Future Leaders Joint CDT-UKSB Virtual Conference

15-17th June 2021

EPSRC & MRC Centre for Doctoral Training Regenerative Medicine

eipETIME Engineering Underpinning Medicine

CDT in Regenerative Medicine

UNIVERSITY OF LEEDS

ADVANCED BIOMEDICAL MATERIALS Centre for Doctoral Training | MANCHESTER 1824 The University of Manchester | The University Of Sheffield.

Doctoral Training Network in Technologies for Healthy Ageing

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Welcome Note

The UK Society for Biomaterials is proud to host this meeting joint with the CDTs working and aligned to this field, this year hosted by the EPSRC/SFI lifETIME CDT. This is a valuable meeting which emphasises both the importance of the work that we do, and the range of topics covered across the network. We will see many highlights over the few days this meeting is held, and we urge you all to continue talking to each other, sharing best practice and ideas to strengthen the research community as a whole.

Networking is always a key component of any conference, and although our meeting again this year is virtual, we have organised the interface to enable a more interactive space. Please do utilise this to meet new people and interact with others who share similar interests. Over the past years the fields or biomaterials and regenerative medicine have evolved with much more emphasis on joint and interdisciplinary working to tackle health-related issues. Topics such as surface science, computational modelling and chemical characterisation are being utilised at the interface with the biosciences, with optimised models of tissue engineered constructs, disease modelling and progression of clinical trials all benefitting greatly. It is our hope that through working as a community we can rapidly progress in our individual areas, being supportive of the early career researchers as they develop their skills, own ideas and establish themselves as independent researchers.

The Centres for Doctoral Training within the areas of Regenerative Medicine and related fields are a nurturing ground for future talent. It is a pleasure to welcome you all to this meeting and we hope that you can all contribute, as well as take away something from participating. Please do connect with our industry partners and sponsors and enjoy the event for the science, the networking and general interaction with others which we miss in these times of virtual working.

Best Wishes,

Paul Roach and Matt Dalby

Future Leaders in Regenerative Medicine: Joint Conference CDTs and UKSB
15th-17th June 2021

DAY 1

09:15	**Arrival online and admittance to conference** **Zoom link:** Future Leaders in Regenerative Medicine: Joint CDT and UKSB Conference **Meeting ID:** 914 7557 8436, **Passcode:** 727658	
09:30	**Welcome**: Prof Matthew Dalby (Director, EPSRC-SFI Joint Centre for Doctoral Training in Engineered Tissues for Discovery, Industry and Medicine)	
09:40-10:25	**Keynote speaker:** Dr Núria Montserrat Pulido, ICREA Research Professor at the Institute for Bioengineering of Catalonia University (IBEC). Title: How to Engineer human pluripotent stem cells to model tissue development and disease	
10:25-10:35	**Move to sessions using breakout rooms (10 min break)**	
	Cell and Gene Therapies	**Bioengineered Models 1**
10:35-12:00	**Chairs:** Mirella Ejiugwo and Aleksandar Atanasov **Patrick Stratham:** Recellularising Decellularised Osteochondral Scaffolds for Cartilage Tissue Engineering **Naomi Phillips:** Investigating the Molecular Identity of Cardiac Progenitor Cells for Applications In Heart Disease **Lydia Beeken:** Assessing the Therapeutic Potential of Corneal Mesenchymal Stem Cells for Ocular Surface Disorders **Matthew Sullivan:** Creation of A Linear Covalently-Closed Hairpin Expression Construct Capable of Effective Gene Transfer Within A Gene Activated Matrix (Gam) **Roxana Sava:** Developing a Potential Combination Therapy for Ischaemic Stroke Using Hydrogel-Delivered Therapeutic Proteins **Poster flashes:** Sebastian Doherty-Boyd Rebecca Davies	**Chair:** Elliot Croft and Ahranee Canden **Hannah Lamont:** Development of an *in vitro* 3D Trabecular Meshwork Model **Devon Crouch:** Cryo-Electrospinning with Poly(E-Caprolactone) Supports Trabecular Meshwork Cell Attachment and Infiltration **Hugo Bell:** Comparing Strut-Based and Sheet-Based Designs in Scaffolds to Enhance Osteoblastogenesis **Nicola Contessi Negrini:** Bioorthogonal Crosslinking and Adhesion of Gelatin Hydrogels **Poster flashes:** Eileen Reidy Caitlin Jackson Jessica Wiseman Lauren Hope Idris Kalokoh Maria Laura Vieri
12:00-14:00	**Break followed by Poster Viewing and Networking via Gather town:** https://gather.town/app/3j1kvU0B2S3qVyOC/JointCDTConference2021 Student will be virtually standing next to their poster.	

DAY 2

09:15	**Arrival online and admittance to conference** **Zoom link:** Future Leaders in Regenerative Medicine: Joint CDT and UKSB Conference **Meeting ID:** 914 7557 8436, **Passcode:** 727658	
09:30	**Welcome**: Prof Abhay Pandit (Deputy Director, EPSRC-SFI Joint Centre for Doctoral Training in Engineered Tissues for Discovery, Industry and Medicine)	
09:35-10:20	**Keynote speaker:** Christine-Maria Horejs, PhD. Chief Editor at Nature. Title: Scientific writing and publishing – insights from a Nature editor	
10:20-10:30	**Move to sessions using breakout rooms (10 min break)**	
	Clinical Advances and Patient Benefits of Applications	**Enabling technologies**
10:30-12:00	**Chair:** Louise Hopkinson and Graeme Pitt **Ralph Murphy:** Targeting and Refining Peripheral Nerve Injury Treatment and Development of Novel Outcome Measures **Sandra Grañana Castillo:** In Silico Modelling for the Prediction of Drug Drug Interactions **Lisa Duff:** A Methodological Framework for AI-Assisted Diagnosis of Active Aortitis Using Radiomic Analysis of FDG PET-CT **Trisha Vikranth:** Decellularised Pleural Membrane Patches for Prolonged Alveolar Air Leaks **Kathryn Malpas:** Unravelling Chitinase-Like Proteins Role in Tissue Remodeling and Regeneration **Poster flashes:** Beatriz (Jacqueline) Solis Davide Verdolino Rebecca Hanson Kathryn Dickins Raissa Barroso	**Chair:** Miguel Ferreira and Mitra Soorani **Naomi Northage:** Efficacy of Plasma Activated Water Disinfection of Flexible Endoscopes **Kern Cowell:** Further Development and Testing of a Novel Automated Decellularisation System **Soraya Williams:** Comparison of Extracellular Vesicle Isolation Processes for Applications in Skeletal Muscle Tissue Engineering **Liam Johnson:** Printed, Skin-Compliant Electrophysiology Electrodes for the Mouse Model **Elfriede Derrer-Merk:** Mitigating Social Connectivity and Support Paradox Through Technology for Older Adults During the Covid-19 Pandemic **Francesco Bacchi:** A Label-Free Biophysical Assay for QC on 3D Cell Cultures **Poster flashes:** Lynsey Steel Gregor Mack Kieran Davies
12:00-14:00	**Break followed by Poster Viewing and Networking via Gather town:** https://gather.town/app/3j1kvU0B2S3qVyOC/JointCDTConference2021 Student will be virtually standing next to their poster.	

DAY 3

09:15	**Arrival online and admittance to conference** **Zoom link:** Future Leaders in Regenerative Medicine: Joint CDT and UKSB Conference **Meeting ID:** 914 7557 8436, **Passcode:** 727658	
09:30	**Welcome**: Prof Matthew Dalby (Director, EPSRC-SFI Joint Centre for Doctoral Training in Engineered Tissues for Discovery, Industry and Medicine)	
09:35-10:20	**Keynote speaker:** Dr Eileen Gentleman, Reader in Bioengineering at Kings College London. Title: Modular Hydrogels in Organoid-Based Disease Modelling	
10:20-10:30	**Move to sessions using breakout rooms (10 min break)**	
	Material Nanopatterning and Properties	**Bioengineered Models 2**
10:30-12:10	**Chair:** Lisa Duff and Lauren Hope **Lydia Styliani Marinou:** Advanced Bioactive Coating for the Bio-Integration of Synthetic Vascular Grafts **Joseph Barnes:** Design, Manufacture and Characterisation of a Dual Antimicrobial-Osteoinductive 'Bone Wrap' **Matthew Culbert:** Advancing Peptide Hydrogels for Intervertebral Disc Repair **Mitra Soorani:** Molecular Dynamics Simulations of Bioactivity Changes Associated with the Incorporation of Copper in Silicate-Based Glasses For Tissue Engineering **Graeme Pitt:** Antimicrobial Coatings for the Prevention of Catheter-Associated Urinary Tract Infections **Poster flashes:** Marcin Gwiazda Thomas Jepson Dominic Mosses	**Chair:** Devon Crouch and Elaine Ma **Leona Ogene:** Graphene Based Bioinks for 3D Orienting Bioactive Articular Cartilage Implants **Kirsten Liggat:** Novel Models of Mammalian Wound Healing and Regeneration **Elliot Croft:** Dissolving Microneedle Arrays: Effect of Polymer Composition on Physiochemical Properties **Kirsty Rooney:** Investigation of Hnf1b-Associated Renal Disease in A Human Kidney Organoid Model **Annamarija Raic:** Round, Reliable Cell Spheroids for Reproducible Cell-Based Assays **Poster flashes:** Aleksandar Atanasov Rachel Furmidge Chanelle McGuinness Laurissa Havins Rebecca Steele Ibrahim Erbay
12:00-13:15	**Break followed by Poster Viewing and Networking via Gather town:** https://gather.town/app/3j1kvU0B2S3qVyOC/JointCDTConference2021 Student will be virtually standing next to their poster.	
13:15	**Closing session**	
14:00	**Chair:** Dr Paul Roach **Gabriel Harrington:** Reproducible Research: Why Should We Care? **Bioactive Materials Journal:** Dr Yang Liu **Award of Prizes and Close**	

DAY 1 Keynote Speaker

Dr Núria Montserrat Pulido

HOW TO ENGINEER HUMAN PLURIPOTENT STEM CELLS TO MODEL TISSUE DEVELOPMENT AND DISEASE

Elena Garreta[1*], Carmen Hurtado del Pozo[1*], Patricia Prado[1], Vanessa Monteil[2,3], Megan Stanifer[4], Steeve Boulant[4], Asier Ullate[5], Felipe Prosper[5], Ali Mirazimi[2,3], Josef M Penninger[6,7], Nuria Montserrat[1,8,9]

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Introduction: The generation of human pluripotent stem cells (hPSCs) derived organoids is one of the biggest scientific advances to understand how human tissues develop and how these get disease.

Methods/**Results**: hPSCs were exposed to three-dimensional microenvironment culture conditions forcing cell to cell contact and cell to extracellular matrix (ECM) interactions in the presence of defined renal inductive signals to generate kidney organoids. Our approach led to the generation of kidney organoids that transcriptomically match second-trimester human fetal kidneys. We then developed a transplantation method that exploits the intrinsic properties of the chick chorioallantoic membrane (CAM) to recreate a soft in vivo microenvironment for organoid growth and differentiation, including vascularization in vivo. These tools have allowed us to exploit our kidney organoid platforms to study first steps of SARS-CoV-2 infection validating the use of a therapeutic compound decreasing viral load. Now, we are exploring on the interaction of systemic conditions impacting on kidney function and disease. Specifically, we are addressing on how a diabetic-like microenvironment impacts on SARS-CoV-2 infection

Discussion: Now we are exploiting our kidney organoid culture platforms to assess kidney disease modeling and the study of kidney morphogenesis. Towards this aim we will explain on the derivation of hPSCs for the inducible expression of Cas9 allowing for targeted genome editing at both pluripotent stage and during kidney organoid generation. Furthermore, we will also discuss on several applications that include the amenability of kidney organoids for COVID19 research applications.

Cell and Gene Therapies

RECELLULARISING DECELLULARISED OSTEOCHONDRAL SCAFFOLDS FOR CARTILAGE TISSUE ENGINEERING

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Corresponding author: mnpls@leeds.ac.uk – PhD student (4th year)

Introduction

Autologous chondrocyte implantation has recently received NICE approval in the United Kingdom for the treatment of articular cartilage lesions in the knee and has shown promising results in clinical trials (1). However, there are concerns over the lack of biomechanical support prior to regeneration, inappropriate repair material and a lack of cell retention within the site of implantation (2).

We propose the use of decellularised osteochondral scaffolds, as a 3-D immunocompatible, biocompatible and biomechanically relevant scaffold to support the implantation and healthy homeostasis of chondrocytes. The use of a biomechanically relevant scaffold may allow immediate restoration of the biomechanical function of the tissue, and the cell component aims to improve integration times. Due to the low permeability of articular cartilage, recellularisation below the superficial surface is limited. This study investigates lyophilisation and collagenase treatment as a means of improving the recellularisation of decellularised cartilage.

Materials and Methods

Decellularised cartilage plugs (n = 3) (8mm (d) x 5mm (h)) were either treated with collagenase; lyophilised O/N; 25-G channels or left untreated. Scaffolds were seeded with 1 x 10^6 C20A4 cells (human chondrocyte cell line) and cultured for 7-days. Viability, DNA content and scaffold penetration were assayed at Day 1 & Day 7.

Results and Discussion

Untreated scaffolds showed cell attachment to the superficial layer but no penetration beneath this. However, both collagenase treated and lyophilised samples showed a greater cell density in the superficial and middle zones of the cartilage, as well as on top of the superficial layer. Cells seeded onto scaffolds with 25G needle occupied the channels, but showed little lateral migration. Viability as measured by alamarBlue, was consistent across all groups.

Conclusions

These results give evidence to suggest a degree of recellularisation using both methods and facilitate future investigation into long-term culture with primary chondrocytes to determine matrix deposition from the cells onto the decellularised scaffolds.

References

1. Knutsen, G. et al. JBJS 86, 455, 2004.
2. Kon, E. et al. Bone & Joint Research 2, 18, 2013.

INVESTIGATING THE MOLECULAR IDENTITY OF CARDIAC PROGENITOR CELLS FOR APPLICATIONS IN HEART DISEASE

Naomi Phillips*[1] Neil Hanley[1], Matthew Birket[1], Alberto Saiani[2]
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Introduction.

With an increasing incidence, compounded by the heart's inherent lack of regenerative capacity, heart disease is an increasing burden on health care and a significant cause of death. Human pluripotent stem cell (hPSC)-derived cardiac progenitor cells (CPCs) have therapeutic promise for myocardial regeneration, but their molecular identity remains ill-defined and the conditions required for CPC self-renewal and differentiation are poorly understood.

The drug regulatable transgene system and fluorescent reporters in the Tet-On-*MYC NKX2-5*^{eGFP} *SOX17*^{tdTomato} hPSC line enable expansion of CPCs which can be further differentiated to cardiomyocytes and endothelial cells and visualisation of endoderm and cardiac specification during differentiation. The differentiation potential of progenitor cells depends on their earlier lineage specification, however in heart development this relationship is poorly understood.

Materials and Methods.

To explore how developmental patterning impacts progenitor (including CPC) fate, I varied the signalling gradients of BMP4 and Activin A, mimicking gastrulation-like events that occur during development, to direct differentiation of hPSCs across a mesoderm-endoderm gradient. From this range of populations, progenitors have been expanded and further differentiated to determine their fate potential. I will be investigating the relationship between differentiation potential and genetic and epigenetic identity using RNA-seq and ATAC-seq.

Results and Discussion.

My data so have confirmed specification of populations across the mesoderm-endoderm gradient and their varying capability to expand and induce NKX2.5 in culture. Utilising the dual reporter line I have also uncovered a previously minimally described NKX2.5+ SOX17+ population. The specific identity of these cells, and their potential role in cardiac differentiation, is being examined using populations sorted based on NKX2.5 and SOX17 expression. Using these thoroughly characterised populations from across mesoderm and endoderm specification, I will determine how their genetic and epigenetic identity relates to their differentiation potential by RNA-seq and ATAC-seq analysis.

Conclusions.

By identifying the genes which functionally determine their fate potential, these data will greatly improve our understanding of the molecular identity of CPCs. These advances are an important step towards using CPCs in a therapeutic context.

ASSESSING THE THERAPEUTIC POTENTIAL OF CORNEAL MESENCHYMAL STEM CELLS FOR OCULAR SURFACE DISORDERS

Lydia Beeken,[1*] Cameron Alexander,[2] Felicity Rose,[3] and Laura Sidney[1]
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Introduction

Following severe mechanical or microbial insult to the cornea, the destructive acute inflammatory phase can activate the transformation of keratocytes to scar-forming fibroblasts, causing significant corneal opacity. Although blindness can be reversed with corneal transplant, excessive inflammation leads to surgery waiting times of up to 18 months. Corneal stromal mesenchymal stem cells (C-MSCs) have previously been identified to possess potent anti-inflammatory properties [1], and their incorporation into a regenerative medicine therapy could provide an effective treatment to combat acute inflammation and reduce the wait for surgery. To ensure success in terms of clinical translation, it is vital to understand the tolerance and response of C-MSCs when placed in the toxic microenvironment of an injured ocular surface. This includes determination of any phenotypic and genotypic changes, in addition to assessment of their survival, behaviour and anti-inflammatory response.

Methods

C-MSCs were isolated from human corneoscleral rims. An *in vitro* inflammatory environment was created by incorporating inflammatory factors into the medium. Cell response was analysed through immunocytochemistry, ELISAs, and Live/Dead, PrestoBlue and cytotoxicity assays. The BD Lyoplate™ Human Cell Surface Marker Screening Panel was utilised to determine any changes of the C-MSC phenotypic profile in the different conditions.

Results & Discussion

Inflammatory cocktail addition reduced cell survival, however the remaining C-MSCs demonstrated increased anti-inflammatory potency. Phenotypic profiling of the cells in normal culture conditions provided an insight into marker variability across donors, in addition to providing a reference for phenotypic comparison to MSCs derived from bone marrow, evidenced in the literature. Bone marrow is currently defined as the gold standard source of MSCs, and their similar phenotypic profile to C-MSCs found in this study provides support that the cornea is a suitable source of therapeutic cells. Furthermore, when compared to C-MSCs treated with inflammatory factors, makers both maintained, or demonstrating significant differences in levels of expression could be deduced. The determination of C-MSC phenotype in an *in vitro* toxic microenvironment, provides a basis into understanding the different cellular pathways that would be activated if C-MSCs were administered to an injured cornea.

