{-16}$ S/m for PCL.

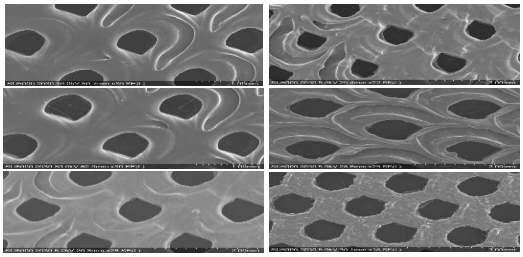

Figure 1: SEM micrographs of PCL 3D structures with (a) 0.25%, (b) 0.5%, (c) 0.75%, (d) 1%, (e) 2%, (f)-5% w/w Graphene

Thermogravimetric analysis (TGA) has been used determine the residual Graphene content after thermal degradation of the PCL.

Conclusion

Honeycomb-like 3D structures have been successfully fabricated from PCL/Graphene mixtures in the range 0.1% to 5% w/w. Above 5% Graphene inclusion, it is found that the structures no longer have the required integrity. Conductivity measurements suggest that the 95%PCL/5%Graphene system has electrical properties that may allow for stimulation of cardiomyocytes therein.

Acknowledgements

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DEVELOPMENT OF A HYDROXYAPATITE-BASED INK FOR THE 3D PRINTING OF BONE TISSUE-LIKE SCAFFOLDS

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Introduction

Additive manufacturing (AM) technologies provide a promising strategy for fabricating custom made and patient specific scaffolds for bone tissue engineering (BTE) (1). Whereas, the development of polymeric filaments and inks for use in 3D printing is advancing rapidly, the same cannot be said for ceramics. The majority of the literature reporting the use of calcium phosphate AM inks revolves around complex mixtures of aqueous-based slurries. Such mixtures are difficult to prepare and must undergo controlled dehydration to from a green structure before sintering. Also, most of them require the use of solvents and binders which may lead to harmful residues along with un-intended chemical interactions such as oxidation or crystallisation (2). The research presented here investigates the use of solvent-free hydroxyapatite-derived inks, based on the use of triglycerides, in order to enable the 3D printing of ceramic-based bespoke substitutes for BTE.

Material & Methods

A pre-sintered grade of hydroxyapatite (HA, Plasma Biotal, UK) was mixed with the triglyceride linoleic acid (TLA, Sigma Aldrich, UK) to form an ink with varying weight to weight ratios. Subsequently, 3D scaffolds were manufactured using an 3D Bioplotter (EnvisionTEC, Germany). SolidEdge CAD software was used to design the required scaffold structures and exported as an STL file to the Biopotter RP software. A low-temperature extrusion head was employed with the ink HA-TLA ink maintained at 22^{o} C during the printing process. The fabricated scaffolds are then sintered to 1200 °C for 3 hours in a Carbolite furnace at a ramp rate of 5^{o}C/min. The resulting scaffolds and inks were characterised in terms of their physical and chemical properties (SEM, micro-CT and TGA).

Results & Discussion

Integral 3D scaffolds where produced using the HA-TLA ink system developed here. A range of scaffolds with various layer geometry and orientation were successfully fabricated. Micro CT analysis of the 3D structures is provided in Figure 1 for a 3D scaffold designed to have a 1mm spacing and a 0-90^{o} layer configuration giving an indicative porosity in the range of 40% with interconnectivity of 98%, which is similar to that of human cancellous bone. These data confirm that the internal structure and associated key parameters, such as porosity, pore interconnectivity and surface area, are all fully representative of the CAD design files employed. SEM analysis was used to evaluate the surface topography and pore size of the constructs. Figure 2(a) shows a that a typical scaffold has an average macro pore size of 502 µm and a strut diameter of 698 µm. Figure 2(b), illustrates the presence of micro pore of 126 µm on the surface of the sintered HA struts.

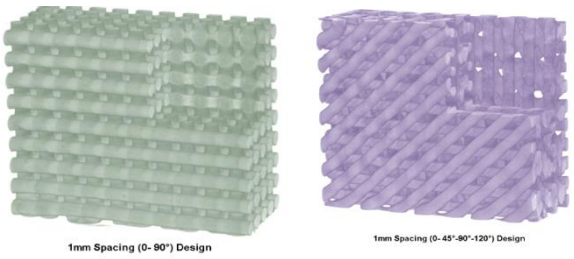

Figure 1. Micro-CT reconstructions of HA-TLA scaffolds.

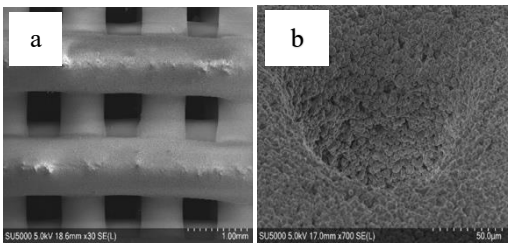

Figure 2. SEM Images of (a) macro-structure and (b) micro-porosity of HA-TLA scaffolds.

