UK Society for Biomaterials

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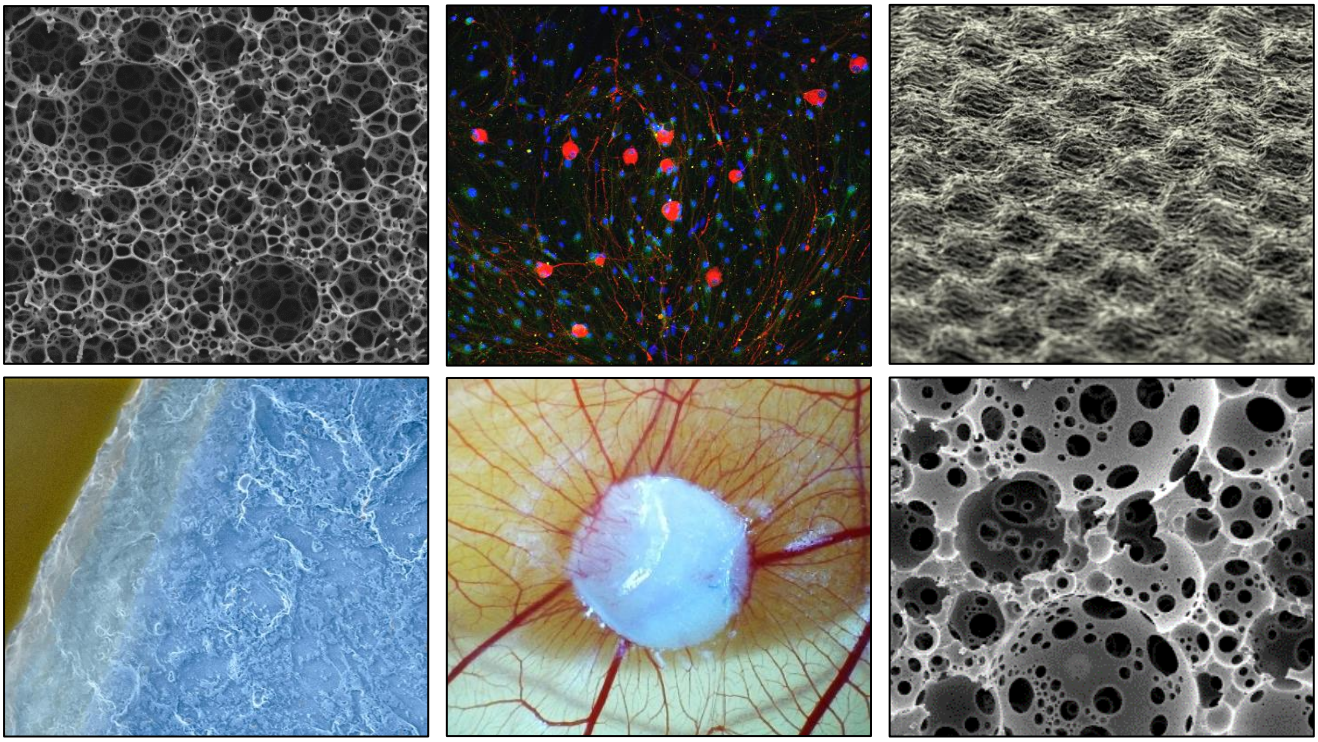
UK Society for Biomaterials

22nd Annual Conference



27th - 28th June 2022

Abstract Booklet

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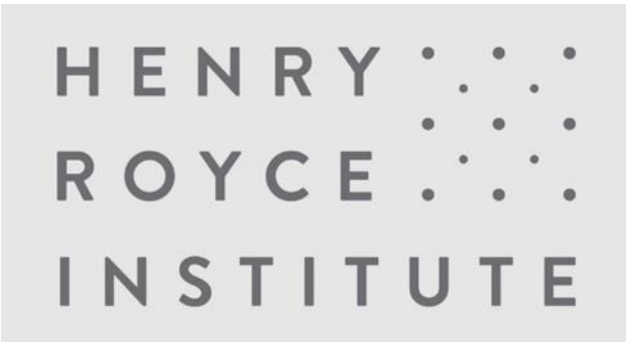

Cover Images
Top Left: Colin Sherborne – PolyHIPE Materials
Top Middle: Caroline S Taylor – Dorsal Root Ganglion Cells
Top Right: Mina Aleemardani – PGS-co-PEG Dermal-Epidermal Junction (DEJ) Scaffold
Bottom Left: Boyang Lui – PCL Films
Bottom Middle: Sarah Shafaat – Polyurethane CAM Assay
Bottom Right: Colin Sherborne - PolyHIPE Materials

UKSB President's Welcome

Dear #UKSB2022 delegates,

It is my pleasure this year to welcome you all to this belated anniversary meeting. The council had planned to hold a 20th anniversary meeting, but with the covid pandemic hitting us, we decided to go virtual for two years and didn't feel that such a platform would be suitable to celebrate such an occasion. This year we are back to physical meetings, and we welcome many of the founding members of the Society. For me personally this is an honour, and I look forward to reminiscing about the Society and previous events.

I attended the 2nd annual meeting of the UKSB when I was a 1st year PhD student, and over the many (more than I'd like to admit) years have seen the field develop. There has been an evolution of biomaterials chemistry and surface science, enabling cell control and improved medical device performance, changes in the way we characterise materials with advanced understanding of the characteristics important at the material-biology interface, and more recently a progression in technologies for the fabrication and modification of biomaterials and devices. This year the programme is as full as ever, covering broadly these areas, and more. It is great to see such interdisciplinary research and various communities working together to tackle current problems and address possibilities of the future.

The Society would be nothing if it did not represent and support its community. In particular, it is always nice to see old friends and meet new ones. Coming back to a physical conference we welcome many early career researchers who may not have attended a physical conference before. The Society has renewed its early career researcher forum, and I hope this will become a strong networking opportunity for the sharing of ideas, skills and expertise - we have a successful lab-2-lab awards scheme to help foster collaborative links, so please do take every opportunity that you can to strengthen your research and your network.

We have a series of prize winners this year who have shown dedication to the field of biomaterials at various career stages. These talks will likely set a scene for not only the advances made, but also the trials and tribulations navigated during the course of the research; it is the latter that provides a base for knowledge, and with it a balanced degree of patience. I'd like to thank all of you this year for your continued work, and support for the UK Society for Biomaterials.

Have a great time.

Best wishes,

Paul Roach

UKSB President

Conference Chair: Professor Cheryl Miller

Local Organising Committee
Dr Victoria Workman
Dr George Bullock
Ms Ohud Hussain
Ms Muntha Alhazmi
Mr Rayan Binduhayyim

UKSB2022 Conference Programme

The Edge
The Endcliffe Village, Endcliffe Crescent,
Sheffield S10 3ED (See **page 85** for details)

Day 1: Monday 27th June 2022

09.00	**Registration and Coffee**	
10.00	Welcome *Prof. Cheryl Miller and Dr Paul Roach*	
Session 1. Innovative biomaterial manufacture	*Chairs: Prof. Cheryl Miller and Dr Victoria Workman*	
10.05	**Keynote: Manufacturing Microspheres from Bioactive Glasses and their Potential Orthobiologic Applications Dr Ifty Ahmed**	**Pg. 9**
10:35	*Flash talks* 1. Laetitia Raynal, University of York, *Developing new functional ligands for protein modification* 2. Luis Marco, Loughborough University *Effects of surface chemistry interaction on neural stem cell derived retinal organoids* 3. Christopher Chong, La Trobe University *Incorporation of arginine-glycine-aspartic acid (RGD) ligands into diphenylalanine (FF) nanofibers scaffolds – exploration of nanoscale properties for tissue engineering* 4. Moira Lorenzo-Lopez, University of Liverpool *Label-free nanoparticle tracking for eye drug delivery* 5. Anna Rhodes, Imperial College London *Using silk fibroin/collagen composite biomaterials to promote in vivo-like healthy and fibrotic gene expression in fibroblasts* 6. Barber, University of York *Investigation of current strategies for N-terminal protein modification* 7. Cagla Erdas, UCL *Understanding the specific role of immunoglobulins in nanoparticle protein corona formation and biological interactions* 8. Ben Almquist, Imperial College London *Biomaterials for rewiring pathologic microRNA signaling in diabetic foot ulcers* 9. Noelia Falcon, University of East Anglia *Investigation into the effect of BMP-12 and TGF-β1 on stimulation of tenogenic markers in serum-free cultured human adipose-derived stem cells*	*Pg. 10-18*
11.05	Coffee/Tea & Posters	
Session 2. Antibacterial Biomaterials	*Chairs: Prof. Raechelle D'Sa and Dr George Bullock*	
11.35	Thomas Swift, University of Bradford *Highly branched biologically responding additives that lead to deformation in polyurethane wound dressings*	Pg. 19
	Man Li, University of Liverpool *Antimicrobial electrospun bandages for the treatment of wound infections*	Pg. 20
12.00	Gold sponsor talk: Elliot Gibson, Biopharma	
12.10	*Flash talks* 10. Siyun Liu, University of Birmingham *Alginate-silica hybrid hydrogels employing novel reagents and a "soft chemistry" sol-gel process*	Pg. 21-27

11. Chloe Stewart, University of Nottingham

Hydrogel formulations to modulate the foreign body response

12. Christopher Merrett, University of Nottingham

3D cell culture: hydrogels capable of in situ functionalisation with a single "click"

13. Saudah Hafeji, University of Leeds

Ankle ligament repair for restoration of function: exploring the application of decellularised grafts

14. Aoife McFerran, Ulster University

Multi-functional layered nanocomposite coatings for bioactive tissue scaffold development

15. Joe Woodley, University of Sheffield

Reduced fibroblast activation on electrospun polycaprolactone scaffolds

16. Joseph Barnes, University of Liverpool

Micro-CT characterisation of a dual antimicrobial-osteoinductive 'bone wrap' using a novel ex vivo chick femur defect model

12:35 *YSF update*

12.45 Lunch & Posters

- Young Scientists Forum (YSF) lunch in bar (upstairs)
- Standard lunch in ground floor

Session 3. Innovative Biomaterial Manufacture

Chairs: Prof. Julian Jones and Dr Annalisa Tirelli

13.45 Keynote: Moving additive manufacturing beyond anatomical customisation **Pg. 28**

Dr Sophie Cox

Nicola Contessi Negrini, Imperial College London Pg. 29

Handheld bioprinting of bioorthogonally crosslinked hydrogels

Amirpasha Moetazedian, University of Birmingham Pg. 30

Microfluidic-based 3D bioprinting to fabricate blood vasculature

14.40 Gold sponsor talk: Dr Marlin Magallanes, Glass Technology Services

Glasses for biomedical applications: from early stage to commercialisation

15.50 *Flash talks* Pg. 31-37

17. Turuvekere Krishnamurthy, Indian Institute of Technology Madras

Tetracycline-loaded isabgol (psyllium) nanoparticles functionalised with hyaluronic acid for accelerating wound healing

18. Jonathan Wilson, University of Nottingham

Calcium phosphate coatings for the degradation control and functionalisation of Magnesium-based alloys

19. George Bullock, University of Sheffield

Peptide-enhanced calcium phosphates for the promotion of bone tissue regeneration in vivo

20. Maria Florez Martin, University College London

Quantifying the effects of Si, B, and Co-releasing bioactive glasses on in vitro bone formation

21. Joshua Lau, University College London

The role of intermittent hypoxia on bone nodule formation in vitro

22. Morgan Thornber, University of Nottingham

Ex vivo spider silk, a novel look at an ancient biomaterial

23. Sahar AlOraibi, University College London

Investigating ionic therapy for Bisphosphonate Related Osteonecrosis of the Jaw

15.15	Coffee/tea & posters	
Session 4. Dental and Orthopaedic Biomaterials		
	Chairs: Prof. Paul Hatton and Dr Dan Merryweather	
15.45	Moresche Bartley, Queen Mary University of London *Electrospun PCL coated with collagen/hydroxyapatite for bone tissue engineering*	Pg. 38
	Eyyüp Karakurt, Brunel University London *Evaluation of biocompatibility of porous titanium-niobium alloys*	Pg. 39
	Joanna Ward, Ulster University *Effects of strontium-substitution in sputter deposited calcium phosphate coatings on the rate of corrosion of magnesium alloys*	Pg. 40
16.25	**Alan Wilson Memorial Lecture: The role of multifunctional polymeric dental biomaterials in tooth restoration** ***Prof. Sanjukta Deb***	**Pg. 41**
16.55	UKSB AGM	
20.00	Conference Dinner at the Mercure Sheffield St Paul's Hotel & Spa (S1 2JE) 7:30 for 8pm	

Day 2: Tuesday 28th June 2022

09:00	**Arrival and coffee**	
Session 5. Biomaterials Chemistry and Characterisation		
	Chairs: Dr Adrian Boyd and Dr Sam Moxon	
09.30	Sudha Varadaraj, Indian Institute of Technology Madras *Multiple cues in acellular amniotic membrane incorporated Embelin for tissue engineering*	Pg. 42
	Aram Saeed, University of East Anglia *Prefunctionalised biodegradable microcarriers for cell culture and cell delivery*	Pg. 43
	Jonathon Curd, University of Nottingham *Customisable peptide hydrogels as 3D biomimetic models of specific tissue microenvironments*	Pg. 44
	Daniel Baines, Lancaster University *Quantitative analysis of fluid content of pH treated whey protein isolate hydrogels in a swollen state using terahertz radiation*	Pg. 45
	Brian Saunders, University of Manchester *Highly-stretchable conductive covalent coacervate gels for electronic skin*	Pg. 46
10.30	Gold sponsor talk: Dr. Vladimir Korolkov, Park Systems UK Ltd *Park Systems AFMs: from resolving exquisite molecular structure in peptides to single capsomere imaging in viruses*	
10.40	Coffee/Tea Break and Posters	
Session 6. Sensing and Controlling Cellular Behaviour		
	Chairs: Dr Paul Roach and Dr Joanna Ward	
11.05	**Larry Hench Young Investigators Prize: Graphene formulations: new ways for manipulating medicine at the nanoscale** ***Dr Tanveer Tabish***	**Pg. 47**
11.35	Akhil Jain, University of Nottingham *Bifunctionalised electric field responsive conductive nanoactuators for glioblastoma multiforme*	Pg. 48
	Pui Lai Rachel Ee, National University of Singapore *Antimicrobial peptide-conjugated graphene-based optical lens for prevention and treatment of bacterial infections*	Pg. 49
	Ben Almquist, Imperial College London *Bioinspired nanotechnology for cellular traction force-mediated delivery of biologics*	Pg. 50
	Nuria Oliva, Imperial College London *Nanotechnology-enabled RNA therapies for tissue engineering and regeneration*	Pg. 51
12.25	Lunch and Posters	
Session 7. *Chairs:* **Addressing Clinical Needs**		
	Chairs: Dr Colin Scotchford and Dr Caroline Taylor	
13.20	**UKSB President's Prize: Biomaterials Solutions for Unmet Clinical Needs** ***Prof. Paul Hatton***	**Pg. 52**
13.50	Devon Crouch, University of Liverpool *Using decellularised human trabecular meshwork as a blueprint for fabricating biomimetic electrospun scaffolds*	Pg. 53
	Jessica Senior, University of Huddersfield *Development of a complex 3D glioblastoma microenvironments*	Pg. 54

	Samuel Moxon, University of Manchester *A 3D bioprinted model of the human intervertebral disc*	Pg. 55

14.30	Coffee/tea & posters

Session 8. Tissue Engineering

Chairs: Dr Jude Curran and Dr Nicola Contessi Negrini

14.55	Aishah Nasir, University of Nottingham *"Chemo-topography" heart-in-a-dish platform for human cardiac modelling*	Pg. 56
	Caroline Taylor, University of Sheffield *Aminosilane coated polycaprolactone scaffolds for peripheral nerve repair*	Pg. 57
	Manohar Koduri, University of Liverpool *Functional nano glucose particle sensor (FN-GPS) in human mesenchymal stem cell (HMSC) environment*	Pg. 58
	Dan Merryweather, Loughborough University *Microfabrication of defined circuits of human neural progenitor cells within a brain-on-chip device*	Pg. 59

15.45	Prizes
16.00	Meeting close

Session 1: Keynote

Manufacturing Microspheres from Bioactive Glasses and their Potential Orthobiologic Applications

Dr Ifty Ahmed

Dr Ifty Ahmed's research has centred on manufacture of resorbable biomaterials for tissue engineering and regenerative medicine applications. His research has spanned developing resorbable phosphate-based glasses and their biocomposites (as resorbable implant materials). Phosphate-based glasses are unique amorphous biomaterials due to their fully resorbable characteristics, which can be made to completely dissolve in aqueous environments with controllable degradation rates (from days, weeks/months to several years). His group has recently developed a process for manufacturing highly porous glass microspheres, which are up to 80% porous with fully interconnected porosity.

Dr Ifty Ahmed is an Associate Professor at the University of Nottingham, based within the Faculty of Engineering, Advanced Materials Research group. He was appointed as Assistant Professor in Sept 2013 and promoted to Associate Professor in Aug 2015. Dr Ahmed's laboratories at Nottingham are equipped with melt-draw and preform draw glass fibre manufacturing processes and he has developed flame spheroidisation processes to manufacture solid (dense) and highly porous microspheres. He has mentored 5 postdoctoral researchers, successfully managed 26 PhDs to completion and has published over 140 journal publications, 6 book chapters and 2 patent applications. He was also the UKSBs first recipient of the 'Larry Hench Young Investigators Award'.

Abstract:

In this talk he will highlight some of the recent work from his group in developing microspheres from bioactive glasses and how he developed porous glass microspheres (from a range of bioactive glasses). He will also showcase some recent work on developing magnetic biomaterials.

Session 1: Flash Poster 1

DEVELOPING NEW FUNCTIONAL LIGANDS FPR PROTEIN MODIFICATION

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*Corresponding author: lr1078@york.ac.uk – PhD student (3rd year)

Introduction

In recent years, it has been shown that synthetic biomaterials are not optimal supports for tissue growth due to their lack of intrinsic biological properties. To improve the biological potency of biomaterials and increase the reach of tissue engineering (TE), functionalisation with proteins has been attempted to enhance the biochemical signalling capabilities of the materials. However, when these proteins are attached via non-specific conjugation chemistries, they suffer a drastic loss of activity. There is therefore a need for generalised chemistries that can conjugate proteins to materials without loss of activity.
Our goal is to develop affinity-guided chemistries that allow proteins to be attached to biomaterials in a highly specific manner, providing these materials with the potent biological functionality required to support tissue growth.[1,2]

Materials and Methods

Peptides identified by phage display to bind to the protein of interest have been synthesized in house on a solid-phase peptide synthesizer using Rink Amide resin to give C-amide terminus peptide.

Results and Discussion

To modify the growth factor FGF-2, a library of peptides has been synthesized to perform ligand-directed chemistry (LD) at a single site on the protein. These peptide ligands are modified at their N-terminus with probes able to undergo LD chemistry such as *N*-acyl-*N*-alkyl sulphonamide (NASA) and *N*-acyl imidazole motifs (**Figure 1A**). Others probes such as photo-affinity probes and fluorophores have also been inserted at the N-terminus to ensure that modification does not affect the binding to the protein. Using analytical techniques such as fluorescence polarization and MS-MS, peptide binding affinities were measured and sites of binding deduced. At a later stage, specific chemical groups will be introduced for further attachment to a biomaterial (**Figure 1B**).

Conclusions

This chemistry has shown that using peptides could have a major advantage compared to classic organic binding probes in order to provide a more precise means to site-specifically modify proteins and allow their conjugation to biomaterials without a loss of activity.

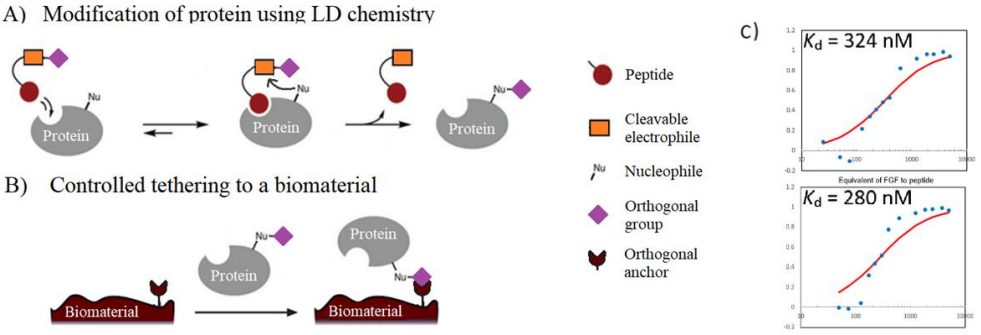

Figure 1: A) Scheme to modify selectively a protein through ligand-directed chemistry (Lg being the binding ligand, Cat the catalyst and Nu a nucleophilic amino acid nearby the binding site), B) Subsequent controlled tethering to a biomaterial C) Fluorescence polarisation binding curves of FGF-binding peptides after N-terminal modification.

References

1. K. Amaike, T. Tamura and I. Hamachi, *Chem. Commun.*, 53, 11972–11983, 2017.
2. K. Shiraiwa, R. Cheng, H. Nonaka, T. Tamura and I. Hamachi, *Cell Chem. Biol.*, 27, 970–985, 2020.

Acknowledgements

L. Raynal and C.D. Spicer are grateful to the Rosetrees Trust for PhD Studentship funding (agreement A2413). We also thank Dr. Dimitris Lagos, Dr. Ed Bergstrom and all the Spicer group.

Session 1: Flash Poster 2

EFFECTS OF SURFACE CHEMISTRY INTERACTION ON NEURAL STEM CELL DERIVED RETINAL ORGANOIDS

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[2] Centre of Biological Engineering, Wolfson School of Mechanical, Electrical and Manufacturing Engineering, Loughborough University, Leicestershire. LE11 3TU. UK
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Introduction:

The physical and chemical characteristics of a determined material are of great relevance for its interactions with biological species. As so, the surface chemistry of a given material plays a pivotal role in the modulation of cells behaviour; However, such responses are not yet fully understood and absolutely characterised. Analysis and understanding of such dependent behaviours are essential when considering different options of new biomaterials in regenerative medicine. Organoid systems are becoming more interesting for clinical applications, however control over their differentiation remains a challenge *in vitro*. Here we demonstrate the use of surface chemistry to dictate changing responses to maturing Retinal Organoids (RO).

Material and Methods:

In this study we show the functional behavior of neural stem cells derived RO within a range of alkane-based self-assembled monolayers presenting different functional groups: NH_2 ((3aminopropyl)triethoxysilane)), phenyl (phenyltriethoxysilane), CH_3 (chlorotrimethylsilane) and OH (plasma treated glass). Briefly 13 mm glass coverslips were coated with 0.1 mM silane solution in dry toluene overnight and let air dry. Surfaces were characterized by water contact angle, XPS and FTIR. The impact of the different surface chemistry upon cell responses was evaluated as per day 15 RO attachment, cell spreading and migration from the organoid. Differentiation, of otherwise non-retinal neurons, was evaluated too through the expression measurement of markers for retinal photoreceptors (CD73) and retinal ganglion cells (Brn3a). Collected data on days 5, 10 and 15 shows the differential attachment and cell development within the organoids through the different surfaces.

Results and Discussion:

Preliminary results show a positive trend of organoids attachment towards more hydrophilic surfaces i.e., (3aminopropyl)triethoxysilane (NH_2) and plasma treated glass (OH). In such surfaces cells have migrated up to 3.8 and 3.5 mm respectively from the edge of the organoid, creating an intricate and complex neural network, in which developmental photoreceptors are present. On the other hand, ganglion cells have not been observed to migrate outside of the organoid, and their numbers decrease with time, in line with the literature. Results show a higher number of ganglion cells on attached RO versus floating samples within the same surfaces, being NH_2 the surface with higher numbers of ganglion cells present in the RO.

Conclusions:

Altogether, the initial results highlight the potential that defined chemical cues have to modulate neural stem cell migration and differentiation withing 3D living tissues. The development of new biomaterials that can offer control of such cell parameters is essential and will have a great impact in the development and scale-up of new cell therapies, particularly allowing the enrichment of the required populations withing the retina.

References:

1. Eiraku M *et al.* Nature. 472(7341),51–56, 2011.
2. Joseph, G *et al.* ACS Omega. 6(30), pp.19901-19910, 2021
3. Marcos L. F *et al.* Journal of Tissue Engineering. 12, 20417314211059876, 2021

Session 1: Flash Poster 3

INCORPORATION OF ARGININE-GLYCINE-ASPARTIC ACID (RGD) LIGANDS INTO DIPHENYLALANINE (FF) NANOFIBRES SCAFFOLDS – EXPLORATION OF NANOSCALE PROPERTIES FOR TISSUE ENGINEERING

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INTRODUCTION

Osteoarthritis (OA) is a degenerative joint condition characterised by destruction of articular cartilage and joint inflammation. Hydrogels and mesenchymal stem cells (MSCs) have emerged as a regenerative solution with the potential to overcome limitations of existing regenerative techniques (microfracture, mosaicplasty, autologous chondrocyte implantation) which typically provide tissue repair of poor quality and have high failure rates. This technique is also significantly less invasive than the most utilised surgical method, total joint arthroplasty (replacement). A well-suited scaffold for cartilage tissue engineering is said to promote cartilage regeneration through accurate mimicry of chemical and biophysical properties of cartilage extracellular matrix (ECM). Fluorenylmethyloxycarbonyl (Fmoc) protected peptides are one family of synthetic peptides identified as attractive building blocks for biocompatible hydrogels, due to their characteristic self-assembly into nanostructures at physiological pH.

METHODS

We utilised a variety of techniques on hybrid Fmoc-FF (diphenylalanine) -RGD (arginine-glycine-aspartic acid) gel systems (3 varieties: 100 % Fmoc-FF, 99 % Fmoc-FF / 1 % -RGD, and 95 % Fmoc-FF / 5 % -RGD) to explore gel-cell biological interactions, nanofibrillar architectures and mechanical properties of nanofibrils at both a nano- and meso-scale. Selected assays included biomass degradation studies, transmission electron microscopy (TEM) and quantitative nanofibrillar analyses, MSC culture, rheology, small-angle x-ray scattering (SAXS) and cell viability studies (live-dead fluorescence microscopy, resazurin metabolic assay).

RESULTS and DISCUSSION

We were able to demonstrate that -RGD ligands are incorporated into the nanofibrillar structure of hybrid Fmoc-FF hydrogels in a concentration-dependent manner, and confer enhanced cell-supporting properties than pure 100 % Fmoc-FF gels alone. Surprisingly, greater incorporation of RGD occurred at lower concentrations (i.e. 1 %) with distinct biophysical features seen in nanofibrillar morphologies visualised on TEM, and through quantitative measures – this appears due to tendencies for Fmoc-RGD peptides to preferentially co-aggregate with Fmoc-FF peptides to form hybrid nanofibrils at lower RGD concentrations. At higher RGD concentrations (i.e. 5 %), more structurally unstable and softer gels were produced and this appears to be linked to tendencies of RGD to incorporate less within growing nanofibrils and instead self-aggregate amorphously. Heterogeneous mixtures of Fmoc-FF nanofibrils and RGD aggregates result in incomplete gelation and premature degradation of 95 % gels. The inclusion of RGD also enhanced the viability of MSCs upon these gels for up to 6 days, with improved cell survival seen in 99 % gels.

CONCLUSION

We have identified that 99 % Fmoc-FF, 1 % -RGD gels may offer a suitable balance of gel stability, RGD ligand availability for cell adhesion, and avoid disordered, unstable gels seen with greater RGD; properties are desirable for tissue engineering. Gel stability can be linked to RGD content. Our findings are expected to have direct implications for optimising Fmoc-FF / -RGD scaffolds for cartilage tissue engineering.

Session 1: Flash Poster 4

LABEL-FREE NANOPARTICLE TRACKING FOR EYE DRUG DELIVERY

Moira Lorenzo López[1*], Professor Eann A. Patterson[1], Dr Victoria Kearns[2] and Dr Jude M. Curran[1].
[1]School of Engineering / University of Liverpool, [2]Institute of Life Course and Life Science / University of Liverpool.
[*]Moira Lorenzo López: m.lorenzo-lopez@liverpool.ac.uk – PhD student (1st year).

Introduction: Drug delivery to the back of the eye is still a challenge for the treatment of sight-threating conditions such as diabetic retinopathy. Despite the optimization of some drug delivery systems, the current treatment of diabetic retinopathy is based on monthly intravitreal injection of drugs (1), which is often compromised due to poor patient adherence to the treatment and an increase of complications from the intravitreal administration route. Advances in nanomedicine hold potential for the stabilization of the drugs and to increase their bioavailability in the eye (2). For this to be successful models are required, which characterise the diffusion of nanoparticle delivery vehicles through the vitreous body and to the cell layer. Here we present the development of a label-free real-time tracking technology, based on caustics, which can be applied to developing pre-clinical screening models which are designed to assess the diffusion of nanoparticles through biological solutions and interactions with adherent cell surfaces. The data presented characterizes the diffusion of nanoparticles in a vitreous humour- like environment and provides a mechanism for optimising drug delivery to the back of the eye for the treatment of diabetic retinopathy.

Materials and Methods: As the vitreous humour is a highly viscous gel containing a vast amount of proteins such as collagen and glycosaminoglycan (hyaluronic acid), in order to validate the real-time, label-free nanoparticle tracking system for different -negatively coated- gold nanoparticles sizes (20 nm, 40 nm and 100 nm) we used different viscous media as a simplification of the vitreous humour (0%, 25%, 50% and 75% of glycerol in deionized water). Their diffusion coefficient at low concentration values (10^7 particles/mL) was recorded with a Zeiss inverted optical microscope, where the phenomena of caustics enable the localization and tracking of nanoparticles by the formation of signatures several orders of magnitude larger than the particles (3). The data were analysed using TrackMate software to obtain experimental values for their diffusion coefficient, which were then compared to the theoretical values obtained from the Stokes-Einstein equation.

Results and Discussion: From the comparison of the theory and experimental values of the diffusion coefficient, we can conclude that, on the one hand, the Stokes-Einstein equation overpredicted the diffusion of 20 nm and 40 nm gold nanoparticles at this low concentration, while on the other hand, the experimental diffusion coefficients for 100 nm gold nanoparticles seem to agree with the theoretical values at the same concentration of 10^7 particles/mL. At low concentrations nanoparticles are mostly surrounded by the liquid molecules in the solution, thus lowering the probability of particle to particle interactions, hence, it is postulated that these collisions can be neglected, which results in lower value for the diffusion coefficient. We here present some insights on the possible existence of a critical size at 100 nm -for negatively coated gold nanoparticles- for the Stokes-Einstein equation deviation, at low concentrations of 10^7 particles/mL, in different viscous media.

Conclusions: The data presented in the paper demonstrates that caustics is a platform technology that can be used to characterize the diffusion of clinically-relevant concentrations of nanoparticles in an array of solutions in real time. The data will be used to enhance the design and development of pre-clinical testing regimes and vehicles for drug delivery.

References:

1. Wang, W. and Lo, A.C.Y. International Journal of Molecular Sciences. 19:6, 2018.
2. Kim, H.M. and Woo, S.J. Pharmaceutics. 13:108, 2021.
3. Patterson, E. A. and Whelan, M. P. Nanotechnology. 19:105502, 2008.

Acknowledgements: Funding bodies; Doctoral Network in Technologies for Healthy Ageing and The Engineering and Physical Sciences Research Council.

Session 1: Flash Poster 5

USING SILK FIBROIN/COLLAGEN COMPOSITE BIOMATERIALS TO PROMOTE *IN VIVO*-LIKE HEALTHY AND FIBROTIC GENE EXPRESSION IN FIBROBLASTS

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Introduction

During normal wound healing, transient myofibroblast activation is necessary to enable wound closure via physical contraction and for the synthesis of new extracellular matrix (ECM) elements[1]. In contrast, fibrosis is characterised by persistent myofibroblast activation[1], and is a hallmark of several skin diseases, including scleroderma, keloidal and hypertrophic scarring, all of which are detrimental to patients' quality of life[2]. TGF-ß1 signalling is widely accepted as an important driver of fibrosis, but inhibition of this pathway has caused side effects such as auto-immunity and carcinogenesis[3]. Hence, there is a need to improve our understanding of the biology underlying fibrosis in order to identify novel anti-fibrotic targets and develop more effective therapies. Conventional *in vitro* 2D culture systems facilitate abnormal cell morphology and disturb cellular gene and protein expression, and thus, they are not biologically representative of an *in vivo*-like milieu[4]. Our aim was to develop a model that more accurately recapitulates the structural and mechanical landscape of the physiological dermis microenvironment. We adapted a 3D *ex vivo* dermis model originally developed by Vidal *et al*.[5] to recapitulate both healthy and fibrotic skin microenvironments. This model is comprised of a composite silk fibroin/collagen gel; collagen provides a familiar extracellular environment whilst silk preserves the mechanical integrity of the matrix[5].