Conclusion

Here we present initial steps into the use of C-MSCs as a cell therapy for ocular surface disorders, demonstrating increased secretion of anti-inflammatory factors from C-MSCs when treated with inflammatory agents, and identification of key similarities and changes in phenotypic profile of the cells. These results provide an insight into the development of a C-MSC therapy with increased potency, and an understanding of the cell response to the injured ocular surface.

References

Morales, MLO., *et al., World journal of stem cells* 2019; 11:84.

CREATION OF A LINEAR COVALENTLY-CLOSED HAIRPIN EXPRESSION CONSTRUCT CAPABLE OF EFFECTIVE GENE TRANSFER WITHIN A GENE ACTIVATED MATRIX (GAM)

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Introduction: By acting as a template, scaffold biomaterials possess significant influence over the behaviour and fate of cells in contact with it. As such, GAMs – loaded with therapeutic genetic payloads, hold significant potential as regenerative devices for tissue regeneration. However, current GAMs are limited by the specific vehicle/vectors which carry and deliver the nucleic acid payloads (e.g. plasmid (pDNA). This includes properties such as vector size, immunogenicity, gene transfer efficiency, and subsequent transgene expression at the site of interest. Interestingly, an alternative expression construct (MIDGE) was developed by Schakowski *et al.,* described as a linear covalently-closed Hairpin loop Expression Vector, of which has the capacity for two-four fold greater transgene expression levels as well as higher transfection efficiencies (1, 2). Through the novel GAM electrophoretic nucleic acid loading method used in this project, a similarly generated vector could have the potential for greater controllability with a GAM system whilst simultaneously providing improved transgene transfection and expression, hence the aim of this project.

Methods: Osteogenic and/or Chondrogenic plasmids expressing BMP6 and TGF-B3 respectively (pCAG-BMP6/ pCAG-TGF-B3) were first linearized using the type IIs restriction enzyme Esp3I. Thus, the unnecessary bacterial elements can be effectively eliminated (ampicillin resistance & origin of replication (Ori)) whilst exposing the gene expression cassette with a self-designed 5' and 3' base overhang (promoter sequence, coding sequence, termination signal). Annealing and ligation steps followed, using T4 DNA Ligase (5U/ul), in the presence of a complimentary 5' and 3' phosphorylated hairpin oligonucleotides (in 10X Excess). Through 5'-3' exonuclease resistance (T7 DNA Polymerase- 10,000U/ml), confirmation of successful hairpin attachment was attained. Isolation was performed via chloroform extraction.

Results & Discussion: Two different Linear covalently-closed Hairpin Expression Constructs were generated through the novel type IIs restriction enzyme method, achieving high vector yields using 8-fold less hairpin excess compared to Schakowski *et al* (1). Current work is ongoing to incorporate these constructs within a previously developed mineralised agarose hydrogel GAM using a novel electrophoretic loading method particularly amenable to linear expression construct payloads. Moreover, using nanoparticle cation-DNA complexation and Polyethylenimine (PEI) integration, scaffold mediated cellular differentiation towards osteogenic and chondrogenic lineages can be achieved.

Conclusions: In summary, unique chondrogenic and osteogenic expression constructs were generated in which linear vector performance within a GAM biomaterial could be studied. Through such a combination of tissue engineering techniques and the above molecular engineering (to create advanced nucleic acid payloads), it is hoped the capacity to provide biocompatible 3D niches that induce or support tissue integration and regeneration will be greatly improved.

References: 1. Schakowski *et al*, Molecular Therapy, Volume 3, Issue 5, 793 – 800, 2. Schakowski *et al*, In Vivo 21, 17e23.

DEVELOPING A POTENTIAL COMBINATION THERAPY FOR ISCHAEMIC STROKE USING HYDROGEL-DELIVERED THERAPEUTIC PROTEINS

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Introduction. With no available treatments for brain tissue repair and functional recovery in patients with lost or impaired neurological functions, stroke therapy remains an important unmet clinical need. The lack of structural support in the stroke cavity resulting from dead tissue clearance by immune cells and insufficient endogenous tissue repair dramatically limit brain functional recovery after stroke. Repair processes, such as microglial switch from pro-inflammatory to pro-regenerative phenotype, angiogenesis and neurogenesis, are potential targets to be considered when designing novel regenerative therapies[1]. For example, soft hydrated biomaterials called hydrogels may offer the necessary structural support and deliver pro-regenerative therapeutic agents for improving their half-life[1,2]. To explore a novel hydrogel-based combination therapy, we tested the biocompatibility of two novel self-assembling peptide hydrogels (SAPHs) with the brain tissue, and their capacity to release the angiogenic vascular endothelial growth factor (VEGF). Furthermore, we have assessed the reparative properties of tumour necrosis factor-alpha stimulated protein-6 (TSG-6), a microglial modulator and multifunctional protein known to mediate anti-inflammatory effects of mesenchymal stem cells (MSCs)[3].

Materials and Methods. To study the biocompatibility of two novel SAPHs with the brain tissue, PeptiGel-Alpha2 and PeptiGel-Alpha4 hydrogels were injected intra-cerebrally in healthy mouse brains, and microglial activation as a measure of brain inflammation, as well as phagocytosis and neuronal apoptosis were assessed by immunohistochemistry (IHC). Levels of recombinant human VEGF(rhVEGF) released by the hydrogels following incubation in PBS at 37°C for six weeks were assessed by enzyme-linked immunosorbent assay. To study the effect of TSG-6 on brain repair and functional recovery in a mouse model of ischaemic stroke, rhTSG-6 was injected intra-cerebrally 5 days post-stroke, and wellbeing and motor asymmetry were assessed up to 28 days post-stroke. Levels of microglial activation as well as proliferating endothelial cells were assessed by IHC on brain sections.

Results. The PeptiGel-Alpha2 hydrogel, unlike PeptiGel-Alpha4, did not induce microglial activation nor neuronal apoptosis in vivo, therefore is biocompatible with the brain tissue. Both hydrogels induced the recruitment of phagocytic cells indicating a clearing mechanism. The hydrogels also released rhVEGF in a sustained manner *in vitro*, and ongoing experiments are testing the bioactivity of this angiogenic growth factor. These findings support the use of PeptiGel-Alpha2 as a temporary scaffold for brain repair and VEGF release system. In parallel, our *in vivo* study revealed a trend toward improved wellbeing as assessed by burrowing test and animal weight following rhTSG-6 administration into the stroke cavity. Furthermore, a trend toward decreased microglial activation and increased endothelial cell proliferation has been observed, which indicates that TSG-6 has beneficial tissue repair properties post-stroke.

Conclusion. Our work describes a novel brain biocompatible SAPH hydrogel with VEGF release capacity suitable for angiogenic applications following brain injury. In parallel, we have tested for the first time the reparative properties of TSG-6 administered sub-acutely in the stroke infarct, and the results have shown the potential this anti-inflammatory and pro-regenerative protein has as a therapeutic tool for the treatment of stroke. Ongoing work is testing the efficacy of TSG-6 and VEGF as a dual combination, assisted by a SAPH-based delivery system, which is also aimed to structurally support tissue repair post–stroke. VEGF and TSG-6 will be delivered by the PeptiGel-Alpha2 hydrogel into the stroke cavity 5 days post-stroke. The animal wellbeing, motor asymmetry, cerebral blood flow, microglial activation, angiogenesis, and neurogenesis will be assessed up to 28 days following stroke. The findings of this study will reveal the therapeutic potential of VEGF/TSG-6/PeptiGel-Alpha2 hydrogel combination for structurally supporting tissue repair and enhancing the endogenous brain regeneration processes following stroke.

References

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2. Mohtaram, N. K., *et al*. Biomed. Mater. **8**, 022001, 2013.
3. Day, A. J. & Milner, C. M. Matrix Biol. **78**–**79**, 60–83, 2019.

DEVELOPING A RELIABLE SYNTHETIC NICHE FOR HSC MAINTENANCE

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Introduction

Haematopoietic stem cells (HSCs) are responsible for the process of haematopoiesis, the continuous production of blood and immune cells through differentiation, proliferation and maintenance of the stem cell pool[1]. Haematopoietic homeostasis is maintained by the microenvironment the HSCs reside in, termed the bone marrow (BM) niche, which uses a complex variety of stimuli to maintain a self-renewing population of multipotent HSCs[2]. Maintaining a population of multipotent HSCs *ex vivo* would allow the stem cells to be studied more readily. This could provide information on HSC malignancies which lead to leukemia, as well as insight into how the BM niche operates as a whole[3]. Treatments and causes of BM-related diseases could also be investigated, including therapeutic CRISPR gene edits of HSCs[4]. Unfortunately, HSCs rapidly proliferate and differentiate when cultured under standard *in vitro* conditions, making them difficult to study. The solution to this is the development of a system that mimics aspects of the in vivo niche microenvironment capable of maintaining a self-renewing, multipotent, long-term HSC (LT-HSC) population *in vitro*[3]. Such systems have been developed in the past[5,6], but have their flaws, namely low reliability and/or difficulty in recovering the HSCs from the model. This project aims to overcome these issues using an artificial, peptide-based hydrogel system with limited batch-to-batch variability. This artificial BM niche will also be highly tunable, allowing us to optimize it for a variety of applications.

Materials and Methods

This project aims to develop a reliable 3D system for the maintenance of LT-HSCs *in vitro*. This system will use poly (ethyl acrylate) (PEA)- and fibronectin (FN)-coated surfaces functionalised with growth factors. PEA causes FN to adopt a fibrillar conformation, allowing synergistic signalling by the fibronectin's RGD domain and bound growth factors which causes mesenchymal stem cells (MSCs) cultured on the coated surface to adopt a niche-like phenotype (nestin+) and produce cytokines that promote HSC maintenance[7]. Synthetic, peptide-based hydrogels (PeptiGels) will be layered on top of the MSCs, providing mechanical stimuli mimicking niche ligands and BM stiffness. A similar system in which the PeptiGel was replaced with collagen gel has been shown to promote nestin expression in the MSCs and maintain a population of LT-HSCs *in vitro*[6]. The PeptiGel-based system will, however, have limited batch-to-batch variability, use no animal-derived products and have tuneable stiffness, charge and ligand presentation. As this system is developed, several techniques will be used to characterise the MSCs and HSCs in this system, namely in cell western, immunofluorescence microscopy and ELISA.

Results and Discussion

Currently we are investigating the effect on MSC phenotype of 5 gels with a range of stiffnesses (1 kPa – 10 kPa) and differing charges. We will then further adapt the gels to present niche ECM proteins such as FN. Once we have characterized the MSC-peptigel niche, we will introduce HSCs and begin optimising the system for their maintenance.

Conclusion

The development of reliable, synthetic niches capable of maintaining a population of HSCs *ex vivo* promises to revolutionise our understanding of the bone marrow niche. This research could also provide insight into the causes and potential treatments of bone marrow-related disorders such as leukaemia.

Acknowledgements

lifETIME ESPRC-SFI CDT in engineered tissues (EP/S02347X/1). Carol-Anne Smith for technical help.

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A COMPARISON OF FETAL BOVINE SERUM EXTRACELLULAR VESICLE (FBS-EV) DEPLETION PROTOCOLS AND THEIR EFFECT ON UMBILICAL CORD MESENCHYMAL STEM CELLS (UC-MSCs)

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Introduction

Extracellular vesicles (EVs) are small membrane bound particles, capable of recapitulating the beneficial abilities of their parental cells (1). EVs derived from umbilical cord mesenchymal stem cells (UC-MSCs) can be used to counter inflammatory disorders such as rheumatoid arthritis, whilst overcoming safety concerns and changes in cell phenotype related to living or engineered cell therapies. However fetal bovine serum (FBS) provides a source of bovine derived EV contamination to those harvested from cell culture (2). Several protocols have been established to minimise this including use of serum free media (SF), ultracentrifugation (UC), or the lesser used ultrafiltration (UF). We aim to compare each FBS-EV depletion protocol, assessing their efficiency at removing contaminating FBS-EVs and the resulting impact on UC-MSCs grown in culture.

Materials & Methods

FBS (Life Technologies, Paisley) was subjected to each FBS-EV depletion protocol; UC by 18-hour spin at 120,000g (2) and UF using Amicon Ultra-15 100kDa filters (3). 10ml was subjected to EV isolation using differential UC by spinning at 2,000g for 20 minutes, 10,000g for 30 minutes and 100,000g for 70 minutes (4). The concentrated EV pellet was assessed by tunable resistive pulse sensing (TRPS) and BCA assay to determine particle count and protein concentration. UC-MSCs were cultured in complete culture media (DMEM +1% pen-strep +10% FBS) before switching to EV collection media of +10% FBS, +10% UC-FBS, +10% UF-FBS, or without FBS (-FBS) 48 hours prior to harvest before assessing morphology, population doubling time (PDT), and presence of identifying MSC surface markers. Furthermore, expression of IDO mRNA before and after 25ng/ml IFN-y activation was quantified by RT-qPCR.

Results and Discussion

Each FBS-EV depletion protocol proved to significantly lower the protein concentration and particle count, with UF proving to be the most efficient with 10.22% particles remaining ($p<0.0001$), as opposed to the 21.72% of UC ($p<0.01$). However, UF seemed to eradicate protein levels entirely with only 0.89% remaining, whilst UC retained 29.85% of the original protein content. This suggests that whilst UF is more efficient at removing contaminating FBS-EVs, it fails to retain supporting proteins as well as the UC method.

In culture, UC-MSCs displayed a clear change in phenotype under different EV collection media conditions. Protein rich conditions (+FBS and +UC-FBS) displayed a more cytoplasmic and spread phenotype, whilst a lower protein content (+UF-FBS and -FBS) displayed a more spindle-like phenotype. Whilst a spindle-like phenotype is associated with rapidly self-renewing MSCs (5), PDTs revealed a trend towards slower cell growth, that which was only significant in serum free conditions ($p<0.01$). Despite slower growth, all conditions met the minimal criteria established by the international society for cell therapies (ISCT) that state MSCs must be defined by the expression of >95% positive surface markers (CD73, CD90 and CD105) and <2% negative surface markers (CD14, CD19, CD34, CD45 and HLA-DR) indicating no effect based on the use of varying EV collection media (6). However, the assessment of IDO mRNA revealed a trend in which lower contribution from FBS supplementation caused a decrease in the level of IDO upregulation upon IFN-y stimulation. This suggests that EV collection media and the presence of FBS impacts greatly on the immunomodulatory potential of UC-MSCs but, reassuringly, does not eradicate it completely.

Conclusion

UF-FBS proved to be the most efficient FBS-EV depletion protocol, however this comes at the loss of FBS derived protein that supports cell growth. Although this does not affect MSC phenotype assessed by surface markers, the ability to upregulate IDO was altered, indicating the input level of supporting components derived from FBS affect their immunomodulatory potential.

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Bioengineered Models 1

DEVELOPMENT OF AN *IN VITRO* 3D TRABECULAR MESHWORK

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Introduction

Glaucoma is a leading cause of irreversible blindness worldwide, with cases estimated to increase globally by 74% in 2040. Primary Open Angle Glaucoma (POAG), the most prevalent subset, occurs highly in patients that express pathological increases in intraocular pressure (IOP), subsequently causing optic neuropathy and vision loss. IOP maintenance is regulated by the Trabecular Meshwork (TM) which becomes fibrotic during POAG, however, relevant 3D *in vitro* models of the TM are still in its infancy. The aim of this study is to develop a more biological relevant 3D model by constructing a particular connective tissue region of the TM known to contribute to POAG pathogenesis, the Juxtacanalicular tissue.

Materials and Methods

Human trabecular meshwork cells (HTMC) were cultured within type I rat tail collagen hydrogels, containing alterations in collagen fiber size and density by confined plastic compression (PC) [1]. HTMC were embedded in type I rat tail collagen gels before undergoing varying rates of PC and cultured for 7 days. HTMC-laden collagen constructs were assessed through collagen contraction and live cell imaging. Post incubation, constructs were cryosectioned and stained for immunoreactivity to α-SMA, fibronectin, laminin, collagen type IV and TGF-β2 by immunohistochemical (IHC) staining. Collagen fibers were fluorescently probed prior to PC [2] and visualized through confocal imaging for observation of resulting collagen architecture produced.

Results and Discussion

The degree of collagen contraction by the first 24 hours (10-50% of original size) was modulated by the optimization of PC, with fully hydrated HTMC/Collagen constructs presenting the highest rates of contraction (50%) compared to PC counterparts. All HTMC/Collagen constructs stained positive for relevant profibrotic (α-SMA, TGF-β2) and extracellular matrix proteins (ECM) (laminin, fibronectin collagen type IV) following IHC staining. An average decrease by 20% in relative pro-fibrotic expression was observed in PC collagen compared to fully hydrated collagen gels. Ultrastructural analysis of acellular collagen constructs after PC had shown formation of anisotropic, hierarchical collagen architectures. Further live cell imaging of HTMC/collagen constructs, 7 days post incubation, presented heightened cellular and ECM alignment throughout the PC collagen compared to fully hydrated HTMC/collagen constructs.

Conclusion

The rate of collagen contraction, which is induced by cellular forces, was manipulated through PC optimization of fully hydrated type I rat tail collagen gels. The study also presented that PC to HTMC/Collagen constructs did not produce unwanted pathological characteristic related to fibrosis through increased in α-SMA, TGF-β2 expression, and established ECM deposition and integration of relevant proteins that are present *in vivo*. Distinct alterations in collagen architecture were noted after PC, with increased cellular and ECM organization of PC HTMC/collagen constructs observed compared to fully hydrated counterparts.

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CRYO-ELECTROSPINNING WITH POLY(ε-CAPROLACTONE) SUPPORTS TRABECULAR MESHWORK CELL ATTACHMENT AND INFILTRATION

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Introduction

The trabecular meshwork (TM) is a highly porous, ocular tissue located in the anterior chamber that drains fluid from the eye, which allows intraocular pressure to be maintained[1]. However, the TM can become obstructed by cellular debris, which raises this pressure and causes compression of the optic nerve. This leads to irreversible sight loss known as primary open angle glaucoma. Electrospinning is a fibre production technique which can be exploited to create a multi-layered, porous network likened to the TM's architecture by varying its processing setup. Yet densely packed, submicron-sized fibre meshes typically result in reduced pore size which can restrict cellular infiltration. Incorporating dry ice into the electrospinning process can overcome this restriction as ice crystal formation increases the fibre deposition area, which therefore increases the pore size. This study investigated cryo-electrospinning with poly(ε-caprolactone) (PCL) as a potential adaptation on conventional electrospinning to create a biomimetic TM model with enhanced pore size to support infiltration of trabecular meshwork cells (NTM_5).