Conclusion

A solvent-free ink based on various w/w ratios of hydroxyapatite and triglyceride linoleic acid has been demonstrated to be capable of forming advanced and complex anisotropic 3D structures, via additive manufacturing technology. The system lends itself to the development of custom-made calcium phosphate-based scaffolds to treat complex critical sized bone defects within orthopaedic surgery

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DEVELOPMENT OF A BIOPROCESS FOR THE EXPANSION AND DIFFERENTIATION OF IPSCS

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Introduction

Neural precursor cells (NPCs), derived from human induced pluripotent stem cells (hiPSCs), are a promising cell source which offer unprecedented opportunities for personalised drug screening, disease modelling and regenerative medicine applications. 1,2 However, the avenue towards the sustainable commercial use of hiPSC derived NPCs is hindered by the lack of robust and reliable large-scale bioprocess designs. Therefore, here we aim to develop a bioprocess integrating the expansion and differentiation of hiPSCs into NPCs, whilst employing bioreactor platforms to reduce the cost and labour investment compared to the conventional planar approach.

Materials and Methods

In order to determine the optimal seeding density, hiPSCs were seeded on vitronectin coated plates at various seeding densities ranging from 2x104 to 6x104 cells/cm2 . Growth kinetics and glucose profiles were recorded over 5 days. hiPSCs, were then differentiated to NPCs as monolayers. Cell morphology and glucose consumption were monitored. Furthermore, since hiPSCs are adherent cells, 8 commercially available microcarriers without additional coating were screened for their ability to support cell attachment and expansion.

Results and Discussion

Cells achieved 80% confluency after 3 days in mTeSR Plus when seeded at 4x104 and 6x104 cells/cm2 , whilst the lower cell density of 2x104 cells/cm2 required 4 days. Cell survival indicated by cell viability at day 1 after single cell passaging, was higher when cells were seeded at 4x104 and 6x104 cells/cm2 compared to the lower seeding density. Nonetheless, after day 2 in culture, the specific growth rates of cells at all seeding densities were similar. On the other hand, the differentiation of hiPSCs and the emergence of NPC populations were indicated by the formation of neural rosettes after 9 days in neural induction medium. hiPSCs were also shown to have successfully adhered onto Cytodex 3 and Cytodex 1 (GE Healthcare), Cultispher G and Cultispher S (Sigma) and Collagen (SoloHill PALL) microcarriers by live-dead staining and phase contrast imaging.

Conclusion

The optimal seeding density was found to be 4x104 cells/cm2 . Neural rosettes formed within 9 days in neural induction medium (Stem Cell Technologies). Additionally, only certain microcarriers were found to support the attachment of hiPSCs. Further work will be conducted to investigate growth profiles of hiPSCs on the different microcarriers, followed by the development of a one-step bioprocess for the production of NPCs from hiPSCs.

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DESIGNING AN ANGLED INTERFACE FOR AN IN VITRO FLEXOR DIGITORUM PROFUNDUS ENTHESIS MODEL THROUGH HUMAN HISTOLOGICAL INVESTIGATION

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Introduction

Interfacial tissue engineering (ITE) endeavours to reproduce the soft-hard tissue interface for potential implantation in diseased or traumatised tendon/ligament-bone regions. ITE primarily targets commonly injured large joints, such as the rotator cuff or anterior cruciate ligament,[1] with native morphological replication of models overlooked in favour of cellular or materials research. Trauma at the flexor digitorum profundus (FDP) insertion onto the distal phalanx (DP) in the hand, the most frequent closed flexor tendon injury,[2] shares similar treatment challenges as large joint enthesis injuries, and our group is designing an *in vitro* ITE model particular to this anatomical region. Since the angle of tendon fibres at the tendon-bone enthesis are an important mechanical consideration in physiological function and pathogenesis,[3] we have investigated FDP enthesis tendon fibre angle histologically, aiming to design and produce a more morphologically accurate *in vitro* model.

Materials and Methods

48 fresh frozen human cadaveric fingers from 6 body donations to The University of Edinburgh Medical School were dissected to obtain isolated samples of the FDP attachment to the DP. 10µm mid-sagittal sections through the enthesis were prepared by wax histology, stained with toluidine blue, and digital scans analysed with ImageJ software for the tendon fibre angle at the mineralised-unmineralised interface (tidemark). Inter-observer reliability was assessed. An angled culture shelf and separate mold were designed with Tinkercad software and 3D printed to create this angle at the tendon-bone interface in an *in vitro* model using a rat fibroblast-seeded fibrin tendon analogue and a brushite bone anchor. Early investigations are underway on the feasibility and reproducibility of the novel model.

Results and Discussion

The mean tendon fibre angle (±SEM) for all fingers (3 male, 3 female; mean age 79.3, range 73-91) at the mineralised-unmineralised interface was 30.08^{o} (±0.64^{o}). Cronbach's α for reliability between 2 observers was high at 0.91. A 30^{o} angled culture shelf for a 6-well plate created a cured silicone base layer in the wells at a reciprocal 30^{o} to the plate, but allowed the forming tendon analogues to be cultured horizontally without gravity or slippage effects. The specially designed mold produced the negative space in the silicone layer for the brushite anchor to be positioned at 30^{o} to the horizontal tendon analogue while encompassing a larger circular area for contraction of the fibroblast-seeded fibrin gel. Careful mutual design of the size and shape of the bone anchor through similar Tinkcad mold creation and 3D printing is essential for the anchor to be held firmly at the correct angle whilst preventing the tendon analogue attaching to unintentionally exposed surfaces.

Conclusions

Through quantitative morphometric analysis of tendon fibres at the human FDP enthesis, we have designed and created an *in vitro* tendon-bone model with enhanced anatomical characteristics. This anatomical approach to ITE design could improve the clinical applicability of *in vitro* models of any enthesis region.