Materials and Methods

To synthesise the gels, silk fibroin (4.5-18 w/v %) was mixed with rat tail collagen (1-5 mg/ml), and cell culture medium (DMEM + 10% FBS). Silk was crosslinked with 20 mg/ml horseradish peroxidase and 5% hydrogen peroxide for 4 hours at 37°C. Storage (G') and loss (G") moduli of human skin and gels were measured as a function of oscillatory strain at 37°C with a rheometer using a cross-hatched geometry and a normal force of 0.1N. Following a 24-hour serum starvation period, fibroblasts were activated into myofibroblasts by administering 5 ng/ml TGF-ß1 every day for 3 days. To characterise cellular gene expression, mRNA was extracted, converted into cDNA, and selected genes were amplified using commercially available primers.

Results and Discussion

Using a biomimetic approach, we originally sought to explore the role of collagen I in determining the gel's mechanical properties, as one of the hallmarks of fibrosis is increased collagen production[1]. Unexpectedly, we observed only non-significant changes in storage modulus when collagen content was varied. In fact, we demonstrated that the silk fibroin component of the hydrogel is much more influential on storage modulus. A range of formulations were investigated, with 13-16% silk-2.5 mg/ml collagen gels best approximating the storage modulus of human skin (242-466 Pa). Gel formulations with higher silk content conferred increased stiffness, but also induced failure at lower oscillatory strains compared with formulations with lower silk content. This is also true of scarred tissue, which is stiffer, but weaker than healthy tissue[6]. Interestingly, fibroblasts and myofibroblasts did not show any significant difference in *COL1A1* gene expression when cultured in standard well plates, whereas some gel formulations elicited significant upregulation of *COL1A1* in myofibroblasts. Current studies aim to further elucidate the origin of this.

Conclusions

In this work, we show that silk fibroin enables mechanical tuneability of this model system, rendering it a good candidate to investigate fibrotic skin disease. We expect to use this model as a biomimetic, high-throughput screening platform for novel therapies to reverse fibrosis.

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Session 1: Flash Poster 6

INVESTIGATION OF CURRENT STRATEGIES FOR N-TERMINAL PROTEIN MODIFICATION

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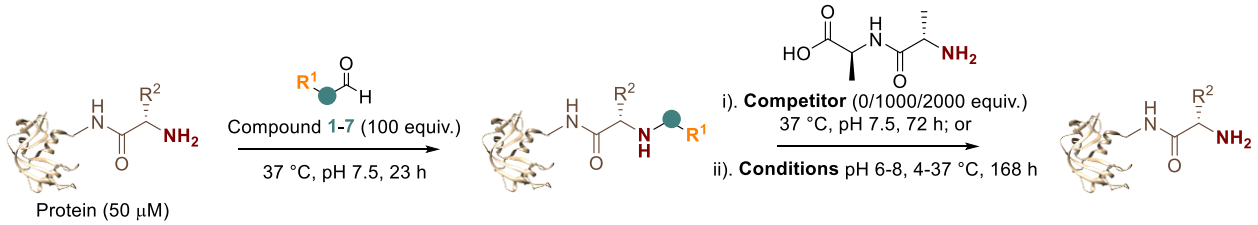
Introduction

N-terminal targeting has emerged as a powerful means to functionalise proteins, for example in the synthesis of protein-polymer conjugates for tissue engineering.[1] However, selectivity is often poor or the conjugates formed suffer from instability;[2] identifying the most suitable N-terminal modification strategy for an intended biomaterials application is therefore critical. We have undertaken a detailed comparative study of leading N-terminal modification strategies to provide key insight into the formation and utility of the resultant protein-conjugates, and a reference point from which these properties can then be improved.

Materials and Methods

A range of N-terminal targeting small molecules were screened against a panel of representative proteins under standardised conditions, and the stability of the protein conjugates to competitive nucleophiles and a range of conditions such as temperature and pH was assessed. We also studied the kinetics of conjugation on model peptides via ^{1}H NMR spectroscopy to calculate forwards and reverse kinetics of modification, and to gain an understanding of factors governing the selectivity.



Results and Discussion

Critically, all N-terminal modification strategies were found to exhibit slow kinetics and some extent of reversibility, with reaction efficiency and selectivity found to be highly protein dependent: there is no “one size fits all” approach to N-terminal protein modification.

Conclusions

This work highlights the need for the screening of a toolbox of complementary N-terminal modification strategies to ensure optimal properties are achieved for a given target protein and application. Next, we hope to build upon this work as a platform to develop new modification strategies that address current limitations.

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Session 1: Flash Poster 7

UNDERSTANDING THE SPECIFIC ROLE OF IMMUNOGLOBULINS IN NANOPARTICLE PROTEIN CORONA FORMATION AND BIOLOGICAL INTERACTIONS

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Introduction:

Nanoparticles (NPs) have great potential as targeted theranostic therapies[1]. The efficacy of targeting is, however, poor with a systematic review reporting that only 0.7% of targeted nanoparticles reach their intended target [2]. This may (in part) be due to the formation of a protein corona (PC) surrounding the NP causing changes to the NP physicochemical properties and causing non-target specific cellular interactions[3]. Understanding the importance of the protein corona on cellular interactions and developing in vitro models that can better predict how these NPs will interact in vivo, is urgently needed to help the translation of nanomedicines. Currently, the majority (97%) of in vitro studies investigating NPs-cell interaction use 10% foetal bovine serum (FBS). Compared to adult human serum, FBS has a different protein composition, the serum proteins have different Amino Acid sequence and, importantly, has low immunoglobulin content (Igs)[4]. Igs may play an important role in the size, aggregation, and NPs-cell interactions. This study investigates the importance of Igs (in the protein corona) in determining cellular interactions.

Materials and Methods:

Gold nanoparticle (AuNPs) uptake by human dermal fibroblasts (HDFs) was analysed in the presence and absence of Igs in HS and FBS using TEM and quantified with ICP-OES. To determine if uptake is caused by changes to the physicochemical properties and/or via immunoglobulin Fc receptor-mediated uptake we blocked the Fc receptor of professional phagocytic cells and evaluated the changes in the uptake.

Results and Discussion:

The composition of the media FBS, HS or Igs depleted HS changed the size and surface chemistry of the NP PC. AuNP cellular uptake significantly decreased in FBS compared to HS ($P < 0.001$). Igs depleted HS also caused a substantial decrease in uptake ($p=0.006$) (Fig. 1). The results on blocking the FcRs on immune cells will provide insight on whether the changes in cellular uptake are due the observed physicochemical changes or due to receptor-mediated uptake.

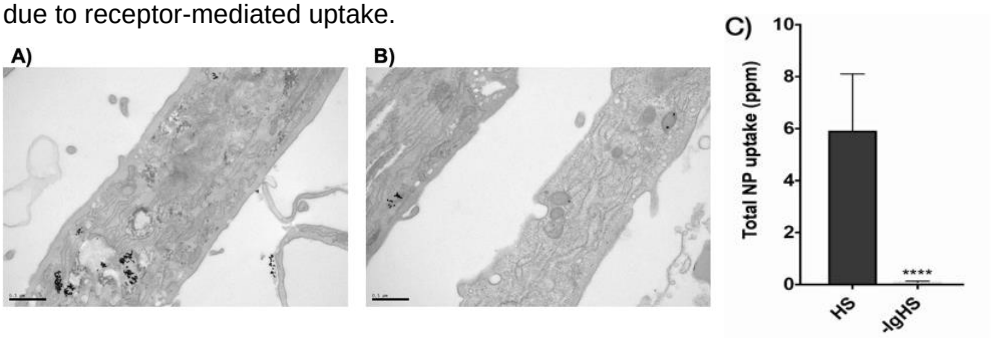

Figure 1: *TEM of AuNP cellular uptake in HS (A) and Ig depleted HS (B). AuNPs cellular uptake of NPs was much reduced in Ig depleted serum as measured by ICP-OES (C).*

Conclusions:

This study demonstrates the role of Igs in AuNP cellular uptake. It also highlights the need for more accurate and standardized in vitro models.

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Session 1: Flash Poster 8

Biomaterials for Rewiring Pathologic microRNA Signaling in Diabetic Foot Ulcers

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Introduction

Diabetes is an ever-increasing burden on society that carries with it a host of detrimental complications. Diabetic foot ulcers (DFU) are the leading cause of non-traumatic lower leg amputation, consume 1-3% of healthcare budgets in developed countries, and are painful, demoralising, and socially isolating. Despite their impact, there is still much that we do not understand about DFUs, the associated biological changes, and how to best address those changes to drive healing. Here, we explore how diabetes changes microRNA signalling to drive apoptosis in clinically amputated DFUs, and how we can design a materials-based delivery system to address these changes.

Materials and Methods

Nanostring sequencing data of cells DFU versus healthy site matched cells were analysed via a computational workflow including R, Ingenuity Pathway Analysis, and KEGG. Amputated DFUs and site matched healthy tissue were obtained via the Imperial College London Tissue Bank (HTA license 12275 and subcollection RSM-BA-15-062, REC Wales Approved Consent Forms 12/WA/0196) to establish in vitro cultures. Candidate microRNAs from the computational workflow were examined for functional behaviour, such as proliferation using RNAiMAX or (β-amino ester)s (pBAEs). Proteomics was conducted at the RPPA core at MD Anderson Cancer Center to elucidate the impact of microRNA delivery, with subsequent analysis done via Ingenuity Pathway Analysis.

Results and Discussion (unpublished, data not shown)

Sequencing data generated from clinical DFUs identified an overexpressed microRNA that is found at elevated levels in amputated DFUs. Interestingly, overexpression of this microRNA in cells derived from DFU patients drives apoptosis, in contrast to cells from healthy individuals, where it drives proliferation. Proteomics and pathway analysis suggests significant changes to cell signaling pathways that drive this differential response. To address this issue, an injectable, shape-filling pBAE-based nanoparticle/hydrogel system was developed to deliver microRNA inhibitors. This delivery system is shown to efficiently inhibit this pathological microRNA signaling.

Conclusions

We have identified a novel microRNA that is overexpressed in clinical DFUs. We show that the presence of diabetes drives a functionally divergent change that results in this microRNA driving the apoptosis of diabetic cells. To address this, we have developed a pBAE-based controlled delivery system to efficiently deliver microRNA inhibitors to mitigate this pathological signaling.

Acknowledgements

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Session 1: Flash Poster 9

INVESTIGATION INTO THE EFFECT OF BMP-12 AND TGF-β1 ON STIMULATION OF TENOGENIC MARKERS IN SERUM-FREE CULTURED HUMAN ADIPOSE-DERIVED STEM CELLS

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Introduction: Within the orthopaedic research field, 33 million musculoskeletal disorders are reported in the United States alone every year. It is estimated that more than half of these disorders are related to tendon tissue injuries, which are only set to increase, due to an ageing population and the prevalence of active lifestyles [1]. While tendon injuries could be treated with conservative treatments or surgical interventions in severe cases, both strategies fail to fully recover the tissue, leading to longer rehabilitation processes for patients. Therefore, alternative treatments such as stem cell-based therapies are becoming attractive revenues due to their regeneration potential. In addition, stem cells can be differentiated to other phenotypes in vivo or under physical and/or chemical cues in vitro. ADSCs are at the forefront of stem cell therapy due to less invasive isolation procedures, higher yield in cell number after purification, and excellent immunomodulatory properties. Growth Factors (GFs) such as Transforming Growth Factor β (TGF-β) and Bone Morphogenetic Proteins (BMPs) have been investigated as effective tools for driving tenogenic differentiation of stem cells. Although several studies have highlighted the promising effect of such GFs in the tenogenic differentiation of ADSCs [1, 2, 3], the use of animal-derived serum is a common feature. Serum is an unknown mixture of proteins and GFs and limits the application of cell-based therapies to humans. Therefore, the emergence of a well-defined method for differentiation of ADSCs toward tendon tissue is still lacking. Herein, we describe the tenogenic effect of BMP-12 and TGF-β1 in ADSCs cultured in *in vitro* serum-free conditions [4]. This culture system provides an important insight into serum-free culture conditions in stem cell differentiation protocols. In addition, we investigated the alignment of ADSCs in 3D scaffolds created from photopolymerisable materials with a pre-defined hierarchical topography, in order to evaluate a tendon-like scaffold for tendon tissue repair.

Materials and Methods: ADSCs were serum starved *in vitro* and treated with BMP-12 or TGF- β1 in the presence or absence of Ascorbic Acid (AA) in different doses and at different time points as the differentiation factor. After specific time points, the viability of ADSCs was analysed by Live/Dead staining and MTS metabolic activity assay. Then, the messenger RNA (mRNA) for key tendon markers was analysed by Real-Time Polymerase Chain Reaction (RT-PCR). Likewise, osteogenic, and cartilaginous markers were analysed. The protein expression was confirmed by Western blotting. In addition, the localisation of several markers was evaluated by Immunocytochemistry (ICC) techniques in permeabilised and non-permeabilised cells. For 3D cultures, ADSCs were likewise serum starved and cultured on photopolymerised scaffolds with hierarchical topography in the shape of parallel grooves. Cell alignment was analysed by Live/Dead staining and quantified using a directionality analysis using Image J.

Results and discussion: In this study, we evaluated the tenogenic effect of different GFs on ADSCs. Scleraxis, a transcription factor involved in tendon development, was upregulated at the mRNA level, but the expression was significantly different depending on the GFs used. Collagen type I, the most abundant type in tendon, was greatly induced. Interestingly, deposition of Collagen I on the extracellular matrix (ECM) by the ADSCs was altered depending on the differentiation factor, which was confirmed by ICC. In addition, cell morphology and confluency changed depending on the GF used. Our data suggest that specific environments may be needed for the induction of a tenogenic phenotype and for the potential application of stem-cell therapies. In addition, ADSCs displayed optimal cellular alignment with single directionality, validating the topography of the manufactured scaffold and its feasibility for a tendon-like scaffold, in which cellular alignment is imperative.

Conclusions: Overall, we believe ADSCs represent a promising approach for tendon regeneration and repair. This study emphasises the relevance of cell culture microenvironments for the development of more accurate and representative tenogenic differentiation tools for pluripotent stem cells, to develop efficient future cell-based therapies for tendon repair.

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Session 2

Session 2: Talk 1

HIGHLY BRANCHED BIOLOGICALLY RESPONDING ADDITIVES THAT LEAD TO DEFORMATION IN POLYURETHANE WOUND DRESSINGS

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Introduction

There is a worldwide need for a fast, rapid diagnostic sensor for pathogenic infectious species that can distinguish between microbial species to enable immediate treatment and improve antibiotic stewardship. Highly branched polymer materials that target specific species can be incorporated into biomaterials to produce highly specific swabs. We have demonstrated this via hydrogels for corneal swabbing[1] or polyurethane wound dressings for peri-wound exudate monitoring.[2]

Materials and Methods

Highly branched poly(N-isopropylacrylamide) (HB-PNIPAM), functionalized with vancomycin at the chain ends, acted as a bacterial adhesive and was incorporated into polyurethane foams to form semi-interpenetrating networks. PNIPAM was labelled with a solvatochromic dye, Nile red. The foams were selectively adhesive for *Staphylococcus aureus* (Gram-positive bacteria) compared to *Pseudomonas aeruginosa* (Gram-negative bacteria).

Results and Discussion

It was found that the thermal response of the polymer was dependent on the architecture,[3,4] and temperature-dependent colour changes were observed within the foam. The foams had open pore structures, and the presence of HB-PNIPAM substantially reduced the shrinkage of the foam as the temperature was increased up to 20 °C. The presence of *S. aureus* was indicated by increased fluorescence intensity (590–800 nm) but there was no response to gram negative strains.

Conclusions

Incorporation of HB-PNIPAM as an interpolymer complex through the PU foam resulted in a thermally responsive structure that responded dynamically (within seconds) to changes in its environment. The additive polymer can be tailored to bind different microorganisms and there is strong evidence of specificity in immediate disclosure for gram negative, gram positive bacteria or fungal pathogens.[1]

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Acknowledgement

The PU wound dressing formulation was based on products belonging to Smith and Nephew. Initial funding of this work for Professor Rimmer came from Smith and Nephew.

Session 2: Talk 2

ANTIMICROBIAL ELECTROSPUN BANDAGES FOR THE TREATMENT OF WOUND INFECTIONS

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Introduction

Diabetes affects 4.7 million people and will exceed 5.5 million by 2025 in the UK,[1] representing a significant burden to patients and the NHS. Chronic wounds caused by bacteria-associated infections are one of the most severe complications of diabetes, leading to diabetes-related amputations.[2] Nitric oxide (NO) is an attractive and highly effective antimicrobial that does not foster microbial resistance, in comparison to more traditional bactericidal agents such as antibiotics and antiseptics.[2-3] Delivery of this antimicrobial however is challenging as NO is a reactive gas with a relatively short half-life. This study aims to develop 3D electrospun membranes for antimicrobial wound dressings that are capable of delivering NO therapeutics in a controlled and sustained manner as well as mimic the mechanical characteristics of the skin tissue. Antimicrobial efficacy was evaluated against two common skin wound infection-related bacterial species, *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*). A cytotoxicity test was performed using a stratified skin model developed in house comprised of HaCaT (human keratinocyte cell line) and WS1 (human skin fibroblast cells).

Materials and Methods

Optimised nanofiber membrane layers were fabricated by electrospinning using blends of poly(ε-caprolactone) (PCL) and gelatin in mass ratios of 100:0, 70:30, 50:50 and 30:70, nomenclature as P100, P70, P50 and P30, respectively. The membranes were crosslinked with genipin followed by tethering of the NO-donor, *N*-diazeniumdiolates. The morphology of the samples was determined by SEM and the compositional analysis of diazeniumdiolate-modified samples was undertaken using FTIR. The stability of the crosslinked samples was examined in PBS at 37°C for up to 30 days. Mechanical properties of the samples before and after functionalisation were evaluated by tensile testing. The kinetics of NO release was monitored using a chemiluminescent NO detector at different pHs. The prevention of biofilm formation and eradication of biofilms were evaluated using CFU assays against *P. aeruginosa* and *S. aureus* biofilms over 24 hours. The cell viability was investigated on HaCaT and WS1 cells by a leaching assay.

Results and Discussion

The fiber diameters for the five formulations varied from 316 to 630 nm. The gelatin sample has an average fiber diameter of 550 ± 106 nm. Upon addition of increasing ratios of PCL to polymer blend solutions, the fiber diameters varied but remained smooth in morphology. Changing of the mass ratios of PCL/Gelatin in membranes (for all formulations except pure gelatin) gave materials with appropriate mechanical properties to shield the wound from a physical disruption and were contained in the ideal tensile strength range for skin cell culture and wound dressings. The NO payload in the membranes was directly related to the number of amines (and hence the amount of gelatin) in the blend. Higher NO payloads corresponded with a higher degree of antimicrobial efficacy. The kinetics of NO release from the membranes was dependent on the pH; with lower pH's releasing NO with a burst release mechanism, and physiological pH's resulting in lower but slower NO release. The sample with the highest NO release showed a 2-log reduction in *P. aeruginosa* and *S. aureus* biofilms. No cytotoxicity was observed for electrospun membranes, and an in vitro wound closure assay demonstrated closure within 16 h when tested with a tested with the stratified *in vitro* skin model.

Conclusions

The NO-donor, *N*-diazeniumdiolates were tethered successfully on varying ratios of electrospun PCL/gelatin. The results presented here clearly indicate that these NO-releasing electrospun membranes hold significant promise as wound dressings due to their antimicrobial activity and biocompatibility.

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Session 2: Flash Poster 10

Alginate-silica hybrid hydrogels employing novel reagents and a "soft chemistry" sol-gel process

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Introduction:

"Soft chemistry" sol-gel processing has enabled the development of organic-inorganic hybrid materials for tissue regeneration[1]. Hybrid materials have intermolecular mixing of the organic and inorganic moieties, leading to substantial benefits over traditional composites; however, the harmful chemicals used in their fabrication, and alcohols evolved during sol-gel processing, mean materials must be dried before implantation or cell culture, limiting their use as hydrogels in tissue fabrication. New sol-gel materials and processing technologies therefore need to be developed for bio-fabrication where cells, biomolecules and hydrogels can be geometrically arranged in a desired 3D structure[2]. Here, we report the synthesis of several polyol-modified silane precursors, their use in the fabrication of two types of organic-inorganic hybrid hydrogels and analysis of the physicochemical properties of these materials.

Materials and Methods:

Synthesis of silane precursors: A mixture of tetraethoxysilane (TEOS) and propylene glycol (at 1:4 molar ratio) was heated at 120 °C. The ethanol produced was continuously removed by distillation. The product, tetrakis(2-hydroxypropyl) silicate (PGMS, Fig 1a) was used to make organic-inorganic hybrid materials with alginate.

Synthesis of Class I hybrids (Fig. 1b): PGMS was dissolved in a solution of alginate in water [prepared from sodium alginate (3 g) in water (100 mL)] to provide molar ratios of the organic to inorganic components of 20:80, 40:60 and 60:40. The mixtures were left at room temperature to gel.

Synthesis of Class II hybrids (Fig. 1b): An organosilane crosslinker (patent pending) prepared via a similar route to PGMS was added to a solution of alginate (3 g sodium alginate in 100 mL water) to afford a 1:1 molar ratio of alginate disaccharide monomer to organosilane. The reaction was carried out at 50 °C for 72 hours. Then, PGMS was added to provide organic-inorganic hydrogels with 20:80, 40:60 and 60:40 wt%. The hydrogel solutions were left at room temperature to gel.

The hybrid hydrogel rheology, structure (SEM), degradation and biological properties using osteoblast (SaOS-2) has been investigated. Osteoblast viability on the surface of the hydrogels was assessed using viability, metabolic and proliferation assays.

Results and Discussion:

PGMS, product of the transesterification reaction formed hybrid hydrogels on mixing with alginate. Both hydrogels produced a porous architecture, however Class I exhibited a fibrous foam-like architecture (Fig 1c) whilst a colloidal particle-like morphology was observed for Class II (Fig 1d). The degradation behaviour was also found to be significantly different for the two classes of hybrids. These differences were owing to the type of bonding between the two classes of materials. A strong covalent interaction between the organic and inorganic moieties in Class II facilitated a more intimate interaction between alginate and silica thus improving the properties of the gels. Furthermore, both Class I and Class II hydrogels proved to be suitable for culturing osteoblasts (SaOS-2) with high cell viability.

Conclusions and Future Work:

Organic-inorganic hybrid hydrogels were successfully synthesised employing polyol-modified silanes under physiologically benign conditions. Future work will focus on using the hydrogels for 3D bio-fabrication to investigate their possible application in bone-tissue engineering.

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Session 2: Flash Poster 11

HYDROGEL FORMULATIONS TO MODULATE THE FOREIGN BODY RESPONSE

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Introduction: The aqueous environment and diverse tailorable properties of hydrogels lends them to be of great benefit in formulating depot injections for controlled protein delivery. (1) However, all biomaterials that persist in the body for a prolonged period of time are subject to the foreign body response (FBR), which eventually culminates in fibrotic encapsulation that is reported to impede drug release. (2) This process is complex and unpredictable, remaining an ongoing problem for implantable biomaterials despite many modification strategies to evade the immune system (3). Our limited understanding as to how the FBR responds to biomaterials and the subsequent impact on sustained drug delivery is largely due to a lack of cell culture models that can recapitulate the FBR *in vitro*. Thus, we developed an *in vitro* 3D co-culture system to study how the dynamic cellular events of the FBR may impact controlled protein release from hydrogel depots.

Materials and Methods: We present our work in two parts; development of an *in vitro* co-culture model to screen the FBR and synthesis of modified injectable hydrogels to modulate fibrotic capsule development.

Part 1: Human primary monocyte-derived macrophages (n=4 donors) were cultured on polyethylene terephthalate (PET) in the presence of macrophage-colony stimulating factor (M-CSF) and interleukin(IL)-4 to form foreign body giant cells (FBGCs). FBGCs are a key feature of the FBR and stimulate fibroblasts to produce collagen during fibrotic encapsulation. (2,3) FBGCs were characterised by bright-field microscopy and image analysis with secreted cytokines and growth factors quantified by ELISA. To establish a co-culture, we first performed a series of indirect co-cultures with macrophages and human foreskin fibroblasts to understand the effects of paracrine signalling between the cell populations. Finally, we incorporated a 3D matrix (Manchester BIOGEL) to our model to mimic the *in vivo* tissue environment and are currently optimising conditions to produce a 3D direct co-culture model to study the FBR to hydrogel depot formulations.

Part 2: We studied the FBR to PLGA-PEG-PLGA hydrogels, which can form gels *in situ* and have been utilised for sustained protein delivery applications. (4) To modulate collagen production during the FBR, PLGA-PEG-PLGA copolymers were functionalised with a peptide sequence inspired by inflammatory molecules naturally present in the ECM. Following incubation with the free peptide, fibroblast activity was analysed by profibrotic gene expression and macrophage secreted cytokines were quantified with ELISA. We introduced maleimide moieties onto PLGA-PEG-PLGA, which were reacted with cysteine residues of the peptide. The extent of peptide functionalisation was varied from 0% to 100% to produce a range of profibrotic hydrogels.

Results and Discussion: Up to 97% of macrophages cultured on PET in the presence of M-CSF and IL-4 fused into FBGCs after 25 days of culture, the largest of which contained up to 48 nuclei. Of the cytokines and growth factors analysed, PDGF-BB, which stimulates collagen production from fibroblasts (5), was significantly increased ($p<0.01$) at day 14 when cells were cultured on PET + IL-4 compared to control cells on TCPS (682.2 ± 398.1 (SEM) pg/mL vs. 10.0 ± 17.4 (SEM) pg/mL). Increased PDGF secretion correlated with early stages of macrophage fusion and suggests a polarisation bias towards tissue repair activities. PLGA-PEG-PLGA and resultant hydrogels were deemed non-toxic to cells; however, a 2D model resulted in excessive dilution of hydrogels, thus we adapted to a 3D model system. DOSY and H^1-NMR confirmed successful polymer maleimidation and peptide conjugation, respectively. We anticipate that tailoring hydrogel peptide functionalisation enables us to modulate subsequent collagen deposition. Moreover, as cells were unable to adhere to the native hydrogel surfaces, peptide functionalisation also provides potential cell attachment sites.

Conclusions: We successfully identified conditions that resulted in a high level of macrophage fusion, which may stimulate collagen deposition from fibroblasts due to the production of growth factors that participate in tissue repair and matrix remodelling. Hydrogels were modified with a profibrotic peptide to create a series of biomaterials that may modulate the development of the fibrotic capsule *in vitro*. By combining our 3D FBR model with profibrotic hydrogels, we hope to enhance understanding of how the fibrotic capsule may influence sustained protein release from injectable hydrogel depots.

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Session 2: Flash Poster 12

3D CELL CULTURE: HYDROGELS CAPABLE OF *IN SITU* FUNCTIONALISATION WITH A SINGLE "CLICK"

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Introduction

There is an increasing demand for methods to recapitulate the *in vivo* extracellular matrix to model the stem cell niche and tumour microenvironment for regenerative medicine and drug discovery. There is growing evidence to suggest that two-dimensional cell culture does not simulate the extracellular matrix effectively. 3D hydrogel scaffolds produced from self-assembling peptides offer an intriguing alternative for they provide structural homogeneity, are of sustainable, non-biological origin and are therefore, 3R compliant.

Materials and Methods

The self-assembling peptide hydrogel, FEFEFKFK, has been shown to facilitate the incorporation of soluble biologically relevant matrix material to mimic tissue-specific environments to aid in cell directionality and specialisation.[1] To assist in creating a fully customisable and reproducible microenvironment the peptide has been extended at its C-terminus with a non-proteinogenic L-azidohomoalanine (L-Aha) residue.[2] This creates a single point of covalent ligation amenable *via* "click" chemistry between the self-assembling peptide and biological matrix molecules of interest bearing the complementary alkyne moiety. By utilising the strain-promoted azide-alkyne cycloaddition, the system permits the end user to modify the 3D hydrogel at different time points, including in the presence of cells, creating a complex and dynamic cell culture scaffold.

Results and Discussion

The work to date has involved the synthesis of various bio-orthogonal linkers to covalently tether relevant matrix material to the 3D hydrogel; for example, the cell adhesion motif RGD. Furthermore, the addition of the "clickable" matrix material at different time points in the gelation procedure — both before and during gelation — has been investigated with promising results. The synthesis of "clickable" glycosaminoglycans; for example, heparin, is also being explored to add to the "toolbox" of matrix molecules capable of covalently modifying the hydrogel with end user-defined levels of functionalisation.

Conclusions

The current work has shown encouraging results towards a fully user-defined, reproducible and 3R-compliant scaffold with novelty in the method and chronology of delivering the desired matrix material. This in turn will permit dynamic and highly complex 3D *in vitro* environments to advance our current understandings in disease and developmental biology.

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Session 2: Flash Poster 13

ANKLE LIGAMENT REPAIR FOR RESTORATION OF FUNCTION: EXPLORING THE APPLICATION OF DECELLULARISED GRAFTS

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Introduction

Ligament integrity is directly associated with ankle stability. Nearly 40% of ankle sprains result in chronic ankle instability, affecting biomechanics and potentially causing osteoarthritis. Ligament reconstruction may be required to restore stability and avoid this degenerative pathway, but first a greater understanding of natural ankle ligament behaviour is required.

To restore stability, ligament replacement may be required. Autograft (from the patient) or allograft (from a donor) use is limited by donor-site morbidity and inflammatory responses respectively. Decellularised porcine grafts could address this challenge, as decellularisation removes cellular material to prevent acute immune responses, while preserving graft mechanical properties.

Materials and Methods

Several porcine tendons were evaluated to identify suitable candidates for decellularisation. The viscoelastic properties of native tissues were assessed using dynamic mechanical analysis (DMA), followed by ramp to 'sub-rupture' at 1% strain/s, and further DMA. Multiple samples (n=5) were taken along the graft to assess variation in properties along the tendon.

Results and Discussion

When identifying suitable porcine tendons, a lack of literature on human ankle ligaments was identified. Inconsistencies in measurement methods and properties reported makes comparison between studies difficult.

Preliminary testing on porcine tendons suggested there is little variation in viscoelastic properties along the length of tendon. Testing also suggested strain rates of 1%/s sub-rupture was not large enough to affect viscoelastic properties (no changes in storage or loss moduli or $\tan\delta$). Further testing is underway to improve upon low initial sample numbers and confirm these results, with varying strain rates to identify suitable sub-rupture sprain conditions.

Conclusions

This work highlights need for new data on human ankle ligaments to address the knowledge gap and identify suitable replacement materials. Future work will generate this data and decellularise porcine tendons of similar dimensions. Collagen damage will be investigated using histology and lightsheet microscopy, and viscoelastic changes through DMA. A combination of mechanical testing and imaging techniques will allow comparison of decellularised porcine tendons to native versions of the same tissue, to examine whether decellularisation affects properties. Human ankle ligaments will also be characterised and their dimensions recorded, to verify the suitability of the selected porcine graft.

Session 2: Flash Poster 14

MULTI-FUNCTIONAL LAYERED NANOCOMPOSITE COATINGS FOR BIOACTIVE TISSUE SCAFFOLD DEVELOPMENT

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Introduction

As the current 'gold standard', autograft procedures exhibit a high success rate but post-procedure infection[1], donor site complications[2] and limited grafting material for these procedures highlight that an engineered "off-the-shelf" solution is required. Tissue engineered scaffolds must match the physico-mechanical properties of surrounding bone; however, current scaffolds often sacrifice porosity in order to meet the desired mechanical strength. Through deposition of a mechanically robust thin film, it is possible to tailor mechanical properties without significantly sacrificing porosity. This work investigates the substitution of nanoclay (NC) into other exponentially growing Layer-by-Layer (LbL) systems for applications in functional coatings.

Materials and Methods

1 wt.% of PEI (Polyethylenimine), PAA (Polyacrylic acid), PDDA (Poly (diallydimethylammonium chloride)) and 0.5 wt.% of powdered NC and Alginate (ALG) were prepared in deionized water. 0.5 wt.% of powdered Chitosan (CHI) was prepared in lactic acid solution (1% v/v). Substrates were prepared by washing in 1 M of NaOH and rinsed with 18.2MΩ deionized water. Glass slides and Polyurethane foams were then coated with $(PEI/PAA/PEI/NC)_n$, PEI-$(PDDA/PAA/PDDA/NC)_n$ and $(CHI/ALG/CHI/NC)_n$. Coatings were examined using Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray (EDX), Stylus Profilometry, Instron Compressive Testing and Micro-CT. As the limiting cytotoxic properties presented by PEI[3] cause critical drawbacks for its use, 7-day *in vitro* studies were performed to assess the cytotoxicity of the coatings.

Results and Discussion

Results suggest that (PEI/PAA/PEI/NC) and PEI-(PDDA/PAA/ PDDA/NC) coatings exhibit similar thicknesses, growth regimes and coating conformity, whereas (CHI/ALG/CHI/NC) presented a substantial increase in the coating thickness. Resazurin Reduction Assay results indicate improved cell viability with coatings deposited with PEI-(PDDA/PAA/ PDDA/NC) and (CHI/ALG/CHI/NC).