Materials and Methods

PCL (12% w/v) was dissolved in 1,1,1,3,3,3,-hexafluoroisopropanol, stirred continuously for 48 h and electrospun using the following parameters: needle voltage +15 kV, collector voltage -4 kV, flow rate 1 ml/h, distance 17 cm and rotating mandrel 100 RPM. 0.001% w/v rhodamine was added immediately prior to electrospinning to allow visualisation of the fibres during confocal microscopy analysis. On a separate run, the mandrel was packed with dry ice (cryo-PCL). Collected cryo-PCL fibres were immediately placed in a vacuum desiccator over night to allow complete water removal without structural collapse. PCL and cryo-PCL scaffold structural properties were compared by scanning electron microscopy (SEM; fibre diameter) and micrometer measurements (scaffold thickness).

Attachment of NTM_5 cells (40,000 cells/cm^2) to PCL and cryo-PCL scaffolds was assessed 4 hours post-seeding by PicoGreen DNA assay. Infiltration of NTM_5 cells following 7 days culture on PCL and cryo-PCL scaffolds was assessed by confocal microscopy. Cells were stained with FITC-Phalloidin and DAPI to visualise cell cytoskeleton and cell nuclei, respectively. Orthogonal views of representative images were used to analyse the depth of cell infiltration.

Results and Discussion

Cryo-electrospinning had no impact on fibre diameter (0.63 ± 0.41 µm cryo-PCL; 0.76 ± 0.50 µm PCL). However, thicker fibre scaffolds were obtained for cryo-electrospinning compared to conventional electrospinning (60.05 ± 2.16 µm cryo-PCL; 33.16 ± 1.95 µm PCL). Fabrication of these thicker scaffolds is owed to ice crystal formation on the mandrel's surface creating larger pores during fibre deposition (Figure 1). The method of fibre fabrication had no effect on NTM_5 cell attachment with 68.2% of seeded-cells attaching to PCL compared to 68.0% on cryo-PCL scaffolds. After 7 days in culture, greater NTM_5 cell infiltration was observed with the thicker, more open cryo-PCL scaffold, with cells reaching depths of 32.25 ± 8.17 µm compared to 5.11 ± 1.03 µm for PCL. This data demonstrates the ability to create larger pores that remain supportive of cell attachment whilst also allowing cells to penetrate to the scaffolds' core.

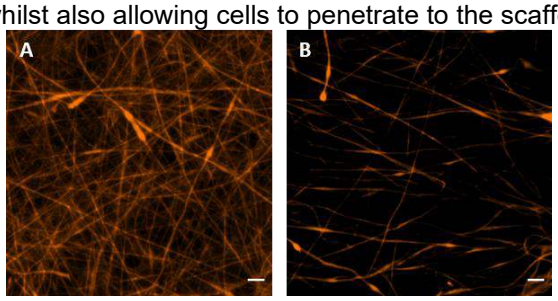

Figure 1: Confocal images of electrospun fibres (orange) of (A) PCL and (B) cryo-PCL. Scale bar = 20 µm

Conclusion

Cryo-electrospinning PCL significantly enhanced scaffold thickness compared to conventionally electrospun PCL and did not affect fibre diameter. Both scaffolds were supportive of cell attachment but the cryo-PCL's larger pores encouraged NTM_5 cell infiltration deep within its fibre network. Cryo-electrospun scaffolds will be further characterised to determine their utility as biomimetic models of the TM.

Acknowledgements

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COMPARING STRUT-BASED AND SHEET-BASED DESIGNS IN SCAFFOLDS TO ENHANCE OSTEOBLASTOGENESIS

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Introduction

Titanium (Ti) porous scaffolds are widely researched due to their excellent mechanical and osteoconductive properties[1] for regenerative medicine applications. Although progress has been made in ascertaining desired stiffness (derived from porosity) and optimum pore size (for osteoinduction) to enhance load-bearing bone healing, it remains unclear what pore design can accelerate osseointegration. Working within the mechanical and osteoconductive boundaries, to prevent stress-shielding and counteract aseptic loosening, different designs were explored to find what geometrical cues can assist the total commitment of pre-osteoblasts. Additive manufacturing was employed to realise these designs. Selective laser melting is a laser-based, powder bed fusion manufacturing technique capable of precisely fabricating these complex porous Ti implants. These porous constructs have been reported to fine-tune stiffness to mimic that of bone, whilst also providing a physical platform on which cells can adhere to, proliferate throughout, and differentiate and deposit new bone[2]. Research has predominantly focused on strut-based, repeating, lattice designs[2,3], however, less work has been reported on sheet-based designs, and a study that compares both from a biological performance stance is missing. Therefore, we present the biological characterisation of two scaffold types; triply periodic minimal surfaces (TPMS) and trabecular-like (TL), compared to a cubic strut-based lattice (CL), traditionally used as porous scaffold. This study is supported by mechanical characterisation to confirm the scaffolds performance meets the requisites for a bone replacement medical device. TPMS have been shown to distribute stress and strain uniformly, meaning bone ingrowth and surrounding bone will experience the same mechanical loads[4]. TL scaffolds were designed to recapitulate the controlled, yet disordered, nature-inspired environments of which osteoblasts are known to favour, a step-away from regimented lattices[5].

Materials and Methods

MC3T3-E1 Mice Pre-Osteoblasts were used to assess their Attachment, Proliferation and Differentiation on the scaffolds. PrestoBlue™ was used to evaluate metabolic activity over 13-days and determine the phenotypical switch from proliferation to differentiation. A cell seeding efficiency was calculated to quantify the number of adhered cells after 24-hours. The 4-MUP reaction was used to quantify ALP content at days 14, 21 & 28, and ascertain which design promoted osteoblasts progression further along the differentiation pathway. Mechanical suitability of the scaffolds was confirmed via compressive testing.

Results and Discussion

Cellular Attachment – All TPMS and TL scaffolds provided a suitable framework for cells to attach to, confirming non-cytotoxicity. The open structure in CL resulted in fewer cells attached after 1d. Cellular Proliferation – Increasing metabolic activity on all scaffolds over 13 days confirmed they were all suitable structures without significant difference between them. A switch from proliferation to differentiation was observed between days 9 and 11 for TPMS and TL scaffolds, unlike CL. Osteogenic Differentiation – Cells cultured on TL-Smooth were observed to progress furthest along the differentiation pathway, followed by the two TPMS and the CL. On the contrary, TL-Sharp reported a decrease in ALP from d14 to d28, indicating it could not sustain cell maturation.

Conclusion

Scaffold design has an impact on cell attachment, proliferation and differentiation. Open structures (e.g., CL) suffer from cell attrition in early time points. All scaffolds proved to be non-cytotoxic and able to sustain proliferation, regardless of their pore design. In the extended differentiation study, TL-Smooth outperformed the rest as the most suitable structure to enhance osteoblastogenesis, suggesting cells prefer strut-based, trabecular-like smoothed structures over ordered lattice surfaces or those with sharp corners.

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BIOORTHOGONAL CROSSLINKING AND ADHESION OF GELATIN HYDROGELS

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Introduction

Gelatin hydrogels are widely used in tissue engineering thanks to their extracellular matrix biomimetic structure. Cytocompatible crosslinking strategies are required to stabilize the gelatin hydrogel structure at physiological temperature (i.e., $T>T_{sol-gel}$) and to control their physico-mechanical properties to guide a desired cell response [1]. Here, we tune the properties of gelatin hydrogels via modulation of their bioorthogonal crosslinking and we show the possibility of adhering hydrogels for potential compartmentalized co-culture.

Materials and Methods

Gelatin derivatives were synthesised by decoration either with tetrazine (Gel_Tz) or norbornene (Gel_Nb) and their degree of modification (DOM) was measured by ^{1}H NMR [2]. Gelatin hydrogels 8% w/v where prepared by mixing the gelatin derivatives obtained with different DOM at different Tz:Nb ratios (R). The rheological properties were investigated during the hydrogel crosslinking by a rheometer (storage modulus G', loss modulus G"). The swelling of the hydrogels was measured up to 2 weeks at 37 °C. Compressive mechanical properties were tested by applying a hysteresis cycle up to 30% strain. Human dental pulp stem cells (hDPSC) were 3D cultured in the hydrogels up to 2 weeks. Cells viability was investigated by live/dead staining after embedding cells in the hydrogels; cell metabolic activity over the culture period was measured by AlamarBlue™ assay; cell morphology at the end of the culture was analysed by F-actin/nuclei staining. *In vitro* enzymatic degradation was tested by immersing hydrogels in 1 U mL^{-1} collagenase solution. Finally, possible adhesion between hydrogels was tested by placing in contact samples prepared with excess Tz and with excess Nb; adhesion was tested by qualitative observation and by measuring the adhesion strength after 2 weeks of immersion in culture medium.

Results and Discussion

The DOM of Gel_Tz and Gel_Nb was successfully varied between 5, 10, and 15%. Hydrogels prepared with different DOM and R crosslinked following different kinetics (i.e., higher DOM and equimolar R lead to faster crosslinking reaction). Crosslinked hydrogels were stable in PBS at 37 °C for up to 2 weeks. Higher swelling was observed for hydrogels prepared with lower DOM and non-equimolar R, which in turn lead to modulation of the hydrogel mechanical properties (i.e., E varied between 0.5 and 5 kPa). Lower DOM and non-equimolar R resulted in lower mechanical properties. Viable hDPSC were embedded in the hydrogels (viability >85%) and increase in metabolic activity was observed for cells cultured in all the prepared formulations. Higher increase of metabolic activity and evidently more elongated morphology was observed for cells cultured in softer hydrogels (i.e., E < 1 kPa). All hydrogels were enzymatically degraded and the degradation kinetic varied depending on the DOM and R used to prepare the hydrogels. Finally, hydrogels prepared with excess Nb and with excess Tz were successfully adhered by placing them in contact and their adhesion was maintained over 14 days of simulated cell culture conditions. These hydrogels showed double the adhesion strength compared to hydrogel controls prepared with equimolar R and put in contact one with the other.

Conclusions

The physico-mechanical properties of bioorthogonal crosslinked gelatin hydrogels were successfully tuned by solely varying the bioorthogonal crosslinking conditions. Cytocompatible hydrogels were shown to support hDPSC encapsulation and proliferation, and to guide cell elongation based on the tuned mechanical properties of the hydrogels. Successful maintenance of adhesion between hydrogels during cultures showed the possibility of using the prepared hydrogels as potential compartmentalized 3D co-culture platforms [3].

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ASSESSING THE VIABILITY AND GROWTH OF 3D COLORECTAL CANCER MODELS IN ALGINATE VERSUS COLLAGEN HYDROGELS.

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Introduction

Colorectal cancer (CRC) is the third most common cause of cancer related deaths worldwide[1]. Despite the development of multiple therapeutics, the survival rate for patients with late-stage CRC remains very low. When colorectal cancer is

Materials and Methods broken down into its four Consensus Molecular Subtypes (CMS), CMS4 tumours, which are rich in mesenchymal stromal cells (MSCs), are shown to have the worst disease-free progression survival[2]. Understanding interactions that take place in the tumour microenvironment (TME) is essential for developing novel therapeutics. Much of the research in the past has focused on 2D monolayer cultures or *in vivo* studies, however, successful clinical translation of therapeutics derived from these methods has been sub-optimal. More recent studies are turning towards developing effective 3D models of CRC that are clinically relevant and can recapitulate the TME, bridging the gap between *in vitro* and *in vivo* work. 3D models can incorporate hydrogels to mimic an extracellular matrix (ECM) like component. This project focuses on analysing proliferation and viability of colorectal cancer spheroids in alginate and collagen gels.

HCT116 and HT29 cells, human CRC cell lines, were cultured as 3D spheroids in alginate or collagen gels. To recapitulate stromal dense tumour microenvironments, spheroids incorporating human MSCs (hMSCs) were established. Spheroids were cultured for 14 days and analysed by Alamar blue, CyQuant and stained with calcein AM/propidium iodide to analyse metabolic activity, proliferation and cell viability, respectively.

Results and Discussion

For HCT116 cells, calcein AM and propidium iodide staining (viability analysis) showed that with and without hMSCs, HCT116 spheroids were viable in both alginate and collagen gels. This suggests that both the collagen and alginate gels provided an environment which preserved cell viability and facilitated growth for at least 14 days. Alamar blue results showed an increase in metabolic activity of HCT116 spheroids, suggesting that cells continued to proliferate between Day 1 and Day 14. CyQuant analysis confirmed these results, showing that HCT116 proliferation increased from Day 1 to Day 14 for cells cultured in both alginate and collagen. For both Alamar blue and Cyquant analysis, metabolic activity and proliferation of cells was higher in collagen gels compared to alginate gels. These results were validated using a second CRC cell line, HT29. HT29 is a CMS3-type epithelial colorectal cancer cell line. Alamar blue staining showed no significant change from Day 1-14 for HT29 spheroids with or without hMSCs. For CyQuant analysis, results showed an increase in proliferation from Day 1-14 in collagen gels but a trend towards a decrease in proliferation with alginate gels. Addition of hMSCs did not have a significant impact on cell viability, proliferation or metabolic activity for either cell line.

Conclusions

Developing a clinically relevant 3D model of CRC will be essential for the development of therapeutics for CRC patients in the future. In order to create a 3D *in vitro* environment that mimics certain aspects of CRC *in vivo*, it is important that cells can survive and proliferate in the ECM-like component used to create the 3D environment. This project shows that HCT116 cells, a CMS4-type CRC cell line, remained viable and proliferated in both alginate and collagen gels over a period of 14 days. HCT116 spheroids also appeared to proliferate at a higher level in collagen gels compared to alginate. These results were validated using a second CRC cell line, HT29 cells. Spheroids formed using HT29 cells appeared to proliferate in collagen gels but not in alginate gels. Taken together, our results suggest that collagen hydrogels provide an environment that promotes viability and proliferation of 2 different CRC cell lines, suggesting that this may be a better ECM-like hydrogel component to use in future studies.

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LAB-BASED PRODUCED VASCULARISED TISSUE FOR IN VITRO LAB ON A CHIP MODELS OF METASTATIC CANCER

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Lab-on-a-chip devices are developing to closely mimic the structural and biological complexity of human tissue. Thus, becoming increasingly popular as an alternative to animal testing for pharmaceuticals. However, the lack of perfusion through vasculature within current models remains to be a challenge, reducing the physiological development capacity for tissue-on-a-chip models. This project utilises high internal phase emulsion templating combined with 3D bioprinting techniques to create an *in vitro* vascularised tissue via fabrication of a porous PCL PolyHIPE scaffold. Initial results demonstrate the fabrication of microporous structures (20-50 µm) using high internal phase emulsion templating. Via 3D printing the microporous PCL polyHIPE in lattice structures, macroporous structures (190-390 µm) were incorporated, critical for mature vascular formation. The use of such porous networks will allow for vascular outgrowth to occur in which cell, cell spheroids and small biopsies can be placed and resulting cell behaviour can be analysed. This will be further developed to overcome the short comings in current models and provide an *in vitro* alternative to *in vivo* testing platforms. Furthermore, this *in vitro* model will be used to explore cancer models. Cancer is a becoming a huge social and economic burden on society, being the most significant barrier to life expectancy in the 21st century. In particular, the model will be able to explore metastasis of cancer which is responsible for 90% of cancer related deaths.

ORGANOTYPIC SPINAL CORD INJURY MODEL WITH SURGICAL GRADE BIOMATERIAL IMPLANTATION: HUMAN/MOUSE *EX VIVO* TISSUE COMPARISON

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Research for spinal cord injury (SCI) is dominated by the use of live animal models. In an attempt to mimic the clinical features of SCI, significant injury is inflicted, resulting high levels of suffering for the large number of animals required for therapeutic testing. The initiative to alleviate such suffering has led to the implementation of the 3 R's (Replacement, Reduction and Refinement), encouraging an increase in research dedicated to the development of suitable neural tissue cultures *in vitro*. Mice models are used extensively in SCI research, suggesting that *in vitro* mouse models are an ideal replacement as human *in vitro* SCI models are rare; particularly *ex vivo* tissue systems. Therefore, the development of both models is crucial. *Ex vivo* organotypic injury models represent a unique research tool combining the advantages of cell culture with the benefits of *in vivo* models, with the ability to retain *in vivo* cytoarchitecture and recapitulate pathological processes. This study is the first human organotypic transecting SCI model and the first to compare *ex vivo* human and mouse tissue responses to a neurosurgical grade scaffold. Human and mouse organotypic SCI slice models, with implantation of DuraGen Plus™ matrix into the injury site were evaluated via three parameters affecting neural regeneration: glial scar formation, axonal outgrowth and microglial infiltration. Our results demonstrate consistent cellular responses within both models together with observation of potential regenerative benefits of implanted DuraGen, such as: morphological disruption of the astroglia scar, support of axonal outgrowth and biomaterial-residing microglial cells assuming a quiescent-like state.

DEVELOPING A 3D MODEL OF THE BONE MARROW ENDOSTEAL NICHE FOR DRUG SCREENING IN *MLL*-REARRANGED ACUTE MYELOID LEUKAEMIA

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Introduction

Acute myeloid leukaemia (AML) is the most common acute leukaemia in adults, with 3,000 new cases per year in the UK (Cancer Research UK, 2019). AML is caused by the acquisition of mutations in haematopoeitic stem and progenitor cells, causing leukaemic stem cells (LSCs) to arise. LSCs remodel the bone marrow niche, and interact with resident stromal cells to create a protective environment that renders them quiescent. As a result, these LSCs can be resistant to chemotherapy, leading to AML relapse. This occurs in approximately 50% of patients. Circumventing this protective bone marrow microenvironment is key to treating AML. Preclinical AML studies rely on 2D human cell studies, genetically modified animal models, and patient-derived xenograft models. However, 2D cell culture is far removed from the *in vivo* situation, and animals such as mice are physiologically and genetically different to humans. Therefore, the aim of my project is to develop a biologically relevant *in vitro* 3D model of the bone marrow endosteal niche for drug testing against AML. Having reviewed currently available materials, I have chosen to utilise 3D printing to make an alginate/gelatin hydrogel containing AML cell lines and spheroids composed of HS5 bone marrow fibroblasts. This would be printed on top of an osteoblastic monolayer to reflect the endosteal bone marrow niche. As proof of concept, I will use this 3D niche system to explore the efficacy of cyclin dependent kinase (CDK) 2/9 inhibitor fadraciclib, alone and in combination with novel therapies. Potential combinations with fadraciclib include venetoclax, a BCL-2 inhibitor; cytarabine, an antimetabolite; and azacytidine, a hypomethylating agent.