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Acknowledgements

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BIOENGINEERING 3D MICROENVIRONMENTS TO STUDY MECHANOTRANSDUCTION AND VASCULARIZATION IN BONE REGENERATION

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Introduction

Bone is a high vascularized tissue so engineering successful novel bone biomaterials must involve not only the formation of new bone tissue but also promote the creation of a microvascular environment resembling the uninjured tissue. In the present work, VEGF is presented tethered to poly-ethyl acrylate (PEA) to induce vascularization. This polymer has been shown to allow solid-phase presentation of GFs through material surface-binding mimicking the stem cell microenvironment and allowing low and controlled dose administration of signals[1]. In addition, the current work is focused in the application of nanovibrational stimulation or 'nanokicking' (NK) in order to study stretch-activated ion channels, gated specifically by mechanical force in mesenchymal stem cells (MSCs). This mechanical stimulation have shown a strong osteogenic response in two-dimensional (2D) and three-dimensional (3D) conditions [2,3].

Materials and Methods

Fibrin gel in vitro angiogenesis assay was used to evaluate tube formation of HUVECs in 24-well plates. Briefly, after FN (20 µg/ml) and VEGF (25 ng/ml) coatings, cells were seeded at a density of 10,000 cells/cm2 complete endothelial cell medium and left at 37 °C overnight. Then, fibrinogen and thrombin were added to the plates which were placed in CO2 incubator at 37 °C for 1 hour to polymerize. After clotting, fibrin matrix was covered with endothelial complete growth medium that was changed every 48 hours. Plates were nanostimulated during this period by a bioreactor at 1000 Hz and 30nm. After this, immunostaining was carried out for CD31 and phalloidin to evaluate protein expression and tubular formation.

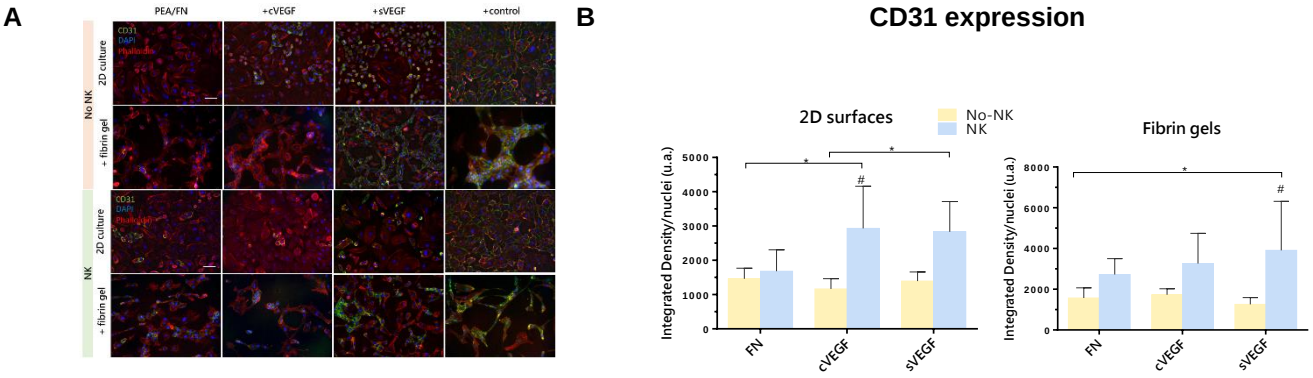

Figure 5. **A**- Immunofluorescence images of ECs monocultures after 7 days under stimulated (NK) or unstimulated (No-NK) conditions. Scale bar: 100 µm. cVEGF = Surface VEGF-coated; sVEGF = soluble VEGF in media. **B**- Quantification of CD31 expression of image A. Statistical significances attributes to values p<0.05 as determined by two-way ANOVA with Tukey's multiple comparisons test (***** p-valor between groups; **#** p-valor within the same group).

Results and Discussion

CD31, an adhesion molecule that is expressed in intracellular EC junctions, plays a crucial role in the formation and maintenance of vessels as well as in forces transduction. In 2D conditions, ECs do not form any tubular-like structure and expression of CD31 is only significantly higher between stimulated and unstimulated groups in coated-VEGF surfaces. When fibrin gels are present, only soluble VEGF showed statistical differences, although an increased general trend is noticeable for NK samples.

Conclusion

The material-based system allows engineering a model for angiogenic and osteogenic growth factors binding. With an in vitro angiogenesis assay, CD31 analysis suggests that nanovibrations themselves do not seem to induce tubule formation but does it appear to increase CD31 expression. We could propose that this will make the cells more receptive to MSCs, but not affecting vasculature formation per se. Thus, co-cultures with MSCs are being carried out in order to further investigate this concept.

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EXAMINATION OF THE SUITABILITY OF LIPIODOL AS A CONTRAST AGENT FOR POLYETHYLENE BIOMATERIALS

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Introduction

Ultra-high molecular weight polyethylene (UHMWPE) is a bearing material used for almost all joint replacement designs. However, UHMWPE has a limited X-ray attenuation, which means early diagnosis of failures such as dislocation, bearing fracture and wear can be challenging because the position of the part cannot be identified (Figure 1). The presence of the polyethylene bearing can be confirmed using embedded metallic radiographic markers; but it has been shown that the radiographic markers can increase the risk of fracture and failure[1]. We have used a novel method to enhance the radiopacity of polyethylene by diffusing an FDA-approved contrast agent into the surface of the polymer. The aim of this study is to find an optimal radiopacity level, where the part can be identified in a radiograph while retaining the static material properties.