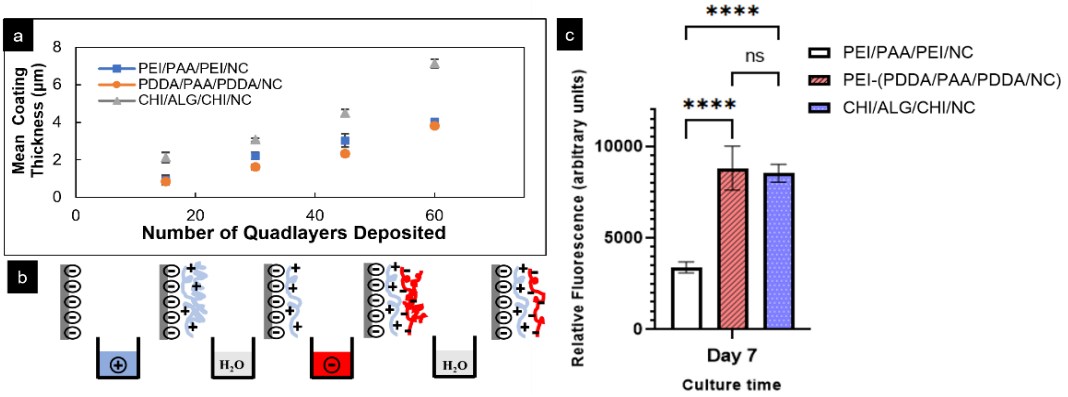

Figure 2 *a) Mean coating thickness of (PEI/PAA/PEI/NC)n, PEI-(PDDA/PAA/PDDA/NC)n and (CHI/ALG/CHI/NC)n coatings as a function of deposited quad layers, b) schematic diagram of LbL process and c) Resazurin Reduction Assay results as an indication of U-2 OS cell viability on exposure to each coating type and cultured for 7 days.*

Conclusions

The incorporation of NC into exponentially growing layers, including PDDA/PAA and CHI/ALG, to improve mechanical properties was successfully demonstrated. Initial *in vitro* studies suggest that substituting PEI has significant benefits on cell viability.

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Session 2: Flash Poster 15

REDUCED FIBROBLAST ACTIVATION ON ELECTROPSUN POLYCAPROLACTONE SCAFFOLDS

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Introduction

Fibroblast activation *in vivo* is characterised by increased proliferation, migration and extracellular matrix (ECM) deposition, as well as changes to gene expression. These changes occur in response to tissue damage and represent the transition from low activity fibroblast to fully differentiated myofibroblast (1). In 2D culture stiff plastic surfaces give rise to partially activated fibroblasts which rapidly proliferate and express low levels alpha smooth muscle actin (α-SMA). Previous work in collagen hydrogels indicated that 3D biomaterials could be used to reduce these activation characteristics, creating a more appropriate *in vitro* culture environment (2).

In this work we fabricated a micro-fibrous polycaprolactone scaffold to thoroughly characterise fibroblast activation in a 3D environment. Understanding the culture features which control fibroblast behaviour represents a key step towards developing tissue engineered models with the incorporation of stromal support cells like the fibroblast. Furthermore, as deregulated fibroblast activation is responsible for fibrotic diseases and implicated in some cancers, models that help us to understand and regulate fibroblast activation are of great interest.

Materials and Methods

Scaffolds were fabricated using an in-house electrospinning rig at the University of Sheffield, School of Clinical Dentistry. Medical grade polycaprolactone was deposited as a non-woven micro-fibrous matt. Scaffolds were fully characterised in terms of fibre diameter, angle and porosity using SEM imaging and mercury intrusion porosimetry. Fibroblasts were also cultured on standard tissue culture plastic for comparison. Cell viability and DNA quantification was conducted alongside Ki67 immunolabelling to quantify proliferation and cell cycle progression. Quantitative polymerised chain reaction (qPCR) was used to investigate the expression of genes involved in cellular senescence, cytoskeletal myofibroblast markers and key ECM proteins.

Results

Cell viability and DNA quantification indicated that fibroblasts were less proliferative when grown on scaffolds than plastic. Live dead staining indicated that excessive cell death was not responsible (data supported by Ki67 immunofluorescence imaging). The proportion of Ki67 negative (G0) cells increased on electrospun scaffolds. Fibroblasts grown on scaffolds did not show elevated p21 mRNA levels as was observed in senescent cells, indicating that Ki67 negative cells were quiescent and not senescent. α-SMA mRNA expression was reduced in fibroblasts grown on electrospun scaffolds compared with those grown on plastic. TGF-β stimulation (known to induce myofibroblast differentiation) stimulated increased α-SMA expression levels for both plastic and scaffold cultured cells compared to untreated cells. Finally, we observed a reduction in the mRNA levels of ECM genes FN1, VCAN, Col1A and Col3A in scaffold grown fibroblasts.

Discussion

This study was prompted by our observation that 3T3 fibroblasts grew relatively slowly on electrospun PCL scaffolds that had previously supported the growth of a wide variety of cell lines. PicoGreen and PrestoBlue viability assays indicated that primary oral fibroblasts also displayed reduced growth rates. To explore this further we carried out Live/Dead staining, Ki67 immunofluorescence and qPCR to quantify senescence associated gene expression. These data suggested increased cell death and cellular senescence were not responsible for the observation of reduced growth, suggesting that instead fibroblasts were entering a quiescent *in vivo* like state. ACTA2 qPCR supported the hypothesis that fibroblasts are maintained in an inactive state. This was also supported by a reduction in the expression of ECM genes expressed by activated fibroblast populations grown on plastic or stimulated *in vivo* in response to tissue injury.

Conclusion

Results indicate that our micro fibrous polycaprolactone scaffold can reduce fibroblast activation in vitro. This scaffold can be used to investigate the mechanisms controlling fibroblast activation with future work investigating our ability to control fibroblast behaviour through scaffold design. Unlike hydrogels, electrospinning offers a high degree of control of individual features from fibre size to porosity to stiffness and surface modification.

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Acknowledgments

Many thanks to the Engineering and Physical Sciences Research Council (EPSRC) who funded this project.

Session 2: Flash Poster 16

MICRO-CT CHARACTERISATION OF A DUAL ANTIMICROBIAL-OSTEOINDUCTIVE 'BONE WRAP' USING A NOVEL *EX-VIVO* FOETAL CHICK FEMUR DEFECT MODEL

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Introduction

As the global population ages, more people are suffering from the effects of ageing and trauma. More years are lived with musculoskeletal disability than any other long-term condition. While osteoporosis is the most prevalent, there are more than 200 musculoskeletal conditions which affect a quarter of the UK adult population. Additionally 300,000 NHS patients acquire a healthcare-associated infection (HCAI) each year [1] most of these are related to an implanted medical device. To reduce the £4.76 billion annual burden that musculoskeletal conditions place on the NHS[1], it is necessary to develop materials which aid the repair of tissue as well as have inherent antimicrobial properties. -NH_2 chemistries have proven to be effective in inducing osteogenic differentiation of hMSCs[3] in standard *in vitro* models; this technology will be used to produce a Bone Wrap – a degradable scaffold designed to work with external fixation. The scaffold will enhance osteointegration at a localised site, whilst minimising the prevalence of bacterial infection. We are incorporating a novel *ex vivo* foetal chick femur defect model which utilises microCT to assess the effectiveness of the osteogenic modifications to explore and optimise the efficiency of our bone wrap to induce new bone.

Materials and Methods

3D scaffolds were electrospun from five polymer blends containing different ratios of Gelatine : PCL (100% G, 75:25, 50:50, 25:75, 100% PCL). These were then modified to incorporate a Nitric Oxide releasing antimicrobial donor [3], which reveals an osteogenic amine group when eluted. Intact femora and tibia were removed from freshly killed Dekalb white chick foetuses after 11 days incubation and cleaned of all muscle tissue. A defect was then made in the bone collar region and wrapped with the scaffold under test as a control, while the contralateral bone was wrapped with the corresponding NO-modified blend to add antibacterial and osteogenic properties. Samples were cultured for 14 days, then fixed before being surface dried and packed in polystyrene inside a humidified syringe for microCT scanning. A Bruker SkySkan 1272 was used for whole bone scans, and a Zeiss Xradia 620 for break site analysis. SkySkan images were visualised and analysed numerically using CTRecon and CTVox, while Xradia images were visualised using Drishti.

Results and Discussion

The benefits of using both complimentary uCT scanners is evident and will be presented – the high throughput nature of the SkyScan allows fast whole bone scanning and numerical analysis allowing for large experimental sample sizes, which can be enhanced by the high-quality visualisations possible with the Zeiss Xradia 620 scanner. The data shows the changes in proportion of bony material to cartilaginous material, which can be used to demonstrate the effect that the different scaffold blends, and the Nitric Oxide modification, have on osteogenesis. The figure below shows the enhanced image quality that the low-throughput Xradia scanning provides over the SkySkan:

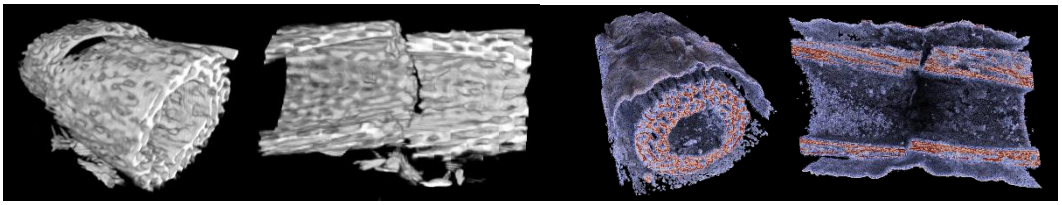

Figure 3: Break site uCT cropped regions and cross-scetions: LEFT - SkyScan 1272, RIGHT: Xradia 620

Conclusion

Two separate microCT scanning systems have been used in a complimentary way to allow high throughput numerical analysis and high quality representative imaging of the site of interest. μCT analysis has been proven to clearly show the defect region visually and numerical threshold volume analysis demonstrates the change in osteogenic capabilities exhibited by different material blends and modifications.

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Session 3: Keynote

Moving additive manufacturing beyond anatomical customisation
Dr Sophie Cox

Dr Sophie C. Cox is a Senior Lecturer in the School of Chemical Engineering and the Healthcare Technologies Institute (HTI) at the University of Birmingham. Her vision is to improve patient quality of life by innovating new medical devices with unprecedented functionality. To achieve this, she brings together expertise in advanced manufacturing and biomaterials. These translational activities are underpinned by basic science focused on understanding the biological response to biomaterials and unearthing mechanisms of action.

Dr Cox leads a research group focused on exploiting additive manufacturing techniques to create customised medical devices. In this talk she shall share a number of case studies highlighting approaches the team have developed to enhance the functionality of personalised bone implants. Alongside this Dr Cox will discuss how 3D printed porous structures may be used as platforms for the manufacture of acellular therapies. Lastly she shall give a brief overview of work that the team did during COVID that exploited both 3D printing and biomaterials science to improve PPE efficacy.

Session 3: Talk 1

HANDHELD BIOPRINTING OF BIOORTHOGONALLY CROSSLINKED HYDROGELS

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Introduction

Classical bioprinting strategies are currently hindering clinical application of bioprinted scaffolds, due to the time-consuming and complex "fabrication-to-application" route. Handheld bioprinting devices for *in vivo* bioprinting represent an interesting alternative as surgeons can use them to directly print scaffolds on injured body sites, greatly simplifying bioprinting procedures and allowing to tailor the shape of the printed scaffold tothe injured tissue[1]. Only few examples are described in literature, due to challenges in developing bioinks suitable for *in situ* bioprinting and in engineering easy-to-handle portable printers[2]. Here, we develop new bioinks that crosslink *in situ* after mixing hydrogel precursors and a microfluidic printer head used to print the inks in a handheld device to potentially allow for *in vivo* bioprinting surgical procedures.

Materials and Methods

Alginate (Mw = 56000, KIMICA Corporation, ALG) and gelatin (type B, gel strength = 240-270 g, Rousselot Biomedical, GEL) were functionalized with norbornene (ALG_Nb)[3] and tetrazine (GEL_Tz)[4], respectively, using carbodiimide chemistry. ^{1}H NMR spectroscopy was used to measure ALG_Nb and GEL_Tz degree of modification. Hydrogels were prepared by mixing the precursors (inverse electron demand Diels−Alder reaction between Tz and Nb) at different concentrations (i.e., 2, 4, and 6% w/v, for GELALG_2, GELALG_4, and GELALG_6) and the evolution of the rheological properties after mixing was tested by a rheometer (Netzsch Kinexus Ultra+). Crosslinked hydrogels were immersed in PBS at 37 °C to test their stability and swelling (n = 5). Printability studies were performed by loading hydrogel precursors into two separate syringesand by injected them into a microfluidic passive Herringbone mixer mounted on the printer head of a commercial 3D printer (Allevi 3). Optimization of printing parameters was performed by printing hydrogels by varying the precursors concentrations and the printer writing speed. Data were analyzed by ANOVA ($p<0.05$).

Results and Discussion

ALG_Nb and GEL_Tz were successfully synthesised, as evidenced by the appearance of their characteristic signals in the NMR spectra, with comparable degrees of modification (0.1 $mmol_{tetrazine/norborne}/g_{gelatin/alginate}$). Hydrogels formed after mixing ALG_Nb and GEL_Tz precursors at different concentrations. G' values increasefor all hydrogels after mixing, indicating ongoing crosslinking; hydrogels prepared with higher concentrations reached higher G' values at plateau (80-2000 Pa; GELALG_6 > GELALG_4 > GELALG_2) and crosslinked faster (60-85 min: GELALG_6 < GELALG_4 < GELALG_2). Crosslinked hydrogels were stable in PBS at 37
°C, confirming their crosslinking; hydrogels prepared with lower concentrations were characterized by higher weight variation (2000-4000%; GELALG_2 > GELALG_4 > GELALG_6). Hydrogel precursors loaded in two separate syringes were extruded via a passive microfluidic mixer in predefined patterns. All formulations couldbe extruded passing through the microfluidic chip that mixed them to promote the crosslinking. Printed hydrogels show qualitatively good printing resolution (i.e., good shape fidelity replicating the pattern), and spontaneously crosslinked after mixing and printing, suggesting their potential use as bioinks for manual *in situ* 3D bioprinting. Hydrogels with higher concentration, printed with higher writing speed, were characterizedby better-defined printed filaments and will be further investigated by *in vitro* test and handheld printing.

Conclusions

Hydrogels were prepared by bioorthogonal crosslinking between ALG_Nb and GEL_Tz. The crosslinking kinetics of the hydrogels was tuned by varying concentration and hydrogel precursors were successfully bioprinted using a passive microfluidic mixer in well-defined shapes.

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Session 3: Talk 2

MICROFLUIDIC-BASED 3D BIOPRINTING TO FABRICATE BLOOD VASCULATURE

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Introduction

The most substantial healthcare challenge facing the UK is the inevitable transition towards ageing population. Organ transplantation as the gold-standard treatment has saved many lives and millions of pounds for the NHS; however, every day, 4 people in the UK die while on the waiting list. The development of new and effective artificially engineered organs and tissue grafts without the need for immunosuppression will therefore be necessary to ensure we maintain and improve the healthcare nationally and globally. 3D extrusion-based bioprinting has emerged as a promising 3D printing technology for development of more advanced and functional organs and tissues, as well as drug development, and 3D in vitro disease models[1,2]. However, current developments are not able to recapitulate the heterogeneity and complexity of organs and tissues (Fig. 1A). Recent studies[1,2] have demonstrated the potential use of microfluidic-based 3D printing by allowing more control and precision over the organisation of biomolecules, cells and material thus, enabling fabrication of complex 3D constructs. However, the traditional fabrication method for such devices is labour-intensive and expensive, limiting its widespread use. Herein, we proposed an agile and novel manufacturing pipeline based on COntinously Varied Extrusion (CONVEX)[3] design approach in extrusion-based printing to develop integrated 3D-printed microfluidic chip nozzles, based on microfluidic mixers and hydrodynamic flow focusing components (Fig. 1B) with the potential to fabricate blood vessels.

Materials and Methods

FullControl GCode designer software[4] was used to extrude a single layer of acrylonitrile butadiene styrene (ABS) with complex passive mixer component using an Ender 3 3D printer. The ABS channels were cast into polydimethylsiloxane (PDMS) before flushing with acetone. To achieve co-axial hydrodynamic flow focusing of calcium chloride-Pluronic solution by 2wt% sodium alginate solutions, the calcium chloride channel had a smaller diameter than sodium alginate channels (Fig.1B).

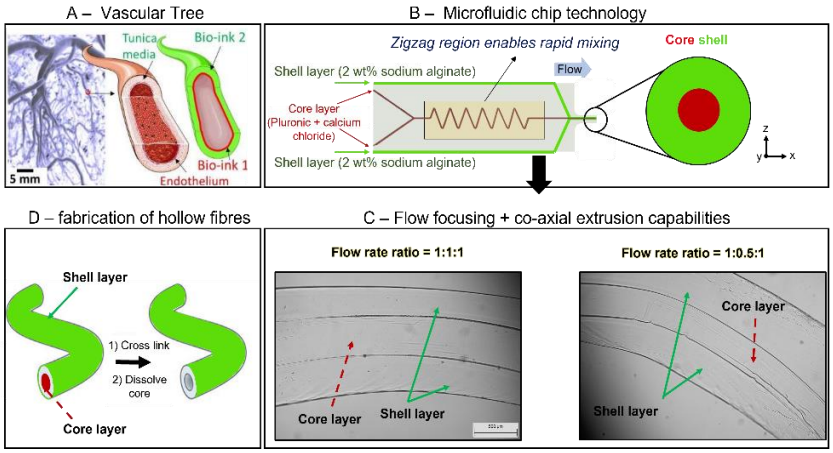

Figure 4: To recreate natural blood vessel (A), a novel microfluidic chip nozzle for extrusion printing platform developed (B). Optical micrographs indicated the flow focusing and co-axial extrusion capabilities of the newly developed nozzle (C). The hollow fibres obtained by removing the core layer after cross linking (D).

Results and Discussion

Direct GCode scripting allowed to incorporate complex passive mixer region (zigzag region. Fig.1B), enabling on-fly mixing of two fluids (core fluid with red dye). Furthermore, flow-focusing capabilities was achieved through cross junction design, resulting in dynamic variation of the diameter of the core fluid (30-40% of total width of channel) by changing the flow rates of shell bioink (Figure 1C). The important aspect of the newly developed 3D printed microfluidic nozzle was fabrication of multi-shell hollow fibres similar to that of the blood vessel. The core bioink layer maintained its structure since sodium alginate (shell layer) cross-linked upon extrusion inside the calcium chloride bath. Although few microfluidic printhead with co-axial extrusion capability are commercially available, the diameter of core fluid cannot be varied dynamically to imitate the changing diameters of blood vessels. On the other hand, our agile manufacturing pipeline is capable of fabricating 3D-printed microfluidic rapidly and consistently at significantly lower cost suitable for recreation of the complexity of blood vessels.

Conclusions

An agile manufacturing pipeline has been developed to fabricate novel microfluidic chip nozzles suitable for 3D bioprinting of complex tissues and organs. Microfluidic chip nozzles allowed dynamic and precise control of the diameter of core/shell hydrogels. Future work will integrate the microfluidic chip nozzle on a 3D printer to fabricate complex 3D structures containing cells.

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Acknowledgements: This research was funded by the EPSRC (EP/V051342/1).

Session 3: Flash Poster 17

TETRACYCLINE-LOADED ISABGOL (PSYLLIUM) NANOPARTICLES FUNCTIONALIZED WITH HYALURONIC ACID FOR ACCELERATING WOUND HEALING

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Introduction

Wound healing normally occurs in four overlapping stages - haemostasis, inflammation, proliferation, and remodelling. In chronic wounds, this timely sequence of repair is disrupted due to various factors, infection being one of the leading causes. We have developed tetracycline-loaded isabgol (psyllium) nanoparticles (NPs) functionalized with hyaluronic acid for accelerating the healing of chronic wounds. Isabgol is a biocompatible polysaccharide that has been successfully used in our previous studies for wound healing applications. The Isabgol NPs were functionalized with hyaluronic acid (HA), a glycosaminoglycan that participates actively in various wound healing stages to accelerate the wound healing process. Tetracycline is a broad-spectrum antibiotic that will prevent infection at the wound site.

Methodology

Isabgol nanoparticles were developed by the emulsification method. The NPs were functionalized with HA from metabolically engineering *Lactococcus lactis* by physical adsorption and glutaraldehyde crosslinking. Tetracycline was loaded into the NPs by adding it into the isabgol gel before NP preparation. The size and zeta potential of the NPs was estimated by dynamic light scattering technique. The particle morphology was studied using a scanning electron microscope. The functionalization of HA on Isabgol was confirmed by FTIR spectroscopy. The encapsulation efficiency and the loading capacity of the NPs were determined by estimating the concentration of tetracycline in the NPs using UV spectrophotometry. *In vitro* drug release measurements were carried out using dialysis membrane (12000–14000 Da) in pH 7.4 phosphate buffered saline and samples were analyzed using UV spectrophotometry. The minimum inhibitory concentrations of the NPs against the Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria were quantified using resazurin microtiter assay. The cytotoxicity of the NPs against L929 fibroblast cells was assessed by MTT assay. Cellular uptake of the NPs was studied by loading rhodamine-B into the NPs and observing uptake by fluorescence imaging.

Results and Discussion

The size and zeta potential of the NPs were found to be ~125 nm and ~35 mV, respectively. The size range allows cellular internalization and ensures escape from mononuclear phagocyte recognition and immune system clearance. The large negative charge prevents agglomeration of the NPs. FTIR spectroscopy confirmed the functionalization of HA on the NPs and the presence of tetracycline. SEM micrographs of the NPs showed spherical shape. The drug encapsulation efficiency and loading capacity was found to be 90% and 30%, respectively. The *in vitro* release study showed sustained release of tetracycline from the NPs. The minimum inhibitory concentration of the tetracycline-loaded NPs was 3.8 µg/ml against *E. coli* and 1.9 µg/ml against *S. aureus*. The NPs were found to be non-toxic against L929 fibroblast cells. The NPs also showed excellent cellular internalization in L929 fibroblast cells.

Conclusion

Tetracycline-loaded Isabgol HA nanoparticles were successfully prepared and characterized. These non-toxic nanoparticles showed excellent cellular internalization and antibacterial activity. The nanoparticles can release the drug in a sustained manner to the wound site and promote wound healing. Further, *in vivo* wound healing efficacy of the nanoparticles needs to be assessed for clinical applications in treatment of diabetic and infected wounds.

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Acknowledgments:

We sincerely thank Prof. Guhan Jayaraman and Mr. Sharath S. for providing us with purified hyaluronic acid from metabolically engineered *Lactococcus lactis.*

Session 3: Flash Poster 18

Calcium Phosphate Coatings for the Degradation Control and Functionalization of Magnesium-based Alloys

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Introduction

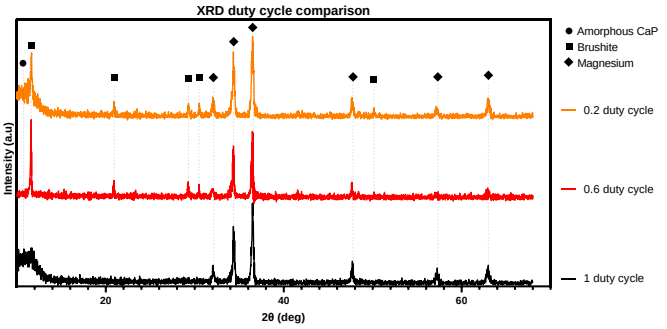
Magnesium and its alloys have attracted interest due to their mechanical properties being similar to that of bone (Mg: ρ~1.7g/cm^3, E~40-45GPa. Bone: ρ~1.2g/cm^3, E~10-40GPa [1]) and the potential to degrade completely in the body. This would then remove the need for implant removal surgery, removing costs and risks associated with removal. However, the corrosion rate of such implants needs controlling to enable continued structural support during bone fracture healing [2]. This research investigates the benefits of coating Mg alloys such as WE43 with CaP coating (crystalline brushite) based on an electrochemical deposition and the potential for combining this coating method with a PVD approach, using an iron-doped phosphate glass, to further improve degradation life.

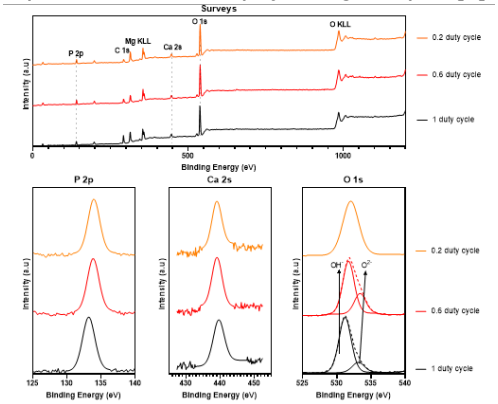
Materials and Methods

Mg WE43 alloy discs (10mm x 1mm) were used as substrates to deposit a CaP coating via wet electrochemical deposition in an electrolyte solution. Deposition time was 20mins with applied voltage -3V and duty cycle was varied in steps of 0.1 to obtain optimal deposition of crystalline structure. A phosphate glass coat was deposited via radio frequency magnetron sputtering at 60W, 24h, 5rpm to supplement the CaP deposition. SEM/EDX, XRD and XPS were used to detail the morphological, chemical and structural properties. Coating influence on cell behaviour was investigated by neutral red assay with MG63 osteoblast-like cells cultured in DMEM media.

Results and Discussion

Varying the duty cycle (time charge is applied per second) of CaP electrodeposition resulted in an optimal deposition at 0.6 duty cycle giving a crystalline brushite deposit whilst maximising deposition rate. Brushite is a precursor for Hydroxyapatite, which will likely delay degradation due to its relative insolubility in aqueous solution at physiologicial pH [3].





XPS survey scans show similar deposition compositions across samples with subtle alterations in peak intensity and position. Concerning the high resolution spectra, there was a noticeable difference in the deconvolutions of the O 1s peak showing that varying the duty cycle will result in different chemical states: O^{2-} and defective oxide OH^-, with higher OH^- for the 1 duty cycle compared to the 0.6 (84.6% vs. 72.6%).

Conclusions

Varying the duty cycle of the electrodeposition shows 0.6 to be the optimum as it balances coating thickness with obtaining a crystalline deposition to delay degradation and a reduced proportion of defective oxides relative to higher duty cycle parameters. This coating will be used in conjunction with deposition of sputtered phosphate glass which is anticipated to augment the CaP coating and control degradation time further.

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Session 3: Flash Poster 19

PEPTIDE-ENHANCED CALCIUM PHOSPHATES FOR THE PROMOTION OF BONE TISSUE REGENERATION *IN VIVO*

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Introduction

Bone tissue regeneration is a challenge in orthopaedic and oral surgery. Graft materials are often used to aid regeneration, with autografts the gold standard due to their osteoinductive properties. Autografting, however, requires a second surgical site which brings risk of surgical complications and donor site morbidity. Animal-derived and synthetic calcium phosphate (CaP) graft materials are also used, however are osteoconductive, providing a scaffold for growth alone. Osteoconductive grafts have been enhanced through the inclusion of proteins as orthobiologics, however the use of these has been controversial, with side effects reported in high number [1]. Our work aims to enhance the bone regenerative capacity of a synthetic calcium phosphate (CaP) medical device through the attachment of an osteogenic peptide. We aimed to examine the safety and efficacy of the peptide-enhanced CaP *in vivo*, and examine the shelf life and long term peptide retention *in vitro.*

Materials and Methods

Carboxylic acid functional groups were grafted onto clinically used CaP granules via plasma deposition of acrylic acid. An osteogenic peptide was then attached to the surface using carbodiimide chemistry. The biocompatibility of the enhanced CaP was assessed in rabbits, with empty defects, non-enhanced and peptide-enhanced CaPs compared at 4- and 12-week time points. Efficacy was assessed in a critical size defect model in skeletally mature sheep, with empty defects, non-enhanced and peptide-enhanced CaPs compared at 13- and 17-week time points. MicroCT and histology were used to assess healing in both *in vivo* studies, with samples of the brain, liver, lung and kidneys also assessed via histopathology in the rabbit study. To assess the long-term retention of the surface peptide, peptide-enhanced CaPs were incubated in PBS at 37 °C for 29 weeks, with HPLC used to assess peptide retention. To examine shelf-life, peptide-enhanced CaPs were aged according to ASTM guidelines with time-points representing 1 and 2 years at room temperature, with biological performance assessed through in vitro cell culture with rat bone mesenchymal stem cells over 21 days, and materials characterisation of the aged device performed with XPS and XRD.

Results and Discussion

The peptide-enhanced CaP was demonstrated to be biocompatible in rabbits, with no negative effects seen in bone through microCT or histopathological analysis, or in histopathological analysis of the sampled organs. The peptide-enhanced CaP promoted healing in critical size defects in skeletally mature sheep, with increased bone regeneration compared to empty defects. Peptide was retained on the surface of the CaP *in vitro* over the length of the study, with only a small percentage of peptide released into the PBS supernatant. This data demonstrates the stability of the peptide bonding process, suggesting a methodology that will allow for dosage control at the intended site of action and reduce the risk of translocation of the biologically active molecule and therefore the potential for side effects as seen in orthobiologics.

Conclusions

We have developed a peptide-enhanced calcium phosphate medical device for improved bone regeneration that has shown considerable promise *in vitro* and *in vivo*, through the attachment of an osteogenic peptide to clinically available calcium phosphates.

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Acknowledgements The authors would like to thank Ceramisys Ltd for providing the calcium phosphates, and Versus Arthritis and the Medical Technologies Innovation and Knowledge Centre for funding this research.

Session 3: Flash Poster 20

QUANTIFYING THE EFFECTS OF SI, B, AND CO-RELEASING BIOACTIVE GLASSES ON IN VITRO BONE FORMATION

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Introduction

Impaired healing of large bone defects and non-union fractures remain amongst the most prevalent clinical challenges, especially among the elderly[1]. Controlled ion release (including silicate (Si), boron (B) and cobalt (Co)) from biomaterials, like bioactive glasses (BGs), has demonstrated promise to induce bone regeneration[2], via enhanced osteoblast proliferation, as well as increased expression of bone related genes and proteins (e.g. alkaline phosphatase, osteocalcin)[3,4]. There remains, however, uncertainty on how these bioactive glass ions effect biomineralisation (the formation of extracellular mineralisation collagen) as well as a lack of quantitative analysis of the concentration dependent effects of these ions on bone nodule formation *in vitro*. This study investigates the role of Si, B, and Co ions and BGs, on bone formation using an *in vitro* model and a quantitative multi-disciplinary characterisation approach (morphological, biochemical, ultrastructural characterisation) to understand the differing effects of these ions on biomineralisation.

Materials and Methods

Primary osteoblasts isolated from neonatal Sprague-Dawley rat calvaria bone were cultured in α-MEM supplemented with foetal bovine serum, 2mM L-glutamine, antibiotic/antimitotic, 2 mM β-glycerophosphate, 10nM dexamethasone, and 50µg/mL ascorbate. Once confluent, they were treated with different mediums under normoxic conditions for 21 days. Conditions included: sodium metasilicate (0.5mM, 1mM, and 2mM), boric acid (0.5mM), and melt derived BGs manufactured in collaboration with Julian Jones (Imperial College London) and Aldo Boccacini (Germany-University of Erlangen-Nuremberg). They include cobalt (12.5µM and 25µM), boron (0.3mM, 0.5mM, and 0.7mM), and sodium metasilicate (0.5Mm and 1mM) BGs. Bone nodule formation was quantified through alizarin red (AR) staining and interferometry, with biochemical composition observed through Raman spectroscopy and transmission electron microscopy. Ion compositions of each BG were measured by ICP-OES.

Results and Discussion

All SiBG dissolution products increased the % area of calcium staining (AR), compared to cells treated with bone supplements alone. Interestingly Si (sodium metasilicate), or B (boric acid) ions alone did not cause the same significant increases in nodule area, suggesting the importance of the combination of ions SiBGs. The composition of SiBG that had the biggest increase in bone nodule formation did not contain B or Co ions. The % area covered by calcium whilst useful, does not allow 3D analysis of the bone (interferometry) or provide biochemical analysis (Raman) of the type of biomineralisation. Interferometry, evaluating height of bone nodules, and looking at the 3D structure showed results similarly to those seen in AR. The benefits of different bone nodule characterisation approaches, including biochemical, ultrastructural, and microstructural quantitative data will be presented and discussed. These results quantify the effects of SiBG, BBG, and CoBG ions on bone nodule formation *in vitro*. B or Co may still have important roles in different stages of bone nodule formation *in vivo* (e.g. inflammation, soft callus formation or angiogenesis).