Materials and Methods

Fadraciclib was added to MOLM-13 AML cells at 0.5xIC50 (concentration which induces 50% cell death), IC50 and 2xIC50, alone and in combination with venetoclax, cytarabine or azacitidine, at 0.5xIC50, IC50 and 2xIC50 concentration in liquid culture. Cell viability was analysed using fluorescence-activated cell sorting and trypan blue exclusion staining after 24, 48, and 72 hours. The drug combinations with the highest level of synergy will be selected for use in the 3D model.

Results and Discussion

These preliminary data indicate that fadraciclib is a potent cytotoxic in AML, and that the apoptotic effect increases when added in combination with other cancer therapies such as azacitidine. Next, I aim to repeat these experiments in 2D co-culture with HS5 cells, prior to testing the drug combinations in our 3D endosteal bone marrow model. Aspects which will require optimisation include degradation of the gel to allow cell extraction for gene expression and protein analysis; crosslinking time; and determining whether it is possible to directly print on top of the osteoblast monolayer.

Conclusions

These preliminary data highlight the efficacy of fadraciclib in 2D culture with chemotherapy drugs. However, these combinations must be tested in the humanised 3D model to determine their ability to circumvent the bone marrow's protective effect.

Acknowledgements

I would like to thank my supervisors, Professor Mhairi Copland, Professor Helen Wheadon and Dr Catherine Berry, and Professor Matthew Dalby, coordinator of the LifETIME CDT programme, for their support and guidance through my PhD studies. Additionally, I would like to thank the LifETIME CDT cohorts across Glasgow, Birmingham and Galway, in addition to my colleagues in the Paul O'Gorman Leukaemia Research Centre and Centre for the Cellular Microenvironment.

USING NITRIC OXIDE AS A RELIABLE MARKER TO DETECT MACROPHAGES' ACTIVATION STATE

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Introduction

Nitric oxide is a reactive and small gaseous molecule that is involved in every system in our body and plays a variety of critical roles in human health. When active, it acts as an inducer of nitric oxide synthase in the cells, which is critical for macrophage protection against various types of infection such as viruses and bacteria.

Materials and Methods

Induce monocytes into macrophages (M0)

THP1 monocyte cells were incubated in 96 well plate, with FBS RPMI 1640 media and PMA added to differentiate cells to M0. Different cell number used are 10,000 20,000 and 50,000 cells/well.

Induce M0 to M1

12 wells for each cell number and Inducers used are IFN-r (20ng/µl) and LPS (1µg/ml) to activate M0 phenotype, M1.

Media collected for cell viability (CCK8 assay) and NO analysis

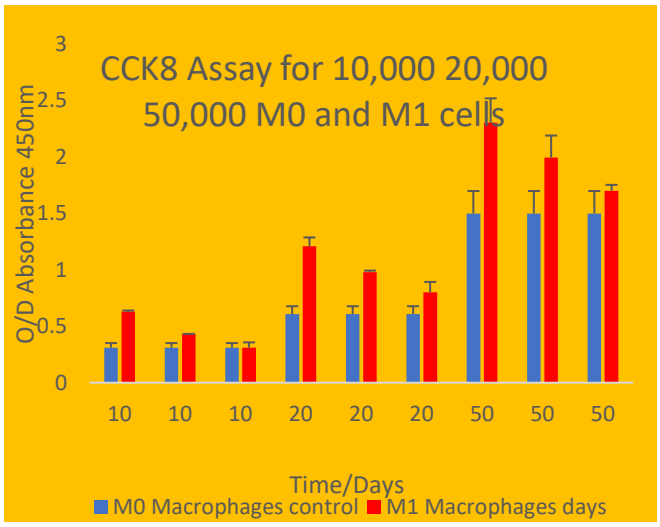
Media collected on this day 2, 4 and day 6 culture were used to analyse for NO release by the M0 and M1 macrophages through Griess assay.

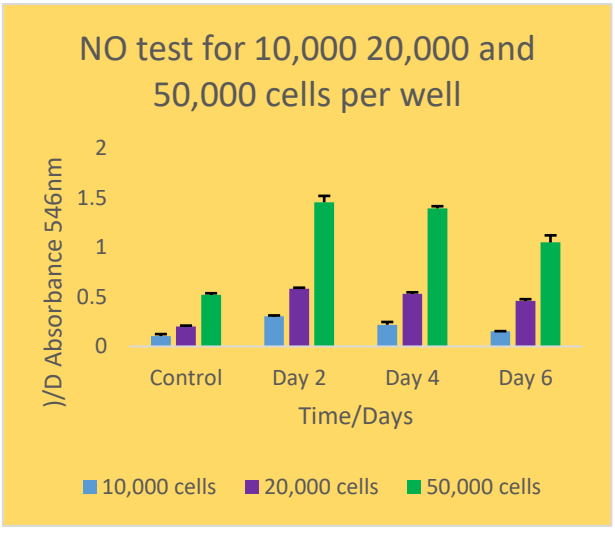
Cell morphology observation

Cell morphology were observed via visible light microscopy.

Results

CCk8 assay and Griess buffer test were both done for the M0 cells 10,000 20,000 and 50,000 and M1 cells 10,000 20,000 and 50,000





Conclusion

LPS+IFN-r activates inactive macrophages M0, turning them into active macrophages M1. M1 cells have an extended morphology compared to M0 cells, which are spherical in shape. M1 cells produce more NO than M0 cells because they are more inflammatory. Longer culture times result in fewer M1 cells and reduced NO output, although the number of M0 cells remains constant as the number of days increases. The NO assay correlates with cell viability.

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ALGINATE-BASED HYDROGELS FOR STEM CELL CHONDROGENESIS

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Introduction

Osteoarthritis (OA) is a debilitating degenerative joint disease characterised by pain and progressive deterioration of articular cartilage and underlying bone. The exact reasons for the disease initiation and progression are still not fully understood and a deeper knowledge of these mechanisms is essential for the development of future and more efficient therapies. Prior preclinical testing has been shown to be a poor predictor of drug efficacy in clinical trials. However, more recent advances in 3D tissue-mimic models suggest that this is a promising approach to study diseases progression and improve drug screening. Our work focused on the development of a hydrogel-based 3D model (using alginate as scaffold biomaterial) mimicking cartilage tissue to facilitate the study of OA-related pathologies. Alginate is a natural polysaccharide extensively studied for biomedical applications and tissue engineering, as it is inexpensive, non-toxic and biocompatible. Three different stiffnesses of alginate gels were developed to determine the optimal setting for the differentiation of murine mesenchymal stem cells (MSCs) to chondrocytes.

Materials and methods

Sodium alginate powder was UV-sterilised (30 minutes, 254nm light) and dissolved in DPBS. Alginate disks were obtained following the protocol by Sharma et al. (2016) [1]. Discs were analysed with an Anton Paar MCR-302 rheometer and a parallel plate geometry was used to perform amplitude sweeps. Compact bone MSCs were extracted from C57Bl6 mice (6-10 weeks old) and cultured following the protocol by Zhu et al. (2010) [2]. MSCs were characterised using flow cytometry. Passage 4 and 5 cells were detached with Accutase, counted and resuspended in the appropriate volume of alginate solution and cell culture media prior discs' gelation. Live/Dead assay was used to determine cell viability inside the gels. Chondrogenic differentiation of MSCs was induced using high-glucose D-MEM supplemented as described in Zhu et al. (2010) [2]. For RNA extraction hydrogel discs where dissolved using a 50 mM EDTA, 55 mM Sodium Citrate and 0.1% glucose solution. After 15 minutes, the suspension was centrifuged, the pellet washed once with DPBS and resuspended in 1 mL TRIzol. Samples were stored at -70°C for future RNA extraction.

Results and discussion

1%, 3% and 5% (w/v) sodium alginate solutions were used to create gels discs with an elastic modulus (G') of 2.8±0.8 kPa, 17±3.1 kPa and 43.1±8.1 kPa respectively. This range of gel stiffnesses has been previously used to drive stem cells differentiation into a chondrogenic phenotype. UV sterilisation had no effect on gels' mechanical properties. Murine MSCs from compact bone were harvested and characterised using flow cytometry. At passage 4, ≈80% of cells were $CD31^-/CD45^-/CD31^-$. Of these, ≈85% were $Sca\text{-}1^+/CD44^+/CD29^+$, but just 18% of these were also $CD105^+$. Passage 4 and 5 MSCs were seeded into the alginate gels at a concentration of 1 million cells ml^{-1}. Viability of seeded MSCs was ≈90% at Day 0, with no significant reduction at Day 7 and no significant difference between alginate concentrations either. The slow diffusion of calcium ions into the gel solutions and the presence of media during the crosslinking process may explain such high cell viability.

Conclusion

Alginate gel discs of three different G' values were successfully synthesised. Murine MSCs from compact bone were characterised and used at passage 4 and 5, highlighting low abundance of CD105+ cells as previously reported. A viability assay showed high cell survival in alginate gels, with no significant difference between gels' stiffnesses. Gene expression of chondrogenic markers will be quantified at Day 7 and Day 14 in gels with chondrogenic medium and compared to standard culture medium. These data, together with histochemistry analysis to quantify collagen and proteoglycans deposition at Day 21, will determine the most suitable gel stiffness for chondrogenesis. The chosen concentration will be further investigated for the development of an osteochondral 3D model to study OA-related mechanisms.

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DAY 2 Keynote Speaker

Christine-Maria Horejs

SCIENTIFIC WRITING AND PUBLISHING – INSIGHTS FROM A *NATURE* EDITOR

Christine-Maria Horejs
Chief Editor, Nature Reviews Materials

I this talk, I will provide an editorial perspective on publishing with *Nature* journals, discuss editorial processes, provide tips and tricks for writing first-class research papers and Review articles, and discuss careers in scientific publishing.

Clinical Advances and Patient Benefits of Applications

IN SILICO MODELLING FOR THE PREDICTION OF DRUG DRUG INTERACTIONS

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Introduction

A drug-drug interaction (DDI) can be defined as the modulation of the pharmacologic activity of one drug, the victim, by the prior or concomitant administration of another drug, the perpetrator. DDIs can reduce the efficacy or increase the toxicity of a treatment and lead to adverse events. The challenges related to the management of DDIs are particularly exacerbated in sub-populations of vulnerable patients, such as the elderly. Only a minority of potential DDIs have actually been characterised in clinical studies; many DDIs remain unexplored clinically or cannot be studied due to ethical constraints. In many cases the prescription of complex polypharmacy is supported only by individual judgment of the prescriber or expert opinion instead of a comprehensive evaluation of pharmacological and clinical data. In silico models for DDIs can represent a very effective strategy for the prediction of DDIs magnitude and the rationalise of future studies in vulnerable patients. The aim of this study was to develop a quantitative algorithm for the prediction of rifampicin enzyme mediated induction DDIs.

Materials and Methods

As input data, drug metabolism and pharmacokinetics (DMPK) characteristics were considered, including transporter specificity, fraction metabolised (fm) by enzyme isoforms and protein binding were compiled for the victim drugs (N=205). Later, the fm values were multiplied by the in vitro increase activity fold (E) of rifampicin for each isoform; the integration of this multiplication created the in vitro metabolic metric (IVMM) parameter. IVMM and the rest of potential parameters were included in the statistical analysis which consisted in a multiple linear regression model.

Results

The AUC ratios (with vs without rifampicin) obtained in our model were in agreement with the AUC ratios observed in clinical studies. Three independent variables were retained in the final model: IVMM, fraction unbound in plasma (Fu) and substrate specificity for OATP1B1 transporter. The use of the 2-fold acceptance criteria (AUC predicted vs observed ratio) yielded a successful rate of more than 75% for the final model.

Discussion and Conclusions

Management of DDIs in the elderly is challenging, especially with the increased multimorbidity, polypharmacy and likelihood of adverse events that characterises this age cohort. The model provides a screening tool to predict potential DDIs, utilising accessible in vitro data which could prove advantageous in early drug development when DDI clinical studies are not yet performed. Therefore, this algorithm could potentially help facilitate a more rational design of clinical studies evaluating the risk of toxicity and loss of efficacy associated with DDIs.

A METHODOLOGICAL FRAMEWORK FOR AI-ASSISTED DIAGNOSIS OF ACTIVE AORTITIS USING RADIOMIC ANALYSIS OF FDG PET-CT

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Introduction: Aortitis is an inflammatory syndrome affecting the aorta and its major branches [1]. Most cases of aortitis are treated initially with glucocorticoids which carries a risk of toxicity [2], therefore it is important that treatment is based on an accurate diagnosis. Diagnosis of active aortitis can be challenging, particularly for patients who have started treatment, as symptoms and blood tests are non-specific. Therefore, imaging plays a key role. Fluoride-18 Fluorodeoxyglucose Positron Emission Tomography – Computed Tomography (FDG PET-CT) identifies areas of increased glycolytic activity in the inflamed vessel wall and is often used to assess patients with suspected aortitis due to large vessel vasculitis (LVV) [3]. This qualitative grading is based on visual assessment by imaging specialists but this subjective evaluation can be inconsistent [3]. Radiomics is an emerging area of study which involves extraction of more substantial quantitative information from medical images referred to as radiomic features (RF) which may help better understand and stratify disease [4]. The purpose of this study was to evaluate the feasibility and explore the potential utility of RFs extracted from FDG PET-CT for improving the accuracy of detecting active aortitis. [5].

Materials and Methods: The aorta was manually segmented on FDG PET-CT in 50 patients with aortitis and 25 controls. Pyradiomics and SimpleITK were used to extract 107 radiomic features (RF) and SUV metrics from the segmented data. Features were harmonised using the ComBat technique. Mann Whitney U test and Logistic Regression (LR) classifiers were used to evaluate the diagnostic utility of extracted RF and SUV parameters. Three fingerprints combining multiple RFs were constructed: A - RFs with: area under the receiver operating characteristic curve (AUC) ≥0.5, accuracy ≥0.7, Mann Whitney U test p value ≥ $0.05/n$ where n = number of features; B - As A with removal of highly correlated RFs; C used principal component analysis (PCA).

Results and Discussion: Several RFs had high accuracy and AUC scores (85% (84-86% 95% Confidence Interval (CI)) and 0.90 (0.83-0.97) when used individually in LR classifiers. RFs also showed higher diagnostic utility than SUV metrics. Radiomic fingerprints performed similarly: A AUC 0.86 (0.68-1.00 95% CI), B AUC 0.91 (0.80-1.00), C AUC 0.87 (0.74-1.00).

Conclusions: A framework for a radiomics-based approach to support diagnosis of aortitis was outlined. Selected RFs, individually or in combination, outperformed SUV metrics for identifying active aortitis. Subject to confirmation of initial findings in an independent dataset, this framework could support development of a clinical decision tool for objective, standardized assessment of aortitis.

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6.

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DEVELOPING NOVEL OUTCOME MEASURES FOR PERIPHERAL NERVE REGENERATION

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Introduction:
In order to target peripheral nerve injury (PNI) treatment to individuals and patient groups it is important to understand who PNIs effect and how. We currently have no national-level data on the basic epidemiology of this devastating type of injury in order to focus treatments and services to those patient groups in most need.

In those patients with the most significant nerve injuries, where a gap exists between the two nerve ends, an unmet clinical need has been identified in order to replace peripheral nerve autograft repair with bioengineered nerve conduits. Modification and refinement of the currently available nerve conduits are required for the initial phase of this process.

Even in those with less severe injuries, clinical treatments have not progressed for several decades. This is in part, due to the lack of standardised outcome measures that would allow us to effectively compare outcomes between PNI centres. In addition, current clinical outcome measures lack the sensitivity to detect early biological changes after PNI therefore restricting the ability to demonstrate the clinical effectiveness of novel nerve repair solutions. New clinical outcome measures are needed that can identify and quantify early, microbiological changes of nerve regeneration.

Materials and Methods:
1. Nationwide retrospective cohort study analysing trends in PNIs over the past 15-years (2005-2019).
2. Phase I clinical trial of novel, microgrooved synthetic nerve conduit (1).
3. Systematic review of clinical outcome measure use in clinical peripheral nerve regeneration (2).
4. Prospective cohort study comparing end-organ epidermal thickness and sweat duct density changes after hand sensory PNI and repair.

Results:
1. The median, non-birth related incidence of PNI in England is 11.3 (IQR 0.89) persons per 100,000 population. There were a median of 5,401 (IQR 418.5) primary procedures for PNI per year. There is an increasing trend of birth-related facial-nerve injury across the study period. Male-to-female incidence remained constant at 2.2 - 2.9 : 1. The commonest age group for PNI were patients in their third decade (incidence 2.95/100,000, IQR 0.29). The upper limb was the most common site of injury with a median of 5788 (IQR 369.5) FCEs per year.

2. 17 patients had a Polynerve device fitted with 14 patients completing the study follow-up. No increased risk of infection or abnormal wound healing was reported.

3. A total of 96 studies were included (15 RCTs, 8 case-control studies, 18 cohort studies, 5 observational studies, and the remainder were case series or retrospective reviews). A total of 56 individual outcome measures were identified, utilized across 28 different countries and 7097 patients. Ten core domains were defined: sensory subjective, sensory objective, motor subjective, motor objective, sensorimotor function, psychology and well-being, disability, quality of life, pain and discomfort, and neurotrophic measures.

4. A total of 28 patients have been recruited thus far (recruitment ends May 2021) with a target of 37 patients to be recruited. Patients are followed for a 6-month period after sensory nerve repair in the hand. Figure 4 is an image of a patient's hand skin demonstrating the end-organ structures (sweat ducts and epidermal thickness) measured in hand sensory nerve injury.