Materials and Methods

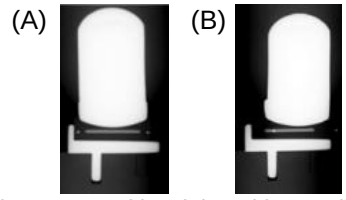

Figure 6. Position (A) and issues like overhanging (B) are difficult to diagnosed of polyethylene bearing cannot be identified using X-ray imaging due to the low X-ray

Un-irradiated medical grade 4 mm thick UHMWPE sheet (GUR1050, Celanese, Oberhausen, Germany) was machined into tensile test samples in accordance with ISO-572 Annex A. An elevated temperature was applied to facilitate the diffusion (85°C, 105°C, 125°C) for 12 h, 18 h and 24 h to achieve a range of radiopacities. The melting point and crystallinity of the samples were quantified using DSC (TA instrument, DSC 250, 20°C-200°C, heat rate= 10 °C/min). Each sample was imaged using a µCT scanner (X Tec, XT H 225 ST, Nikon Metrology UK Ltd, Derby, UK, 162 kV, resolution 0.2 mm) to quantify the radiopacity. Analysis of the CT data was performed using Simpleware ScanIP (Synopsys, Inc., Exeter, UK (release version 2017). Water, air and untreated polyethylene were used to convert the CT- grayscale to Hounsfield Unit (HU). Tensile tests were conducted at room temperature in accordance with ISO-527 using an electromechanical test machine (Instron 5965) at a rate of 50 mm/min, on; virgin UHMWPE, thermally treated UHMWPE and Lipiodol treated UHWMPE. Five specimens per condition were tested to obtain tensile modulus (E), 0.2% yield strain, ultimate tensile strength (UTS), toughness and elongation at failure.

Results and Discussion

The results of our study (Figure 2) confirmed that treating polyethylene with Lipiodol does not alter the crystallinity of the polymer (p=0.56) while it enhances the X-ray visibility of the samples (from 110 HU to 1200 HU). In terms of mechanical properties, there was no significant alteration in the yield strength (p=0.07) and ultimate tensile strength (p=0. 3) of the Lipiodol treated, thermal treatment and virgin UHMWPE samples provided the treatment temperature was less than 125 °C. However, there was an slight increase (2%) elongation at failure (p=0.049) of the samples. It has been shown that other clinically avialable oil-infused UHMWPE (e.g. vitamin-E) has a similar plasticising effect on polyethyene, but the effect can be mitigated through irradiation-induced crosslinking[3].

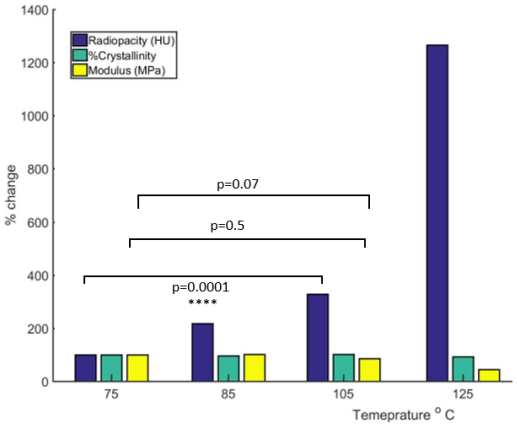

Figure 7. The radiopacity, crystallinity, and elastic modulus of the untreated polyethylene samples compared to those treated with Lipiodol under varying conditions.

Conclusions

The aim of this study was to identify the optimal level of radiopacity. Based on the current result the optimal radiopacity can be achieved at by treatment at 105 °C for 24 h (approx. 400 HU). Under these conditions the contrast agent (Lipiodol) does not alter the crystallinity or the tensile properties of the samples, and are comparable with untreated polyethylene. Radiopaque UHWMPE is a promising material for medical use; however, the differences observed in the toughness and ductility will be investigated further as part of future work to assess the safety of the material.

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NOVEL POROUS STRUCTURES FOR ENHANCED OSTEOINTEGRATION IN ORTHOPAEDIC DEVICES

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Introduction

Initial fixation is a key factor that determines the long-term success of implantable orthopaedic devices. To improve initial fixation, and reduce the prevalence of aseptic loosening, a method for optimising the surfaces of orthopaedic devices to enhance osteointegration has been developed. This is achieved via production of porous titanium wireframe structures using Selective Laser Melting (SLM) technology. Accelerated bone ingrowth, enabled by interactions between the surface and the surrounding tissue, allows for mechanical interlocking of the device. Previous work introduced a software algorithm that allowed the surface of structures to be roughened, which increased the coefficient of friction between the surface and bone tissue. Here, we have further developed this algorithm to allow for directionally biased surface roughening. This could allow for easier device insertion and decrease micro motion, while still supporting cell adhesion and promoting osteogenesis.

Materials and Methods

Test specimens with an array of surface features (Fig. 1a) were produced using SLM. Specimens were inserted axially into synthetic bone material (*SawBones*) at a rate of 1mm/s, and then pulled out at the same rate. Maximum push-in and pull-out force (N) was recorded. Specimens were rotated using a custom-built rig. Maximum torque force (Ncm) was recorded. Specimens were seeded with 2.5, 5, 7.5 and $10x10^5$ Green Fluorescent Protein-transfected Mg-63 Osteosarcoma cells and incubated at 37°C for 4h. Cell adhesion and distribution were observed using confocal microscopy (Fig.1b).