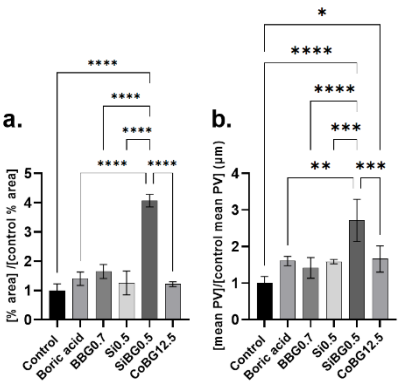

Figure 5. Biomineralisation area of SiBGs with similar Si levels (0.5mM), normalized to control (bone supplements alone). (a) % area covered by AR staining and (b) mean PV (peak to valley, i.e., highest to lowest point in bone nodules)

Conclusion

Standardisation of *in vitro* characterisation of BG on bone nodules allows the development and testing of new bioactive glasses with optimised ion release profiles.

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Session 3: Flash Poster 21

The role of intermittent hypoxia on bone nodule formation in vitro

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Introduction

Oxygen plays an important role in bone regeneration following fractures. Blood vessel rupture at the site of fracture causes a drop in oxygen level (hypoxia) and stabilises hypoxia-inducible factor-1α (HIF-1α), an important factor in bone regeneration [1]. HIF-1α stabilisation regulates important pathways in bone regeneration including angiogenesis, mesenchymal stem cell recruitment, osteoblast-osteoclast crosstalk, and is shown to drastically increase bone formation in mice [2]. However, prolonged hypoxia has been shown to inhibit bone formation [3].The disparity in the bone regenerative effects of hypoxia and HIF-1α may be due to the duration and timing of hypoxia. Therefore, studying the effects of short term hypoxia on bone formation may improve understanding of hypoxia in fracture healing, and identify the optimal duration of hypoxia for bone regeneration and HIF stabilisation therapies. This study aims to investigate the effect of different durations of hypoxia (1% O_2) on bone formation using an in-vitro model with multidisciplinary characterisation on cell behaviour (angiogenic, proliferation, and differentiation), and biochemical (Raman spectroscopy), ultrastructural (TEM), and microstructural (Interferometry) bone formation characterisation.

Experimental Methods

Primary osteoblasts were isolated from calvaria of neonatal Sprague-Dawley rats, cultured in α-MEM medium with 10% foetal bovine serum (FBS), 1% L-glutamine, 1% antibiotics (penicillin/streptomycin). Once confluent, cells were seeded at a density of 60,000 cells per 12 well plate and supplemented with 2 mM β-glycerophosphate, 10 nM dexamethasone, and 50 µg/mL ascorbic acid. Cells were held for in hypoxia (1% O_2) for different durations of time including 1, 3 and 7 days and then transferred to normoxia (20% O_2) until day 21. Hypoxia (1% O_2) and normoxia (20% O_2) for 21 days were used as controls. Proliferation (total DNA quantification), angiogenic response (VEGF ELISA), and alkaline phosphate (ALP) activity of cells were measured. Bone nodules were further characterised using Alizarin Red calcium staining, transmission electron microscopy (TEM), Raman spectrometry and interferometry.

Results and Discussion

All hypoxic conditions exhibited reduced proliferation compared to normoxia after 14 days. After 14 days, hypoxia control and 7-day hypoxia showed similar levels of proliferation (figure 1a). Normoxia VEGF concentration remained low in day 1 and day 7. Hypoxia showed increased VEGF at day 1 compared to normoxia. Hypoxia continued to increase VEGF concentration at day 7. At day 7, 1-day and 3-day hypoxia VEGF decreased back to normoxia levels demonstrating reversibility of VEGF concentration when exposed to normoxia.

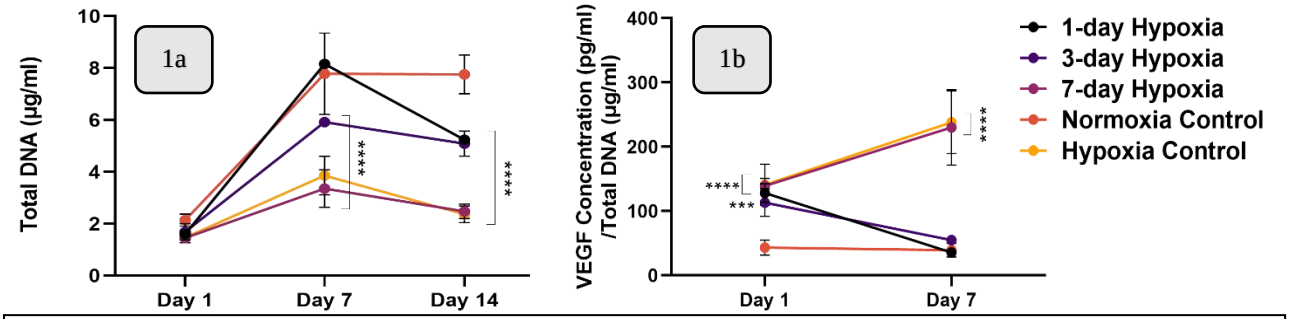

Figure 1. The effect of hypoxia duration on proliferation and angiogenic response. **a)** Normoxia showing the greatest proliferation at day 14, with increasing durations of hypoxia resulting in decreased proliferation. **b)** Normoxia showing lowest VEGF concentration at day 1 and 7. Hypoxia showing highest VEGF concentrations at day 1 and 7. At day 7, 1-Day and 3-Day VEGF concentrations reduced to normoxia levels following exposure to normoxia.

Conclusion

This study develops our understanding of the short term effect of hypoxia on bone that would be mimicked in the natural physiological progression of fracture healing. This study also identifies the optimal duration of hypoxia for bone regeneration.

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Session 3: Flash Poster 22

EX-VIVO SPIDER SILK, A NOVEL LOOK AT AN AINCENT BIOMATERIAL

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Introduction: Spider silk has been referred to as the "Holy Grail" of biomaterials [1] due to its unique combination of both strength and toughness, whilst at the same time being lightweight and biodegradable [2]. Physiologically silk is also highly attractive, it is non-immunogenic in humans [3] biocompatible [4] and has even been used to facilitate and promote nerve regeneration in murine[5, 6].

Understandably these properties have led many researchers to attempt to produce spider silk over the years, using bacterial, yeast and mammalian cells alongside the well-publicised mammary tissues in adult goats. However, these attempts have all met with limited success due to challenges surrounding compromises made in the expression system used, i.e. the formation of large inclusion bodies, transcriptional hairpins, and truncated proteins [7-10]

Materials and Methods: Using novel cell culturing methodologies developed in the Goodacre lab, whole silk glands have been successfully removed from the abdomens of *Larinioides sclopetarius* spiders and maintained in DMEM F-12 cell culture media at 18°C 80% humidity. Glands were observed utilizing stereo microscopy and timelapse imaging. Native spun full length silk proteins were dissolved and re-solidified for the first time using a novel protocol developed in the Goodacre lab utilizing BMIM-AC and other ionic liquids. Silk proteins were observed using Polarized Light Microscopy.

Results and Discussion: Using these methods glands were maintained in culture for periods of up to 1 month, allowing for a consistent production of liquid spider silk dope equaling the volume of the gland every 24 hours. Observing silk solidifying in the culture media coupled with the observations of the successful dissolution and coating of full-length spider silk proteins has allowed for unique insights into the conditions required for silk form and gain it's famous properties.

Conclusions: These novel methodologies allow for insights into the formation of full length, non-truncated spider silk proteins. Shedding light on to the molecular underpinnings of the biomaterial and potentially providing the information necessary to the industrial manufacture of an ancient and elusive biomaterial. This coupled with the ability to dissolve silk and coat new surfaces could open the door to a novel antimicrobial bioincompatible medical implant coating which could have an enormous impact in the age of antibiotic resistance and biofilm formation.

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Session 3: Flash Poster 23

Investigating Ionic Therapy for Bisphosphonate Related Osteonecrosis of the Jaw.

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Introduction

Bisphosphonates (BP) are antiresorptive drugs used to treat a wide range of bone disorders such as osteoporosis. BPs can, however, lead to bisphosphonate-related osteonecrosis of the jaw (BRONJ)[1,2]. Several hypotheses regarding the pathogenesis of BPs have been suggested; including suppression of bone remodelling, re-epithelisation, and impaired angiogenesis, but the mechanism remains unclear[3]. Ion releasing biomaterials, such as bioactive glasses, have been utilised to enhance osteogenic properties and promote bone regeneration[4]. Silicate species (Si ions) have been shown to induce osteoblast proliferation, bone nodule mineralisation, modulate bone matrix proteins, and stimulate angiogenesis and osteogenic differentiation[5,6]. This study will investigate the effects of Si in restoring osteoblast function and regulating bone regeneration following BP intervention. Moreover, the effects of BPs on osteoblasts will also be studied to potentially enhance the understanding around BPs' pathogenesis pathways.

Materials and Methods

Primary calvaria osteoblasts (OBs) were isolated from neonatal 3-days old Sprague Dawley rats and were cultured in α-mem media containing 10% FBS, 1% antibiotics/ antimitotic (AB/AM), and 1% L-Glutamine, and supplemented with 2 mM β-glycerophosphate, 10nM dexamethasone, and 50µg/mL ascorbate. The OBs were treated with zoledronic acid (ZA; 0.067, 0.2, 1.8µM), a type of nitrogen-containing BP, and/or 0.5mM Si (ZA(0.067µM)+Si, ZA(0.2 µM)+Si, ZA(1.8 µM)+Si) for 21 days. The proliferation (total DNA), alkaline phosphate activity (ALP), angiogenic response (VEGF ELISA), and ROS production was measured. Alizarin red for calcium staining, TEM, interferometry, and Raman spectroscopy were used to further characterise in vitro bone nodule formation in response to ZA and/or Si.

Results and Discussion

ZA impaired bone nodule formation in a concentration dependant manner (0.2µM) showed lower bone nodule formation in comparison to ZA (0.067µM), while ZA (1.8µM) completely inhibited nodule formation and was cytotoxic (*Figure 1*). Si treatment restored early-stage bone formation in ZA-treated cells (*Figure 2*). This revealed the potential of ionic therapy in restoring bone function and regulating bone regeneration following BRONJ.

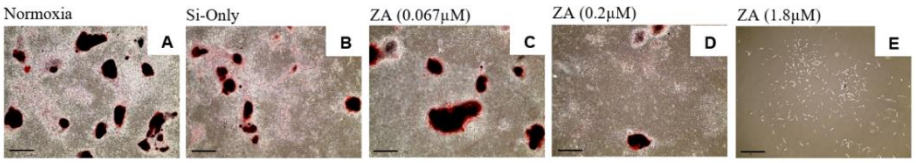

Figure 1 Alizarin red calcium staining showed that higher concentrations of ZA BP has led to a reduction in bone nodule formation in primary rat osteoblast. (Images taken using EVOS™ XL Core Thermo Fischer Scientific; scale bars = 500µm).

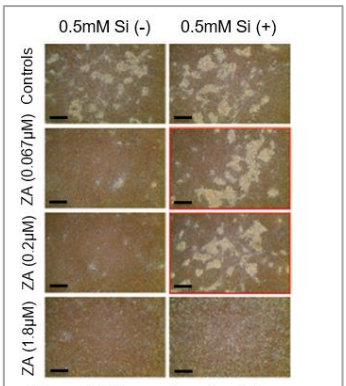

Figure 2 Si ions restored early bone nodule formation in ZA treated osteoblasts. (Images taken using EVOS™ XL Core Thermo Fischer Scientific; scale bars = 200µm).

Conclusion

This study advances our understanding of how Si modulates osteoblast behaviour and may allow the development of biomaterials with patient specific ion release profiles tailored for BRONJ patients.

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Session 4: Talk 1

Electrospun PCL coated with Collagen/Hydroxyapatite for Bone Tissue Engineering

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Introduction

Bone tissue is susceptible to damage caused by trauma and disease resulting in bone loss and bone defects. Autologous bone grafts have been frequently used for bone regeneration however there are drawbacks suchas limited bone supply, donor site morbidity and a long recovery time [1]. To address this limitation and the clinical demand for bone grafts, biodegradable bone graft substitutes have been developed. We therefore developed an electrospun polycaprolactone (PCL) scaffold that has been immersed in a collagen (Col) and hydroxyapatite (HA) coating. The microstructure of electrospun fibres were analysed using Scanning Electron Microscopy (SEM). The cytocompatibility of these electrospun scaffolds was further investigated using the Saos-2 osteosarcoma cell line.

Materials and Methods

15% (wt/v) PCL was prepared by dissolving PCL pellets in chloroform. The PCL solution was loaded into a 10ml Luer-lock syringe with a 16 gauge blunt-tipped needle 37 mm long. Fibres were electrospun onto a rotating collector spinning at 800 rpm with a flow rate of 6ml/hr at 20.5 kV and the needle tip-to-collector distance was kept at 30 cm.

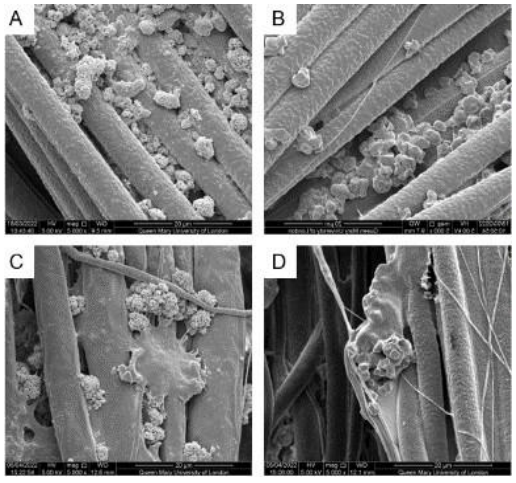
70wt% HA 30wt/v% collagen (Col/HA) coating used two types of HA: spray-dried HA (HA1) comprising agglomerates of nanoscaled needles and sintered HA (HA2) with smoother topography (both d_{50} c. 4µm, purchased from Plasma Biotal Ltd, UK). The HA particles were dispersed in pepsin soluble collagen type I in

0.01 M HCl (purchased from Collagen Solutions Ltd, UK). The PCL samples were sterilised in 70% ethanol and immersed in either collagen (Col) or Col/HA and dried overnight at room temperature. Uncoated PCL wasused as a control.

Saos-2 cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented by 20% foetal bovine serum (FBS) and 1% penicillin/streptomycin and incubated at 37°C at 5% CO_2. Using non-coated 24 well plates, Saos-2 cells were seeded at a density of 800,000 cells/ml directly onto sterilised PCL samples.

Results and Discussion

SEM micrographs (Figures 1A & 1B) show the particle morphology of HA1 and HA2. The HA1 particles display nanoscale needle projections and rough topography (Figure 1A). In contrast, HA2 particles exhibit a much smoother surface (Figure 1B). Within the first day of incubation the cells attached to the fibres using filopodial extensions (Figures 1C& 1D). Cells appeared to adhere to the surfaces of individual and multiple fibres also interacting with the HA1 (Figure 1C) and HA2 particles (Figure 1D) within the Col/HA coating.



Figures 1A-D: SEM micrographs A) Col/HA1 coating and B) Col/HA2 coating on PCL fibres, C) Saos-2 cells attached to Col/HA1 and D) Col/HA2 coated fibres (scale bars =20µm)

Conclusions

Introducing the collagen-HA coating onto the PCL fibres maintained the morphology and size of the PCL fibres. SEM micrographs further showed a good distribution and integration of HA1 and HA2 particles on the surface and in between PCL electrospun fibres. Electrospun PCL fibres produced uniform andaligned fibres which may encourage the attachment and elongation of the Saos-2 cells along their surfaces. Saos-2 cells also appeared to interact with HA particles however further cell viability testing, such as Alamar Blue assay, is required to validate these results.

Acknowledgments
EPSRC is thanked for funding the studentship (EP/N50953X/1)
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Session 4: Talk 2

Evaluation of Biocompatibility of Porous Titanium- Niobium Alloys

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Introduction

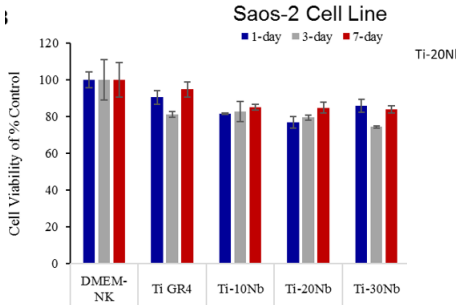
Titanium (Ti) and its alloys are widely used as load-bearing implant materials owing to their low density, acceptable strength properties, and superior chemical stability [1]. They can be generally categorized as alpha (α), alpha (α) + beta (β) and beta (β) alloys, which is dependent on the occurrence of some alloying elements in Ti matrix [2]. Nb is completely biocompatible and a strong beta stabilizer in Ti. Ti-Nb alloys are thus more suitable for implant applications as they exhibit lower elastic modulus than alpha Ti alloys with less stress-shield effect. On the other hand, a porous structure within the load-bearing implant is essential for tissue growth and transport of body fluid, by providing adequate interlocking between load-bearing implant and tissue [1]. The aim of this work is to investigate the effect of Nb concentration on the biocompatibility in vitro for a series of porous binary Ti–Nb alloys with Nb contents up to 30 at. %.

Materials and Methods

Powder metallurgy was selected as production method in this study since the technology can readily generate porous structures, which increase the cell adhesion assay. Ti-(x) Nb alloys of nominal Nb contents (x=10, 20 and 30 at. %) with various porosities (%) were produced using powder metallurgy followed by sintering at 1200 ˚C for 6 h. The biocompatibility of the Ti-Nb alloys was evaluated by the cytotoxicity in accordance with the ISO 10993 for the biological evaluation of medical devices [3].

Results and Discussion

Cell number analysis was performed in cell cultures of 1, 3 and 7days and revealed that the human bone osteosarcoma cell line (Saos-2) was proliferated effectively on the surfaces of the Ti-(x) Nb alloys (at. %) without cytotoxic effects on the cell culture. Although there was an increase in cell proliferation for Ti-10Nb and Ti-20Nb as a function of time(day), nonlinear cell proliferation behaviour was observed for Ti-30Nb. The maximum cell viability was determined on the Ti-10Nb (at. %) alloy for 1 day, which was 94.87 %. In addition, as seen in the SEM image, large area of Ti-20Nb (at. %) surface is almost covered by Saos-2. The adherence of Saos-2 cell line on surface of Ti-20Nb (at. %) after 3 days showed that there was a good integration between the alloy and the cell. Consequently, increasing Nb content did not play crucial effect on the cell proliferation.



	DMEM-NK	Ti GR4	Ti-10Nb	Ti-20Nb	Ti-30Nb
1-day	100,00	90,57	81,66	77,11	85,93
3-day	100,00	81,32	82,73	79,58	74,40
7-day	100,00	94,87	85,24	84,88	83,89

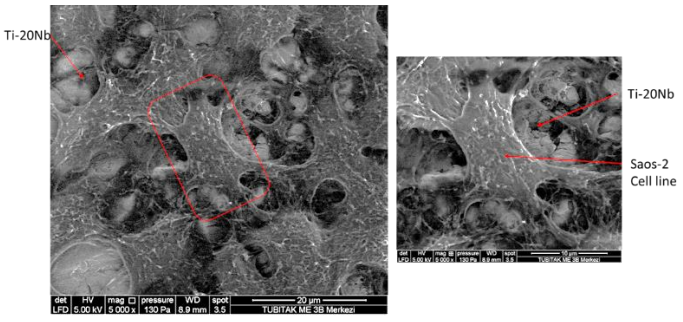


Figure 1. SEM images of Saos-2 cells seeded at **2.5x104** cells on the Ti-Nb alloy disc and incubated for 72 hrs. After that It was fixed with **2.5 % gluteraldehyde** and dried with etOH.

Conclusion

Porous Ti–Nb alloys with Nb contents up to 30 (at. %) successfully fabricated by powder metallurgy as load-bearing. The all Ti-based alloy resulted in more than 75 % cell viability which is required level for the medical devices or load bearing implant.

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Session 4: Talk 3

EFFECTS OF STRONTIUM-SUBSTITUTION IN SPUTTER DEPOSITED CALCIUM PHOSPHATE COATINGS ON THE RATE OF CORROSION OF MAGNESIUM ALLOYS

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Introduction

Fracture fixation devices are commonly made from non-degradable metals such as titanium and stainless steel, that are mechanically mismatched and require removal/revision surgeries [1]. More recently, magnesium (Mg) alloys have been highlighted as an alternative approach[2], providing the appropriate mechanical properties, and the ability to resorb *in situ*. However, they are prone to rapid resorption, and need modification to arrest this phenomenon, until such times as bone has been regenerated. One option for targeting the rapid resorption is implant modification by way of a coating[3]. Calcium phosphate coatings have been investigated thoroughly in this respect, due to the added bioactive element, with particular interest shown recently in substituted apatites[4].

Materials and Methods

Custom magnesium alloys, namely ZEWX and WJK, were manually abraded using 1200 grit SiC paper. Deposition of CaP and SrCaP thin films, was carried out in a custom-built high vacuum sputtering system. Corrosion testing was completed through immersion of samples in simulated body fluid (SBF) with coupons of each Mg alloy (coated with CaP/SrCaP) submerged in 15 mL of SBF and incubated at 37 °C, under atmospheric pressure conditions for a total period of 14 days, with the SBF solution being replaced daily. Samples were subjected to gravimetric analysis pre- and post-corrosion testing based on the weight loss measured, with results were used to calculate the corrosion rate (CR). Post-corrosion, Micro–Computed Tomography was employed to create volumetric reconstructions of the substrate (seen in Fig. 1).

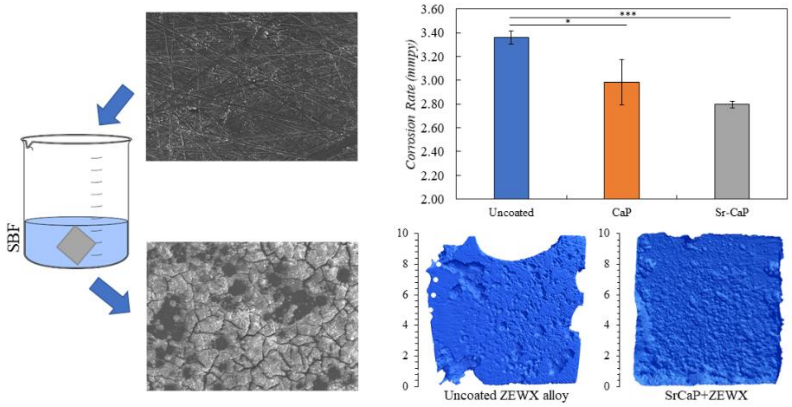

Figure 6 *SEM analysis ZEWX Mg alloy pre- and post-immersion in SBF, with respective corrosion rates plotted, showing significant difference between uncoated and coated substrates.*

Results and Discussion

Calculation of corrosion rate (mmpy) reveals that the CaP and SrCaP coatings act to increase the corrosion resistance of the WJK and ZEWX Mg alloys. This is confirmed by rendered volumetric images which show CaP and SrCaP coated substrates undergo less pitting than the pristine, uncoated Mg alloy.

Conclusions

Application of soluble bioactive CaP and SrCaP coatings onto bioresorbable Mg alloys offers a means to control the rate of corrosion that occurs over the short to medium term. Moreover, while the addition of Sr to CaP coatings does not significantly alter the Mg alloy corrosion rate it does offer the potential to further enhance the attendant bioactivity.

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Session 4: Alan Wilson Memorial Lecture

The role of multifunctional polymeric dental biomaterials in tooth restoration

Prof. Sanjukta Deb

Sanjukta Deb is a professor in Biomaterials Science at the Faculty of Dentistry, King's College London. Her research relates to innovative material-based approaches to restore or repair damaged tissues targeted towards clinical translation. The aim is to utilize biomaterials chemistry to develop tissue analogue materials specifically bone tissue regeneration, bone and dental cements, drug delivery and dental applications.

Abstract

Tooth decay is one of the most prevalent, lifestyle related non-communicable diseases and the current treatments range from restoration of the cavity with biomaterials to surgical excision of the necrotic tooth. Although modern minimally invasive therapies have seen the advent of smart restorative biomaterials that make it possible to restore teeth whilst preserving more tooth tissue, the failure of restorations is still a common occurrence. This is usually addressed by removing the failed restoration and replacing it however this clinical step inevitably results in further loss of tooth tissue, thereby weakening the tooth structure further. There is growing evidence to show that repair of the tooth-restoration complex is favourable over replacement of the entire filling, thus necessitating research into materials that can be used for repair.

A significant proportion of currently utilized procedures in restoring diseased or damaged teeth depend on the close interaction of polymeric materials with dental tissues. This presentation will reflect on both repair and restoration of tooth tissue using organophosphorus monomers and detail on a unique class of material with potential to dynamically interact with tooth tissue and existing restoratives for management of a failed tooth-restoration complex. These poly alkenoate based cements (pRMGIC) are designed using existing tooth adherent cements, namely glass-ionomers with an organophosphorus monomer, 2-(methacryloyloxy) ethyl phosphate, thereby promoting the interaction of the ligating phosphate groups with the glass matrix and the mineral component of the tooth. The pRMGIC cements exhibit superior physical properties than the conventional resin modified glass ionomer cements (RMGIC) with a twofold increase in biaxial flexure strength on ageing whilst their ability to decalcify tooth apatite results in enhanced interfacial adhesion due to chelation with calcium ions of the tooth apatite. Thus, these cements have the potential to be used as adhesive restorative materials and as a repair material for different restoratives, that would benefit both patients and the severely constrained healthcare budgets available.

Session 5: Talk 1

Multiple Cues In Acellular Amniotic Membrane Incorporated Embelin For Tissue Engineering

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Introduction

Amniotic Membrane (AM) has been recast as a potential source of composite biomaterial by various preparation techniques that aim at retaining growth factors and secretome. AM scaffold its topography, its constituting array of bioactive molecules, and modality of application remain nascent. AM incorporated with embelin, a natural benzoquinone compound for neutralizing free radicals, while simultaneously accelerate wound healing.

Materials and Methods:

AM processed from the placenta has been characterized for integrity by histology, bio-degradability, thermogravimetric analysis (TGA), and cytokines analysis determined the presence of growth factors vital for tissue regeneration. The spectroscopic analysis confirmed the synthesized embelin and demonstrated burst release (>80%) from the embelin incorporated AM supported by mathematical modeling. Surface topography and roughness of embelin incorporated AM were examined by scanning electron microscopy and atomic force microscopy respectively. In addition to anti-oxidant activity, the presence of embelin has significantly improved the initial fibroblast cell adhesion and proliferation compared to plain AM and TCPS.

Results and Discussion:

Amniotic Membranes (AM) are an indispensable source as the components of the tissue engineering triad: scaffolds, cells, and bioactive molecules. The sustained degradation rate of AM from our study supports the hypothesis of tissue regeneration aid. The growth factors expressed at high levels and biological regulated proteins such as TIMP-2, ALCAM, CD14, IL 1α, IL1RA. AM along with Embelin is used as a scaffold along with quenching oxygen radicals, fibronectin in the membrane interacts with the fibroblasts *via* the fibronectin-integrin interactions which further ensures the secretion of various growth factors and cytokines contributes to the wound healing process.

Conclusions:

The facile separation and detailed characterization of AM unravel the array of growth factors favorable as scaffold/biomaterial. Embelin that enhances the anti-oxidant property and has been used in combination with naturally sourced biomaterial. This Scaffold helps in depositing collagen to the wound site, increase the strength of tissues, forms cross-linkages between collagen fibers and quenches the oxygen radicals which causes tissue damage.

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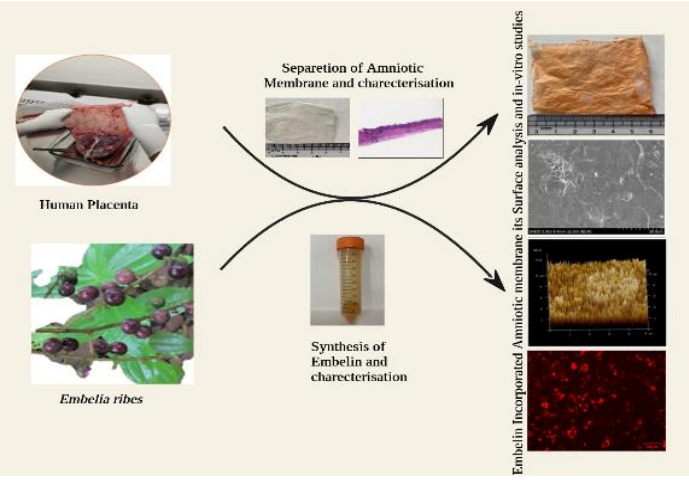

Session 5: Talk 2

PREFUNCTIONALISED BIODEGRADABLE MICROCARRIERS FOR CELL CULTURE AND CELL DELIVERY

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Introduction: Synthetic polyester polymers such as poly (lactic-co-glycolic acid) (PLGA) have been at the forefront of clinical and biomedical applications including drug delivery systems, tissue repair scaffolds, and medical devices. The primary interest in this type of polymers is mainly due to their biocompatible nature and tuneable degradation profile. Despite such advantages, polyester polymers lack cell adhesion moieties which in turn limit their capacity to interact with cellular materials and hamper their applications in the tissue engineering and regenerative medicine field. Several physical and chemical modifications have been explored and developed to allow the insertion of cell adhesion moieties. That said, most of these methods rely upon undesirable post-functionalisation and purification techniques. The post-modification processes are seen as a major drawback, not always defined and require a series of conjugation and purification steps, thus adding to the complexity of the synthesis process and biomedical applications. Herein, we present a novel prefunctionalisation method for the generation of PLGA microparticles with integrated adhesion moieties. This strategy promotes surface cell adhesion at physiological conditions without the requirement for further post-modification.
Materials and Methods: In a well-established Ring-Opening Polymerisation process, 2-2-dimethylaminoethanol was used as an initiator to obtain poly (lactide-co-glycolide-i-dimethylaminoethyl) $PLGA_{DMAE}$ polymers. A standard unmodified PLGA polymer was synthesised and used as a control. The molecular weight distribution, polymer structure, polymer compositions were analysed and compared to the control polymers. Microparticles were fabricated using the membrane emulsion technique. The cell adhesion properties were analysed using fluorescent imaging and SEM.
Results and discussion: GPC analysis showed that the number-average molecular weight (Mn) and polydispersity (PDI) of both PLGA and $PLGA_{DMAE}$ polymers were comparable. In addition, the bulk properties of the $PLGA_{DMAE}$ polymer remained unchanged compared to the control PLGA. Next, the resultant polymers were subsequently used in the fabrication of microparticles via membrane emulsion technique, which allowed control over the morphology and size distribution of the microparticles. Bright-field imaging and SEM demonstrated that there were no significant differences in the morphologies or size distribution between the PLGA and $PLGA_{DMAE}$ microparticles. Then, Adipose-Derived Stem Cells (ADSCs) were co-cultured with $PLGA_{DMAE}$ microparticles to investigate their cell adhesive properties and comparing against the standard PLGA. LDH and LIVE/DEAD assays demonstrated that the surface functionalised $PLGA_{DMAE}$ microparticles maintained a low toxicity profile compared to the unmodified PLGA microparticles. In addition, SEM showed that the ADSCs readily adhered to $PLGA_{DMAE}$ microparticles and exhibited differential cellular morphologies when compared to ADSCs co-cultured with unmodified PLGA microparticles, demonstrating that $PLGA_{DMAE}$ microparticles readily promote adhesion at physiological conditions. Lastly, GPC and SEM displayed that the degradation profile of the new $PLGA_{DMAE}$ was enhanced compared to that of standard PLGA polymers.
Conclusions: Overall, this novel prefunctionalisation strategy enables the successful insertion of cell adhesion moieties to render these biodegradable polyester materials readily available for tissue engineering and regenerative medicine applications

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Acknowledgements:

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Session 5: Talk 3

CUSTOMISABLE PEPTIDE HYDROGELS AS 3D BIOMIMETIC MODELS OF SPECIFIC TISSUE MICROENVIRONMENTS

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Introduction

Current *in vitro* models of development and disease typically fail the recapitulate the complexities of the native extracellular matrix (ECM), leading to a poor extrapolation of *in vitro* findings to *in vivo* relevance. This is a major contributing factor to the high attrition rate of once promising novel drug candidates moving from the lab to the clinic. This has driven the recent interest in the development of more sophisticated 3D *in vitro* models which allow customisation of matrix composition and stiffness. The FEFEFKFK peptide hydrogel is a promising new candidate model platform that aims to address all the shortcomings of current *in vitro* models, by offering end users multiple methods for customising both its biochemical and biomechanical features.

Materials and Methods

The FEFEFKFK peptide hydrogel model platform could already be customised by encapsulating soluble matrix components selected to match specific tissue microenvironments and its stiffness can be controlled independent of matrix composition. To expand on this, two new methods for covalent immobilisation of biomolecules were investigated: sortase-mediated functionalisation and “click” chemistry-mediated functionalisation. By leveraging these two new methods, gels were functionalised with relevant peptides and glycosaminoglycans (GAGs) and their impact on the differentiation potential of encapsulated mouse embryonic stem cells (mESCs) was evaluated, via immunohistochemistry.