Conclusions:
Peripheral nerve injury is common and most often affects the upper limb of young men (20 – 40 years). Polynerve, our topographically enhanced nerve conduit, provides a cost effective, efficient and scalable approach to nerve gap management that enhances peripheral nerve regeneration through effective neurobiological integration in sensory nerve gap injuries <20mm. Lack of consensus on outcome measure use hinders comparison of outcomes between nerve injury centres and the development of novel treatments. Development of a core outcome set will help standardize outcome reporting, improve translation of novel treatments from lab to clinical practice, and ensure future research in PNI is more amenable to systematic review and meta-analysis. Epidermal thickness and sweat duct density changes after PNI may be a more accurate measure of peripheral nerve regeneration in humans than current clinical outcome measures.

DECELLULARISED PLEURAL MEMBRANE PATCHES FOR PROLONGED ALVEOLAR AIR LEAKS

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Introduction

Prolonged alveolar air leaks as post-surgical complications to routine lung resections and biopsies are a significant cause for patient morbidity. Extended duration of chest tube drainage and emergency revision surgeries are the standard approaches for its clinical management [1,2]. Transplantable decellularised pleural patches as adjuncts to conventional intraoperative closure techniques could reinforce the physical barrier, reducing incidence and severity of sustained air leaks. As a tissue engineered treatment modality, it can promote regeneration of the compromised pleura as a bioactive interface, stimulating endogenous repair mechanisms in the patient.

Adopting the classic paradigm of conditioning cells in a bio-instructive microenvironment niche, we focused on cell culture studies, optimising an isolation and expansion protocol for porcine sourced mesothelial cells (PMC) and on biological scaffold derivation, developing a protocol for porcine pleural membrane (PPM) decellularisation and characterisation.

Materials and Methods

Cell culture studies looked at isolation efficiency of trypsin, pronase and direct explant culture for primary mesothelial cells from PPM, followed by their expansion and propagation in DMEM and cFAD medium to optimize culturing conditions favoring a mesothelial phenotype. Light microscopy, immunostaining and morphometric analysis were used to characterise established cell lines.

PPM decellularisation was carried out using a combinatorial approach of physical (freeze-thaw cycles) and chemical (0.5% sodium deoxycholate and 1% Triton-X 100 in 10mMTris buffer) treatments. Decellularised PPM were characterised against native counterparts using histological and nuclear DNA staining, mechanical testing, and membrane thickness estimation.

Results

Pronase digested cell cultures in cFAD medium, exhibited characteristic cobblestone morphology of a mesothelial-like phenotype, staining positive for mesothelin and vimentin. Cellular aspect ratios and population doubling times coupled with light microscopic images of cell morphology through subsequent passages, were indicative of a mesothelial phenotype.

H&E staining of decellularised PPM showed absence of stained nuclei, consistent with significant reduction ($p<0.0001$) of DAPI stained nuclei counts against native controls. Histological studies with Alcian blue staining for glycosaminoglycans and Picrosirius red staining for collagen exhibited comparable staining profiles and intensities in decellularised PPM, suggestive of minimal disruption to structural alignment and composition of the native ECM. Decellularised PPM were estimated to be thicker (218 ± 67µm) than the native controls (145 ± 33µm). Uni-axial tensile testing showed no significant change to the core mechanical properties of the decellularised PPM, evidenced by the estimated Youngs modulus of the treated membranes (804.08 ± 670.49 kPa) being comparable to the native PPM (828.44 ± 177 kPa).

Conclusion

Our pilot study represents a step forward in deriving clinically relevant bioactive ECM scaffolds in the form of decellularised PPM. Future work entails expanding the characterisation regime to include proteomics and ultrastructural studies. Assessing recellularisation potential of the derived scaffolds using the in-house established mesothelial cell lines, will underpin our research focus towards developing proof of concept for a biological scaffold-based therapeutic approach.

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UNRAVELLING CHITINASE-LIKE PROTEINS ROLE IN TISSUE REMODELING AND REGENERATION

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Introduction: Chitinase-like proteins (CLPs) are strongly associated with a wide range of inflammatory diseases, and are particularly implicated in tissue repair and remodelling in the lung during type 2 immune responses [1]. The molecular mechanisms involved in these CLP functions are not fully understood. We have hypothesised that CLP function will be determined by the specific interactions between CLPs and glycosaminoglycans (GAGs), heterogeneous polysaccharides that are components of the extracellular matrix that have well documented roles in the regulation of protein localisation and function. The aim of this project is to determine whether GAGs regulate CLP function during tissue repair.

Materials and Methods: Recombinant CLPs Ym1 and Ym2 (both murine proteins) have been made in bacteria using different methodologies. Ym2 was expressed using BL21 *E. coli* cells and purified using ion exchange and size exclusion chromatography; whilst Ym1 was expressed using SHuffle cells and purified using a nickel resin followed by His-trap, ion exchange and size exclusion chromatography.

Results: SDS PAGE analysis and electrospray ionisation mass spectrometry has shown that the purified Ym1 and Ym2 proteins have the expected molecular weights consistent with the presence of three disulphide bonds. Heparin affinity chromatography has indicated that both Ym1 and Ym2 bind to heparin (a type of highly sulphated GAG made only by mast cells) and do so in a pH dependent manner (with no binding at neutral pH and increasing binding as the pH is lowered). Further biochemical interaction analysis using biolayer interferometry (BLI) has allowed the screening of Ym2 binding to multiple GAG preparations. This has shown for the first time that Ym2 interacts with heparan sulphate (HS), binding with high affinity to this ubiquitous component of the extracellular matrix. Preliminary experiments with Ym1 have also indicated that this CLP is likely to interact with HS. Further BLI studies are ongoing to determine the sulphate specificity of the Ym2-HS interaction and investigate binding to other types of GAG (e.g. chondroitin sulphate and dermatan sulphate).

Discussion & Conclusions: Ym1 and Ym2 have been shown to bind to GAGs (e.g. HS), which are present in lung tissues. The unusual pH dependency of their interactions indicates that binding only occurs under acidic conditions and thus will likely increase during inflammation, perhaps regulating their localization in particular lung compartments. Work is in progress using site-directed mutagenesis of Ym2 to identify the GAG-binding site and generate mutant proteins with altered GAG-binding properties for analysis in *in vivo* models of helminth infection; here we have targeted histidine residues we predict will mediate Ym2's pH-dependent binding to heparin/HS. These studies will further our knowledge of the role that CLPs have in type 2 immune responses.

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MODIFICATION OF DECELLULARISATION METHODS TO ASSESS THE EFFECTS OF SWELLING ON THE MECHANICAL PROPERTIES OF TENDON

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Anterior cruciate ligament (ACL) rupture accounts for 40% of knee injuries [1], requiring 400,000 reconstructions annually worldwide [2]. Decellularised porcine superflexor tendon (pSFT) provides an off-the-shelf, cost-efficient option for ACL reconstruction (ACLR). During decellularisation, phosphate buffered saline (PBS) is used for washing out cytotoxic solutes and reagents, whilst maintaining tissue hydration. It has been shown to increase water content in tendon, swelling the tissue [3]. This has proven to reduce the mechanical properties of tendon [4, 5].

In this study, end stage PBS washes in the standard protocol were substituted with physiological saline solution to determine if tissue swelling could be reduced without negatively affecting its mechanical properties, while achieving the same degree of cellular removal. pSFTs decellularised using the modified protocol were compared to pSFTs decellularised using the standard protocol (PBS) and native tendons. Collagen crimp characteristics were analysed with ImageJ using histological sections stained with Sirius Red, comparing the same three groups. Hydroxyproline, glycosaminoglycan and denatured collagen content were quantified using spectrophotometric assays.

The physiological saline group showed no differences in mechanical properties, except for a significantly higher toe modulus, compared to standard PBS group or native tendons. Tissue dimensions post-decellularisation of physiological saline group were reduced compared to standard PBS group with no statistical differences, yet less swelling was apparent. In addition, histological evaluation showed no traces of nuclei in any group post decellularisation. Crimp period was significantly decreased after decellularisation, thus increased extensibility was observed in the form of increased length between crimp peaks. Crimp amplitude was not significantly different between native and physiological saline groups, yet significantly reduced for the standard PBS group. Crimp angle was reduced for both decellularised groups, compared to native tendons. Quantification of glycosaminoglycans and denatured collagen showed no significant differences between groups. Relative collagen content was significantly higher for physiological saline group compared to standard PBS group and native tendons.

Decellularisation using PBS or physiological saline has no significant effect on the loss of matrix components; however, whether these remain functional should be further studied. Collagen crimp pattern is affected by the removal of cellular content during the decellularisation process. Decellularised pSFTs using the standard or modified process have suitable mechanical properties to act as a viable graft for ACLR.

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UNDERSTANDING THE MECHANISMS OF ACTION OF COLLAGEN-BASED WOUND DRESSINGS

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Introduction

Wound healing is a complex process that involves numerous cell types, cytokines, chemokines, growth factors and extracellular matrix (ECM) components, which work synergistically to achieve healing[1, 2]. It consists in four overlapping phases that occur smoothly to achieve healing[1–3]. When the healing process fails to proceed through these physiological phases, the wound is referred to as a chronic wound[1,2,4,5]. Chronic wounds are a significant global problem, causing patient morbidity and a substantial financial burden on health services worldwide. The rising prevalence of chronic wounds puts increasing pressure on global health services, calling for the development of therapies that can relieve both patients and healthcare systems of this economic and societal burden [6,7].

There are a vast variety of commercially available dressings aimed at managing chronic wounds, making it difficult for healthcare professionals to choose the most appropriate therapy[2]. Collagen-based dressings are a large class of dressings which offer numerous beneficial properties for the treatment of recalcitrant wounds[8-11]. Commercially available collagen-based wound dressings differ in composition but present similar claims. There is a lack of comparative data to differentiate between these dressings and understand their mode of action. This project is set to compare the effects of collagen-based wound dressings using a diabetic murine full-thickness wound model.

Materials and Methods

The dressings chosen for analysis were a control non-woven dressing (3M, Saint Paul, MN, US), 3M™ Promogran™ Protease Modulating Matrix and 3M™ Promogran Prisma™ Wound Balancing Matrix (3M, Saint Paul, MN, US), Puracol® (Medline Industries Inc., Northfield, IL, US), ColActive® Plus (Covalon Technologies Ltd., Mississauga, Ontario, Canada) and UrgoStart® (UrgoMedical, Chenôve, France), a synthetic dressing with similar claims to collagen-based dressings. Thirty-six diabetic (*db/db*) female mice received two full-thickness excisional wounds. Wounds were treated with a pre-moistened control or collagen dressings (12 wounds per group). Dressings were changed or re-applied after 3 days according to the products' IFU. After 7 days, macroscopic images were taken, and wounds harvested. Wounds were bisected and processed for histological and biochemical analysis. Wound width, wound area, and re-epithelialisation were quantified from haematoxylin and eosin stained sections using ImagePro software.

Results and Discussion

Wound area calculated from the macroscopic images for all wounds (12 per group) showed that 3 of the 4 collagen dressings and UrgoStart promote healing compared to the control ($p=<0.05$), achieving 60-70% closure within 7 days. Analysis of wound parameters from histological sections of 4 of the 12 wounds for each dressing revealed greater re-epithelialisation compared to the control, albeit owing to the limited numbers of wounds analysed so far this increase in re-epithelialisation was not significant. Granulation tissue area, wound length and re-epithelialisation varied amongst the collagen dressings but no significant differences were observed. Full analysis of all 12 wounds for each dressing is underway.

Conclusion

Our preliminary results show that collagen-based dressings promote healing of diabetic murine wounds to a greater extent than a non-collagen control dressing. The rate at which these dressings promoted healing differed between dressings. Full analysis of all 12 wounds for each treatment group may provide differentiation between the type of collagen dressing and effect on healing. Future work will determine if the composition of these different collagen-based dressings differentially impact specific cell types within the wound including keratinocytes, fibroblasts, and immune cells. These results may help healthcare professionals with a greater understanding of how collagen-based dressing can modulate healing of chronic wounds.

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A NOVEL 3D OSTEOCHONDRAL SCAFFOLD WITH MECHANO-IDENTICAL PROPERTIES OF THE NATIVE TISSUE FOR *IN SITU* TISSUE REGENERATION

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Osteoarthritis (OA) is a chronic, global, degenerative disease of synovial joints. Most prevalent in load-bearing joints, OA is the most common musculoskeletal condition, form of arthritis and cause of pain and disability. The increasing prevalence of obesity, sedentary lifestyles, OA in younger populations and an ageing population will increase OA incidence and the psycho-socioeconomical burdens associated. Osteochondral (OC) tissue is a composite system comprising of articular cartilage (AC), underlying subchondral bone and a complex interfacial zone between them. Grafting of OC tissue poses challenges including limited supply, immune rejection and ethical considerations. Surgical treatments (e.g., autologous OC transplantation) can only be used in non-weight bearing joints and normal cartilage function is rarely restored. Traditionally, health-care providers passively await the final 'joint death' necessitating an irreversible total joint arthroplasty (TJA) procedure, the clinical gold standard treatment, however full joint function isn't always restored and presents long-term complications including aseptic loosening, infection, morbidity rates following surgery and functional failure. Revision surgery is complex due to the weakened surrounding tissue and association with additional short- and long-term complications. Revision surgery rates are increasing and there are significantly greater risks that individuals under 60 will require revision surgery within 10 years resulting in younger patients living with a lower quality of life for several decades. This project aims to develop and produce an "off-the shelf" scaffold to provide a novel means of preventing TJAs through effective and economical tissue regeneration, treating OC defects and OA and restoring lost joint function. A biomimetic multiphasic scaffold with a gradual interface will provide necessary mechanical support and ensure successful integration for long-term functionality by permitting separate osteogenic and chondrogenic factors, simultaneously. This "smart" tissue engineering (TE) scaffold will contain a trabecular bone-regenerating region (BRR), a subchondral plate region (SPR) and an articular cartilage-regenerating region (ARR).

Polyhydroxyalkanoates (PHAs) are a relatively novel family of biopolyesters produced by bacteria under various environmental and often unbalanced growth conditions, offering fantastic potential as a sustainable, environmentally friendly, renewable resource for the successful fabrication of TE scaffolds. The structural, mechanical, physical, biocompatibility and biodegradability properties of PHAs can be optimised by altering biosynthesis conditions, blending with other PHAs and surface modifications to support cell adhesion and ultimately tissue regeneration. The scaffold will be manufactured from blends of Poly(3-hydroxybutyrate) and poly(3-hydroxyoctanoate-*co*-3-hydroxydecanoate) utilising 3D printing for the BRR phase and electrospinning for the ARR phase. Investigation of varying PHA blends will determine the optimal composition for the desired mechanical and degradation properties. These fabrication methods demonstrate the potential to produced functionalised scaffolds with controlled micro- and nanoscale biomimetic features. Recent bioprinting techniques enable fabrication of scaffolds with more personalised biomimetic multifunctional architectures by converting clinical imaging data into computer-aided design (CAD) files and designing anatomically accurate scaffolds. Cold plasma deposition of allylamine and binding of cell homing and chondrogenic factors will further enhance the scaffold surface to promote regeneration of articular cartilage through recruiting, binding and promoting chondrogenesis of stem cells. Scanning Electron Microscopy will be used to examine morphological characteristics and microstructure of the scaffold and to study the morphology and distribution of cells growing on the substrate as well as quantifying osteoblasts and chondrocytes at suitable time periods (e.g., 1, 4, 7, 14 days) to examine cellular adhesion, formation, division and proliferation.

Biocompatibility will be assessed *in vitro* utilising Live/Dead cell viability assays histological and immunohistochemical analyses. RT-PCR will be used for analysis of gene expression of major osteogenic and chondrogenic proteins of mesenchymal stem cells (MSCs) and MG62 chondrocytes for the BRR and MSCs for the ARR, evaluating suitability for cell growth and appropriate tissue formation. Chondrogenic and osteogenic differentiation can be enhanced through a complex interplay of biochemical cues. Osteocalcin, alkaline phosphate and collagen type I will be used as osteogenic biomarkers, while aggrecan and collagen type II will be chondrogenic biomarkers. *In vivo* animal models will be crucial to evaluate the biocompatibility of the scaffolds and chosen bioactive factors for clinically relevant feasibility and safety evaluations. Mechanical characterisation is required on a macro-, micro- and nanoscale to evaluate scaffold suitability for OC TE: macroscopical three-point bending will determine Young's Modulus (YM) during 'whole joint loading' of the scaffold; Vickers microhardness indentation performed at different locations will determine YM of the BRR, SPR and ARR individually; nanoindentation utilising Scanning Probe Microscopy will determine the strain elastic modulus at varying planes and create topographical images of the fibre surfaces. Confined/unconfined static compression testing will enable direct measurement of YM and yield strength and observation of deformation behaviour which should demonstrate three distinct regions: a linear elastic region; the offset yield strength; the densification and crushing of the scaffold. Fatigue performance will be evaluated by dynamic compression testing to determine scaffold lifetime under physiological conditions.

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THE DEVELOPMENT OF DRUG-ELUTING ELECTROSPUN DEVICES FOR CLINICAL APPLICATIONS IN GYNECOLOGY

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Introduction

Gynaecological conditions are extremely common, affecting up to an estimated 20% of people with a uterus (1); they can be at best irritating and at worst life limiting. Whilst pessaries are effective for the delivery of drugs in some conditions, they are not applicable for all and have known drawbacks. The multi-disciplinary team supervising this project have already successfully developed Electrospun polymer devices that have proved effective in the delivery of drugs to the mouth (2), and it is believed that this technology can be adapted to address unmet needs in gynaecology as well. Topical drug delivery is preferable to systemic treatments in many gynaecological interventions, however given the nature of gynaecology this can be exceptionally challenging. Development of a drug-eluting, smart patch that can respond to local conditions within the uterus and release drugs topically could help solve these issues. Gynaecological conditions that would benefit from direct and sustained drug delivery include infections (e.g Candida albicans) and lichenoid reactions in the vagina, endometriosis in the uterus, and various gynecological cancers. The aim of this research is therefore to design, fabricate, characterise and evaluate an electrospun patch to help meet this need in gynaecology.