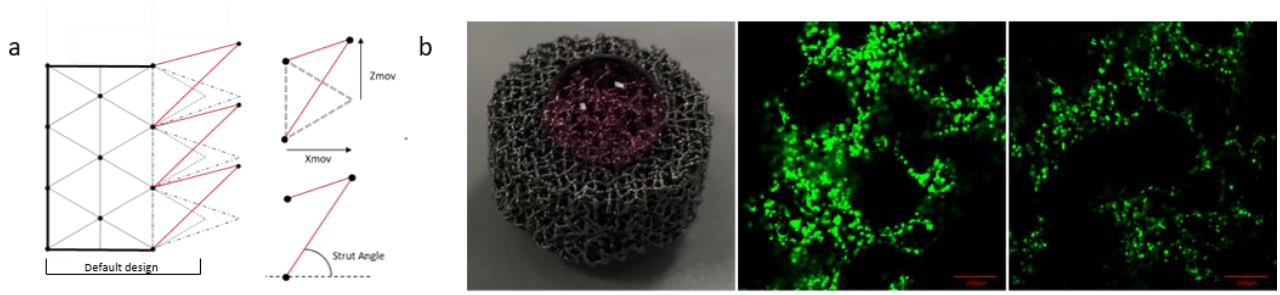

Figure 1: a. Schematic representation of surface manipulation. **b**. Ø10x5mm specimen seeded with media containing cells (left) and confocal microscopy images showing adhered cells (middle, right). Scale: 200µm. 10x magnification.

Results and Discussion

Data obtained demonstrates that maximum force required for axial push-in, pull-out and torque is largely dictated by interference. As interference increases, the force required becomes greater in response. Specimens with a Z movement of 0.4 and 0.5mm required greater force during push-in despite their increased lower strut angles. This may be explained by the fact that struts do not appear to deflect upon insertion (not expected) but rather cut through the synthetic bone material. Lower struts with smaller angles receive greater support from upper struts and aid cutting. This cutting phenomenon also explains the markedly reduced forces required to pull-out specimens. When push-in and pull-out data are displayed alongside lower strut angle there is no obvious trend, although when push-in/pull-out are considered as a ratio (i.e. easiest to push-in and hardest to pull-out), an angle of 48° (Z0.2, X0.15) is the optimum variable in this regard. Confocal microscopy has shown that the surface supports initial cell adhesion and validates our seeding method for use in future *in vitro* experiments.

Conclusions

We have developed a method for producing novel, reproducible surface features to increase mechanical fixation in porous orthopaedic medical devices. Initial data has shown mechanical interference to be a key factor in device fixation, although the effects of directional bias are less clear. We have also shown that the surface supports initial cell adhesion. Future work will further investigate the osteoconductive potential of this novel surface design.

Acknowledgements

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UNDERSTANDING CELLULAR UPTAKE OF SILICATE SPECIES IN BONE CELLS

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Introduction

Silicate based bioactive glasses (BG) are used clinically to regenerate bone [1] [2]. Previous literature has demonstrated the therapeutic importance of BG dissolution products for bone repair and regeneration [3]. There remains, however, a lack of understanding on how soluble silica species interact with cells. This includes how silica species are internalised (and possibly excreted), the intracellular concentration and location of these ions. A greater understanding of silicate cellular internalisation will help in the optimisation of bioactive glass ion release rates for more precise control of cell behaviour. As such, this study aims to investigate silicate ion uptake dynamics in osteoblasts whilst examining some the roles these ions may play in bone regeneration.

Methods and Materials

Osteoblast-like (SaOS-2) cells were cultured at 10,000/cm^2 in McCoy's Glutamax 5A medium containing 1mM sodium silicate hydrate. To assess if cytotoxicity was induced by different silicate concentrations and uptake inhibitors, proliferation, metabolic activity and cellular morphology were assessed by total DNA, Alamar blue and light microscopy assessment, respectively. Quantification of Si uptake was performed by ICP-OES, following lysing cells in 1M nitric acid at 85°C and filtration (after 6-96 h cell culture). Inhibition of silicate uptake mechanisms were evaluated by the use of chemical inhibitors of the sodium bicarbonate co-transporter protein by S0859 (Merck). To locate intracellular Si, cultured cells were fixed in 2% paraformaldehyde resin, stained with 1% osmium tetroxide. Scanning Transmission electron microscopy (STEM) (JEOL 2100 Plus) was used to image the cells and energy dispersive X-ray spectroscopy (EDXS) was used to locate and quantify Si content. Si uptake experiments were performed in triplicate. Data was statistically analysed using a Tau-Thompson assessment for anomalies and a student's t-test for statistical significance.

Results and Discussion

A total increase in intracellular Si uptake was observed up to 48h (whilst the amount of uptake rate per hour decreased over time) (fig. 1A/B). Upon the replacement of Si containing serum with a control (no Si containing media) the concentration of Si decreased ($p<0.05$), suggesting the excretion of Si from the cells (Fig. 1C). The inhibition of sodium-bicarbonate co-transporter protein decreased ion uptake (without effecting cell number) suggesting a potential mechanism of uptake (Fig 1D). STEM/EDX point analysis showed Si to be evenly distributed within the cell. Higher concentrations were seen to be localised within vesicle-like structures suggesting an active movement of Si throughout the cell.