Results

We have demonstrated that sortase-mediated immobilisation of an RGD containing peptide leads to a measurable impact on the differentiation potential of encapsulated mESCs. We have also provided an initial proof of concept for “click” chemistry-mediated immobilisation of the GAG heparin and its impact on mESC differentiation. In a final experiment, we were also able to demonstrate that multiple functionalisations could be performed, by combining different methods, to create a more complex gel environment which has a unique impact on encapsulated cells.

Conclusions

We have demonstrated the validity of two new methods for covalent immobilisation of relevant biomolecules. Combined with the previous methods for customising with soluble matrix additions and for independent control of matrix stiffness, the FEFEFKFK peptide hydrogel model now offers end users a greater degree of freedom in how they approach model design. This will allow them to create increasingly complex gel environments which better represent the native ECM of specific tissue microenvironments, leading to more reliable results obtained in drug discovery and investigative research.

Session 5: Talk 4

QUANTITATIVE ANALYSIS OF FLUID CONTENT OF PH TREATED WHEY PROTEIN ISOLATE HYDROGELS IN A SWOLLEN STATE USING TERAHERTZ RADIATION.

Daniel K. Baines, PhD student[1], Hungyen Lin, PhD[2], Timothy E.L. Douglas, PhD[3], Karen L. Wright, PhD[4]
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Introduction

The ability of Whey protein isolates WPI) to form gelatinous structures has led to utilisation in the biomedical sector. The gelation, caused by peptide denaturing and aggregation, commonly induced through thermal activity, has provided topical applications, surfaces for cellular proliferation and drug delivery mechanisms (Mayorova et al., 2021). However, the use of WPI, particularly *in vivo,* exposes the protein mixture to the changing variables and conditions in a living organism. A chronic wound environment exposes WPI hydrogels to pH levels between pH4-pH9 (Kumar and Honnegowda, 2015). Results from a previous investigation have suggested that WPI hydrogels react differently when introduced into different pH conditions, with a defined change in the sorption abilities of the hydrogels. Understanding the sorption capacity of hydrogels therefore provides a basis for determining how the hydrogels would react in the presence of bodily fluids as wound exudate. The results from a previous investigation suggested that WPI hydrogels contract under pH4 conditions, losing fluid content as time increases However, under ph7 and 9 conditions the hydrogels react differently. Hydrogels introduced to both pH7 and 9 condition present absorption qualities with absorption increasing relevant to the ph. Here we apply an approach using terahertz radiation previously demonstrated by (Kudlacik-Kramarczyk et al., 2021) to quantitatively analyse the fluid content and the relative sorption properties of WPI hydrogels.

Materials and Methods

WPI Hydrogel solutions were formed to the concentration of 40% (10gWPI/ 25mL MilliQ solution) and were homogenised. The prepared solutions were injected into a PTFE liquid cell with dimensions 30mm length x 10mm width at a thickness circa 250 µm. The samples were then formed in 90^{o}C H_2O, for 5 minutes, ensuring gelation. Post gelation, the sample was placed into 5mL of the relevant solution (pH4, pH7.4 (PBS) or pH9). At 1-minute intervals the sample was removed from the solution, any surface solution dried and was simultaneously imaged using THz camera and the mass measured. The terahertz measurements were taken and calculated in accordance with Beer-Lambert Law (Kudlacik-Kramarczyk et al, 2021)

Results

For the pH4 variable, results suggest a decrease in fluid content of 58.53% from minute 1 to minute 5 amounting to an overall decrease in fluid content of 2.23mg. However, as suggested by the preliminary investigation both the pH7 and pH9 variables increase in fluid content, during the same time period with an increase of 43.35% (1.49mg) and 45.25% (2.02mg), respectively.

Discussion

Although still in its infancy, our data suggests that through the analysis of the data it could potentially be possible to ascertain through non-invasive methods the state of healing within the wound. For instance, in the chronic wound state with pH levels as high as pH9, the hydrogel would reach its maximum sorption capacity. However, a healed wound would present normal skin pH levels of circa 4.5 with the data suggesting a contraction and loss in water content displayed by the hydrogel. However, currently there are limitations to this potential such as distinguishing between the water content of the hydrogel layer and the water content of the wound or limb itself. One solution would be a material that would not alter the hydrogel sorption properties but would block terahertz, providing a definitive line between the hydrogel and the limb.

Conclusion

We have presented preliminary data on the quantitatively analysis of the solution content of the WPI hydrogel and additionally quantify their sorption capacities using terahertz imaging. Our results show that there is a difference between the swelling properties when the hydrogel samples are introduced to different pH conditions and that this variation could be quantified using terahertz radiation.

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Acknowledgements

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Session 5: Talk 5

Highly-stretchable conductive covalent coacervate gels for electronic skin

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Introduction

Highly stretchable electrically conductive hydrogels have been extensively researched in recent years, especially for applications in strain and pressure sensing, electronic skin and implantable bioelectronic devices. The most common method to prepare high-performance hydrogels involves in situ free-radical polymerization. Herein, we present a new cross-linked complex coacervate approach to prepare conductive hydrogels that are both highly stretchable and compressive.

Materials and Methods

The gels investigated in this study involve a complex coacervate between carboxylated nanogels (NGs) and branched poly(ethylene imine) (PEI), whereby the latter is covalently crosslinked by poly(ethylene glycol) diglycidyl ether (PEGDGE). Inclusion of graphene nanoplatelets (Gnp) provides electrical conductivity as well as tensile and compressive strain sensing capability to the hydrogels. The structure is depicted in Fig. 1(A).

Results and Discussion

In this study we demonstrate that judicious selection of the molecular weight of the PEGDGE crosslinker enables the mechanical properties of these hydrogels to be tuned. Indeed, the gels prepared with a PEGDGE molecular weight of 6,000 g/mol defy the general rule that toughness decreases as strength increases. The conductive hydrogels achieve a compressive strength of 25 MPa and stretchability of up to 1500%. These new gels are both adhesive and conformal. They provide a self-healable and strain sensing electronic circuit (Fig. 1(B)), report human motion (Fig. 1(C)), while exhibiting low cytotoxicity (Fig. 1(D)). Our new approach to conductive gel preparation is efficient, involves only preformed components and is scalable[1].

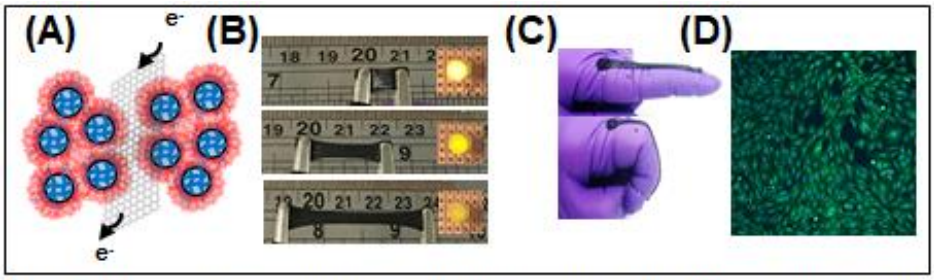

Fig. 1. (A) Coacervate structure, **(B)** Strain sensing, **(C)** Movement sensing, **(D)** Cell challenge experiments.

Conclusions

We report a facile new protocol for the preparation of highly stretchable electrically conductive gels that defy the toughness-strength paradox. Remarkably, addition of Gnps not only confers electrical conductivity but also further increases gel toughness and strength, enabling gels to be stretched to up to 1500% of their original dimensions. Moreover, gel preparation is efficient, cost-effective and amenable to industrial scale-up.

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Session 6: Larry Hench Young Investigators Prize

Graphene formulations: New ways for manipulating medicine at the nanoscale

Dr Tanveer Tabish

Dr. Tanveer A. Tabish is British Heart Foundation (BHF) Advanced Fellow and Principal Investigator in Nanomedicine at the Radcliffe Department of Medicine and Department of Engineering Science at the University of Oxford. He has previously held research positions at the Imperial College London, University College London, and University of Exeter. He spent almost 8 years in biomaterials, nanomedicines and bionanophotonics, leading teams developing novel diagnostic (linear and nonlinear optical) and therapeutic (photodynamic, photothermal and medical gas therapies) techniques that advance analytical capabilities and therapeutic interventions for drug discovery and nanomedicines development. Tanveer has made major scientific breakthroughs and significant contributions to the development of graphene-based anti-cancer nanomedicine, and he has extensive experience in pre-clinical translation of novel nanomaterials of different sizes and shapes for disease diagnostic and therapeutic purposes. He interweaves the scientific fields of nanomedicince, biomaterials, tissue engineering, bioengineering, and biochemistry to develop 'personalised' solutions based on light-mediated therapeutic modalities that utilise nanotechnology in treating many diseases such as cancer, wound healing, cardiovascular diseases, and central nervous system disorders. He has published over 55 research papers in high impact-factor peer reviewed scientific journals and filed one patent. Tanveer has also delivered over 10 invited lectures at many different international conferences and meetings worldwide, particularly in the USA (major recent meeting include: MS&T). He has secured over three quarter of a million pounds in research funding to support his cutting edge and novel programme of research.

Abstract:

Nanostructured graphene is a versatile two-dimensional carbon-based nanomaterial for medical applications since the structure, molecular weight, size-dependent luminescent features, and chemical composition, as well as bioconjugation/functionalisation, can be precisely controlled. This talk will provide an overview of our recent developments on the design of graphene nanostructures to interface with cells (using both pharmacological and genetic approaches), tissues and living systems as well as human biopsy material for cancer diagnosis and treatment in a safe and targeted single procedure. This talk will also describe how different derivatives of graphene (such as graphene oxide, reduced graphene oxide, nanopores, porous nanosheets, quantum dots and foam) can be accumulated within diseased cells selectively while remaining non-toxic to surrounding cells under the dark environment, with innovative synthesis strategies, structures, and properties, enabling first-in-field multimodal imaging and therapeutic options. Besides expanding our knowledge of the fundamental crosstalk between graphene, disease diagnosis and therapeutic index, this lecture will also provide novel insights into the strategies for preparing mitochondria-targeted graphene to destroy the cell's powerhouse in a targeted and safe fashion. Targeting mitochondria with graphene may represent a significantly important therapeutic target and may potentially transform the conventional therapeutic interventions both philosophically and practically.

Session 6: Talk 1

Bifunctionalised Electric Field Responsive Conductive Nanoactuators for Glioblastoma Multiforme

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Introduction: Glioblastoma Multiforme (GBM) is a fast-growing and aggressive brain tumor which classified as "Hard-to-Treat" by cancer research UK. The mainstay of treatment for GBMs is surgery, followed by radiation and chemotherapy, which are invasive and only improves the 5-year survival rate by 6 months. The main reasons behind the low efficacy of these current GBM treatments is low drug bioavailability and tumor heterogeneity, which fuels resistance to these therapies. Thus, new modalities for specific elimination of GBM cells in patients are urgently needed.

Materials and Methods: In this work, conductive gold nanoparticles (GNPs) were functionalised with two clinically relevant redox active biomolecules using EDC-NHS carbodiimide chemistry. These bifucntionalised GNPs were characterised using an array of analytical techniques. The ability of these bifunctionalised GNPs to induce apoptosis upon application of alternating current electric fields (AC EFs) was studied in a heterogenous population of patient derived GBM cells.

Results and Discussion: *In vitro* studies demonstrated that high frequency AC EFs were most effective in inducing caspase-3 mediated apoptosis in GBM cells by stimulating redox reaction on the surface of bifunctionalised EF responsive nanoactuators. Furthermore, visualisation of the intracellular trafficking in live cells under the influence of AC EFs revealed enhanced uptake and proton sponge-based endosomal escape of these bifunctionalised gold nanoactuators in primary GBM cells.

Conclusions: Our findings provide significant evidences that electric fields can be used to establish communication with charged molecular machineries within the cells to actuate their behaviour and maintain homeostasis. This technology will lead to new paradigm in electric field mediated therapy, tissue engineering and regenerative medicine.

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Session 6: Talk 2

ANTIMICROBIAL PEPTIDE-CONJUGATED GRAPHENE-BASED OPTICAL LENS FOR PREVENTION AND TREATMENT OF BACTERIAL EYE INFECTIONS

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Introduction

Graphene, as a two-dimensional hexagonal lattice of the carbon atom, displays remarkable electrical, mechanical and chemical properties as well as excellent compatibility with biological systems. In contrast to classical chemical and mechanical exfoliation methods to prepare graphene, chemical vapour deposition (CVD) technology has recently enabled the production of large area, high quality transparent graphene with extensive uses in electronics and biomedical devices[1]. Contact lenses (CL) are the most widely prescribed biomedical device in the world and it has been found that CVD graphene coating on CL offers several advantages such as electromagnetic wave interference shielding and dehydration protection[2]. Besides dryness and dehydration, long-term wearing of CL also results in ocular infections via biofouling but it is currently unclear if CVD graphene, alone or with antimicrobial peptides (AMP) modifications, can efficiently protect against them.

Materials and Methods

First, a monolayer graphene coating was synthesized using CVD method and transferred onto CLs materials such as polymethyl methacrylate (PMMA) and polydimethylsiloxane (PDMS). Next, a series of AMP-conjugated CVD graphene-coated PMMA and PDMS were prepared for comparison. Peptide functionalization was performed by three sequential steps: oxidization, carboxylation and peptide conjugation. The amount of conjugated AMP was quantified using the 3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde (CBQCA) protein quantification assay. The materials were then characterized for light transmittance, contact angle and protein deposition. Anti-fouling properties of the modified materials were evaluated via colony counting, XTT and LIVE/DEAD biofilm viability assays, while biocompatibility tests were performed using human corneal fibroblasts and epithelial cells.

Results and Discussion

AMP were successfully conjugated on CVD graphene CL surfaces in the range of 2 to 4 μg/cm^2 and had no impact on light transmittance of CL materials (99%). Compared against commercial CL, AMP-conjugated CVD graphene-based CL had contact angles of less than 45° and protein deposition of less than 13 μg/cm^2 tested within 16 h, indicating greater transparency and acceptable wettability and protein deposition. Using confocal microscopy, *P. aeruginosa* was observed to form an intact biofilm on surfaces without AMP, but not on AMP-conjugated graphene. This observation was supported by lower colony counts over 24h to 48h on the latter. When tested against human corneal fibroblast and epithelial cells, cell viability was greater for CVD graphene-coated PDMS (80%) than PMMA (60%).

Conclusion

All in all, our study highlights the potential of AMP- conjugated CVD graphene coating on CL to minimize bacterial infection and prevent the occurrence of infections associated with biofilms.

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Session 6: Talk 3

Bioinspired Nanotechnology for Cellular Traction Force-Mediated Delivery of Biologics

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Introduction

Tissue repair is driven by a complex spatiotemporal process that removes damaged tissue while also building up its replacement[1]. Recently, we have developed a bioinspired method for the controlled delivery of therapeutics that relies on cellular traction forces to activate and release the bound therapeutics, which we call TrAPs[2]. Here, we explore how this platform can be used to drive therapeutic angiogenesis and work to understand how it can be used in vivo as a therapeutic delivery system.

Materials and Methods

TrAP synthesis was done via a Mermade 6 oligonucleotide synthesiser. In vitro sprouting assays were performed using microvascular endothelial cells on cytodex beads and imaged via a Leica Stellaris 5 lightsheet microscope. In vivo evaluation was done using a tissue defect model in Wistar rats and characterized using multiple methods.

Results and Discussion

(unpublished, data not shown)
TrAP-functionalised collagen are shown to robustly promote the sprouting and migration of endothelial cells in vitro, providing sustained pro-angiogenic signaling over days to weeks. Subsequent in vivo testing confirms that TrAPs are functional in vivo and can drive changes to the early stages of tissue repair.

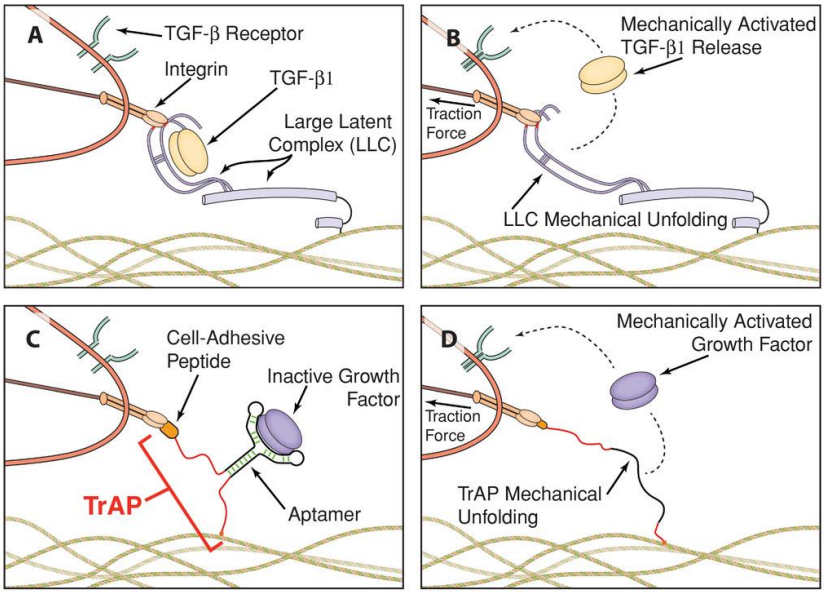

Figure 1. Bioinspired traction force activated payloads (TrAPS) for

Conclusions

TrAPs provide a versatile platform for enabling cell-specific activation of therapeutics for new opportunities in spatiotemporal modulation of tissue repair. Here, TrAPs are used to facilitate the sprouting and growth of blood vessels, opening new opportunities for dynamically modulating therapeutic angiogenesis.

Acknowledgements

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Session 6: Talk 4

NANOTECHNOLOGY-ENABLED RNA THERAPIES FOR TISSUE ENGINEERING AND REGENERATION

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Introduction

RNA therapies have promising applications in tissue engineering and regeneration, as they can temporarily promote or suppress the expression of proteins involved in key regenerative processes, without irreversible DNA modifications. However, RNA's instability and limited intracellular delivery have hampered the successful translation of RNA therapies to the clinic [1]. Poly(β-amino ester)s (pBAEs) have been used as nucleic acid delivery vectors to cancer cells, and shown to enable clinical translation [3]. However, non-viral intracellular delivery to primary cells is particularly challenging, and efficient approaches, like electroporation [2], are not suitable for clinical translation. We have optimized pBAEs to deliver RNA to difficult-to-transfect primary cells, such as dermal fibroblasts (HDF) [4], keratinocytes (HKc), chondrocytes (HCh) and mesenchymal stem cells (MSCs), all highly interesting targets for tissue engineering and regeneration purposes.

Materials and Methods

Transfection efficiency of primary human cells (HDF, HKC, HCh and MSC) was optimised by tuning the polarity of the backbone pBAE polymers and the ratios of arginine and histidine-ended pBAEs. mRNA encoding the reporter gene EGFP (Stratech) was encapsulated, and efficiency of transfection was measured after 24 hours by fluorescence microscopy and flow cytometry, compared to commercially available control JetMessenger. Therapeutic siRNAs were delivered to MSCs, and silencing was measured after 24 hours by RT-qPCR.

Results and Discussion

The optimal pBAE formulation for HDFs, which we named C6RH, is composed of a non-polar backbone and a 3:2 arginine to histidine ratio, and showed over 80% transfection efficiency of HDFs compared to 50% obtained with JetMessenger (Figures A, B & G) [4]. Moreover, we observed very low toxicity, with over 85% cell survival. Similar optimisation process was conducted with HKc and HCh, both notoriously difficult-to-transfect cells, obtaining 93% and 87% transfection efficiency, respectively (Figure C-G, unpublished data). We also explored the delivery of therapeutic siRNAs to MSCs, as part of an ongoing study identifying key genes that promote ossification in soft tissues after injury. The delivery of a target siRNA using optimised pBAE nanoparticles showed high silencing efficacy and overall prevention of bone formation.

Conclusions

We have established a pipeline to optimise transfection efficiency of pBAE nanoparticles to difficult-to-transfect human primary cells like HDF, HKc, HCh and MSCs. These nanoparticles are clinically translational [3], and their bioavailability can be enhanced by incorporating them into hydrogels for local and sustained delivery [3,4]. Previous work had focused on delivering RNAs as cancer therapeutics. Through in depth understanding of pBAE chemistry and cell biology, this work expands the applicability of this platform to the tissue engineering and regenerative medicine arenas, opening a new avenue to tackle complex human diseases like chronic wounds, osteoarthritis and organ fibrosis, amongst others.

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Session 7: UKSB President's Prize

Biomaterials Solutions for Unmet Clinical Needs

Prof. Paul Hatton

Despite tremendous advances in medicine over the past half century, frequently made as a result of the translation of discoveries in biomaterials science into successful medical devices, many challenges and unmet clinical needs remain. Paul Hatton has worked at the interface between biology and materials science for over 30 years, leading on translational projects directed towards improved treatments for patient, industry and societal benefit. His presentation will review these briefly before focussing on a recent programme in transmucosal drug delivery that illustrates the critical role of "team science" in modern biomaterials research.

Session 7: Talk 1

USING DECELLULARISED HUMAN TRABECULAR MESHWORK AS A BLUEPRINT FOR FABRICATING BIOMIMETIC ELECTROSPUN SCAFFOLDS

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Introduction

Glaucoma is the leading cause of irreversible blindness worldwide and is linked to raised intraocular pressure (IOP)[1]. The trabecular meshwork (TM) is essential for regulating aqueous humour outflow. Yet, in glaucoma, a drastic reduction in cellularity is associated with blockages of the porous TM resulting in elevated IOP. This causes compression of the optic nerve head resulting in death of retinal ganglion cells and, ultimately, irreversible vision loss[2]. Current treatments include topical pharmacological agents (low clinical efficacy due to poor patient adherence) or surgery to create drainage channels (hypotony and fibrosis are common postoperative complications requiring further intervention). Synthetic scaffolds that mimic the TM's fine-scale anatomical 3D hierarchical architecture could be utilised as a novel tissue engineered intervention for TM replacement or as a 3D *in vitro* model for glaucoma research. Using X-ray computed tomography (X-CT), we have captured high resolution 3D images of decellularised human TM (Fig.1) and the information from these images provides a blueprint for the design and fabrication of scaffolds that recapitulate TM structure. Here we outline current fabrication processes – electrospinning and melt electrowriting (MEW) – to create 3D scaffolds that aim to mimic TM architecture.

Materials and Methods

Poly(ε-caprolactone) (PCL; 12%w/v) and poly(lactic-*co*-glycolic acid) ($PLGA_{8515}$; 8%w/v) were dissolved separately in 1,1,1,3,3,3-hexafluoroisopropanol. In the first run, PCL was electrospun with and without the presence of dry ice in the collecting mandrel to enhance overall porosity due to ice crystal formation on the mandrel's surface (i.e. cryo-spinning). In second ($PLGA_{85:15}$) and third (PCL) runs, flow rate and voltage were manipulated partway through the electrospinning process in an attempt to decrease fibre size and generate a hierarchical scaffold. In the fourth run a gradient change in fibre diameter was attempted by first electrospinning $PLGA_{8515}$, followed by spinning PCL directly on top (PLGA:PCL). In the fifth and final run, a MEW PCL cross-hatch scaffold (fibre spacing 250µm) was attached to the collector plate and PCL electrospun directly on top. All scaffolds were imaged using scanning electron microscopy and X-CT. For each run fibre diameters were measured using Fiji ImageJ software (v2.3.0/1.53).

Results and Discussion

For Run 1, cryo-spinning PCL resulted in a highly porous scaffold, compared to the more compact, conventional electrospun PCL. Manipulation of spinning parameters mid-spin resulted in a decrease of median fibre diameter for the $PLGA_{8515}$ scaffold (Run 2) from 2.36 µm (Interquartile range (IQR) 0.53 – 2.69 µm) to 1.29 µm (IQR 0.39 – 1.89 µm). Whereas, the change in median fibre sizes of the PCL scaffold (Run 3) were negligible (0.66 µm and 0.75 µm) and did not create a hierarchical scaffold. The PLGA:PCL scaffold (Run 4) resulted in integration of both synthetic polymers creating a dual-phase structure. In the final run, electrospinning on top of a MEW scaffold (Run 5), a multi-layered structure was generated with electrospun fibres conforming to the cross-hatch architecture and lying across and partially through the open pores of the 3D structure.

Conclusions

By adapting the materials used and electrospinning process, we have generated multiple scaffold structures that aim to mimic the hierarchical nature of the TM. Future studies will continue to optimise this mimicry to determine the suitability of these scaffolds for cell replacement therapies or novel *in vitro* mimetics.

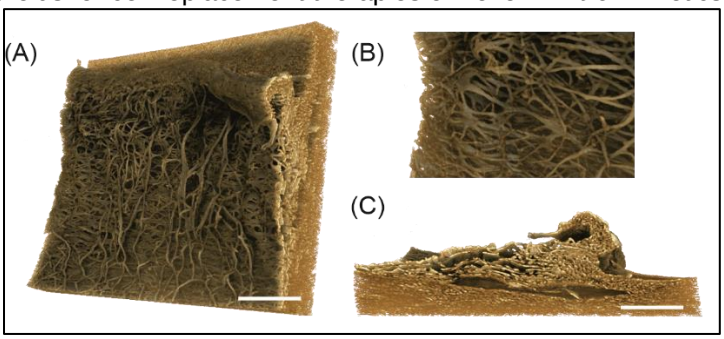

Figure 1: X-ray CT images of human decellularised trabecular meshwork (A) front view, (B) front-on magnified, (C) cross-section view. Scale bar = 50µm.

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Session 7: Talk 2

DEVELOPMENT OF COMPLEX 3D GLIOBLASTOMA MICROENVIRONMENTS

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Introduction

Glioblastoma (GBM) brain tumours in patients account for poor prognosis and dismal survival rates due to their highly aggressive capacity to rapidly migrate to other regions of the brain. In vivo animal models used to investigate experimental treatments for GBMs usually involve surgical intracranial implantation of tumour cells into mice or rats, with some displaying severe side effects such as stroke post-surgery. Furthermore, there are major doubts regarding the usefulness of such in vivo models. This has been questioned over time where many anti-cancer drugs are shown to be effective in mice but fail to produce the same response in humans[1]. Here we describe the development of a 3D bioprinted model of the GBM physical and chemical environment to produce a more physiologically representative system in which candidate drugs are tested.

Materials and Methods

Cell lines - Glioma cell lines U87 and U251 derived from human GBM were used in this study. Knockdowns of anti-migratory and pro-migratory genes (ARHGAP12 and ARHGAP29 respectively) were used to examine their roles in the actin polymerization pathway in cancer cell migration. All cells were intrinsically tagged with green fluorescent protein (GFP) for ease or tracking. Cells were grown in DMEM supplemented with 10% foetal calf serum and penicillin/streptomycin in a CO_2 incubator. U87 and U251 wild type and knockdown spheroids were generated in 96-well ultra-low adherence plates (Nunclon®).

3D Migration assay - A non-cell-adhesive agarose hydrogel was fashioned in the shape of a cube containing an empty channel at its core. Single spheroids were then suspended in type I collagen to form a bioink which was subsequently printed within the agarose channel void to form migratory tracts. The distribution of collagen was organised to have low, intermediate, and high-density regions within the construct, replicating the complexity of native GBM. 3D models were then cultured under control conditions (media only) or treated with anti-migratory drugs (CCG-1423, rhosin or combination) (Figure 1 left).

Light-sheet and confocal microscopy - Using light-sheet microscopy, migration was imaged live every hour between time points 0hrs and 24hrs and data was collected in the form of z-stacks and time-lapse videography. For confocal microscopy, constructs were cultured over 48 hours, fixed with 4% PFA overnight and stained for F-actin (phalloidin-TRITC, ECM Biosciences) with a DAPI counterstain.

Results and Discussion

In models where migration is promoted, actin is vastly upregulated and cells assume a migratory mesenchymal phenotype, whereas under anti-migratory drug treatment, cells are amoeboid in shape with a dramatic reduction in actin expression and consequently limited migration velocity (Figure 1 right).

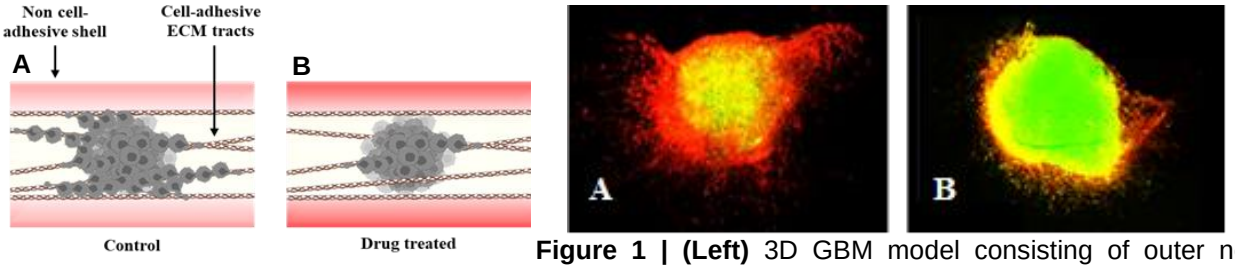

Figure 1 | (Left) 3D GBM model consisting of outer non-cell-adhesive shell and inner collagen migratory tracts under **A)** control conditions and **B)** anti-migratory drug treated conditions. (**Right**) Confocal micrographs of anti-migratory knockdowns exhibiting **A)** enhanced migration and mesenchymal phenotype under control conditions and **B)** anti-migratory effects under combination drug culture (actin - red, intrinsic cell stain - green).

Conclusions

Here, we have demonstrated that it is possible to develop biologically-relevant GBM models that capture the anisotropic nature of the tumour microenvironment using multi-layer biopolymer engineering. The ultimate goal of this research is to develop technology that can help provide personalised treatments for glioblastoma and subsequently improve patient outcomes.

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Acknowledgements

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Session 7: Talk 3

A 3D BIOPRINTED MODEL OF THE HUMAN INTERVERTEBRAL DISC

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Introduction

The intervertebral disc (IVD) aids the flexible movement of the spinal column by providing vertebra with support against mechanical loads[1]. It is broadly comprised of two distinct tissue regions. At the core, the IVD contains a soft and gelatinous nucleus pulposus (NP) populated by spherical NP cells embedded in a polysaccharide-rich matrix. Conversely, the outer annulus fibrosus (AF) of the disc is much stiffer and comprised of elongated AF cells in an aligned, fibrous matrix[2]. Degeneration of the disc is a common problem with age being a major risk factor. Progression of IVD degeneration leads to chronic pain and can result in permanent disability. Repairing the damaged IVD is often impaired by an inability to regenerate the two distinct regions of the disc simultaneously. This study aims to investigate if a newly developed suspended hydrogel bioprinting system[3] could be employed to fabricate full-scale analogues of the IVD for tissue regeneration and disc disease modelling.

Materials and Methods

A biphasic hydrogel construct was fabricated via suspended layer additive manufacturing (SLAM). Constructs were generated containing distinct regions of gellan gum polysaccharide (gellan) and type I collagen to recapitulate the NP and AF regions respectively. The printed constructs were subsequently analysed with transmission electron microscopy (TEM) to evaluate gel microstructure. Additionally, human NP and AF cell lines were incorporated into the relevant regions of the construct and cultured for 7 and 14 days. Live/dead assays were utilised to assess cell viability at d1 and d7 and phalloidin staining was used to evaluate cell morphology at d7 and d14. Additionally, cell tracking dyes were employed to evaluate cell migration within the construct and fluorescent histology was utilised to screen for extracellular matrix depositions after 14 days of culture.

Results and Discussion

Constructs were successfully fabricated with distinct regions of gellan and type I collagen in order to mimic the structural gradients within IVD tissue microenvironments[4]. Moreover, cell-laden IVD constructs were bioprinted without compromising cell viability. Human NP cells embedded within the “NP-like” gellan gum region of the construct retained a spherical morphology resembling what is observed in vivo. Within the collagenous “AF-like” region, human AF cells exhibited an elongated morphology and showed evidence of regions of cell alignment. Further inspection of these regions with transmission electron microscopy revealed the presence of aligned collagen fibres within the construct. Fluorescence microscopy also demonstrated that pre-labelled NP and AF cells did not migrate through the gel structure and, instead, remained in the appropriate regions of the printed structure. Moreover, NP cells demonstrated evidence of matrix remodelling with deposition of hyaluronic acid into the surrounding environment.

Conclusions

These data highlight the potential to utilise SLAM for the generation of constructs that can reflect the heterogeneous nature of the IVD for disease modelling and regenerative medicine applications. This model will now be utilised to study the influence of environmental factors such as hypoxia, mechanical load and ECM composition on IVD degeneration.