Materials and Methods

The delivery of therapeutic nucleic acids (TNA) has already proved effective in treating a range of conditions, including various different cancers (3), but as yet there is limited research that focuses on utilising these treatments for gynecology. Given the potential for TNA, both realised and not, this project's initial aim is to develop an electrospinning technique that can successfully produce TNA-containing fibre patches without reducing the efficacy of the TNA. Related research has already gone some way to demonstrating the protective capabilities of coaxially electrospinning but as yet there is no reliable or simple way of producing TNA-loaded electrospun patches. Initial trials will utilise poly[2-(dimethylamino)ethyl methacrylate-*block*-di(ethyleneglycol) methyl ether methacrylate (poly-DMAEMA) as the carrier for the TNA, given it have proven use in gene delivery in a vesicular structure. Analysis of these data will then be used to determine the next steps to be conducted in the research.

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OPTICAL DETECTION OF AIRBORNE FUNGAL PATHOGENS

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Introduction: *Aspergillus spp*. are asexual, saprophytic fungi that are ubiquitous in the environment and are more commonly known for their persistence in agricultural crops. From a clinical perspective, species such as *Aspergillus fumigatus* are responsible for triggering a range of conditions known as aspergillosis, predominantly affecting immunocompromised patients where invasive aspergillosis (IA) can lead to 40–50% mortality in leukaemia patients. Even in immunocompetent individuals, chronic pulmonary aspergillosis (CPA) causes a serious clinical challenge (80% 5-year mortality) with both CPA and IA requiring the use of long-term azole anti-fungal treatment. The overuse of these treatments has promoted an increase in resistant *A. fumigatus* isolates. As a result, oral therapies such as triazoles, which are the only class of oral antifungal with activity against *Aspergillus spp.*, are no longer effective. To effectively manage and reduce infection by *A. fumigatus* suitable monitoring and detection techniques are required to prompt decontamination protocols and preventative measures. The Sentinel system is a series of networked environmental monitoring systems featuring an interchangeable, selective biomimetic growth surface to identify *A. fumigatus* by its growth characteristics. The film will be imaged by a commodity camera, the images processed by machine-learning algorithms, and diagnostic information shared wirelessly. The focus of the CDT research is to create this selective element, making a thin film growth niche to germinate *A. fumigatus*, which can be mass produced.

Materials and methods: The organism will be grown in media of varying composition to investigate the boundaries of growth that impose a stress response in a form of phenotypical mutation. Stress response will be explored by changing temperature, nutrients, trace elements, pH in combination with polymer membranes of varying structure, type, colour/opacity, pore size, surface charge, topography and thickness. Morphological changes should be visible at 20x magnification and lower with optical microscopy. To increase specificity to resistant strains, anti-fungal compounds will be incorporated into the niche and sensitivity will be explored alongside stress response to identify the lowest concentration of spores in the shortest period of time.

Results, Discussion and Conclusion: The adaptability of *Aspergillus* species allows it to grow on an array of surfaces and environments, therefore by changing its growth conditions stress responses are initiated causing morphological changes in cell colour, structure and shape. By exploring its natural response to varying environmental conditions and nutrient sources a sensitive and specific niche can be developed, allowing faster growth and the developed of an identification criteria. In this way, decontamination protocols can be initiated to prevent infection outbreaks of population at risk, whilst the presence of *A. fumigatus* is confirmed much earlier than conventional microbiological techniques.

Enabling Technologies

EFFICACY OF PLASMA ACTIVATED WATER DISINFECTION OF FLEXIBLE ENDOSCOPES

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Introduction

The use of flexible endoscopic devices has become a major feature of modern clinical practice, both for diagnostic applications and surgical interventions. Endoscopic procedures are conducted with reusable devices, the nature of which are an ideal environment for biofilm development inside the narrow lumen of the endoscope channels. The fact that current reprocessing methods are ill suited to eliminate biofilm formation increasing the potential for cross-patient contamination highlights the need for a new approach to disinfection. In this study, the use of Plasma Activated Water (PAW) was examined for the decontamination of narrow lumens, mimicking those found in modern endoscopic devices. When cold gas plasma interacts with water an abundance of highly reactive chemical species are created directly in the liquid medium, resulting in a solution known as PAW. Several past studies have shown that PAW is able to penetrate and eliminate biofilm contamination, making it an extremely effective and convenient antimicrobial agent. This work explores the possibility to utilise PAW as a new endoscope decontamination technology.

Materials and Methods

In this study, a flow system was created to contaminate endoscope surrogate test pieces prepared from translucent Teflon tubing (Masterflex tubing, 2 mm and 6 mm ID, Fisher Bioblock Scientific, Illirch, France) with a mixed species biofilm produced from repeated rounds of cultivation and rinsing. Test pieces were first filled with tryptic soy broth (TSB) containing 1% human serum and kept at 37°C for 24 h, so that organic matter could attach to the surface of the channels, increasing bacterial adherence potential. The media was drained from the test pieces and the system rinsed with sterile water. Fresh TSB was then inoculated with *Staphylococcus aureus USA300*, *Staphylococcus epidermidis 1457*, *Pseudomonas aeruginosa PA01* and *Escherichia coli Bw25113* and circulated through the system at a rate of 100 ml/min for 45 min. Following draining of the contamination media, the system was rinsed with sterile water and cultivated with discharged bacterial liquid from a previous stage. The contaminated test pieces were left to incubate for 24 h at 37°C. The system was rinsed, and control test pieces were removed for analysis of biofilm formation. A low-temperature air plasma device was placed above a stirred volume of distilled water to produce the PAW at a rate of 10 ml/min. Under such conditions PAW was generated indirectly as a result of longer-lived reactive oxygen and nitrogen species arriving at the water surface, including O_3, H_2O_2, NO, N_2O and NO_2. For comparison with PAW, a commercially available buffered peracetic acid currently used for endoscope disinfection was prepared at a concentration of 2%. Disinfection of the contaminated test pieces involved circulation of either PAW or buffered peracetic acid through the system at a flow rate of 100 ml/min. The system was then rinsed, and the test pieces used for biofilm analysis.

Results and Discussion

Initial results showed a 3/3.5 log reduction of biofilm contamination in lumens with a 6 mm and 2 mm diameter, respectively, after a 20 min PAW treatment. By switching to a multiple rinse approach, whereby 2x10 minute PAW treatments gave up to a 6 log reduction. Critically, the multiple rinse method for PAW disinfection not only improved results but also is more representative of current endoscope reprocessing methods where multiple stages of disinfection are used. In comparison, the commercial buffered peracetic acid resulted in a 6-log reduction in biofilm in 2 mm and 6 mm lumens after a 2x10 min treatment, showing no statistically significant difference with the PAW treatment. Crystal violet staining revealed the presence of organic matter in both PAW and peracetic acid treated test pieces. This resulted in regrowth in both cases suggesting the need for an extra step to remove this, e.g., manual brushing step.

Conclusions

These results have shown that PAW is a very promising method for disinfection of contaminated endoscopes in comparison to currently used methods, however there is a need for refinement of the PAW treatment to optimise disinfection and cut down reprocessing times. Further work will focus on establishing the optimum PAW generation conditions, characterising chemical composition to identify mode of action, identifying impact of PAW on endoscope materials, and exploring how PAW could aid or replace stages of endoscope reprocessing.

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FURTHER DEVELOPMENT AND TESTING OF A NOVEL AUTOMATED DECELLULARISATION SYSTEM

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Introduction

Decellularisation is the removal of cells and DNA from biological tissue and has been regarded as a solution to the host immune reaction and poor regeneration that can occur from the use of xenografts and allografts [1]. To allow the clinical uptake and commercialisation of decellularised tissue there are several issues that need to be addressed. The current small batch, open processing methods used to produce decellularised scaffolds are labour and time intensive, requiring daily solution changes and taking up to six weeks to complete. Previous work has been completed to manufacture a scalable, automated, closed system to decellularise bone plug grafts. Two decellularisation processes were used: the standard 42-day process and a shortened 13-day process. The system successfully decellularised porcine bone grafts using both processes but had a few minor reliability problems. The aim of this study was to manufacture a second more reliable system and test the system could successfully decellularise porcine bone tissue before moving on to decellularising allograft tibial bone.

Materials and Methods

An updated automated decellurisation system was designed and manufactured with new pumps, valves, and electronic components. The components were enclosed in a modified aluminium case and the Arduino code and user interface was restructured to be more user friendly. To test the updated automated decellularisation system, bone plugs were obtained from porcine femurs. Half were decellularised using the thirteen-day decellularisation process in the updated system, and half of the bone plugs were left as native controls. To assess the quality of the decellularised bone plugs, DNA and fat concentrations were quantified to ensure successful decellularisation, H&E and DAPI stains were used to identify any remaining cells or cellular material, sterility broths were used to ensure both processes were completed aseptically, and removal of cytotoxic reagents was evaluated using both extract and contact cytotoxicity tests.

Results and Discussion

The new system successfully completed the full decellularisation process with no reliability issues. The decellularised bone plugs sufficiently reduced the DNA concentration to a level previously determined to be decellularised [2] and there were no histological sign of cells or cellular material. The results indicate that the new automated decellularisation system can successfully decellularise porcine bone tissue, matching the previously designed system. Future work will involve using the updated system to decellularise allograft tibial bone using the new 13-day process with the possibility of further optimising and shortening the decellularisation process.

Conclusions

An updated and more reliable automated decellularisation system successfully removed DNA and cellular material from porcine femoral bone, following the success from this study the system will be used to decellularise allograft tibial bone.

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COMPARISON OF EXTRACELLULAR VESICLE ISOLATION PROCESSES FOR APPLICATIONS IN SKELETAL MUSCLE TISSUE ENGINEREING

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Introduction

Evidence suggests skeletal muscle (SkM) myotube derived extracellular vesicles (EVs) drive SkM processes, indicating their regenerative potential. However, therapeutically aligned studies are limited by the ability to efficiently obtain high yield purified populations from minimal sample volumes in a cost-effective manner.

Materials and Methods

This study compared isolation methods to understand EV output variation using Nanoparticle tracking analysis (NTA), BCA protein assay, western blots (CD9, CD63, Alix, Annexin A2), ExoELISA's (CD63 and CD81) and nano flow cytometry (NanoFCM) (CD9, CD63, CD81). To further understand advantages and limitations (e.g. efficiency, cost, scalability, purity) a survey was internationally distributed via Qualtrics within the EV community. EVs were isolated from C2C12 mouse myoblast cells by ultracentrifugation (UC), polyethylene glycol (PEG) precipitation, Total Exosome Isolation Reagent (TEIR), an aqueous two-phase system (ATPS) utilising PEG and dextran, and size exclusion chromatography (SEC). ATPS was repeated with multiple washes of the top PEG phase for additional purification.

Results

TEIR displayed the highest particle concentration (1.15E+09) followed by UC (8.46E+08) and PEG precipitation (7.11E+08), with purity (particles per µg protein) 7.16E+05, 1.22E+06 and 6.90E+05 respectively. SEC and ATPS showed lower sample purity, 1.12E+05 and 1.99E+05 and particle counts of 4.57E+08 and 6.31E+08 respectively. ATPS with repeat washes displayed the lowest particle counts (2.71E+08) and highest purity (2.01E+06). EV markers were present in all isolations but with distinctly variable profiles.

Discussion and Conclusions

This high-throughput comparison study indicates that methods withdraw myogenic EV-enriched fractions with variable purity and marker profiles. This could have significant implications for defining SkM processes and EV applications in tissue engineering.

PRINTED, SKIN-COMPLIANT ELECTROPHYSIOLOGY ELECTRODES FOR THE MOUSE MODEL

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Introduction

Using electrocardiograms (ECG) to monitor the electrical activity of the heart within rodents plays a crucial role in cardiovascular science and discovery biology. Demand for which has been amplified following the wide-spread adoption of transgenic mice in pre-clinical science, where knock-out models for human disorders provide crucial insights into disease aetiology and potential therapeutics. However, currently ECG acquisition in conscious, free-moving animals is reliant on the surgical implantation of rigid, in-flexible biopotential recording devices. This places considerable burden on the animal, weighing up to 20% the animal's body weight and requiring a 2-week recovery period. Development of a non-invasive, flexible and skin-mounted recording system would dramatically refine cardiovascular research; both expanding the potential scope of research questions, and also improving animal welfare. However, integrating an ECG with mouse skin requires research to identify suitable interface materials, which can support a good electrical contact when challenged with the mouse model's highly compliant skin mechanics, and dense pelage.

Materials and methods

In this work several design strategies for interfacing with mouse skin were explored, each analogous to an electrode previously reported successful for on-human use. The probes were fabricated by screen printing silver/silver-chloride onto either temporary tattoo paper, attached using polyvinyl acetate (PVA); or, 25 µm thick polyethylene naphthalate (PEN), attached using an adhesive polyurethane (PU) backing, or attached with conductive gel. To better understand the difficulties presented by mouse skin, these electrodes were then compared for performance on humans and mice. This involved charichterisation of the skin-contact impedance when connected to ex vivo mouse skin samples, or pig skin samples (acting as a stand-in for human skin). Furthermore, we compared the interface's ability to acquire good quality ECG signals in both humans and anaesthetised mice, assessing their capability for yielding usable metrics concerning cardiac activity.

Results and Discussion

Results found all the tested designs were able to collect ECG signals from unconscious mice, even when placed over some residual fur following shaving. For which, metrics for heart rate variability could be obtained and identified in all design variations. Notably, gel interfacing electrodes yielded the clearest ECG waveform features, a result which corroborated to the lowest contact impedance scores on mouse skin. Seemingly this was because the gel ensured a good resistive connection through the residual fur. In contrast, temporary tattoo electrodes demonstrated the highest skin impedance, likely because the temporary tattoo adhesive (PVA) introduced another insulating layer between the interface and skin.

Conclusions

This work suggests printed, flexible biosensing electrodes could offer a viable strategy for establishing an electrical connection with mouse skin. Further adaptation of the material solutions described might enable the creation of more sophisticated tools for monitoring the mouse model in pre-clinical research.
Furthermore, additional research into the long-term durability of these materials when attached to free-moving animals could provide a foundation in engineering a complete, wireless, skin-integrated ECG for mice.

MITIGATING SOCIAL CONNECTIVITY AND SUPPORT PARADOX THROUGH TECHNOLOGY FOR OLDER ADULTS DURING THE COVID-19 PANDEMIC?

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Introduction: COVID-19 shook the world in 2020. Older people were especially impacted with higher morbidity, mortality, and advice to stay at home. We investigated how older adults' social interaction and communication changed within familiar relationships during the pandemic.

Method: We investigated in a qualitative longitudinal study how people in the United Kingdom aged 65 and over experienced the COVID-19 pandemic. Participants (n=33) were recruited via a large-scale nationally representative survey (https://www.sheffield.ac.uk/psychology-consortium-covid19). We asked how life had been pre-pandemic, how they experienced the first and second wave of infection, and how the way they communicated changed. The data were analysed using constructivist grounded theory.

Discussion: We found that the family support system had been interrupted, that there were changes in the methods of support, and that the feeling of belonging was challenged. Before the pandemic, the use of technology was less important for many older people. Physical interaction was highly important to experience a meaningful life. The pandemic disrupted the well-established way of interaction and communication apart and the participants had to be creative in new ways for communication and exchange of support. Many of them challenged themselves to become familiar with new technologies like video calls. Although the use of technology increased during the pandemic, participants found it could not replace the 'gold standard' and the technology does not have the emotional equivalence of embodied presence. Burtholt et al. (2020) suggest that proximity to at least one family member reduces the risk of loneliness. The question for new technology is – "how is it possible to create ways that allow physical interaction and meaningful communication whilst social distancing measures remain in place?"

A LABEL-FREE BIOPHYSICAL ASSAY FOR QC ON 3D CELL CULTURES

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Introduction

Tumour spheroids are widely used 3D *in vitro* models in cancer research. Nonetheless, the diverse spheroid formation capabilities of different cell lines stress the need for standardization of uniform culture protocols. Gathering precise information on their size, weight and mass density values is crucial to support decisions for protocols optimization [1]. Here a flow-based technology is used to establish a label-free, non-invasive assay for measuring the biophysical properties of colorectal carcinoma (CRC) spheroids.

Materials and Methods

The human certified CRC cell lines HT-29, HCT-15, SW620 and DLD-1, were obtained from the cell bank of the Policlinico San Martino, Genoa, Italy. CRC spheroids were generated in flat-bottom Ultra-Low attachment 96-well plates as previously described [2]. For the analysis with the flow-based technology (*W8 Physical Cytometer)*, CRC spheroids on day 6 of formation were fixed with PFA 4% overnight at 4°C, resuspended in 3.5 mL of Dulbecco's phosphate-buffered saline (DPBS), at low concentration (<200 spheroids/mL), and then analysed as previously described by *Cristaldi et al.* [1]. A minimum of 10 single spheroids was analysed for every test condition; mean values and the related standard deviation were extrapolated from 10 repetitions.

Results and Discussion

Tumour spheroids of four CRC cell lines HT-29, SW620, HCT-15, and DLD-1 were analysed. Experiments were performed on heterogeneously sized spheroids to prove the feasibility of the flow-based assay on cell aggregates ranging from 100 to 200 µm in diameter. HCT-15, DLD-1, and SW620 spheroids displayed round shape with a smooth surface, while HT-29 spheroids showed irregular shape with a rough surface (Figure 1A). As shown in Figure 1B, SW620 and DLD-1 spheroids' weights (ng, left graph) were higher than that of HT-29. Also, their diameters (µm, central graph), were larger than those of HT-29 spheroids. Noteworthy, the mass density was consistently higher in SW620 and DLD-1 than HT- 29 spheroids (fg/µm^3, right graph). As mass density represents a direct parameter to evaluate sample compaction, data agreed with the preliminary microscopic investigation, where HT-29 cells formed loose aggregates, instead of compact tumour spheres.

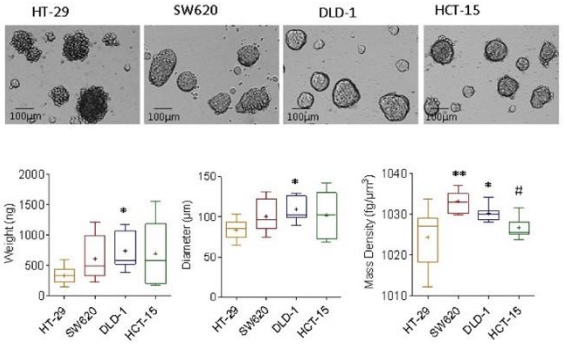

Fig.1. A: Bright-field images of CRC spheroids. B: Measurement of weight, diameter and mass density of CRC spheroids. Statistical analysis was performed using two-tailed unpaired Student's t-test: *p< 0.05, **p< 0.001 vs HT-29, #p<0.05 vs DLD-1.