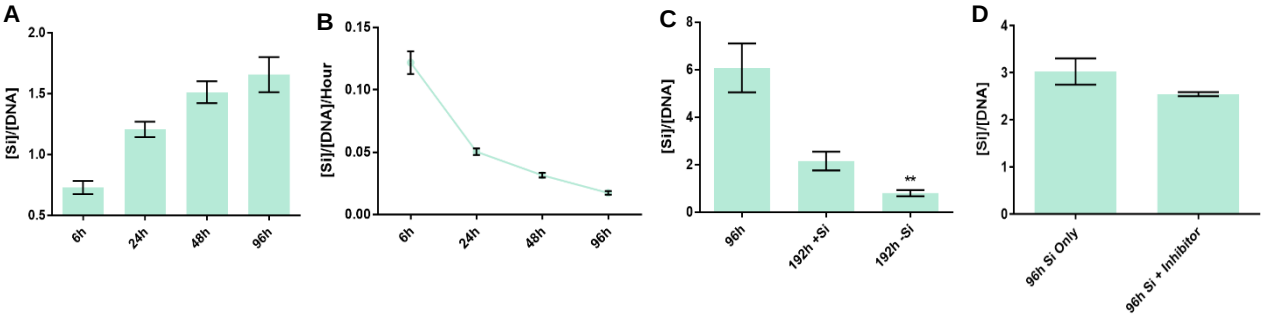

*Figure 1. Si uptake by osteoblast-like SaOS-2 cells as determined by ICP-OES.(A) Intracellular Si concentration increased/µg DNA up to 48hrs, (B) Si ion uptake rate decreases as a function of time, (C) replacing culture medium with Si and non-Si conditioned mediums causes a significant decrease in Si, (D) intracellular Si ion concentration decreases following inhibition of sodium-bicarbonate uptake channels. N=3, error presented as +/- SD, *= P<0.05, **= P<0.01*

Conclusions

Osteoblast-like cells reach a maximum uptake of Si ions over time. Our study suggested that the cells may begin excreting ions at specific stages during their proliferation. Further experiments showed that sodium-bicarbonate channels may play a role in the cellular uptake of these ions. As such, new BG materials should be tailored to release silicate and other ions to target specific cell types dependent on their uptake rates and mechanisms.

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STRUCTURAL AND MECHANICAL CHANGES IN PLLA-BASED POLYMER BLENDS DURING HYDROLYTIC DEGRADATION

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Introduction

Poly-L-lactide (PLLA) is an appealing biomedical implant material, however for certain load-bearing applications such as cardiac stents it suffers several limitations. Among these are its slow degradation time, poor stiffness and strength (requiring PLLA stents to have thicker struts than conventional metallic stents), and tendency for embrittlement during degradation[1]. Here we demonstrate how polyethylene glycol functionalised poly-lactide-co-caprolactone (PLCL-PEG) can be blended with PLLA, in order to control both the degradation timescale, and the structural and mechanical changes undergone during degradation.

Materials and Methods

Polymer blends were made using solvent casting and injection moulding, with mechanical testing carried out immersed in deionised water at 37°C. Degradation behaviour was measured in phosphate-buffered saline at 37°C by pH monitoring. Polymer blends were characterised before and after degradation by Differential Scanning Calorimetry (DSC), X-ray Diffraction (XRD), and Gel Permeation Chromatography (GPC).

Results and Discussion

Long-term degradation tests show strong dependence of degradation time on blend composition, demonstrating the ability to controllably accelerate PLLA degradation via blending. The two blend components (PLLA and PLCL-PEG) do not simply degrade independently of each other, but rather the degradation products released by the fast degrading polymer (PLCL-PEG) catalyse and accelerate the degradation of the slower degrading PLLA component. This is shown by the molecular weight distributions (Fig. 1), where the measured blend degradation is greater than would be expected for independently degrading components.

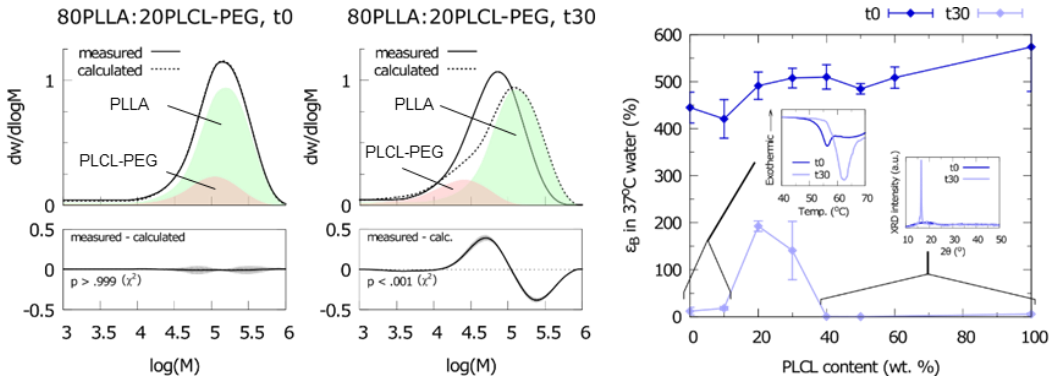

Fig. 1: Molecular weight distributions for PLLA:PLCL-PEG blends before and after 30 days degradation (left and middle). Calculated curves based on linear combination of individual components before and after degradation. Elongation at break (in 37°C water) for PLLA:PLCL-PEG blends before and after 30 days degradation, with DSC and XRD plots inset (right).