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Session 8: Talk 1

"CHEMO-TOPOGRAPHY" HEART-IN-A-DISH PLATFORM FOR HUMAN CARDIAC MODELLING

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Introduction: Cardiovascular disease (CVD) remains one of the leading causes of ill-health and mortality. The ability to derive human induced pluripotent stem cells (hiPSCs) from patients and healthy individuals, and then differentiate into cardiomyocytes (hiPSC-CMs) provides new opportunities to model and understand human diseases. Current approaches produce hiPSC-CMs that lack maturity relative to the adult human heart. The use of biomaterials could improve hiPSC-CM functionality by altering biophysical and biomechanical properties including surface chemistry and topography to better represent the *in vivo* environment.[1] We have previously identified poly(TCDMDA-blend-BA), a novel fully-synthetic substrate for expanding and differentiating hiPSCs into cardiomyocytes at a ~300 fold reduced cost[2], here we present a platform for modelling hiPSC-CMs.

Materials and Methods: In this study, a Chemo-topography: combining a proprietary polymer and micro-topographies to improve hiPSC-CM maturity was identified from screening the TopoChip[3] (~2000 micro-topographies) and polymer (~300 polymer chemistries) micro-arrays.[2,4] Experiments at scale were produced using soft lithography on polystyrene and PRIMO (alveole) technology in tissue culture well-plates. Materials were then functionalised using standard UV polymerisation methods. HiPSC-CM function was assessed using Clyde Biosciences, CellOptiq™ and structure was assessed using high-resolution confocal microscopy where hiPSC-CM material interactions were quantified using Detectron2, Artificial intelligence (AI) guided image analysis and custom structural analytical tools.

Results and Discussion: We identified a synergistic interplay between surface chemistry and topography in the chemo-topography platform, which improves hiPSC-CM maturity. Micro-topographies improved structural integrity of hiPSC-CMs by promoting cell elongation (aspect ratio 6-7) and alignment (sarcomere organisation). AI guided image analysis showed that micro-topography orientation, size and interaction manipulated hiPSC-CMs to desired phenotypes. The discovered polymer coating improved hiPSC-CM function by increasing contraction amplitude and rate. This platform is now being combined with maturation protocols using newly identified media components. Throughput of the system is being improved by converting to well-plate format where cardiotoxicity drug screening assays can be performed. Incorporation of this technology into 3D bioprinting and microfluidic systems will also explore its application as medical devices.

Conclusions: Here, we present a novel "Chemo-topography" platform which can improve human cardiac modelling. We anticipate this will benefit both supply and application of hiPSC-CMs including the replacement of animal models which are known to poorly represent human cardiac physiology.

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Session 8: Talk 2

AMINOSILANE COATED POLYCAPROLACTONE SCAFFOLDS FOR PERIPHERAL NERVE REPAIR

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INTRODUCTION: 2.8% of all trauma patients will occur a Peripheral Nerve Injury. The current treatment for large gap injuries (+20mm) is still autografting, although there is limited donor nerve available and donor site morbidity. For a short gap injury (10-20mm), synthetic Nerve Guide Conduits are used. Therefore, current research focuses on improving NGCs for use in longer gap injuries. Coatings have been used to improve the biocompatibility of synthetic nerve guide conduits, as well as providing surface chemistry, and nanotopography guidance for the regenerating axon. Synthetic coatings, such as aminosilanes, are much cheaper to produce, than natural coatings, have the ability to control chemical group deposition at the sub-micron scale and avoids immune responses.

METHODS: Polycaprolactone films were produced by spin coating and fibres manufactured by electrospinning. Fibres and films were exposed to oxygen plasma and immersed in 3% 11-Aminoundecyltriethoxysilane (CL11) isopropanol solution for 2 hours, washed, and dried overnight. Surfaces were characterised using WCA, Ninhydrin assay, XPS and AFM. NG108-15 neuronal cells, and rat primary Schwann cells were cultured onto for 6 days. Rat Dorsal Root Ganglion bodies were extracted from rat spinal cords, and dissociated into neuronal cell types, to culture for 6 days. Live/dead analysis was used to confirm the biocompatibility of the glass coverslips, for the NG108 neuronal cells and primary Schwann cells, as well as immunolabelling for β III tubulin, NG108-15 neuronal cell differentiation and neurite marker, and for S100, a Schwann cell marker.

RESULTS: WCA, XPS and ninhydrin assay confirmed successful modification of PCL using CL11, confirming the presence of NH_2 groups. AFM analysis confirmed the addition of oxygen plasma, followed by 11-Aminoundecyltriethoxysilane, significantly increased surface roughness, compared to PCL alone. Live/Dead analysis confirmed that both coatings were biocompatible, but that there were higher numbers of live cells cultured on samples modified with oxygen plasma and 11-Aminoundecyltriethoxysilane. Primary Schwann cells cultured on samples stained positively for S100β confirming maintained Schwann cell phenotype. The highest average neurite lengths were found on primary neurons, and NG108-18 neuronal cells, cultured onto samples modified with oxygen plasma and 11-Aminoundecyltriethoxysilane, in which higher numbers of primary neurons and Schwann cells were visualised.

DISCUSSION & CONCLUSIONS: This study confirmed the successful modification of PCL films, and fibres, using 11-Aminoundecyltriethoxysilane modification. The addition of CL11 decreased hydrophobicity of PCL, increased surface roughness, and therefore promoted NG108-15 neuronal cell, primary Schwann cell and dorsal root ganglia attachment, and viability, and neurite outgrowth. Future work will involve applying this treatment to synthetic nerve guide conduits, containing fibres, for investigations *in vivo*.

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Session 8: Talk 3

FUNCTIONAL NANO GLUCOSE PARTICLE SENSOR (FN-GPS) IN HUMAN MESENCHYMAL STEM CELL (HMSC) ENVIRONMENT

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Introduction:

Mesenchymal stem cells (MSC) use glucose-derived pathways for metabolism upon transplantation *in- vivo* [1]. In addition, *in- vitro* studies show MSC osteogenic and chondrogenic differentiation depends on the specific time point of change in glucose levels and introduction of associated differentiation factors. 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. To validate the use of this technology in HMSC culture systems viability and cytotoxic effect of Nano Sensors to monitor glucose levels in real time in vitro was assessed.

Material and Methods

Nano glucose sensors were fabricated using Polystyrene Nano beads (PSB) with surface modified by carboxyl groups (Thermo SCIENTIFIC, W050C) s. For Nano glucose sensors Pluronic F127 (a triblock copolymer), was employed (all are from Aldrich Sigma) and attached onto the surface of PSB by an esterification process. 1 mg of glucose -sensitive red fluorescent molecule alizarin and Boronic [2] was dissolved in 200 µL of 99.5 % pure ethanol. The ethanol containing fluorophore solution was added to the previously prepared 1mL solution of F127 grafted PSB and ultra-sonicated for at least 30 minutes to form functional glucose sensors by hydrophobic interaction is as shown in figure 1

Results and Discussion

Concentrations (0.1, and 1 mg/mL) of each sensor was tested and measured using live cell assays in 96 well plates. Cells cultured in 96 well plates at a seeding density of 2500 cells/ $cm^2$are as shown in figure 2.Synthesized glucose nanosensors show a lesser or no cytotoxic effect with HMSC. The cells encapsulated with nano sensors inside 3D hydrogel system shows comparable cell viability with control. The in-house synthesized FN-GPS was calibrated using the plate reader by incubating the nano sensors with serial dilution of glucose concentration, and demonstrated a good broad dynamic range 0.1mM to 0.1, sensitivity of -0.3223 (au. / (mg/L)) with an accuracy of 85 %. The contour plots for hydrogels were normalized and relative glucose intensity shown in figure 3 by using the measured calibration values.

Conclusion

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

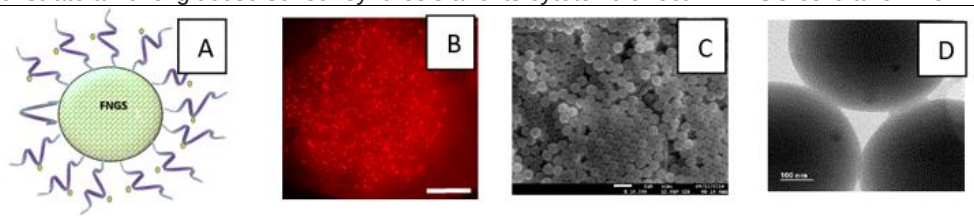

Figure 1. (A) Schematic illustration of FNGPS (B) Fluorescence image of FNGS inside hydrogel (scale bar 500 µm) (C) SEM (D) TEM image of PSBs grafted with glucose fluorophore

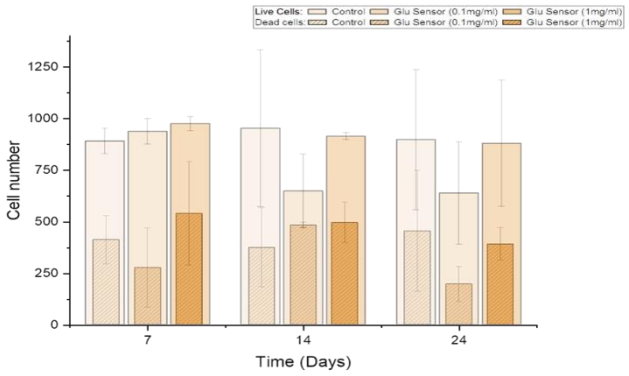

Figure 2: Quantification of cells using ImageJ for Live/dead assay in basal media imaged by confocal microscopy at Day 7, 14 & 24, cultured in normoxia (21% O2, 5% CO2) incubated with different concentrations of glucose nanosensors (0.1mg/mL and 1mg/mL) (N=2, n=2)

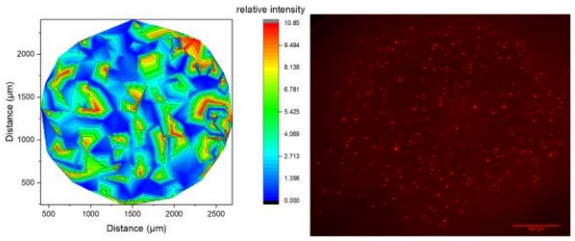

Figure 3: Contour plot of glucose distribution measured using fluorescence based glucose nano sensors (stained in red colour) for HMSCs encapsulated in 3D alginate hydrogels of 1wt% + $CaCl_2$, day 14 scale bar 500 µm.

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Session 8: Talk 4

Microfabrication of defined circuits of human neural progenitor cells within a brain-on-chip device

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Introduction

The controlled generation of defined neural circuitry remains a major hurdle in the development of biomimetic *in vitro* models of the central nervous system. In the body connectivity of specific neuron to neuron interaction defines the function of nervous tissue, however the ability to control connectivity is lost in standard culture formats without the use of chemical or physical patterning to control cell migration and neurite outgrowth [1]. Similarly animal-derived cell sources of neurons and supporting glia are often poor models of their human equivalents, with the human brain representing one of the major areas in which we are biologically differentiated from other mammals [2, 3]. Here we report the use of a microfabricated brain-on-chip device in which microchannels are used to connect segregated human induced pluripotent stem cell-derived neural progenitor cells while restricting their migration to form defined cell networks.

Materials & Methods

Brain-on-chip templates were designed using AutoDesk AutoCAD software. Digital templates were transferred to a direct writer photolithography platform and printed onto silicon chips coated with a 100 μm film of the photoresist SU-8-50 at 368 nm. Printed designs were reverse-replica moulded with Qsil 216 PDMS. PDMS structures were adhered onto laminin and poly-L-ornithine coated glass cover slips. 5,000 cells were loaded into each chamber and the entire PDMS structure submersed in a 50:50 mix of DMEM/F12 and Neurobasal media with N-2 and B-27 neuronal supplements. Once cellular rosettes were observed to form differentiation media was applied consisting of BrainPhys basal medium with 2% v/v SM1 supplement and 10 μM DAPT

Results

Direct photo-writing of SU-8-50 was observed to produce good feature resolution to single micron scale with minimal curving or breakage. Following silanization microculture structures were readily replicated with PDMS. Cells were observed to proliferate freely within the microculture space and were observed to display signs of differentiation by changing of biomarker expression as observed under immunofluorescence imaging.

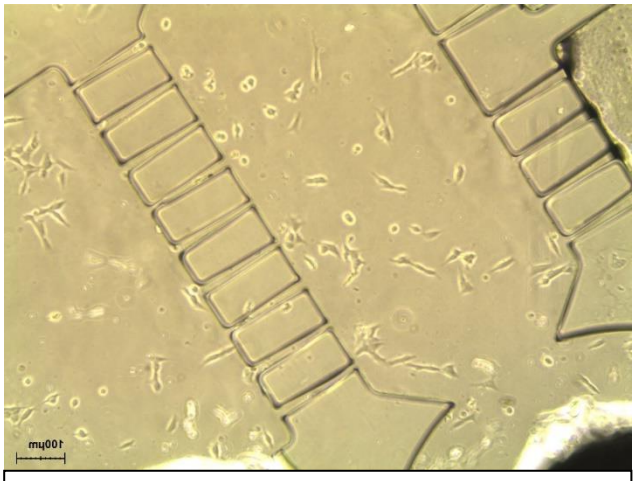

Figure 1 Neuroblastoma cell line in an example microfabricated brain-on-chip device.

Discussion & Conclusions

Human iPSC-derived neuronal progenitor cells were observed to grow readily within a media-immersed microculture structure. Expression of nestin and beta-tubulin expression demonstrated that progenitor cells retained their stem-like state until the application of differentiation media, following which a mature neuronal phenotype was observed to develop over time with increasing expression of beta-tubulin mirrored by decreasing expression of nestin.

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POSTER 24: ELASTOMERIC, BIOADHESIVE AND PH-RESPONSIVE COPOLYMERS BASED ON DIRECT CROSSLINKING OF POLY(GLYCEROL SEBACATE)-CO-POLYETHYLENE GLYCOL

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Introduction

Hydrogels made from synthetic polymers have proven useful for biomedical applications, including cell culture, tissue engineering, and drug delivery. Synthetic hydrogels with amphiphilic structures can possess beneficial characteristics like adjustable mechanical properties and biodegradation, responsiveness to physicochemical dynamics, and bioadhesiveness, all of which have potential biomedical applications.[1] Poly(glycerol sebacate) (PGS) is an example of a synthetic polymer that has exhibited elasticity, cytocompatibility, and biodegradability in a biomedical context. A fascinating aspect of PGS is its resemblance to fatty acids and its modulus, similar to the soft tissue of humans. However, the fact that it is hydrophobic has prevented its use in advanced applications, such as hydrogel fabrication, bioink development, and cell encapsulation.[2,3] We sought to address this limitation by developing novel biodegradable, biocompatible, bioadhesive, highly elastomeric and pH-responsive copolymers by direct crosslinking of the PGS pre-polymer and two types of polyethylene glycol (PEG), without using crosslinking agents. By combining hydrophilic PEG with hydrophobic PGS, we have demonstrated that it is possible to create hydrogels with outstanding bioadhesiveness, elastomeric properties, and pH-responsive swelling.

Materials and Methods

The effect of PEG type and quantity in the production of copolymers scaffolds was systematically examined with regard to their physicochemical properties and biocompatibility in this study. PGS-co-PEG pre-polymers were copolymerised by a two-step procedure: (1) polycondensation of sebacic acid (SA) and PEG in various weight ratios to yield SA/PEG prepolymers, and (2) addition of glycerol and synthesis of PGS-co-PEG pre-polymers.

Results and Discussion

It was found that the copolymers are highly elastomeric, flexible and can be stretched and knotted easily. These elastomers can adhere firmly to different substrates, such as glass, polycaprolactone, polytetrafluoroethylene, silicone, wood and aluminium, with maximum lap-shear strength and adhesion strength reached at 336 kPa and 84 kPa, respectively. Their Young's moduli were in the range of 0.004–0.521 MPa, and copolymers with greater amounts of PEG (PEG≥40%) showed stretching beyond 4-5 times their initial length. The swelling ratio within water increased almost 25 times by adding PEG. In addition, the copolymers had pH-responsive behaviour. Swelling ratios were greater in basic conditions (pH=9.1) and followed the trend pH 9.1> pH 7.4> pH 5. This makes these PGS-co-PEG hydrogels suitable for pH-dependent drug release. Additionally, the structure of copolymers facilitates tuneable biodegradation by changing crosslinking density and PEG content. This work shows that the degradation kinetics accelerated with the addition of PEG, with a direct correlation with the amount of PEG added. The copolymers were also shown to support metabolically active human keratinocytes for at least 7 days, demonstrating their cytocompatibility.

Conclusions

With their multiple functions, these new copolymers show great potential for biomedical applications and controlled drug delivery systems. Further, it is expected that the development of these elastomers will provide new ideas for developing future tissue engineering biomaterials.

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Acknowledgements

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POSTER 25: REPURPOSING ANTI-DIABETIC DRUG (BYETTA) AS A NOVEL HIF-1 STABILSING CHEMICAL

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Corresponding author: g.jell@ucl.ac.uk – Undergraduate (3rd year)

Introduction.

Diabetic patients have impaired bone healing. The risk of a fracture is 47-62% higher in those with inadequately controlled diabetes compared to healthy patients[1] and the length of time for a fracture healing in a diabetic patient is 87% longer[2]. High glucose levels (hyperglycaemia) impair the ability of cells to respond to hypoxia via inhibition of the hypoxia inducible factor (HIF)-1α pathway. Considering the important role of HIF-1α in bone regeneration, targeting the HIF-1α pathway with biochemicals may offer new treatment options. Pereira et al. (2017) demonstrated that exenatide (trade name Byetta), a glucagon-like peptide-1 receptor agonist (GLP-1) restores bone nodule formation in a diabetic model in vitro[3]. Other studies have found that GLP-1 stabilises the HIF-pathway in INS-1 insulinoma cells[4], however it is unclear whether Exenatide mediated bone nodule restoration in hyperglycaemia is due to HIF-1α stabilisation or other cellular mechanisms. Using an in vitro bone nodule formation, we have previously shown that HIF stabilising molecules (CoCl and DMOG), restores bone nodule formation in hyperglycaemia[5]. In this study, we investigate if Byetta can help restore bone nodule formation via the hypoxia pathway in hyperglycaemia, allowing it's possible repurposing to treat impaired fracture healing in diabetic patients.

Experimental methods.

Calvaria pre-osteoblasts were isolated from neonatal Sprague-Dawley rats and cultured α-MEM supplemented with 10 mM dexamethasone, 2 mM β-glycerophosphate and 50 µg/mL ascorbate acid for 21 days. Once confluent cells were exposed to either low glucose (5.5mM), medium glucose (25mM) or high glucose (50mM) environments, cultured in either a normoxia environment or a hypoxic environment. The cells were also treated with either 25 nM or 50 nM of Exenatide conditioned medium, in all glucose concentrations. Proliferation (total DNA assay), ALP activity, angiogenic response (VEGF ELISA), ROS activity (ELISA), glucose uptake and bone nodule formation measured.

Results and Discussion.

Both medium and high glucose environment inhibited formation of bone nodules while hypoxia inhibited formation in all environments. For the first time it was shown the effect of Exenatide on primary osteoblasts in different glucose environments and how it affects the ROS production, angiogenic response and ALP activity.

Conclusion.

Understanding how Exenatide induces bone restoration in hyperglycaemic environments will develop our understanding of the role of HIF-1α in fracture repair. This can be used to make improved treatments for those with impaired bone healing.

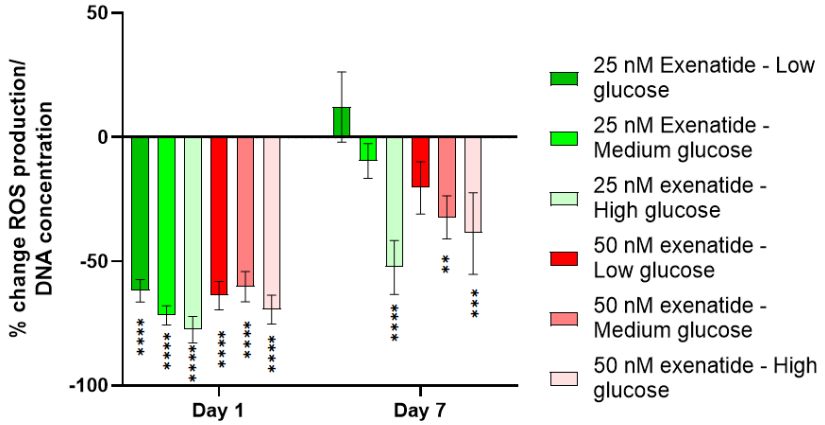

Figure 1: Exenatide reduces ROS production compared to the corresponding glucose environments at day 1 and in higher concentrations on day 7. N=6, **= p ≤ 0.01, ****= p ≤ 0.0001.

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POSTER 26: The Effects of Latanoprost Loading on the Physiochemical Properties of Dissolvable Microneedle Array Patches

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Introduction

Dissolving microneedle array patches (dMAPs) designed for ophthalmic drug delivery require fast dissolution and appropriate mechanical strength for patient comfort and effective application respectively.[1] DMAPs are fabricated from water soluble, biodegradable polymers, such as polyvinylpyrrolidone (PVP), and an encapsulated therapeutic within the polymer matrix.[2] Integration of therapeutic compounds can alter the physiochemical properties of dMAPs; such additives disrupt interchain interactions, introduce changes to surface behaviour and effect chain mobility of polymeric chains within a rigid structure.[3] Consequently, insertion efficiency and drug release are altered by changes in mechanical strength and dissolution kinetics.[4] The aim of this investigation is to mitigate the disruptive impact of incorporating latanoprost, the first line therapeutic for glaucoma, to dMAP physiochemical properties *via* the addition of a plasticiser, polyethylene glycol (PEG).

Materials and Methods

PVP (K30, 40 000 g mol^{-1}) PEG (1 000- 5 000 g mol^{-1}, 5-10 wt. %) solutions (50 % wt.) and dMAPs with latanoprost (50 µg mL^{-1}) were formulated *via* a solvent casting method. The surface behaviour of unloaded and loaded PVP·PEG blends was evaluated *via* contact angle and surface tension measurements. Mechanical strength of dMAPs was measured by employment of increasing force to determine the compression and insertion into corneal tissue *ex vivo*. DMAP dissolution rates, latanoprost permeation and biodistribution in an *ex vivo* eye model was assessed and compared to a topical dose radiometrically using tritiated latanoprost.

Results and Discussion

Surface measurements in PVP·PEG blends emphasised an increase in contact angle (Figure 1A) in drug loaded compositions of PVP_{100}·PEG_0 relative to its unloaded counterpart. The rise in hydrophobicity is related to the amphiphilic structure of latanoprost, creating a hydrophilic-hydrophobic barrier at the air-polymeric solution interface. PVP_{95}·$PEG_{5(5000)}$ formulations saw no change in surface behaviour when latanoprost was incorporated. Compression testing of PVP_{100}·PEG_0 dMAPs showed a reduction in strength upon latanoprost incorporation relative to PVP_{95}·$PEG_{5(5000)}$ which saw no change between its unloaded and loaded compositions (Figure 1B). The similarity within PVP_{95}·$PEG_{5(5000)}$ is related to the hydrophilic nature of PEG, minimising drug interference *via* plasticiser control on the physiochemical properties of the PVP·PEG blends.

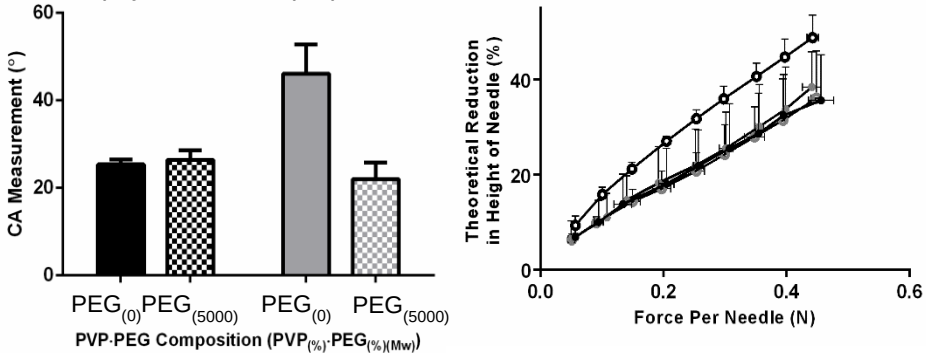

Figure 7: *Latanoprost loading (tiled/ring) effects relative to unloaded (bold/dot) PVP·PEG blends on* ***A:*** *Contact angles (°) and* ***B:*** *Compression testing of PVP_{100}·PEG_0 (black) and PVP_{95}·$PEG_{5(5000)}$ (grey).*

Conclusions

PVP·PEG dMAPs were successfully fabricated as well as latanoprost containing analogues to investigate the effect of drug incorporation on a range of physiochemical properties. Surface behavior measurements and compression testing emphasised the ability of PEG to mitigate the effect of latanoprost on solutions and the mechanical strength of PVP_{95}·$PEG_{5(5000)}$ dMAPs relative to PVP_{100}·PEG_0. Overall, this study demonstrated the importance of PEG in dMAPs to tailor effects of drug incorporation on the physiochemical properties of bespoke dMAPs required for minimally invasive ophthalmic drug delivery.

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POSTER 27: POLYMER–QUANTUM DOTS HYBRID NANOTHERANOSTICS FOR CANCER TREATMENT

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Introduction

Whilst there has been significant breakthrough in cancer therapy systems over the years, current therapeutic systems present clear setbacks, such as their toxicity and damaging effects to human body cells and the host.[1] Futhermore, tumour cells can be very resistant to conventional therapies. One new promising approach is called nanotheranostics that can be applied to noninvasively discover and target image biomarkers and further deliver treatment based on the biomarker distribution.[2] Combined with a method called Layer-by-Layer (LbL), an approach to develop an ultrathin film on solid support by alternate exposure to positive and negative species with spontaneous deposition of the oppositely charged ions [3], the design of a promising new nanotheranostics system becomes clear. The aim of our work was to incorporate nanomaterials called Quantum Dots (QDs) and cancer drugs in a shell-like type structure with a LbL film encapsulating it to derive an overall therapeutic system. The need for easy-to-fabricate, safe and non-toxic therapeutic LbL systems has driven the research field towards the use of Carbon Quantum Dots (CQDs). Their bioimaging with green, non-toxic, biocompatible, and attractive quantum properties present as distinctively advantageous over conventional/metal-based quantum dots which has been used in cancer therapy but present clear setbacks such as their toxicity.

Materials and Methods

The generation of CQDs were procured via a thermochemical process as reported elsewhere [4], in which the biomass (walnut shell and chitin) was decomposed in an inert environment at a temperature range of 600-700^{o}C. The products (liquid and solid) were then incubated in the hydrothermal carbonization autoclave to produce CQDs, ranging in size between 3-10nm. These dots were then analysed using an array of techniques such as TEM, zeta potential, FTIR-ATR and photoluminescence to confirm the morphology, appearance, and structure of the dots. The next stage was to use these dots as the base for the LbL procedure and using poly(allyamine hydrochloride) (PAH) as the polycation and poly(sodium styrene sulfonate) (PSS) as the polyanion to create nanocapsules with 7 consecutive oppositely charged layers. Doxorubicin hydrochloride was added in the core. Figure 1 shows the process steps to produce CQDs

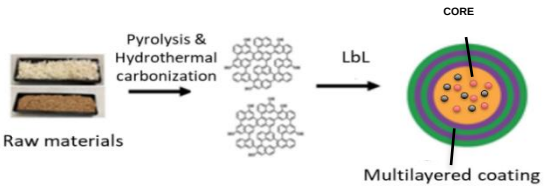

Figure 1: Pyrolysis carbonization method followed by LbL to produce a nanotheranostics system made of CQDs

Results and Discussion

Currently, the synthesis of CQDs in this PhD work has consistently yielded dots that typically fluoresce at ~300nm (blue region of UV light) from each of the different biomass starting materials. Further analytical techniques such as TEM, FTIR-ATR, XPS and Photoluminescence have also furthered the confirmation that the CQDs synthesised are all almost identical and are able to be applied to the LbL step of the PhD.

Preliminary results obtained from the LbL systems that are currently ongoing are confirmed to be progressing successfully. The formation of this nanocoating has been confirmed morphologically (TEM, zeta potential and AFM) and chemically (FTIR and XPS). Successful LbL systems have been manufactured with the formation of 7 nanolayers incorporating the selected drug and quantum dots as core of the nano capsule. The controlled release of the drug during the time was confirmed by UV-Vis spectroscopy with a low initial burst release. Preliminary cell viability tests have also confirmed the cytocompatibility of the CQDs and manufactured LbL nanotheranostics.

Conclusions

The discussion of the ongoing challenges and opportunities associated with a green and sustainable synthesis of CQDs with specific functional groups, so that the next stages in the development of the nanotherapy system to target tumours can commence. Given that the CQDs have been successfully synthesised, and the LbL method has been commenced, the next step will be to complete the layers surrounding the CQDs and cancer drugs. Furthering on from this, this nano-capsule will be tested on cancer cells to determine the successfulness of the nano-capsule.

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POSTER 28: MONOLAYER CHEMICAL VAPOR DEPOSITION (CVD) GRAPHENE COATING AS NOVEL ANTIFOULING SURFACE TO PREVENT CATHETER-ASSOCIATED INFECTIONS

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Introduction

Graphene is a unique carbon material with diverse applications ranging from electronics to biomedicine. Monolayer graphene synthesized by the chemical vapor deposition (CVD) method consists of carbon with a flawless crystal structure. Besides presenting as a continuous, uniform, transparent monolayer, CVD graphene firmly adheres on substrates, is able to withstand sterilization and can be standardized with controllable parameters.[1] In this study, we exploit these extraordinary properties of CVD graphene to develop a novel mechainically flexible, optically transparent coating for biomedical devices which can inhibit biofilm formation and prevent the occurrence of infections associated with biofilms. The ability of microbial species to grow and thrive on the biotic surface result in biofilms that are difficult to eradicate. Biofilm formation on the medical devices is the main cause of infections such as catheter-related bloodstream infections (CRBSI) and catheter-associated urinary tract infections (CAUTI) in nosocomial settings.[2,3]

Materials and Methods, Results and Discussion

We coated polymer substrates (PET and PDMS) with monolayer CVD graphene and verified their composition via ultraviolet photoelectron spectroscopy (UPS) and X-ray photoelectron spectroscopy (XPS). Raman spectroscopy confirmed that the transfer process of monolayer CVD graphene coating from copper foil to polymer substrate was successful and no defects were introduced. Atomic force microscopy (AFM) identified that monolayer CVD graphene coating was even and smooth with a roughness of less than 2 nm on average. Against various types of microbes including P. aeruginosa (Gram-negative bacteria), S. aureus (Gram-positive bacteria), Candida albicans (yeast) and M. abscessus (Mycobacteria), CVD graphene-coated substrates displayed distinct antifouling activity as measured by colony counting and LIVE/DEAD staining. In the presence of serum proteins, the number of microbes attached to the monolayer CVD graphene coating surface was less than the uncoated surface. The evaluation of biocompatibility with mammalian cells indicated the monolayer CVD graphene coating is non-toxic with no hemolytic effect on human red blood cells and greater than 90% cell viability using somatic and stem cells.

Conclusion

Together, our study highlights the potential of CVD graphene beyond electronic devices to biomedical applications.

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POSTER 29: USING BRIDGING FLOCCULATION FOR THE DEVELOPMENT OF A POLYMER-BASED POINT-OF-CARE DIAGNOSTIC FOR TARGETED DETECTION OF DNA

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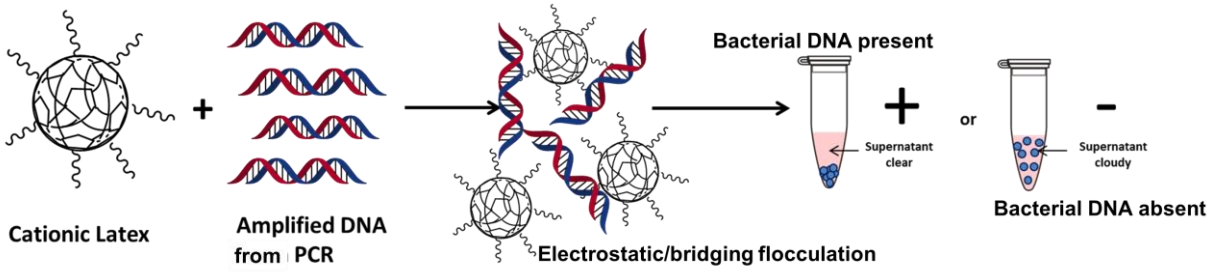
The current gold standard diagnostic for bacterial infections is the use of culture, which can be time consuming and can take up to five days for results to be reported. There is therefore an unmet clinical need for a rapid and cost-effective alternative. This paper demonstrates a method of detecting the presence of amplified DNA from bacterial samples using polymer latexes and widely available equipment, providing an accessible alternative to more expensive DNA detection techniques. If DNA is present in a sample, it's successful amplification results in flocculation of the polymer latex followed by rapid sedimentation, thus giving a clear visual result. The sensitivity and speed of the test has been investigated using a combination of disc centrifuge photosedimentometry, UV-Visible spectrophotometry and aqueous electrophoresis. To-date, label-free detection of DNA has been achieved at amplified DNA concentrations as low as 0.57 ng/µl within 2 hours from the start of amplification to detection.