Conclusions

In summary, this label-free biophysical assay clearly illustrates the cell line-dependent heterogeneity in size and shape of CRC spheroids, although tested under the same experimental conditions, and generated with the same cell seeding number. Of note, these different characteristics can be measured and evaluated to select the most promising cell lines and define the best culture conditions, accordingly.

Acknowledgements

We are grateful to Prof. Poggi and Zocchi for their precious collaboration in achieving these results.

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THE EFFECT OF ELECTRICAL STIMULATION AND HYDROGEN PEROXIDE ON GENE EXPRESSION IN AN IMMORTALISED HUMAN MESENCHYMAL STEM CELL LINE

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Hydrogen peroxide (H_2O_2) is a by-product at the cathode during direct current electrical stimulation (DC ES), and it is now well accepted that H_2O_2 is an essential molecule within the cell for the regulation of processes such as proliferation, differentiation, and migration [1]. DC ES is currently under investigation as a tool to accelerate differentiation in human mesenchymal stem cells; several research groups have proof of its efficacy, but few truly understand the cellular mechanism underpinning the response. On stimulation, there is a marked release of intracellular calcium, changes occur to membrane ion channels, and there are alterations in the cytoskeleton, among other effects [2]. What is not understood is how much of a role the extracellular H_2O_2 is playing on the cellular effects that occur both during and after DC ES. Long term, it is hoped that DC ES could have the potential to accelerate differentiation in the mass production of stem cells, rendering the process more rapid and cost-effective.

Immediate responses to DC ES in hMSCs have not been studied in detail. The gene expression and metabolic activity of hMSCs from an immortalised hMSC cell line, Y201, will be studied after 1 stimulation period of 30 or 60 mins of electrical stimulation in the presence and absence of catalase, an enzyme that breaks down H_2O_2. In previous experiments, hMSCs from 2 donors (Donor A = M, 19Y, unknown race; Donor B = M, 24Y, Hispanic) were used, both with highly contrasting results. Donor A's bone marrow-derived hMSCs showed consistent and significant increases in metabolic activity after 4 days of DC ES, whereas Donor B showed either no change in metabolic activity, or significant decreases after 60 minutes of stimulation. Studies on gene expression also gave contrasting results. In the use of the new cell line, it is hoped that some consistency can be achieved, and that the role of H_2O_2 will become clear.

It is hoped that this work can help inform researchers working in the field of electrical stimulation the extent to which H_2O_2 contributes to the observed response. Furthermore, it may allow the fine-tuning of the extracellular environment to maximise the potential of DC ES as a tool in tissue engineering.

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Declaration: I took a six month interruption between July 2020 and January 2021. I have also moved to a new building which has presented challenges in the ability for progression with my work. I no longer am able to work with the cells I was previously, and so have begun working with a new cell line. I cannot predict the response to DC ES until I am able to carry out the experiment, which I will be doing the week commencing 12th of April 2021.

DEVELOPMENT OF A NOVEL, SMART, AND DISPOSABLE TEAR COLLECTION DEVICE FOR POINT-OF-CARE OCULAR AND SYSTEMIC DISEASE DIAGNOSIS

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Introduction

The most recent World Health Organisation (WHO) report estimates globally that 2.2 billion people from preadolescence suffer from vision impairment, and over half of these suffer eye conditions such as dry eye disease; glaucoma; and diabetic retinopathy[1]. The outer eye surface is populated by a precorneal tear film layer (7-10 µL) composed of tears that contains thousands of biomarkers with correlating concentrations to a myriad of ocular diseases. Moreover, exploitation of the blood-ocular barriers has enabled in recent years the diagnosis of systemic diseases including cancers and motor pathologies. In order to analyse these film biomarkers for diagnosis, tears are collected conventionally by methods such as Schirmer test filter strips; microcapillary glass tubes; and cellulose absorbent sponges, which remain suboptimal. High variability between achievable sample volumes; types of tears collected; tear biochemistry; tear proteome; and the necessity for post-processing to extract the tear sample, justify the lack of a standard protocol for tear collection and analysis for accurate disease diagnosis and staging. Therefore, there is a need to develop next-generation tear collection devices that are reliable and indicative of diseases to enhance the diagnostic potential of tears.

Project Overview

In partnership with Menicon Co., Ltd, a global contact lens manufacturer, this research sets out to develop and test a novel tear collection device composed of biomaterial polymers that is able to remain under the lower eyelid while simultaneously capturing a low volume of tear fluid. Establishing requirements of a new collection device gears the research direction towards the use of contact lens substrates, and stimuli-responsive biomaterials which have shown microfluidic potential for capturing and storing biological fluids. The project scope can be broken down into four key milestones: (1) development and characterisation of a prototype smart-based collection device with microfluidic components in wet and dry-lab environments; (2) integration of the device into industry partners analysing system by a microfluidic channel for investigating the sample; (3) establishment of an interface permitting fluid transfer between the device and analysis system; and (4) clinical studies on human participants for device efficacy. Findings from such work will allow for tear film biomarkers derived from the ocular system and body to be correlated with baseline values for point-of-care diagnosis.

Expected Results

It is expected that a prototype collection device without electronics will be developed inexpensively using microfabrication techniques, and it is capable of collecting and storing between up to 500 nL of basal tears under non-turbulent flow conditions. Furthermore, incorporating a stimuli-responsive biomaterial, while being comfortable to the wearer and non-obtrusive to vision, will prevent sample evaporation and ensure the device is safe to use. After extensive *in vivo*, *in vitro*, and *in silico* experiments it is hypothesised that the miniature device will remain mechanically stable during wear in the lower eyelid and not cause ocular irritation or corneal damage, ultimately leading to transfer of the collected sample volume to the analysis system following device removal. Analysis will result in the identification of numerous biomarkers and their concentrations for correlating to a variety of ocular and systemic diseases for diagnosing, monitoring, and staging clinical conditions. When compared against conventional collection methods, the device will collect tear samples that better represent the biomarkers present day-to-day as it will not require post-processing or reflex tear sampling.

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INVESTIGATING THE ANTI-INFLAMMATORY AND TISSUE-PROTECTIVE PROTIEN TSG-6 AS A REGULATOR OF INTERVERTEBRAL DISC DEGENERATION

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Abstract submitted for poster
Abstract themes: Cell and Gene Therapies, Enabling Technologies

Introduction:

Degeneration of the intervertebral disc (IVD) is a leading cause of lower back pain, and a significant clinical problem. Inflammation mediated by Interleukin-1 beta (IL-1β) and tumour-necrosis factor alpha (TNF-α) drives IVD degeneration through promoting a phenotypic switch in the resident nucleus pulposus (NP) cells towards a more catabolic state, resulting in extracellular matrix degradation. TSG-6 (the protein product of tumour necrosis factor-stimulated gene 6) is expressed during inflammation and has been shown to have anti-inflammatory and tissue-protective effects in a broad range of disease models including osteoarthritis. However, there has been little exploration of TSG-6 in the IVD. A greater understanding of endogenous TSG-6 expression by NP cells in the healthy and degenerate disc will shed light on its role in IVD homeostasis and its potential as a therapeutic target for IVD degeneration. Informative studies of the effects of TSG-6 on NP cells requires the development of *in vitro* 3D culture systems where cells deposit an NP-like matrix.

Methods:

Human IVD samples were obtained from patients undergoing surgery for disc degeneration and associated back pain. NP cells were extracted from IVD samples and cultured in monolayer prior to RNA extraction for qPCR analysis. Formalin-fixed, paraffin-embedded IVD samples were sectioned for RNAscope analysis. NP cells were expanded in monolayer before encapsulation in alginate beads using the $CaCl_2$ gelation method; beads were cultured in chondrogenic media for up to 28 days prior to being fixed and sectioned for histological analysis.

Results:

Using qPCR, we generated a large gene expression dataset and demonstrated that TSG-6 expression is positively correlated with the expression of a range of catabolic factors, including IL-1β and TNF, and negatively correlated with expression of the NP matrix component aggrecan. Exploration of mRNA expression by single cells *in situ* using RNAscope, showed expression of TSG-6 by NP cells in disc tissues with both low and high degenerative grades. A method has been optimised for 3D *in vitro* culture of human NP cells in alginate beads; extensive staining for the matrix component hyaluronan after 21 and 28 days in culture suggested that an NP-like matrix is being deposited in this model.

Discussion & Conclusions:

These data offer an insight into the expression of TSG-6 in the human IVD, both on extracted cells and human IVD tissue, which will be of use to future investigations. The development of a 3D culture model will be a valuable tool for characterising the effects of exogenous TSG-6 on NP cells.

DAY 3 Keynote Speaker

Dr Eileen Gentleman

MODULAR HYDROGELS IN ORGANOID-BASED DISEASE MODELLING

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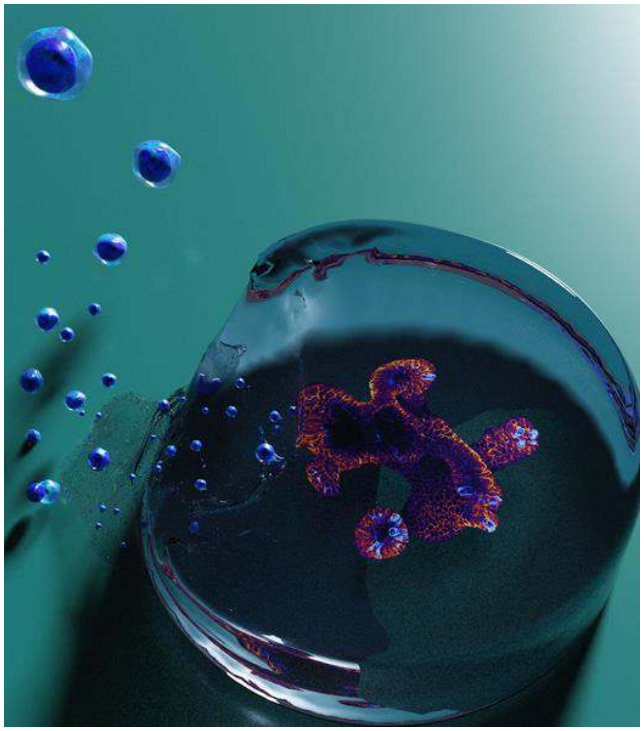
Pathological matrix remodelling plays a central role in many human diseases, but is challenging to study as *in vitro* models often cannot replicate the complex 3D cell-matrix interactions that drive pathologies. In this seminar, I will discuss how we built a 3D model of the human gut that allowed us to uncover an unexpected role for a rare immune cell type called ILC1 in driving gut fibrosis in patients with inflammatory bowel diseases. We used molecular dynamics simulations to design PEG hydrogels that cross-link quickly, but can still mimic the stiffness of normal intestinal tissue. We then co-cultured encapsulated human intestinal organoids with ILC1, and using a combination of atomic force microscopy force spectroscopy and multiple particle tracking microrheology, found that ILC1 drive intestinal matrix remodelling through a balance of MMP9-mediated matrix degradation and TGFβ1-driven fibronectin deposition. Our findings demonstrate the potential of using hydrogels in disease modelling, and open the possibility of unravelling how pathological matrix remodelling contributes to disease.



Material Nanopatterning and Properties

ADVANCED BIOACTIVE COATING FOR THE BIO-INTEGRATION OF SYNTHETIC VASCULAR GRAFTS

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Introduction

The implementation of prosthetic grafts has transformed the management of cardiovascular disease. However, graft failure still remains a persistent issue, that may be caused by anastomotic intimal hyperplasia, thrombogenesis and compliance mismatch[1]. It has been suggested over the years that a complete endothelial layer lining the lumen of vascular synthetic graft might result in higher patency rates and reduced intimal hyperplasia. Here, we propose a bioactive coating – a material-based strategy for the local presentation of appropriate growth factors in order to promote healthy endothelialisation in vivo. Briefly, it comprises an elastomer coating, Poly (Ethyl Acrylate) (PEA), sequentially functionalised with an extracellular matrix protein, fibronectin. Particularly, plasma polymerised PEA (pPEA) has been shown to allow the formation of nanonetworks of fibronectin (FN) fibrils. Such nanonetworks offer high availability of specific binding sites, such as integrin binding sites and heparin II binding domain[2]. Hence, selected growth factors will be locally presented on the coating, acting as the driving force for cell recruitment, adhesion and migration. Finally, the coating is implemented woven PET synthetic grafts to ensure its relevance.

Materials & Methods

A characterization study of the PEA-coated surface included XPS, water contact angle measurement, protein adsorption and growth factor binding assays. Additionally, blood leak tests were conducted to further explore the effect of PEA coating on the fabric. Initial cellular experiments focus on the effect of VEGF and FGF and their potential combined effects on human umbilical vein endothelial cells (HUVECs) – cell proliferation and adhesion, qualified and quantified with immunostaining of the actin cytoskeleton and membrane proteins. Cell migration studies are conducted under static conditions, with the use of barrier inserts.

Results & Discussion

Investigations show that pPEA is effectively coated on the complex topography of the fabric. Additionally, it appears to impact significantly the properties of the fabric. More specifically, pPEA-coated fabric samples appear more hydrophobic than the uncoated fabric. It is also important that pPEA/FN-coated grafts are becoming hydrophilic compared to pPEA only coated fabric, which allows better cell adhesion and viability, also seen in the cellular experiments. To this end, cell viability and proliferation are improved with the presentation of the growth factors from our system. Additionally, growth factors enhance cell-cell communications. Finally, initial cell migration studies show how growth factors do increase migration speed.

Conclusions

The proposed system, PEA coating functionalized with FN and growth factors, appears to provide the conditions for increased cell spreading, proliferation and adhesion. It promotes cell migration with increasing speed between the controls and the studied system.

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Acknowledgements

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DESIGN, MANUFACTURE AND CHARACTERISATION OF A DUAL ANTIMICROBIAL-OSTEOINDUCTIVE 'BONE WRAP'

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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 [1]. While osteoporosis is the most prevalent, there are more than 200 musculoskeletal conditions which affect a quarter of the UK adult population[1]. Additionally 300,000 NHS patients acquire a healthcare-associated infection (HCAI) each year[3]; 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. This project aims to develop polymer brush chemistries to provide direct control of material induced, cell adhesive and bacterial anti-fouling properties. 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 optimising an experimental workflow including *in vitro* and *ex vivo* characterisation methods which will be used to fine-tune the mechanical, cytocompatible, and cell-instructive properties of the 'bone wrap'. The protocols include fluorescent and electron microscopy, colorimetric assays and the optimisation of an innovative *ex vivo* foetal chick femur defect model.

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 scaffolds were characterised using water contact angle.

For microscopy, primary hMSCs were seeded onto scaffolds at an optimised density of 20,000 cells/cm^2 and cultured in basal medium for up to 28 days, at 37°C, 5% CO_2. Confocal imaging samples were fixed using paraformaldehyde, then stained for with phalloidin for actin, and DAPI for cell nuclei. For SEM, cells were fixed using glutaraldehyde, then dehydrated in ethanol and chemically dried using HMDS, before sputter coating with gold and imaging.

For the chick femur model, femora were isolated from E11 white chicken eggs, which were fractured near the midpoint using a scalpel. The defect site was then wrapped in an electrospun scaffold using different techniques to support the structure before culturing in basal medium for 14 days. Samples were then fixed using paraformaldehyde and analysed using μCT.

Results and Discussion

Water contact angles demonstrated that gelatine produced a borderline hydrophilic mesh that was not stable over 10 seconds. Increasing the PCL content makes the mesh more hydrophobic, and more stable over time on contact with water. Similarly, 100 % gelatine and 25:75 G:PCL samples degraded too quickly to be stable for cell culture, or imaging.

Representative confocal images of hMSCs cultured on the scaffolds showed that the stable scaffold blends are not cytotoxic. After 7 days in culture, all scaffold blends had affected the cellular response. The hydrophobic nature of 100% PCL scaffolds led to clustered cytoskeletal formation. 50% and 75% PCL blends promoted even cell coverage and well-developed cytoskeletal formations as shown through f-actin staining. Cell nuclei staining suggests infiltration of the less hydrophobic scaffolds by the cells, which is confirmed by SEM images. μCT analysis has been proven to clearly show the defect region and new bone formation.

Conclusion

A comprehensive testing regime has been developed for testing of cell-instructive modifications to a range of electrospun degradable scaffolds, including material characterisation and biological response analysis. Initial results show the foetal chick femur defect model is a promising technique to prove the effect of future modifications to the osteoinductive and bone regeneration capabilities of the dual-action scaffolds. This will allow optimisation of the cellular control when developing the osteoinductive and antimicrobial layers, in conjunction with the more standard *in vitro* cellular assays previously presented.

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Acknowledgements

Electrospun scaffolds were manufactured by PhD student Man Li, for research into antimicrobial implants.

ADVANCING PEPTIDE HYDROGELS FOR INTERVERTEBRAL DISC REPAIR

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Introduction

Lower back pain affects 80% of adults[1] and costs the UK £12 billion per annum.[2] Degeneration of the intervertebral disc is one of the main causes of lower back pain and starts during the teen years. Current treatment options include physiotherapy and cognitive exercises followed by spinal fusion which are expensive and have a limited efficacy. Self-assembling peptides have been evidenced as effective nucleus augmentation devices because they can be injected via a minimally invasive technique, gel in situ and restore biomechanical properties to the disc. Controlling and altering the primary structure of the peptides determines the chemical and mechanical properties of the hydrogel. This project looks at developing a new primary structure (P_{11}-34) to combine the advantageous properties of previously investigated hydrogels. Each peptide is injected simultaneously with a glycosaminoglycan, chondroitin sulphate which reduces the critical concentration for self-assembly. Chondroitin sulphate is present in the healthy intervertebral disc and increases the osmotic pressure of the nucleus pulposus allowing the disc to swell with water and resist load.

Materials and Methods

Three peptides, all 11 amino acids in length, are being investigated for their use as nucleus augmentation devices. Current testing is looking at the cytotoxicity, rheology profile and self-assembling properties of the new peptide. Cytotoxicity testing is being conducted with direct contact, extract, and indirect contact assays with BHK and L929 cells. Rheological testing is investigating the effect of needle length, needle gauge and primary peptide structure on the mechanical properties of the gel.