Important changes in the mechanical properties during degradation are also observed (Fig. 1). The large initial ductility is lost after 30 days degradation for most blend compositions. For blends with low PLCL-PEG content this occurs due to aging (shown by increased T_g and larger associated endothermic peak in DSC) as the relatively homogeneous composition provides little barrier to rearrangement. For high PLCL-PEG content, molecular weight degradation has shortened chain length sufficiently to allow significant rearrangement i.e. crystallisation (shown by XRD), also resulting in embrittlement. These two effects result in a “sweet spot” of moderate PLCL-PEG content that is high enough to prevent aging, but not so high as to cause degradation-induced crystallisation, leading to delayed structural relaxation and embrittlement for these compositions.

Conclusions

Blending PLCL-PEG with PLLA controllably accelerates hydrolytic degradation. In large amounts this leads to crystallisation and embrittlement, however in smaller amounts PLCL-PEG balances faster degradation and resistance to the structural relaxations (aging, crystallisation) that cause embrittlement. These results pave the way towards achieving bioresorbable materials that have (and retain) favourable mechanical properties along a suitable degradation timescale.

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REGULATION OF THE HIF PATHWAY FOR CONTROLLED BONE REMODELLING IN PATIENTS WITH IMPAIRED FRACTURE REPAIR

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Introduction

Diabetic and elderly patients have a lower rate of bone remodelling, more crystalline bone, together with increased risk of fracture and delayed fracture repair [1]. A decrease in osteoclastogenesis and osteoclast activity in these patients may be partly responsible for such pathologies [2]. Novel approaches are needed to encourage healthy bone regeneration in these patients. The cellular response to changes in oxygen pressure (regulated by Hypoxia Inducible Factor 1α, HIF-1α) has been identified as an important upstream regulator of bone tissue regeneration and a regulator of osteoblast-osteoclast cross-talk [3]. Diabetic patients have also been shown to have a decreased response to changes in oxygen pressure due to the inhibition of the HIF pathway in bone cells [4]. Targeting the HIF pathway through the release of HIF mimetics from bioactive glasses may offer a new approach to regulate bone repair in these patients. Here we investigate if three known HIF mimetics (cobalt, DFO and DMOG) can regulate osteoclastogenesis using quantitative in vitro models. These studies will be used to develop new materials that can regulate bone remodelling.

Materials and Methods

An osteoclastic RAW 264.7 sub-clone cell line was generated and cultured in Dulbecco's Modified Eagle Medium (DMEM) GlutaMAX™, supplemented with 10% (v/v) foetal bovine serum (FBS) and 1% (v/v) penicillin streptomycin (P/S), seeded at $3x10^3/cm^2$ and a passage number <20. Cells were cultured with 3ng/ml of RANKL for experimental treatments with the HIF mimetics (Co, DMOG and DFO), a positive control (20ng/ml RANKL) and negative control (0 ng/ml RANKL) were included. The effect of HIF mimetics on osteoclast metabolic activity and proliferation was determined with Alamar® Blue and Total DNA assays. Osteoclastogenesis was determined with TRAP-5b staining and resorption of dentin discs (quantified by coherence scanning interferometry). Echinomycin was used to inhibit the HIF pathway.

Results and Discussion

The HIF mimetics Co (12.5-200μM), DFO (25-100μM) and DMOG (100-400μM) increased osteoclastogenesis as determined by the expression of the osteoclast specific TRAP-5b factor ($p<0.001$). The HIF inhibitor echinomycin, decreased TRAP-5b expression (Fig.1B $p<0.001$) confirming the role of the HIF pathway. Quantification of osteoclast activity on dentine discs could provide evidence of bone resorption enhancement by HIF mimetics.

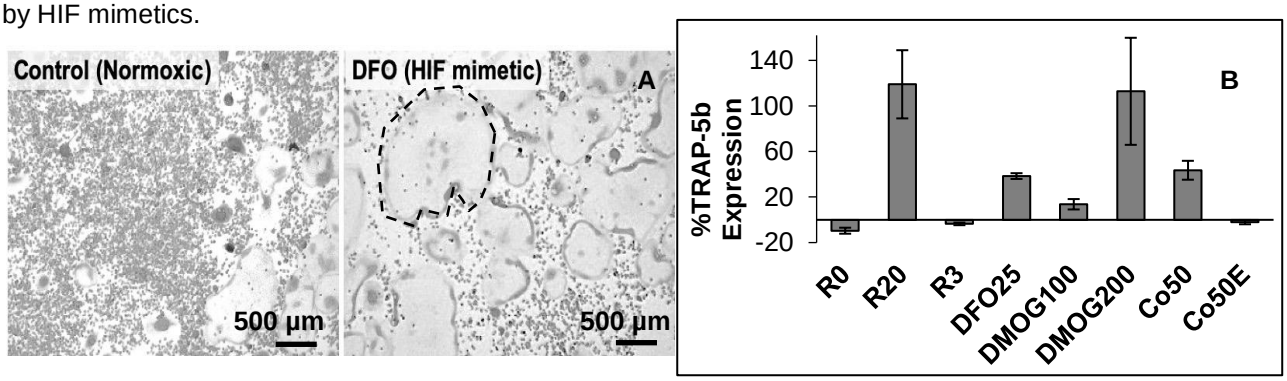

Figure 1. *Osteoclast formation determined by HIF mimetics. (A) TRAP staining of osteoclast RAW 264.7 subclone with DFO (HIF mimetic) vs control (3ng/ml RANKL). An osteoclast is indicated by the dashed shape. (B) %TRAP-5b expression control (0ng/ml, 20ng/ml, 3ng/ml RANKL and 50μM Co + 2nM echinomycin) vs HIF mimetics (25μM DFO, 100μM and 200μM DMOG and 50μM Co). ***p<0.001, n=6 for experimental conditions.*

Conclusions

The release of HIF mimetics (Co DFO and DMOG) increased osteoclastogenesis and the rate of bone resorption. The next generation of bone repair biomaterials can take advantage of this by regulating both bone remodelling (osteoclasts) and bone formation (osteoblasts).