Acknowledgement: Dr Gavin Humphreys, Department of Pharmacy and Optometry, Faculty of Biology, Medicine and Health, University of Manchester.

POSTER 30: EVALUATION OF LATERAL SHEARING INTERFEROMETRY FOR NON-DESTRUCTIVE WHOLE-FIELD MEASUREMENT OF LOCALISED CORNEAL BIOMECHANICAL PROPERTIES

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INTRODUCTION

Worldwide, corneal diseases are a leading cause of preventable irreversible blindness [1]. The highly organised corneal collagen fibre extracellular matrix (ECM) is detrimentally affected by disease, resulting in localised and global, biomechanical tissue weakening, which is not detected until irreversible damage occurs. Keratoconus (KCN) is a degenerative disease, where gradual disruption to the corneal ECM causes the tissue to thin, bulge, and scar, which may lead to permanent blindness in adolescents and young adults. Currently, no device can non-destructively measure the subtle, whole field, corneal biomechanics in real time. The aim was to investigate the suitability of Lateral Shearing Interferometry (LSI) for whole field corneal measurements.

MATERIALS AND METHODS

Porcine corneas were loaded into a modified Barron Artificial Anterior Chamber (AAC), and finely coated with an inert paraffin-based solution, to generate an adequate signal to noise ratio. The AAC was connected to a hydraulic rig with a reservoir filled with PBS, which varied the pressure exerted upon the cornea(s) when the height of the reservoir changed by 5 or 10mm, this simulated biomimetic intraocular pressure (IOP) fluctuations (baseline pressure set at 15.00 mmHg). A customised 45° peripheral mirror was added to the AAC to measure out-of-plane components of the corneal limbus. A bespoke LSI (Dfect Dtect, Laser Optical Engineering (LoE, Ltd)) measured the rate of corneal deformation following controlled pressure fluctuations. Interferometric fringes were produced that were used to determine whole-field biomechanical shape changes following surgical incisions. Using phase stepping techniques and proprietary software (LoE, Ltd), fringe patterns and 3D colour contoured images were produced to demonstrate corneal flexural strain changes.

RESULTS

“Noisy” fringe patterns were generated when the pressure fluctuation was initially applied, which became clearer following corneal settling and stabilisation. This behaviour suggests that the corneal structure has a “damped” response to the pressure pulse applied. The presence of the 45° mirror greatly improved the fringe patterns generated. The device was able to demonstrate whole field biomechanical changes following surgical incision. Following the introduction of a surgical cut, the cornea bulged to compensate for the incision and the curvature steepened; a visible fringe “border” formed around the incision. The incision severed some of the collagen fibres which caused a region of higher flexural strain on the left side (LHS) of the tissue, due to the phase angle of the collagen fibres. Following a pressure increase (15.37 mmHg), more fringe patterns formed, indicative of increased flexural strain. Furthermore, an area of increased rate of corneal displacement was revealed post-incision on the interferograms, consistent with increased strain placed on this area of the cornea by the incision, demonstrating the sensitivity of the device and the capability to detect localised defects.

DISCUSSION & CONCLUSION

The current inability to measure *in vivo* corneal biomechanics in a clinical setting is a barrier for the early detection, diagnosis, and tailored treatments of KCN. The results of this experiment demonstrated that LSI could be a promising technology for corneal biomechanical analysis. LSI provides suitable sensitivity to examine corneal biomechanical integrity when subjected to realistic IOP variations. The use of the peripheral mirror suggested that regional biomechanical changes can be measured across the cornea and limbus. It is hypothesised that the circumferential corneal collagen arrangement may act as a “pressure relief” system using the bias of the collagen fibre axes to provide significant elasticity in a radial axis. When a pressure increase of 0.37 mmHg was applied, the cornea had a potential “damped” response which took several seconds to complete. This suggests that the cornea could be smoothing the surface fluctuation and acting as a damper, as the cornea takes a prolonged period to respond to pressure changes. If this hypothesis is correct, this knowledge may permit ophthalmologists to target treatments to specific areas of biomechanical instability.

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Funding from Fight for Sight/ Keratoconus Group UK Small Grant Award (Ref: 24KE182) is gratefully acknowledged

POSTER 31: Optimisation of cell aggregates in hydrogel structures.

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Introduction

In order to mimic human brain complexity, 3D models have been proposed as a more advanced alternative compared to animal models. Critical challenges remain including biomaterials capable of matching the mechanical properties and extracellular matrix (ECM) composition of neural tissue as well as natural and synthetic scaffolds that support tissue architectures reflective of brain organisation and structure. This study aims to use hydrogels to produce an environment to guide neurite extension and cell migration for the betterment of 3D drug delivery models. Here we report a comparative optimisation study of cell aggregates using natural and synthetic hydrogels which aim to progress 3D tissue structure interactions and replication.

Materials and Methods

Aggregates were produced using the SH-SY5Y cell line at 5000 cell population and plated into V-bottom 96 well plates which were prepared using BIOFLOAT (FaCellitate) to enhance the aggregation process. Agarose gel concentrations of 0.75 to 2.00 % were prepared and plated into a 96 well plate [1]**.** Once gelated, 100ul of maintenance medium was pipetted on top of the gel to produce a working hydrogel. Five synthetic PeptiGels® supplied from Manchester BIOGEL (Alpha 1, Alpha 2, Alpha 4, Gamma 2 and Alpha 2 – RGD) were prepared according to protocol and pipetted into a 96-well plate. 100ul maintenance medium added on top of the gel to gelate and produce a working hydrogel. Aggregates were imaged and analysed using ImageJ software. Key variables were aggregate area, circularity, feret diameter, aspect ratio and roundness. Data was collected on days 4, 8 and 12 to observe aggregate reaction to the gel environment. Calcein-AM/Ethidium Homodimer live/dead assay was used to assess the viability of the aggregates as well as phalloidin for cell morphology.

Results and Discussion

Aggregates stained for cell viability showed varied results based on the host gel. As observed previously, aggregates were healthy in body, yet the outer layer had a higher proportion of compromised cells, possibly due to the external hydrogel environment. In previous optimisation, aggregates maintained a high roundness, and this was observed in the non-adhesive agarose. Rounding of cell bodies were observed to persist. However, the aggregates in cell adhesive PeptiGels®, the aggregate body had spread as it interacted with the synthetic hydrogel. PeptiGels® were found to degrade rapidly which may limit their application whereas the varied agarose concentrations maintained their structure.

Conclusion

The stiff natural gel was found to be the more stable of the two hydrogel types for a supportive cell culture structure for aggregates to maintain their position. The PeptiGels® had various results regarding their influence on the aggregates thus further research is required.

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POSTER 32: ASSESSMENT OF CELL SEDIMENTATION IN LOW VISCOSITY BIOINKS

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Introduction

Suspended 3D Bioprinting, whereby structures are printed within a supporting gel bed, has recently been developed as an improved method in extrusion-based 3D bioprinting. The major advantage of this technique is that it allows the use of low viscosity bio-inks by providing support that prevents collapse of the structure during the printing process. This method allows for the fabrication of constructs with complex geometries from low viscosity biomaterials such as alginate and collagen, as well as improved cell survival due to reduced shear stress on cells throughout the printing process[1]. However, with the printed part remaining in the liquid state and only being solidified once the printing process is completed, there is potential for cell sedimentation to occur, resulting in a heterogeneous cell density within the printed structure. Here, we investigate how the rheological properties of commonly used low viscosity bio-inks can influence this gravity-driven process.

Materials and Methods

Bio-ink preparation – Solutions of 2% sodium alginate (Sigma Aldrich, UK) were autoclaved and cooled to room temperature to form working solutions. PureCol™ EZ Gel (Sigma Aldrich, UK) 0.5% type 1 collagen solution was used as supplied. Alginate-collagen mixtures were prepared by mixing the two solutions at a 1:1 ratio. Solutions containing collagen were kept on ice throughout the experiments to prevent crosslinking. Cell culture media (DMEM + 10% FBS, + 1% Pen/Strep) was used as a control bio-ink at room temperature.

Cell lines - Human dermal fibroblasts (HDFs) were selected for the sedimentation experiments having previously been used in suspended 3D bioprinting. The HDFs were cultured in DMEM (+ 10% FBS, + 1% Pen/Strep) in a 5% CO_2 incubator at 37 °C. When the HDF culture reached 80% confluency, cells were suspended at a concentration of 2 x 10^5 per mL in bio-ink solutions for cell sedimentation assays.

Cell sedimentation assay – Bio-ink cartridges (compatible with an INKREDIBLE Bioprinter, CELLINK) were loaded with 3 mL of bio-ink. At t = 0, 30 and 60 minutes, 50 μL samples were extracted from the upper surface of the bio-ink cell suspension. Cell counts were conducted (n=3) and an average cell number per 50 μL was calculated. Cell sedimentation at 30 and 60 minutes was expressed as a percentage decrease in respect to the cell number at t=0 in the individual bio-ink.

Rheological measurements – Measurements of viscosity were conducted using a 55 mm, 2° cone and plate geometry on a Bohlin Gemini Rheometer at low shear rate to compare viscosity profiles between bio-inks.

Results and Discussion

The viscosity of the different bio-inks appears to have a significant impact on the sedimentation of HDFs. Cells suspended in DMEM sedimented rapidly in the first 30 min, whereas within both bio-inks the HDF's remained suspended at t=30 (Fig 1), despite the 2% alginate having a lower viscosity than the collagen alginate mixture (1.2 Pa·s versus 9.7 Pa·s at a shear rate of 0.001 s^{-1}). After 60 min however, there was significant sedimentation in the 2% alginate bio-ink ($p<0.05$) compared with the alginate-collagen mixture which showed no significant change ($p>0.05$) between t=30 and t=60. Indeed, HDFs in the 2% alginate bio-ink had sedimented to the same extent as those originally suspended in DMEM at t=60.

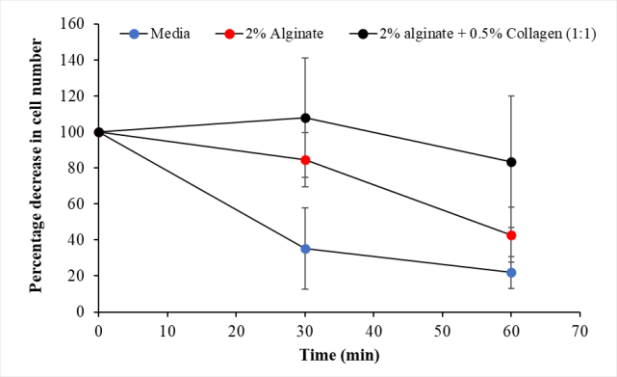

Figure 1 - Cell sedimentation as a function of time in DMEM, 2% Alginate and 2% Alg 0.5% Col 1:1 mixture.

Conclusion

This study highlights the cell sedimentation profiles within various low viscosity bio-inks, which could result in heterogeneous cell densities within printed constructs. This could particularly impact structures printed using suspended additive manufacturing techniques where prints remain in the liquid state until the print process is complete. Considerations regarding cell sedimentation in bio-inks should therefore be taken when designing low viscosity bio-inks for use in suspended 3D bioprinting and a realisation of this is extremely important.

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Acknowledgements

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POSTER 33: ELECTROSPUN PVA MEMBRANES FOR BLOOD SALVAGE

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Introduction

Intraoperative blood loss can regularly exceed 20% of a patient's blood volume across a range of surgical interventions, and in cases of tumour surgeries can reach up to 40% [1-3]. Patient blood management (PBM) aims to minimise the need for allogeneic blood transfusions (ABT) with their associated risks and high costs, and has been shown to significantly improve patient outcomes [4-6]. Intraoperative cell salvage (IOCS), also known as blood salvage, is a form of PBM, where the patient's shed blood is collected during surgery and the red blood cells are returned back to the patient. Surgical oncologists, however, remain skeptical about the use of IOCS, due to concerns about the reintroduction of circulating tumour cells to the patient and the associated potential of metastatic spread. Replacing the current IOCS devices, which separate cells based on size and density, with much more selective immunoaffinity methods offers the possibility to overcome the above limitation. This work is the first stage towards the development of an alternative IOCS configuration, featuring non-woven polymeric supports arranged in a membrane chromatography format. Poly(vinyl alcohol) (PVA) has been the polymer of choice for the creation of the base matrix, due to its FDA approval for medical applications and desirable chemical and biological properties.

Materials and Methods

PVA with 98% and 99% degrees of hydrolysis was electrospun using two different methods (needless DC, and AC) and then heat treated at 180 °C for various durations (1-8 hours), in order to prevent polymer dissolution in aqueous environments. The physicochemical and mechanical properties of the electrospun mats were investigated using FTIR, XRD, ESEM and tensile testing. The most promising materials subsequently underwent cytotoxicity testing with human mesenchymal stem cells, followed by flowthrough experiments in a filtration configuration with a suspension of SY5Y neural blastoma cells and then with defibrinated sheep's blood.

Results and Discussion

Comparison of 98% and 99% hydrolysed PVA membranes produced by needleless DC electrospinning showed that the latter maintained better fibre morphology following saturation in ESEM, irrespective of heat treatment duration, and exhibited a 20% to 60% increase in tensile strength *c.f.* the 98% ones. AC electrospinning created much thicker fibres than the DC method, measuring 624 nm and 281 nm respectively, thereby rendering the supports a very open porous structure, which is highly suitable for the easy passage of large entities such as human cells. Despite the fact that both electrospinning methods did not appear to have a negative impact of the growth of human mesenchymal cells and cytocompatibility results for all samples exceeded 100% compared to controls, subsequent flowthrough experiments using a filtration configuration were carried out with non-woven mats created from 99% hydrolysed PVA by needless DC electrospinning. This was influenced by the established scalability and GMP compliance of the technology, in contrast to AC electrospinning which is still under development. Both SY5Y neural blastoma and defibrinated sheep's blood successfully passed through the un-functionalised membranes, with negligible pressure drops, and minimum mechanical cell entrapment within the 3D fibrous network.

Conclusions

Non-woven membranes were successfully created from 99% hydrolysed PVA using needless DC electrospinning and maintained their fibrous morphology in aqueous environments through cross-linking by heat treatment. The highly porous network and cell friendly nature of the resulting supports enabled the unimpeded passage of two different cell suspensions, thereby paving the way for the creation of a novel blood salvage device for oncological surgeries.

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POSTER 34: ANTIBIOTIC LOADED CALCIUM PHOSPHATES FOR BONE INFECTIONS.

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Introduction

Postoperative infections remain a complication after trauma & implant surgery, with an increase in cases over the past decade, leading to a high burden on healthcare systems. Osteomyelitis is associated with both biofilm formation and bone necrosis; therefore, a treatment is needed which alleviates both issues. Synthetic bone grafts, specifically nanoscale hydroxyapatite (nHA) pastes, have previously been developed to aid in bone regeneration with a high success owing to their similarity to natural bone. nHA is biocompatible, has osteoconductive properties and resorbs at a rate to support the growth of new bone. Currently, antibiotics are either given orally, intravenously which cannot reach avascular areas, or in the form of non-degradable PMMA beads which require surgery to remove. The preparation of a paste pre-loaded with antibiotics could potentially overcome these difficulties, although they have not been reported to date and their stability under gamma-irradiation needs to be tested. The aim of this project is to determine the appropriate concentrations of antibiotics to load into the pastes, and to assess the antibacterial activity and release from the loaded pastes against clinical isolates of bacteria.

Materials and Methods

Since pastes will require sterilisation, diffusion assays were carried out to assess gamma- and non-irradiated antibiotic activity against *Staphylococcus aureus* (clinical isolate from osteomyelitis 6850) and *Pseudomonas aeruginosa* (clinical isolate SOM-1). Minimum Inhibitory Concentrations (MIC) of gentamicin sulphate salt, vancomycin hydrochloride hydrate and tobramycin sulphate (gamma-irradiated and non-irradiated) were also measured to assess if the MIC was altered after gamma-irradiation. Antibiotic-loaded nHA pastes were then prepared and characterised.

Results and Discussion

Disc diffusion assays showed that gamma-irradiated antibiotics had less activity against *S. aureus* and *P. aeruginosa* compared to non-irradiated forms (controls). These MIC results established that a higher concentration of antibiotics was therefore needed after gamma-irradiation to inhibit bacterial growth.

Conclusion

These results suggested that antibiotic loaded nHA pastes could be prepared, but a greater concentration of antibiotic might need to be incorporated to compensate for loss of activity post-sterilisation. Further work will involve cytotoxicity testing against MG63 human osteosarcoma cell line, and antibiofilm testing of the antibiotic loaded pastes.

Acknowledgements

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POSTER 35: ENGINEERED CANCER *IN VITRO* MODEL TO DEMULTIPLEX BIOPHYSICAL CUES IN METASTASIS

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Introduction:
Prostate cancer is one of the most common cancers that affects men, with a prevalence of 26% in the UK in 2014. This is predicted to increase to 29% by 2035 with mortality rates set to increase by 3%. [1] 17–34% of cases are diagnosed at the metastatic stage, primarily to bone tissue. [2] Over the past decade, there has been a shift in the use of three dimensional (3D) models from solely focusing on the regeneration of tissues *in vivo* to developing precision 3D *in vitro* human tissue models. The focus of the latter is to emulate normal and diseased tissues as tools to study biological processes and develop treatment plans. [3,4] The main driving force behind this is the fact that although animal models recapitulate the *in vivo* complexity, such models lack the physiological and genetic similarity to the human conditions being studied. For example, the clinical translation of therapies from animal model testing to clinical applications is very low. [5] As new fabrication processes, such as 3D bioprinting, and bio-compatible, printable materials (e.g. alginate-based hydrogels) are developed, a growing number of studies have reported the development and refinements of 3D *in vitro* modelling's ability to include characteristics particular of human tissues. Importantly, tissues' extracellular matrix (ECM) provides both chemical and physical support to cells *in vivo*, and is responsible for regulating cell phenotypes. Any engineered *in vitro* models need to mimic these properties as closely as possible. In this project, I will be focusing on designing precision alginate-based hydrogels with physio-chemical and mechanical properties to model prostate tissue and its tumour sites *in vitro*. During the project, a second target tissue will be engineered and included in a tumour-on-chip platform to better understand the biological signalling pathways behind prostate cancer metastasis and the role of physical cues arising from the changing environment of both the primary tumour and the main site of metastatic growth (i.e. bone tissue). The model developed will enable investigation of the key biophysical factors involved in cell-substrate interaction during prostate cancer metastasis, expanding the current understanding of the process.

Project plan:
As investigation into the different mechanical properties that play a role in cell behaviour and signalling continues, it is increasingly difficult to determine the specific role that each property has in cell-substrate sensing. It has been found that substrate stiffness, viscoelasticity, density, and topography [6] all play roles in directing cell behaviour. In order to demultiplex these individual biophysical cues on cancer metastasis, I aim to develop a 3D *in vitro* model with tuneable biophysical properties and cellular composition, mimicking prostate cancer and bone tissues, allowing the interrogation of their crosstalk. Alginate-based hydrogels will be modified to enable cell encapsulation. A perfusion system (i.e. tumour-on-a-chip) with modifiable flow, nutrient, and oxygen delivery to mimic circulation will include the cell-containing hydrogels. Response of encapsulated prostate cancer cells to different biophysical stimuli, their extravasation, and metastasis to bone-like recipient will be investigated to fill current knowledge-gaps. This will inform further model optimisation, giving rise to realistic tissue models with the potential for direct pre-clinical application in early detection, drug screening, and therapy intervention.
Throughout the project, a library of alginate-based hydrogels will be optimised to aid the fabrication of 3D *in vitro* models. Bioprinting will enable the generation of 3D prostate cancer *in vitro* models at several stages of tumour progression, from which, study of phenotypic changes in prostate cancer cells and their interaction with the metastatic growth site (i.e. bone) will be possible (e.g. a co-culture with osteoblasts and osteoclasts.)
The prostate-to-bone engineered model will use biomaterials to match properties of target tissues (mechanical and chemical) and capture physiologically relevant tumour features (shear stresses, pH, and oxygen-levels).

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POSTER 36: NOVEL SYNTHESIS OF CLASS I AND CLASS II GELATIN-BASED ORGANIC-INORGANIC HYBRID HYDROGELS

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Introduction

Hydrogels are an attractive choice of scaffold material for bone tissue engineering. The use of natural hydrogels, *e.g.*, gelatin, however, is restricted by their poor long-term mechanical properties. One strategy to improve mechanical properties is to form hybrid organic/inorganic co-networks, resulting in synergy of the properties of the two components.[1,2,3] Hybrid hydrogels can be classified into two classes. In Class I, the matrices are embedded within one another through weak bonds, such as hydrogen. In Class II, the organic and inorganic domains are covalently linked (Figure 1a). Here, Class I and II gelatin- and gelatin methacryloyl (GelMA)–silica hybrid hydrogels were produced using a sol-gel process employing bespoke silane precursors.

Materials and Methods

Synthesis of precursors: The inorganic precursor, propylene glycol-modified silane (PGMS), was synthesised by transesterification of tetraethoxysilane (TEOS) with propylene glycol. A similar reaction was used to form a polyol-modified silane coupling agent (patent pending).

Class I hybrid: 5 wt% GelMA solution was prepared by dissolving GelMA in Dulbecco's Modified Eagle Medium (DMEM) and PGMS was added in an organic:inorganic ratio of 20:80 to produce GelMA-silica (Class I).

Class II hybrids: The coupling agent was added to 50 mg mL^{-1} of gelatin and GelMA in 10 mM $HCl_{(aq)}$. The mixtures were left for 24 h and 72 h at 40 °C to produce inorganically functionalised gelatin (Fn-gelatin) and GelMA (Fn-GelMA), which were separately added to DMEM. PGMS was added (same ratio as Class I) to produce 5 wt% Fn-gelatin-silica (Class II) and Fn-GelMA-silica (Class II) hydrogels.

Hydrogel characterisation: Samples were taken during functionalisation and analysed by FTIR and ^{1}H-NMR spectroscopy. A degradation assay was conducted in PBS, measuring change in mass over time, to determine the mechanical properties of Class I vs Class II hydrogels.

Results and Discussion

Covalent coupling of inorganic silica with a gelatin-based organic network was successfully achieved using the novel coupling agent. FTIR spectroscopic analysis of the hybrids reveals new bands at ~1115 cm^{-1} and 1090 cm^{-1}, assigned to Si–O–Si vibrations, as well as increased intensity of the band at 970 cm^{-1} (Si–O), corresponding to the formation of silica structure.[4] As expected, the degradation rate (mass change) increased for the Class I hydrogel compared to Class II (Figure 1b) demonstrating the stabilising role of incorporating a covalent inorganic crosslinker.[3]

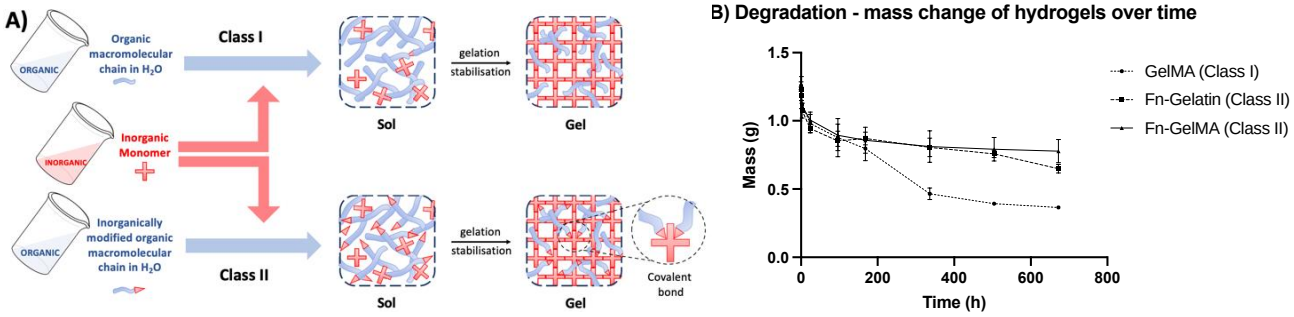

Figure 1) **A**: schematic showing the formation of Class I and Class II hydrogel hybrids, **B**: Degradation demonstrating mass change of Class I and Class II hybrid hydrogels over 28 days.

Conclusions

Gelatin and GelMA were successfully functionalised with a polyol-modified silane coupling agent. Gelatin-silica and GelMA-silica class II hybrid hydrogels were formulated using a sol-gel reaction with PGMS. Class II hybrid hydrogels demonstrated improved long-term stability compared to a Class I GelMA.

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POSTER 37: A NEW HYDROLYTIC ROUTE TO AN EXPERIMENTAL GLASS FOR USE IN BIOACTIVE GLASS-IONOMER CEMENT

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Introduction: Routes to glass-ionomer cements (GICs) have been previously explored through traditional silica-precursors, however, economic routes have not been fully reported. Alkoxysilanes, particularly tetraethyl orthosilicate, are conventionally used as the silica-precursor. They are expensive and have been reported to be toxic on inhalation. The aim of this study was to use sodium metasilicate as an alternative low-cost silica-precursor via a new hydrolytic sol-gel method to synthesize a glass-ionomer and thereafter investigate the mechanical properties and bioactivity of the prepared GIC relative to a commercial GIC.

Materials and Methods: Sodium metasilicate was hydrolysed with hydrochloric acid to form a gel, which was washed with de-ionized water to eliminate sodium chloride, the hydrolysis by-product, elimination was confirmed with dilute silver nitrate solution. The gel was aged and then dried to obtain silica. Silica was thereafter dissolved in citric acid to form a complex used for encapsulating the other glass precursors. Afterwards, a burnout procedure was performed on the silica-citric acid-precursor moiety to obtain glass-ionomer powder. Cements were formed from the as-prepared powder and a commercial powder using a similar polymeric acid solution. The cements were subjected to mechanical testing (compressive, flexural strength and microhardness) and bioactivity tests in simulated body fluid (SBF). The samples were characterised using scanning electron microscopy, energy dispersive X-ray analysis, X-ray diffraction and Fourier transform infrared spectroscopy. Independent samples t-test was used for data analysis with significance set at $p < 0.05$.

Results and Discussion: The experimental GIC exhibited compressive, flexural strength and microhardness of 103.65 ($\pm$4.53) MPa, 17.41 ($\pm$1.69) MPa and 64.10 ($\pm$3.84) KHN, respectively; while those for the commercial GIC were 118.86 ($\pm$1.67) MPa, 21.63 ($\pm$2.36) MPa and 72.45 ($\pm$3.30) KHN, respectively. Compressive strength is the most important property for conventional GICs. The experimental GIC met the minimum ISO 9917-1:2016 specification (100 MPa) for restorative GICs. However, the values obtained for flexural strength and microhardness failed to meet the threshold values (20 MPa and 70KHN respectively) required for restorative GICs. The experimental GIC upon immersion in SBF over a 28-day period compared with commercial GIC by showing good level of hydrolytic stability while nucleating a layer of apatite-like deposits on its surface. This implies a better mineralisation ability and improved chemical bonding to dental hard tissues which are desirable properties of posterior dental restoratives. This is expected to enhance clinical performance of these restorations by reducing the incidence of adhesive failure when used in stress-bearing areas.

Conclusions: This study shows that a reactive glass suitable for glass-ionomer cements can be synthesized by hydrolytic route using sodium metasilicate as starting silica-precursor. The biomineralisation induction capacity of the experimental GIC was superior to the commercially one, however, the mechanical properties were significantly lower than that of the commercial GIC. Current research efforts are still ongoing to further enhance the mechanical properties of this experimental glass-ionomer with beneficial mineralisation properties as this could serve as a candidate dental restorative material.

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POSTER 38: ANTIMICROBIAL PEPTIDES ENCAPSULATED GELATIN MICROPARTICLES FOR THE TREATMENT OF WOUND INFECTIONS

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Introduction Patients undergoing maxillofacial head and neck cancer surgery have an approximately 30-80% risk of developing a wound infection[1]. Currently wound infections are treated by the administration of antibiotics, however, due to rise in antimicrobial resistance there is a drive to reduce the use of antibiotics. Antimicrobial peptides (AMPs) are a promising alternative to antibiotics. AMPs are naturally occurring macromolecules that demonstrate a potent antimicrobial activity against a broad range of microbes, including viruses, bacteria, and fungi[2]. AMPs are part of every organism's innate immune response and act as a first line of defence against infection. As there are a variety of AMP sequences and multiple mechanisms of action, the tendency of bacteria developing resistance is low. The antimicrobial activity of AMPs are hindered under biological conditions as they are susceptible to proteolytic degradation[3]. Development of encapsulation strategies offers a way of circumventing the degradation issue and increasing the residence time. This study investigates two AMPs (nisin & DJK-5), encapsulated in gelatin particles. The influence of the choice of AMPs, particle size, zeta potential value, drug loading and in vitro drug release was studied.

Materials and Methods Gelatin particles were prepared using two different synthesis methods. Method 1 was 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 DJK-5 was then left to incubate with the gelatin particles for 24 hrs to entrap the AMPs onto the particles. Method 2 was a water in oil W/O emulsion method. An aqueous solution of gelatin was added to water to form the W/O emulsion. The aqueous solution was added to ethyl acetate and left to emulsify. The particles were characterised with Fourier-transform infrared spectroscopy (FTIR), zeta potential, particle size analysis and scanning electron microscopy (SEM). The antimicrobial efficacy was tested against *Methicillin-resistant Staphylococcus aureus (MRSA)* and *Escherichia coli (E. Coli)* using the spread plate technique and the colony forming units (CFU) were measured at 4 and 24 hr time points.

Results and Discussion In this study we have developed a procedure to entrap antimicrobial peptides into gelatin particles using an O/W & W/O emulsion method. For method 1 (O/W), The effect of pH on the fabrication and efficiency of encapsulation of the AMP-encapsulated particles was investigated. The gelatin particles were 5384 d. nm in size with a zeta potential of +11.1. Optimised formulations of nisin-encapsulated gelatin particles demonstrated a 3-log reduction in *MRSA* and no reduction against *E. coli.* This is expected as nisin is known to only have efficacy against gram-positive bacteria. DJK-5 -encapsulated particles displayed a 1-log reduction against *MRSA & E. coli* at 0.1 mg/ml. For method 2 (W/O), the influence of several experimental variables (stirs speed, surfactant & temperature) was varied to optimise the encapsulation efficiency of the particles. The gelatin particles had an average size of 139.4 nm zeta potential of -38.3. Optimised formulations of nisin-encapsulated gelatin particles demonstrated excellent antimicrobial efficacy against *MRSA* and DJK-5 encapsulated particles demonstrated excellent antimicrobial reduction against both *MRSA* and *E. coli*.

Conclusions We have developed two methodologies to fabricate and encapsulate AMPs, nisin an DJK-5 into gelatin particles. The O/W particles were larger and less homogeneous compared to the W/O particles, as they were more homogeneous and smaller in size. Both O/W and W/O particles displayed excellent antimicrobial efficacy. In this study we have developed a procedure to entrap AMPs into gelatin particles using a W/O emulsion method. The AMP-loaded particles show to be a promising alternative to antibiotics for treating wound infections from maxillofacial head and neck cancer surgery.

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POSTER 39: Electroactive marine collagen scaffolds are a sustainable biomaterial for developing neural stem cell implants

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Spinal cord injury (SCI) is a serious condition caused by damage to the spinal cord through trauma or disease, often with permanent debilitating effects. Despite a lack of effective treatments for SCI, cell therapy remains as the most promising option. The advent of biomaterials has increased the potential of cell therapy through improving cell survival and retention. Whilst medical grade mammalian collagen biomaterials exist for applications such as wound healing, marine collagen (MC) presents as an attractive alternative to the latter in that it carries no risk of bovine spongiform encephalopathy transmission, has similar immunogenicity and is more sustainable to source. However, it has yet to be tested for neural applications. In this study, we investigated the potential of functionalised electroactive Jellagen®, a MC biomaterial, in maintaining key regenerative features of neural stem cells (NSCs) including viability, proliferation and differentiation. It was hypothesized that electroactive Jellagen® would not affect the key regenerative features of NSCs in our cell culture model.

NSCs were obtained from P0-P3 mice and expanded as neurospheres in culture. The cells were plated and maintained in monolayer medium for 5 days on glass controls, inert Jellagen® and electroactive Jellagen® scaffold slices. A viability assay, proliferation assay and immunocytochemistry were performed to compare the health and characteristics of the cells. A subset of the NSCs were induced in differentiation medium for a further 7 days prior to performing the viability assay, proliferation assay and immunocytochemistry.

Descriptive statistics were obtained and the control and experiment groups were compared to each other using the one-way analysis of variance. A p value of <0.05 was considered statistically significant. Data was analysed from n=3, where n is an individual repeat from a biological litter.

The cells on electroactive Jellagen® substrates showed a high viability. Differentiation was not affected and proliferation followed similar trends in all groups. For the first time we report a protocol to functionalise MC scaffolds with a conductive component and data that suggests electroactive MC is neuro-compatible with murine-derived primary NSCs in culture. The data supports the future exploration and development of MC in neural tissue engineering applications.