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UNIQUE PATTERNS OF ELASTIN DEGRADATION IN ASCENDING AORTIC ANEURYSMS IN BICUSPID AORTIC VALVE PATIENTS

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Introduction

Bicuspid aortic valve patients (BAV) are associated with increased risk of ascending aortic aneurysms. However, is unclear whether matrix degradation varies in different ascending aneurysm aetiologies. Here, we measured the micromechanical and biochemical properties and characterised elastin microstructure within the aortic tissue of two specific aneurysmal groups; BAV with associated aneurysm (BAV-A) and idiopathic degenerative aneurysm (DA). Aneurysmal tissues are compared against control tissue.

Methods

Aortic tissue was obtained from patients undergoing aneurysmal repair surgery (BAV-A; n=15 and DA; n=15). Control tissue was punch biopsies obtained during coronary artery by-pass graft (CABG; n=9). The elastic modulus (E) was measured with nanoindentation for the medial layer. Glycosaminoglycan (GAG), collagen and elastin levels were measured using biochemical assays. Verhoeff Van Gieson-stained sections were imaged for elastin microstructural quantification.

Results

BAV-A had over 20% higher E relative to control and DA. No significance between DA and control due to tissue heterogeneity. Collagen level of BAV-A (36.9±7.4µg/mg) and DA (49.9±10.9µg/mg) was higher compared to the control (30.2±13.1µg/mg). GAG and elastin levels were not significant between the groups. Elastin segments were uniform in controls. Aneurysmal tissues had loss of segments close to the intima and adventitia layers. Although BAV-A and DA had more elastin segments compacted in the media, elastin segments were highly fragmented in DA.

Conclusions

BAV-A has increased stiffness within the aortic wall relative to DA and control tissue. Although elastin levels were equal for all groups, spatial distribution of elastin provided us with a unique profile of matrix degradation for BAV-A. The findings of this work are important for the development of future clinical treatment of BAV-A treatment.

THE APPLICATION OF COLD ATMOSPHERIC PLASMA GAS TO DIRECT WOUND HEALING IN EQUINES

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Introduction

Equines are, generally, excellent wound healers, as they are capable of repairing huge skin and muscle traumas. However, in some cases the healing process becomes compromised which can lead to the wound becoming infected. The typical treatment of equine wounds is through cleaning, debridement and bandaging, but wounds can still become infected. More than 90% of chronic wounds contain biofilms which are resistant to treatments. Our research is centred using cold atmospheric plasma to improve the efficacy of wound healing in equines. The emergence of plasma medicine has provided some hope for advancement in wound closure rates for patients with chronic wounds and some positive clinical results have already been observed. However, the potential to combine antimicrobials with plasma medicine has not yet been widely explored and this study outlines one potential way to combine such therapies.

Materials and Methods

Collagen with and without antimicrobials, was introduced into the discharge of cold atmospheric plasma and the activated materials were deposited onto surfaces to produce a dry and adherent coating. The plasma device was then used to deliver the collagen and antimicrobials into bacterial and eukaryotic cultures and the antimicrobial, cytotoxic and wound healing effects were compared to untreated and plasma only treated reference controls.

Results

Surface analysis using FTIR showed that the plasma deposit retained the chemical features of the dissolved protein and antimicrobials. The plasma deposited collagen was shown to effectively promote re-epithelialisation compared to the control. Although plasma treatment alone enhanced re-epithelialisation, the collagen treatment produced a statistically significant ($p < 0.05$) improvement in the rate of angiogenesis and re-epithelialisation. Plasma deposited antimicrobials showed an increased effect in bactericidal activity when compared with plasma treatment alone. The antimicrobials showed cytotoxic activity to eukaryotic cells at high concentrations, however, this was not seen at low concentrations.

Discussion

High energy plasma devices have been shown to kill cells, cauterize flesh and fragment chemical precursors. The data in this research demonstrated that using the above setup, the plasma-modified collagen formed a surface layer which largely retained the chemical properties of the starting material. Treatment with plasma alone showed a beneficial effect for wound healing, while, higher levels of re-epithelialisation and angiogenesis were demonstrated using collagen. From this research, it can be deduced that exposing fibroblasts to cold atmospheric plasma can induce wound healing factors and these can be improved further by plasma deposited collagen. It has been known for a number of decades that certain naturally forming elements have antimicrobial activity; this has also been shown by cold atmospheric plasma. There has, however, been little research in the combination of plasma and other antimicrobials. These investigations showed that plasma treatment alone had bactericidal effects and this effect was further improved by the deposition of antimicrobials via the plasma.

Conclusions

The aim of this study was to determine the antimicrobial and wound healing effect of plasma deposited materials which had not been explored in depth previously. We determined that combining both an antimicrobial and biological therapy with a plasma treatment showed promising results in the treatment of chronic wounds and demonstrated the potential in combining biological therapies with plasma deposition for targeted delivery and enhanced healing.