POSTER 40: Nitric Oxide (NO) Loaded Gelatin Nanoparticles as Antimicrobial Therapeutics

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Introduction

Antimicrobial resistance (AMR) is an emerging global threat, and if no action is taken is estimated to result in 10 million deaths a year, costing $100 trillion by the year 2050[1]. The overuse of antibiotics since their discovery 80 years ago, has led to the development of multidrug resistant bacteria (MDR). As such, there is a need to develop new therapeutic agents which do not lead to AMR. Nitric oxide (NO) is an effective broad-spectrum antimicrobial agent and is endogenously produced by the immune system in response to invading pathogens. NO has several mechanisms by which it can inactivate microorganisms and hence has not been shown to lead to the development of MDR bacteria[2]. Despite its promising antimicrobial function, as NO is a highly reactive free radical with a short half-life, delivery to the site of infection is challenging. Nanoparticle drug delivery systems can be used for the controlled release of NO to the target tissue in therapeutic doses. Gelatin nanoparticles (GNPs) are promising drug delivery vehicles due to their biocompatibility[3], ease of fabrication and ability to control the release of drugs. In this study, the successful loading of *N*-diazeniumdiolates, a NO donor onto GNPs (GNP/NO), was demonstrated and the loading efficiency, release kinetics were assessed. The antimicrobial efficacy of GNP/NO was assessed against two bacteria and a fungal species. The cytotoxicity of the nanoparticles using a standard ISO-10993 protocol.

Materials and Methods

GNPs were fabricated using the two-step desolvation method, followed by the loading of *N*-diazeniumdiolates (GNP/NO). Their physicochemical properties were assessed using Dynamic Light Scattering (DLS), Fourier-Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopy (SEM). Chemiluminescence was used to determine the NO payload and kinetics of release of the GNP/NO. The antimicrobial efficacy of GNP/NO was tested over a 24 h period against *Escherichia coli* (9001)*, Staphylococcus aureus* (12891) and *Candida albicans* (1363). The cytotoxicity of the GNP/NO were assessed using both direct contact and leaching assays against a L929 cell line.

Results and Discussion

GNPs were synthesised at pH 3.5 and were found to produce homogenous spherical particles in the range of 200-250 nm. Particle size was confirmed via DLS and SEM analysis. The FTIR spectrum displayed peaks characteristic of *N*-diazeniumdiolates at both O-N-N-O (1380 cm^{-1}) and N-O (1278 cm^{-1}) confirming its successful loading into the GNPs. Chemiluminescence analysis was used to analyse the release rate of NO from the GNPs. An initial burst, followed by a sustained release over 24 h was observed. Antimicrobial testing revealed GNP/NO to cause a significant reduction in viable bacteria after 4 h, and further reduction at 24 h for all tested microorganisms. Cytotoxicity assays demonstrated GNP/NO to have limited cytotoxicity when tested against L929 cells.

Conclusions

This current study has demonstrated the successful development of *N*-diazeniumdiolate loaded GNPs with antimicrobial capabilities. The functionalised particles displayed excellent antimicrobial properties after 24 h of exposure and showed limited cytotoxicity. Further investigation is warranted to assess the stability of GNP/NO over time, and the cytocompatibility of GNP/NO *in vivo,* ensuring the feasibility of GNP/NO for use as an effective biomedical application for the treatment of hospital acquired infections.

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POSTER 41: BIOGENIC SYNTHESIS OF ZnO NANOPARTICLES USING *ANNONNA MURICATA* PLANT LEAF EXTRACT AND ITS ANTI-CANCER EFFICACY

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Introduction

Green nanoparticles have gotten a lot of attention because of their potential applications in tissue regeneration, bioimaging, wound healing, and cancer therapy[1]. The physical and chemical methods to synthesize metal oxide nanoparticles have an environmental impact, necessitating the development of an environmentally friendly green strategy for nanoparticle synthesis[2-4].

Materials and Methods:

Zinc acetate dihydrate (0.02 M) was added to 50 mL of water and kept under constant stirring. Then, 1 mL aqueous leaf extract of *Annona muricata* (Am) plant was mixed with the above solution. Later, 2 M NaOH was added to reach the solution pH = 12 and stirred for 2h. The resulting pale white precipitate was obtained after centrifugation at 15000 rpm and dried at 60˚C in a hot air oven overnight.

Results and Discussion:

Am-ZnO NPs were evaluated using UV/Visible spectroscopy, FTIR spectroscopy, X-Ray Diffraction, DLS, and Zeta potential. Nanoparticles had an optical absorbance of 355 nm and a net negative surface charge of ~ - 2.59 mV. TEM characterizes the Shape and size of the nanoparticles. The obtained Am-ZnO NPs are biocompatible and hemocompatible in nature. These nanoparticles caused an anti-cancer therapeutic effect in MIA PaCa2 and MOLT4 cancer cells by inducing oxidative stress, and a change in mitochondrial membrane potential leads to programmed cell death. Further, we observed a reduction in the size of lung cancer spheroids with doxorubicin as a positive control.

Conclusions:

The Am-ZnO NPs were physico-chemically characterized by different spectroscopy and microscopy techniques. The FTIR and UV-visible spectrum exhibited the preparation and optical nature of Am-ZnO NPs, respectively. HR-TEM analysis displayed the nearly spherical shape of Am-ZnO NPs. The zeta potential measurement presented the net negative charge on the surface of NPs. These NPs can be used broadly because of their biocompatible and hemocompatible nature. Furthermore, the fabricated Am-ZnO NPs showed strong anti-cancer activity on both 2D and 3D tumor models. Subsequently, the Am-ZnO NPs treated cancer cells underwent programmed cell death with depolarization in their MMP.

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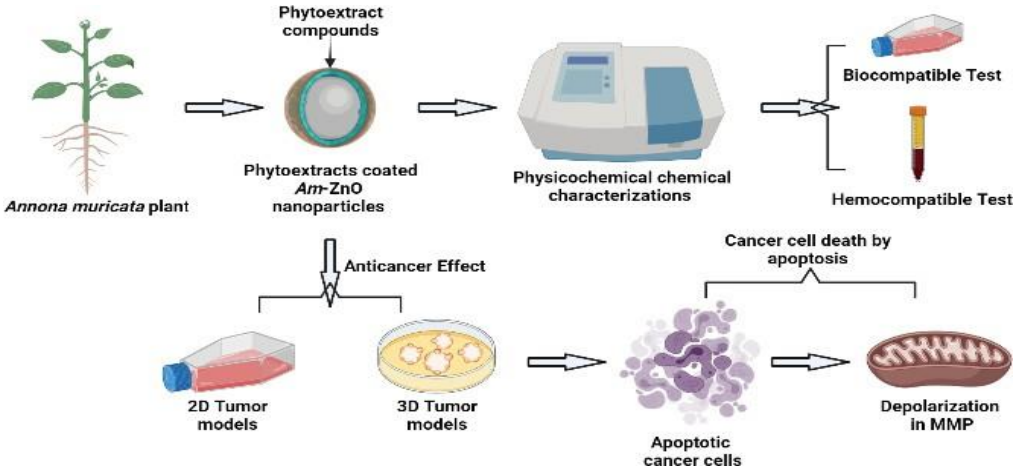

POSTER 42: CHARACTERISATION OF NATIVE AND DECELLULARISED PORCINE TENDON UNDER TENSION AND COMPRESSION: A CLOSER LOOK AT GLYCOSAMINOGLYCAN CONTRIBUTION TO TENDON MECHANICS

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Introduction: Decellularised porcine superflexor tendon (pSFT) has been demonstrated to be a suitable scaffold for anterior cruciate ligament reconstruction [1]. While the role of collagen in tendons is well known, the mechanical role of glycosaminoglycans (GAGs) is less clear and may be altered by the decellularisation process.

Objectives: To determine the effects of decellularisation on pSFT GAG content and mechanical function and to investigate the consequences of GAG loss in tensile and compressive loading.

Materials and Methods: pSFTs were decellularised following previous techniques [2]. For GAG removal, native pSFTs were treated with chondroitinase ABC (ChABC; 0.1U/mL, 72h). Cell and GAG removal was validated using histology and quantitative assays. Native, decellularised and ChABC treated groups (n=6) were biomechanically characterised. In tension, specimens underwent stress relaxation and strength testing using previous protocols [1]. Stress relaxation data was fitted to a modified Maxwell-Weichert model to determine time-dependent (E_1 & E_2) and time-independent moduli (E_0). The toe and linear region moduli (E_{toe}, E_{linear}), in addition to tensile strength (UTS) and failure strain were determined from strength testing. In compression, specimens underwent confined loading conditions (ramp at 10 s^{-1} to 10% strain and hold). The aggregate modulus (H_A) and zero-strain permeability (k_0) were determined using previous techniques [3]. Data was analysed by one-way ANOVA with Tukey post-hoc test to determine significant differences between test groups ($P<0.05$).

Results and Discussion: Quantitative assays showed no GAG reduction post-decellularisation, but a significant reduction after ChABC treatment. H_A was only significantly reduced in the ChABC group, proving GAGs play a key role in bearing compressive loads. k_0 was significantly higher for the ChABC group compared to decellularised, suggesting permeability is affected differently by decellularisation processes and enzymatic treatment for GAG removal. E_0 was significantly reduced in the decellularised group compared to native and ChABC groups, while E_1 and E_2 were not different between groups. E_{toe}, E_{linear}, UTS and failure strain were not different between groups, suggesting GAG loss does not adversely affect pSFT mechanics under tension.

Conclusions: Decellularisation does not affect GAG content or impair mechanical function in pSFT. GAG loss adversely affects pSFT compressive properties, revealing major mechanical contribution under compression, but no significant role under tension.

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POSTER 43: TOWARDS A XENOBIOTIC FREE POLYETHYLENE GLYCOL BASED COLONIC CRYPT ARRAY

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INTRODUCTION

The ability to model the physiology of the human colonic epithelium *ex vivo* holds great potential to accelerate research into the molecular mechanisms which govern human health and disease, facilitate high through-put drug discovery, and drive precision medicine strategies for complex diseases such as cancer. The break-out biomimetic models that have currently been acting as the work horse for such research include human colonic organoids and organ-on-a-chip systems. However, the translation of said research is somewhat occluded by a reliance on modelling human tissue within animal derived extracellular matrix microenvironments, as well as in some systems; a lack of consistent structural mimicry of the highly invaginated architecture of the *in vivo* epithelium. To overcome these principal limitations: we present formative attempts at synthesising a xenobiotic-free polyethylene glycol-based colonic crypt scaffold.

METHODOLOGY

Polyethylene glycol (PEG) hydrogels were synthesised via Michael type addition reactions between 4 arm PEG thiol, and 4 arm PEG maleimide terminated monomers. A stamp containing colonic crypt like protrusions was 3D printed and used to micromould a PEG hydrogel scaffold. Scanning electron microscopy and cyro-scanning electron microscopy were undergone on the stamp and resultant PEG scaffold respectively. Rheological assessment was undergone on a variety of candidate polymer formulations. LIVE/DEAD viability assays were undergone on Caco-2 cells cultured on the colonic crypt scaffold.

RESULTS

The bulk mechanical properties of a xenobiotic free tetra arm polyethylene glycol thiol/maleimide hydrogel were fine-tuned via alterations in polymer concentration and molecular weight to approximate estimates of the *in vivo* stiffness of the colonic stem cell niche. The candidate polymer formulation was diffusive, as indicated via the diffusion of fluorescent 10 kDa dextran through the hydrogel. Integration of RGD on the surface of the colonic crypt scaffold was undergone as a means to provide adhesive cues for Caco-2 cells. The colonic crypt scaffold demonstrated a favourable cytotoxicity profile when used for the culture of Caco-2 cell lines.

CONCLUSION

The colonic crypt scaffold is a tractable model which can be readily modified in regard to its mechanical and biochemical properties to optimise cell culture. The xenobiotic free nature of the polymer overcomes the risk of hydrogel mediated immunogen and pathogen transfer as well as the batch-to-batch variability associated with organotypic models which rely on Matrigel as a means to facilitate culture. This proof of principle study lays the groundwork for future planned studies utilising the platform to facilitate the culture of patient derived organoids as monolayers.

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POSTER 44: THE USE OF CERAMIC-MODIFIED POLYESTER BLENDS FOR THE MANUFACTURE OF A MULTI-FUNCTIONAL BILAYER MEMBRANE FOR THE REGENERATION OF PERIODONTAL TISSUE

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Background: Guided Bone Regeneration (GBR) is a regenerative therapeutic procedure that has been widely studied for its effective role in treating periodontal disease. Different membranes are currently used for GBR, However, they have limitations in terms of space maintenance, early/late absorption, mechanical properties, and the need for a second operation to remove non-resorbable membranes. As a result, they are less effective to completely fit for purpose (1). Current research reported by our group has demonstrated the potential of using a new polyester blend (Floreon) for bone regeneration applications (2). This partnership has evolved further, and we now collaborate with Floreon Ltd. to test the biomedical potential of using the new polyester blend to manufacture an innovative GBR synthetic bilayered membrane.

Objective: The aim of this study was to fabricate a bilayer membrane made of ceramic modified Floreon and to evaluate its mechanical, physicochemical and biological performance.

Methods: A bilayered membrane was fabricated which consisted of (i) porous bottom layer using electrospinning and Floreon reinforced with different ceramic concentrations; we hypothesise this membrane would preserve the bone socket; (ii) smooth layer of pure Floreon on top (physical barrier) obtained by spin-coating technique. PLA membranes were also produced as controls. Characterisation was performed for biophysical and mechanical properties through Fourier-transform infrared spectroscopy, differential scanning calorimetry, scanning electron microscopy, porosity, wettability, tensile testing. The cell viability, degradation and bioactivity were also studied.

Results: The bilayer nanofibrous membranes exhibited excellent mechanical properties initially as well as after one month in PBS; Moreover, the electrospun Floreon membranes reinforced with ceramics exhibited an increase in pore diameter and fibre size which satisfies the ideal properties needed for GBR membranes. Floreon polyester blends have proven to be biocompatible and comparable to PLA controls and are a promising platform for the manufacture of complex membranes for bone regeneration, in our case, a GBR bilayer.

Conclusion: Our combination of manufacturing techniques (electrospinning & Spin-coating) has proven to be a promising method for the development of new GBR membranes. This work demonstrates the potential of using Floreon and ceramic-modified Floreon for dental applications, and the advantages of modifying these blends with Bio-ceramic components.

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POSTER 45: POROUS POLY(GLYCEROL SEBACATE)-BASED SCAFFOLDS FOR AUTOLOGOUS FAT GRAFTING

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Introduction

Autologous fat grafting (AFG) is commonly used for reconstructive surgeries to replace soft tissues. However, this transplanted fat has poor long-term survival and thus costly additional procedures are often required[1]. As such, there is a clinical requirement for an improved fat graft that can sustain tissue volume following transplantation.

Poly(glycerol sebacate) (PGS) is a soft yet tough synthetic elastomer - closely mimicking the mechanical properties of adipose tissue. Following the methacrylation of PGS (PGS-M), it can be photocrosslinked, and, by altering the degree of methacrylation, its mechanical and biodegradation properties can be tuned to match the requirements of the target tissue[2].

Materials and Methods

Polymerised high internal phase emulsion (polyHIPE) was used to create porosity within PGS-M polymer. Following UV curing, scaffolds were visualised using scanning electron microscopy. Adipose-derived stem cells (ADSCs) isolated from human tissue were seeded onto the scaffolds, and a resazurin reduction assay was performed to assess cell metabolic activity.

Results and Discussion

Using polyHIPE templating, a highly porous structure was made from PGS-M. Scaffolds were manufactured with varying pore sizes, which could be increased by decreasing the spin speed during emulsion templating. Scaffolds porosity was greater than 74%, the maximum packaging density of uniform spherical droplets. PGS-M scaffolds were highly flexible and exhibited elastic recovery following compression with a 25N load, displaying similar mechanical properties to adipose tissue. A steady increase in metabolic activity of ADSCs cultured on PGS-M scaffolds was observed over the course of cell culture, suggesting that the cells were attaching to and proliferating on the scaffolds, and that the scaffolds were not cytotoxic to the cells.

Conclusions

Here we demonstrate that adipose derived stem cells attach and proliferate on PGS-M polyHIPE scaffolds. Following further development, we envisage that these scaffolds may have the potential to be used in combination with adipose tissue to supplement fat grafting and promote the long-term survival of transplanted adipose tissue.

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POSTER 46: THE ROLE OF VANCOMYCING DOSING PROFILES ON ANTIMICROBIAL RESISTANCE DEVELOPMENT

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Introduction

Antimicrobial resistance (AMR) is rapidly raising in clinical settings with some authors predicting a global health-care burden of $100 trillion, and affect 10 million lives annually by 2050 [1,2]. Their high populations, rapid division cycles, genomic plasticity and ability to transfer genetic information between species confer bacteria a high adaptability, endangering antibiotic therapies. Coupled with a misuse of antibiotics both in healthcare and agriculture, a limited number of new molecules in the regulatory pipeline and a lack of novel mechanisms of action to hinder resistance, concerns over the coming of a post-antibiotic era are on the rise [3]. To ensure that such bleak future does not come to pass, it is critical that the biomaterial community actively takes action. While novel molecules will be required, their research and implementation in clinical settings may take decades, thus, understanding the effect of current dosing regimens in AMR to modify current policies should be a priority to protect antibiotic therapies. A prime example is vancomycin which historically was considered as a last resort molecule due to its adverse effects [4]. Nevertheless, the rise in AMR and better tracking of renal functions to ensure its safe uptake, is making vancomycin a more common treatment against severe Gram-positive infections. Molecular mechanisms of resistance to vancomycin have already arisen which combined with controversial dosing regimes may risk its future use. In this work, the influence of different regimes of vancomycin on AMR development of gram positive and negative species were studied *in vitro* under static and dynamic conditions.

Materials and Methods

S. aureus Newman and *E. coli* O44:H18 strains were subjected to three different vancomycin regimens: a single 15µg inoculation, two 7.5µg dosages and three 5µg treatments. Colony forming units, MIC/MBC for a panel of four antibiotics and flow cytometry analysis were performed to estimate susceptibility to antibiotic resistance. Variations in populations and mutations were analysed by further studying different colonies of the treated cultures while a bioreactor was used to assess differences between static and dynamic seeding.

Results and Discussion

Vancomycin dosing resulted in a significant effect on both species, however, its influence on the Gram-negative species was heavily subdued when compared to *S. aureus*. Intermittent dosing was more effective than a single bolus inoculation, although slight variations in treatment heavily influenced AMR. Two doses of 7.5ug were less effective than other intermittent strategies, rapidly selecting for more vancomycin resistant cells which also became less susceptible to gentamicin. Interestingly, *S. aureus* seemed to be more effectively treated with ciprofloxacin after three vancomycin dosages, while Kanamycin was less effective after a single or double treatment.

Conclusions

Overall, this study shows that vancomycin dosing has a significant influence on antimicrobial resistance which can translate into variations in susceptibility to other molecules. While more work is still necessary to optimize vancomycin treatment and the molecular pathways responsible for AMR, the rise in effectiveness of intermittent dosing and their synergy with other antibiotic treatments are already invaluable tools that could guide clinicians to limit AMR development.

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Acknowledgments

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POSTER 47: NANOSCALE ZINC-SUBSTITUTED HYDROXYAPATITE WITH ANTIMICROBIAL PROPERTIES FOR BONE TISSUE REGENERATION

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Introduction

Bone infections (e.g. osteomyelitis & periodontal disease) are all caused primarily by bacterial pathogens. There is therefore interest in the development of antimicrobial biomaterials, but while extensive research into promising technologies has been published this has not yet resulted in successful clinical translation. Nanoscale hydroxyapatite (nHA) is one class of biomaterial with great potential given that - while existing commercial formulations do not have antimicrobial properties - a promising modification based on adding silver has been published[1].

Aim & Objectives

The aim of this research was to investigate the substitution of calcium with zinc in nHA using a rapid mix wet chemical precipitation method developed in Sheffield[1-3]. The specific objectives of the work presented here were to firstly design and fabricate a systematic series of Zn-substituted materials (0 to 20 mol%), and then characterize these products using X-ray diffraction (XRD), transmission electron microscopy (TEM), and thermogravimetric analysis (TGA).

Materials & Methods

Zinc substituted nHA was prepared (0, 5, 10, 15, and 20 mol.% zinc) using the modified rapid mix wet chemical precipitation method[1-3] Solutions of phosphoric acid (H_3PO_4) and zinc hydroxide $Zn(OH)_2$ were prepared with different Zn concentrations and mixed at room temperature. The suspension formed was washed several times until conductivity reached plateau, and the product was dried and ground into a powder form. A proportion of the powders were sintered at 1000 °C for 2 h to evaluate thermal stability prior to characterization.

Results & Discussion

Zinc oxide (ZnO) and brushite ($CaHPO_4 \cdot 2H_2O$) were detected in addition to nanoscale hydroxyapatite, and morphological changes in the nHA precipitates were detected using TEM with evidence of greater agglomeration after incorporation of zinc. Thermal decomposition had occurred more readily with greater zinc content, suggesting the crystal lattice may be less stable due to replacement of Ca by Zn.

Conclusion

Preliminary data suggested successful production of nanoscale Zn-substituted hydroxyapatite using a rapid mixing wet precipitation method (not previously reported). The HA precipitates tend to agglomerate and lose crystallinity with Zn substitution.

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POSTER 48: POLYMER NANO-ISLAND COATING FOR SMALL DIAMETER DRUG ELUTING STENTS

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Introduction

Cardiovascular disease (CVD) is the leading cause of mortality around the world[1]. Drug Eluting Stent (DES) is developed by coating Bare Metal Stents (BMS) with anti-proliferative drugs to overcome the problems of BMS. And although DES has better outcomes and it is the current gold standard for CVD patients, it still suffers from late Stent Thrombosis (ST) and poor endothelialisation. Surface engineering techniques to improve endothelialisation while retaining blood compatibility are still a major unmet therapeutic need in clinical world.
Studies have shown that the modifications on the surfaces at nanoscale can influence the cellular response[2]. Therefore, the aim of this study is to develop a novel drug eluting stent platform via nanotechnological approaches which can release drugs and antibodies from a stable and biocompatible polymer, inhibit ST formation while enhancing endothelialisation.

Materials and Methods

We have developed a preliminary approach of organosilane treatment prior to the polymer coating. For organosilane treatment 5 different functional group have been used as $-CH_3$, $-NH_2$, -SH, -F and methacrylate. 13 mm Glass coverslips were immersed in toluene which had 100 µM of each organosilanes for an overnight, washed with toluene 3 times and airdried. Surfaces were characterized using Water Contact Angle (WCA). Human Aortic Smooth Muscle Cells (HASMCs) were cultured in Medium 231 supplemented with SMGS. HASMCs were seeded onto organosilane modified coverslips as well as a plain glass control at density of 60,000 cells / mL and cultured at 37° C in 5% CO_2 incubator for 7 days. Methylene Blue staining data was obtained after 7 days to monitor cell proliferation.

Results and Discussion

Preliminary results of WCA shows that the surface treatment with organosilanes have been successful and all the silane groups showed different WCA results compared to control group which means we have achieved to modify the surface. The methylene blue staining pictures of HASMCs also shows that the cells are behaving differently on each individual surface. The best surface coverage obtained by -F and $-NH_2$ groups which are even higher than plain control glasses (Fig 1).

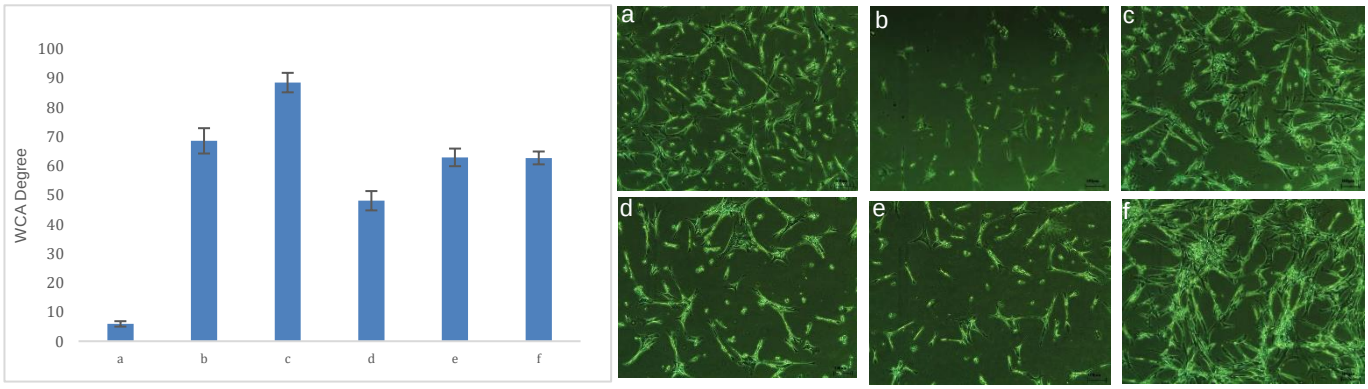

Fig. 8. Water Contact Angle results (n:10 with error bars representing +/- St Dev) and HASMC cells on organosilanized surfaces with 100 µm scale (a: control, b: $-CH_3$, c: $-NH_2$, d: -SH, e: methacrylate, and f: -F)

Conclusions

The initial results showed that the proposed silane treatment is modifying the surfaces successfully and on every surface the cells behave uniquely. These modifications will be continued by coating the surfaces with polymer as well as drugs and antibodies. At each step the HASMCs and HUVECs cell spreading and proliferation will be monitored to obtain the best surface modification for the cells around the stented area of cardiovascular arteries with the aim of developing a novel DES system which will enhance endothelialization and inhibit smooth muscle cell proliferation.

POSTER 49: KNOWLEDGE TRANSFER PARTNERSHIP – CELL TESTING TO ASSIST DEVELOPMENT OF NOVEL BIOMATERIALS

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Introduction: The Knowledge Transfer Partnership (KTP) scheme is specifically targeted to support collaboration between an Industrial partner (Lucideon Ltd) and an Academic Institution (Queen Mary University of London, QMUL) based around the transfer of knowledge from the academic partner to the industrial setting to help businesses to improve their competitiveness and productivity through the better use of knowledge, technology and skills within the UK knowledge base. Key to the successful transfer and embedding of this knowledge from the Academic team into the Industrial setting is the role of the KTP associate who manages and assumes responsibility for delivery of the programme of work to meet the aims of the partnership. The QMUL-Lucideon KTP aim is delivery of an *in vitro* cell culture facility to enhance the commercial activities of the Healthcare Division of Lucideon. An in house *in vitro* test facility will have several commercial benefits; provision of holistic biomedical materials physiochemical, mechanical and biological characterisation, to enhance existing consultancy provision for clients in the healthcare sector, while also facilitating the innovation and validation of Lucideons' own biomedical materials interests to enhance commercial consultancy and licensing opportunities to increase revenue streams. The Academic Institution QMUL, has significant track record in the development and pre-clinical characterisation of biomedical materials and translating these findings into commercially successful clinical use. Specifically, their past experience in *in vitro* pre-clinical evaluation of biomedical materials ideally places the team at QMUL to support the KTP associate to significantly accelerate the process of specifying and embedding a Biomedical materials *in vitro* test facility at Lucideon. A key part of this process is the KTP associates involvement in the production and characterisation of a variety of candidate test materials, driven by the interests of Lucideon, working with Lucideon healthcare, sales and marketing teams. This was then followed by the KTP associate undertaking the first series of *in vitro* tests at QMUL to transfer the academic knowhow into a series of robust protocols and standard operating procedures (SOPs) suitable for guiding the operation of an *in vitro* facility within a commercial setting to good manufacturing practices.

Materials and Methods: Candidate test materials, have been produced for *in vitro* testing. This includes a series mono and biphasic calcium phosphates and hybrid polymer/bioactive glass composites incorporating novel glass compositions, developed at Lucideon. Prior to the first tranche of *in vitro* testing physical characterisation was also performed at Lucideon. In order to develop the protocols and SOPs for future cell culture experiments at Lucideon the KTP associate will run a progressive series of biological characterisation tests at QMUL using L929 Fibroblasts and Human Mesenchymal Stem Cells (hMSCs). The first tranche of *in vitro* testing includes assessment of materials cytotoxicity and pro-osteogenic capacity. Concomitant to these tasks, a cell culture laboratory has been designed for installation at Lucideon's new site at Stone, Staffordshire. Commissioning of the facility, including steps towards accreditation, is anticipated to begin in the first quarter of 2023.

Results and Discussion: The aim of the KTP is to enhance Lucideon's Materials Testing and Consultancy portfolio. The revenue stream comprising commercial confidential consultancy; public funding engagement and platform technology development leading to the sale of licences, royalty agreements.

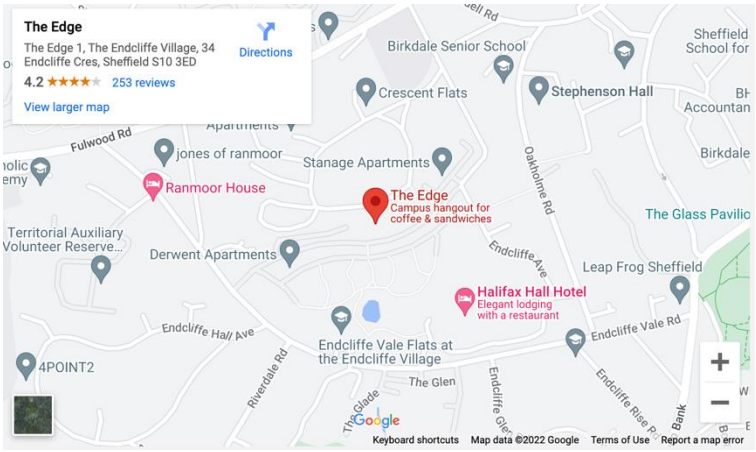
The project will also bring about novel materials and IP that can be taken forward. As well as IP it is expected that academic papers will be published. Whilst the cell culture facility could have been built by Lucideon without the KTP, the KTP has allowed an experienced person, the KTP Associate, to be embedded at Lucideon to work full time on the project. This person also liaises with experts in cell testing to introduce the correct SOPs. The KTP Associate has a Personal Development budget to allow them to obtain the skills required to both deliver the project and to enhance their career progression.

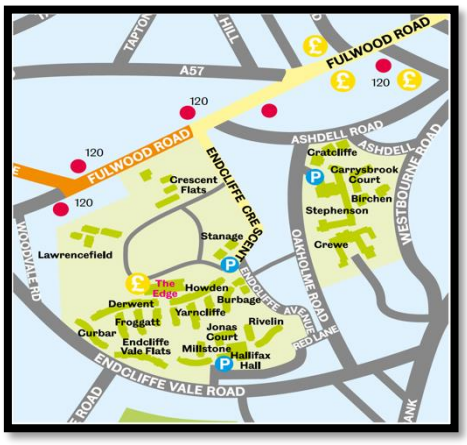
Conclusion: Knowledge Transfer Partnerships are an advantageous way for Academic Institutions to work together with Industry. The Industrial Partner gains knowledge from the Academic Partner through the associate to deliver something that the Industrial Partner does not have. This particular partnership will deliver a cell testing facility to Lucideon to increase the consultancy portfolio and widen revenue streams.

Acknowledgements: Knowledge Transfer Partnerships (KTPs) aim to help businesses to improve their competitiveness and productivity through the better use of knowledge, technology and skills within the UK knowledge base. This KTP project (012231) was funded by UKRI through Innovate UK.

Maps

Conference Venue: The Edge @ The University of Sheffield





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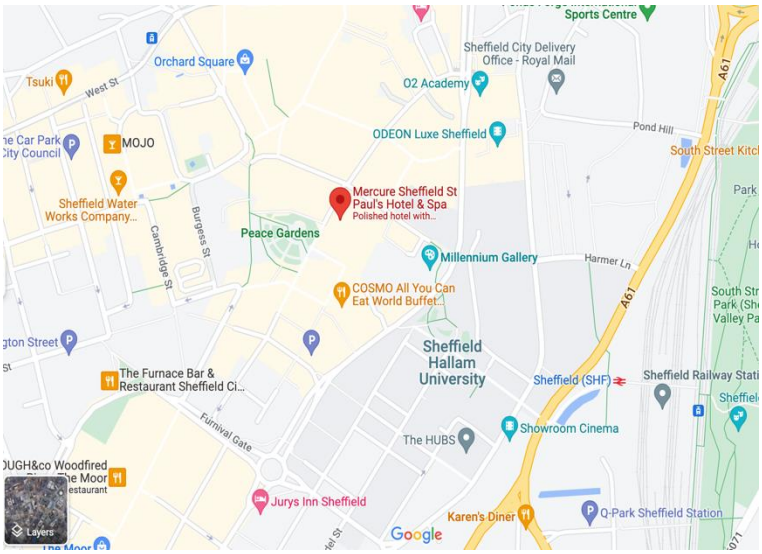

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