Results and Discussion

Cytotoxicity testing has shown little evidence of cytotoxicity however there is evidence of a cell specific response to the hydrogels with BHK cells exhibiting a smaller level of ATP production and L929 cells exhibiting a larger level of ATP production when compared to the respective negative controls. Rheology data has shown that the length and gauge of the needle has no significant effect on the viscoelastic properties of the hydrogels, but the peptide primary structure can alter the storage and loss moduli.

The different peptides can be used with chondroitin sulphate to form hydrogels with suitable properties for nucleus augmentation. The change in peptide structure alters the storage and loss moduli whilst maintaining the viscoelastic properties of the hydrogel. The cytotoxicity results show the possibility of a cell specific response to the hydrogels.

Conclusions

The three peptides investigated can be used to form hydrogels with suitable properties for nucleus augmentation. Future work will investigate which peptide forms the most suitable hydrogel by completing the remaining cytotoxicity testing as well as determining the rate of any hydrogel disassembly.

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MOLECULAR DYNAMICS SIMULATIONS OF BIOACTIVITY CHANGES ASSOCIATED WITH THE INCORPORATION OF COPPER IN SILICATE-BASED GLASSES FOR TISSUE ENGINEERING

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The active surface of silicate-based bioactive glasses (BG) has been widely used for different medical applications due to its strong active response in contact with a physiological medium [1]. The present project focuses on copper-containing silicate-based glasses (Cu-BG) for use in soft tissue engineering. Previously, the addition of copper ions into the basic BG composition have been only studied experimentally to investigate their effect on biological tissue [2] [3] [4]. To the best of our knowledge, this is the first time the effect of copper on bioactive behaviour of BG is studied via molecular dynamics (MD) simulation. The aim of the project is to describe the effect of copper incorporation into the BGs to establish a better understanding of the relationship between the microscopic structure and the bioactive behaviour of the glass. The simulations are carried out on a series of SiO_2-Na_2O-CaO-Cu_2O-P_2O_5 compositions. The amount of Cu_2O is increased at the expense of Na2O. An empirical fitting of classical interatomic force fields suitable for Cu^{+}-O and Cu^{2+}-O was performed using GULP [5] to reproduce the $AlCuO_2$, Cu_2O, $CuSiO_3$, and $Na2Si_3O_8$ crystal structures. The polarization is included via the shell model. Classical MD simulations of the above compositions were performed, and the partial pair-distribution functions, coordination numbers and their distribution are used to study the effects of copper integration on the local environment of cations and network connectivity. In the next step of this project, we will focus on the fabrication and characterization of the Cu-BG for the development of dermal scaffolds.

Keywords: bioactive glass; bioactivity; molecular dynamics; simulation; copper; structure properties; tissue engineering; scaffolds

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ANTIMICROBIAL COATINGS FOR THE PREVENTION OF CATHETER-ASSOCIATED URINARY TRACT INFECTIONS

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Introduction

Catheter-Associated Urinary Tract Infections (CAUTI's) are responsible for 17% of Healthcare Associated Infections in the UK, costing the NHS £99m annually.[1] Current treatments involve high doses of antibiotics (e.g. Nitrofurantoin) but this is problematic given the rising cases of antimicrobial resistance.[2] Nitric oxide (NO) is a potent broad-spectrum antimicrobial which reacts with reactive oxygen species (peroxide, superoxide) to generate reactive nitrogen species (RNS).[3] These RNS have several methods of bacterial inactivation such as lipid peroxidation, protein disfunction and DNA modification.[3] Given the multimechanistic action of NO, it is unlikely that bacteria will develop resistance, thus making this a highly effective alternative to antibiotics.[3]

Methods

Pristine PDMS was oxygen functionalized followed by self-assembly of mercaptopropyl trimethoxysilane (MPTMS) on the hydroxylated surfaces. The surfaces were immobilised with aminodextran macromonomers, followed by NO immobilization. Samples were characterised using Fourier transformed infrared spectroscopy (FTIR) and water contact angle (WCA). NO release was monitored using a Sievers 280i Nitric Oxide Analyser. Antimicrobial efficacy was investigated against P. mirabilis, E. faecalis and E. coli at 4 and 24 hours.

Results

FTIR was recorded to confirm each step in the reaction pathway (pristine PDMS, oxygen functionalised and silanised). Evidence of successful silanisation can be seen via the presence of the broad peak a 3300cm-1, attributed Si-O. Each of the steps in the reaction pathway were corroborated with WCA. NOA confirmed tethering of NO. Antibacterial efficacy was tested against 3 species of urobacteria. A 3 log reduction in the colony forming units was observed against P. mirabilis and E. coli, alongside a 2.5 log reduction against E. faecalis after 4 hours. After 24 hours, complete kill was observed against P. mirabilis and E. coli. A 1 log reduction was obtained vs E. faecalis after 24 hours, due to bacterial regrowth.

Conclusions

FTIR, WCA and NOA data provided evidence of successful NO functionalization of the surfaces. The functionalized surfaces show highly effectiveness against the tested strains at 4 hours and 24 hours for P. mirabilis and E. coli. Efficacy against E. faecalis was not as effective after 24 hours.

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SYNTHESIS OF NANOPARTICLES OF POLY (9, 9' - DIOCTYLFLUORENE) FOR POTENTIAL APPLICATION AS MATERIALS FOR USE IN RETINAL PHOTORECEPTORS

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Introduction

The increased prevalence of different types of eye retinal diseases such as retinal pigmentosis and age-related macular degeneration (AMD) has had a large impact on the growth in blindness cases in the global population. Therefore, one of the key challenges in regenerative medicine is to develop a novel approach for functional eye retinal prostheses. Applications of current systems based on photoactive inorganic semiconductors such as silicon photodiodes (SPD) have many limitations regarding requirements for complex electrical circuits, and external power supply and use of rigid inelastic structures with low biocompatibility. [1] Moreover, the generated electric signals from SPD are insufficient to efficiently transmit and trigger the neuron retinal ganglion cells (RGC). An alternative approach is to use consolidated films of conjugated polymer nanoparticles (CNPs) such as derivatives of poly 9, 9' - dioctylfluorene (PFO), that have maximum light absorption in the range of the natural blue S-cones photoreceptors. Light absorption by the CNPs leads to excitations of the CNP that are capable of triggering the retinal ganglion cells (RGC) and improve the visual perception. PFO CNPs are semiconducting organic materials with unique required properties such as photosensitivity, mechanical, optoelectronic properties, high light absorption, extinction coefficient, and are capable of electrical stimulation of the RGC cells. [2] In this study, we report a research strategy to synthesise and characterise the PFO CPNs for potential future application in the retina implant system.

Materials and Methods

The PFO CNPs were synthesised through the Suzuki-Miyaura cross-coupling reaction of the an aryl-boronate ester with aryl-dibromide monomers using a palladium catalyst ($Pd(PPh_3)_4$) and dispersed in miniemulsion. [3] The addition of the sodium dodecyl sulfate (SDS) as a surfactant stabilizes the CNP dispersion. The reaction mixtures were emulsified with three different total times of ultrasonication (2, 4 and 8 minutes). The process of the polymerization was conducted overnight at 72°C. The obtained CNPs dispersions were characterised using various techniques such as UV/Vis Spectroscopy, Dynamic Light Scattering (DLS), Gel Permeation Chromatography (GPC), to investigate the influence of the different applied sonication times on selected physical properties and morphology.

Results and Discussion

The evaluation of the optical properties using UV/Vis spectroscopy of all analysed PFO CNPs dispersions revealed the maximum absorbance peak at 376 nm. Additionally, it was noted the appearance of a peak at 440 nm with lower intensity, this peak corresponded to the presence of the β-phase ordered morphology of the backbone PFO polymer. The results of the hydrodynamic diameter and polydispersity index (PI) obtained from DLS measurements show the tendency to decreasing the average particle diameter and polydispersity towards at longer time of the performed ultrasonication. The size of the PFO CNPs was significantly reduced from 177 nm for 2 minutes ultrasonication to 92 nm for the longest sonication time (8 minutes). In addition, the extended sonication time considerably narrowed the normal distribution of the diameter of the particles and consequently, it ensures the formation of smaller nanoparticles with a low polydispersity (PI = 0.16). Determination of the polymer molecular weight was performed by the GPC, it was observed the variations of the number average molecular weight (M_n) from 3200 to 5300 g/ml, which were increased with longer sonication time. The values of the polydispersity index (PDI) represented as a ratio between the weight average molecular weight (M_w) and M_n were in the range from 1.9 to 2.9.

Conclusions

We have demonstrated a successful strategy to synthesise the polyfluorene nanoparticles in the range of diameter below 100 nm using the Suzuki-Miyaura cross-coupling reaction in the miniemulsion stabilized by SDS surfactant. It was confirmed that longer sonication times lead to a significant reduction in the hydrodynamic diameter of the formed nanoparticles. Overall, these results provide a solid foundation to obtain uniform CNPs dispersion with appropriate optical properties for future application as the photosensitive platform to imitate the function of the blue S-cones retinal photoreceptors.

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PRODUCTION OF A PHOTOCURABLE POLYHYDROXYALKANOATES FOR LIVER TISSUE SCAFFOLDING IN AN ORGAN-ON-CHIP DEVICE

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Perhaps more than ever, there is a great impetus for science, and biomaterials in general to strive for a greater sense of sustainability in research. My PhD project, which involves the production of Poly (3-hydroxybutyrate-co-3-hydrovyvalerate) or PHBV, and its subsequent use as a cell supporting scaffold, will come from a renewable, bacterial source. After transesterification with a further chemical agent to allow it to be 3D printed into a cell-supporting scaffold, this complex can ultimately be incorporated into a three-dimensional latent flow Organ-on-chip device, for drug testing on the cell/tissue sample.

Further to the sustainable production aspect, the potential that this concept has, in the field of drug testing, proposes a solution to the age old issue of animal and tissue testing on new chemical compounds, by providing a small-scale, harm-free and more accurate model.

In order to make the polymer suitable for stereolithography, based upon a previously conducted regime (Hirt et al. 1996), transesterification with ethylene glycol, will produce photocurability in the PHBV. Without such reaction, the polymer be unable to be printed otherwise, as its glass transition temperature is far too low. Following production of the scaffold, and prior to cell seeding, the printed polymer will be subject to a multitude of tests, including mechanical analysis and thermogravimetric analysis, to uncover information regarding its tensile properties and transition temperature points, and overall suitability as a cell-supporting scaffold.

The scaffold is intended to be seeded with a soon to be chosen human liver cell line, due to the liver's importance in the metabolic pathway. Its response and integration and therefore biocompatibility of the scaffold will be measured in time using a Live/Dead assay.

The physical properties of this scaffold are intended to be similar to those of other polyhydroalkanoate, polycaprolactone, and hydrogel scaffolds, i.e. malleable, yet supportive, with a structure as hollow as possible, and of course biodegradable.
In all, this project aims to adapt the existing yet new technology of Organ-on-chip, whilst advancing on the little explored area of photo curing PHBV.

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INVESTIGATING THE HIERARCHAL EFFECTS OF MULTIFUNCTIONAL 3D BIOMIMETIC SCAFFOLDS ON CELL BIOLOGY

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Introduction

Technology for modulating stem cell behaviour via production of 3-dimensional cell culture substrates is currently lacking. This is because existing technologies fail in their ability to mimic the varied biological cues introduced to cells and tissues *in vivo*. Such failure results in highly variable and immature tissue engineering outputs which lack the features of the *in vivo* counterparts. Manufacture of bespoke multifunctional scaffolds using photocurable materials with known effect on cell biology can solve this[1]. Multifunctionality includes defined microtopography, mechanical and surface chemical properties, and conductivity profiles. All such properties have shown an effect on cell behaviour, but it is anticipated that such effects may be decoupled from one another and thus evidence of hierarchy or synergy described[2,3,4].

Methodology

Scaffold topography and geometry is controlled here using computer-aided designs and the BMF Microarch S130 high resolution 3D printer. Mechanical properties are controlled through the addition of flexible co-polymers also identified through cell adhesion arrays[1]. Surface chemistry is characterised through Raman Spectroscopy (RS) and Atomic Force Microscopy (AFM). Conductivity is controlled through the addition of conductive nanoparticles and co-polymers to monomer resins and assessed using Electrical Impedance Spectroscopy (EIS) and Cyclic Voltammetry (CV). IPSC-CMs are produced using established protocols.

Results

RS and AFM has identified no phase separation of co-polymers. To determine whether mechanical properties can be controlled, young's modulus was analysed for Pentaerythritol Triacrylate (PETrA) as the major and minor component (70:30) of a co-polymer structure with Ethylene glycol dicyclopentenyl ether acrylate (EGDPEA). Co-polymer resins featuring varying ratios of tri:monoacrylate monomers do not result in varying structure mechanical properties. PETrA homopolymer and co-polymers showed highly similar Young's Modulus with all analysed structures between 116 and 117kPa. EGDPEA alone showed a modulus of 31kPa. Inclusion of conductive carbon nanoparticles (MWCNTs) to PETrA homopolymers significantly decreases young's modulus vs naïve (96.47kPa vs 116.47kPa, p=0.0002). However, MWCNTs are not fully dispersed as shown by RS and AFM and EIS data with no effect on conductivity seen compared to naïve PETrA (14.82kΩ vs 15.24kΩ at 100kHz). Adding a conductive co-polymer, Polyaniline (PANI), reduces impedance vs naïve PETrA (14.08kΩ vs 27.49kΩ at 100kHz), and materials begin to affect the electrochemical performance of the circuit. It is currently unknown whether PANI affects material mechanical properties

Conclusion

Mechanical properties of materials are not affected by the ratio of monoacrylate:triacrylate groups in the resin. Multi-walled Carbon nanotubule (MWCNTs) inclusion renders the material more elastic, possibly due to the interference of MWCNTs with polymerization thus affecting chain length or crosslinking. MWCNT inclusion does not have the desired effect of modulating material electrochemical properties due to lack of particle dispersion throughout the structure. Addition of doped PANI to homopolymer resins shows significant effect in modulating material electrochemical properties. PANI appears well dispersed throughout printed structures and this interconnectivity affects electrical performance. EIS data suggests that PANI may render structures able to perform as bipolar electrodes. RS data concludes that scaffold surface chemistry is unchanged by inclusion of co-polymers, representing an opportunity to decouple cell-surface interactions and substrate mechanical properties when assessing biological effects.

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Bioengineered Models 2

GRAPHENE BASED BIOINKS FOR 3D PRINTING BIOACTIVE ARTICULAR CARTILAGE IMPLANTS

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Articular cartilage (AC) has a poor capacity for self-repair, consequently defects are one of the major causes of immobility and poor quality of life for millions of individuals worldwide. Growth factors from the TGF-β and BMP signalling families are key regulators of the production and maintenance of the articular cartilage phenotype and consequently are a feature of many in vitro protocols for chondrogenesis. However, their use in regenerative therapies is limited by their short half-lives and low protein stability. In this project we aim to use Graphene Oxide (GO), a 2D carbon nanomaterial, to deliver a chondrogenic growth factor to human pluripotent stem cell derived chondroprogenitors within 3D printed hydrogel/scaffolds to generate articular cartilage tissue.

Methods: In order to determine an appropriate concentration range of GO to use throughout this project we began with investigating the cytotoxic effects of GO-cell exposure. A human chondrocyte cell line (TC28a2) was chosen as a model to assess the cytotoxicity of 3 sizes of Graphene oxide (large (2-8µm) small (25nm-1.5µm) and ultra-small (10nm-590nm) before moving on to human embryonic stem cell (hESC) derived chondroprogenitors. For further characterisation of cell- material interactions, cell uptake and plasma membrane interactions were investigated via confocal microscopy with graphene oxide and live-cell imaging with Cell Mask green membrane stain. We also investigated the effects of GO on TGF-β signalling activity within the human chondrocyte cell line using a SMAD2/3 dependent reporter developed by Dr Steven Woods (Humphreys *et al.*, 2020).

Results to date show no cytotoxicity of graphene oxide with Tc28a2 cells after 72 hours. Confocal microscopy provided novel insight into the interaction between GO and a chondrogenic cell line indicating uptake was size dependent with only large GO being taken up noticeably. Interestingly we have found that GO alone was able to significantly increase TGFβ signalling within the human chondrocyte cell line which could reduce the amount of growth factor required within our final GO construct. Further work is required to determine the mechanism behind this effect and whether or not it is sustained.

Conclusion, this project seeks to bring together established protocols to introduce GO into our biomaterials, capable of guiding hESC differentiation towards articular chondrocytes by the spatio-temporal delivery of a chondrogenic growth factor.

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NOVEL MODELS OF MAMMALIAN WOUND HEALING AND REGENERATION

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Introduction

It has long been of clinical interest to stimulate scarless wound healing after traumatic injuries. Healing of composite tissue injuries in complex structures such as the hands is particularly difficult to coordinate. The resultant scarring can be debilitating, as fibrosis of the tendon and skin layers leads to loss of digit functionality. No animal models of hand injury including tendon currently exist in the literature. We describe a new model of composite tissue injury, including tendon, in the mouse digit. We assess its utility in comparing healing phenotypes between wild type mice and MRL/MpJ mice, which show regenerative ear healing[1]. Additionally, we have developed an *ex vivo* culture system of mouse eyes to examine three corneal injury types varying in severity, which demonstrate both scar-free healing and eschar formation. This system will facilitate further examination of the underlying causes of epithelial scarring in mammals.

Materials and Methods

In vivo digit and ear punch: An observational study of a microsurgical murine composite tissue injury of the digit in C57/Bl6 (wildtype) and MRL/MpJ (regenerative healer) mice was performed and assessed for healing over 42 days. The injury involved excision of the neurovascular bundle, tendon and skin between the distal interphalangeal joint and the proximal digital crease. Concordant ear punches were made to assess the regenerative capacity of the mice during their healing. Tissue was harvested at day 7, 14, and 42, along with unwounded contralateral controls. Digits were decalcified in EDTA, and both tissue types were processed into wax. *Ex vivo* eye injuries: C57Bl/6 and MRL/MpJ mice were enucleated. The eyes were injured using three methods: epithelial scraping using Vannus scissors, and partial thickness and full thickness burns using a Bovie cauteriser. The eyes were stained for LIVE/DEAD (Invitrogen, cat no. L3224) and imaged on a stereo microscope over a period of 7 days. Healing of each injury model was assessed both from wholemount examination and from sectioning and staining with haematoxylin and eosin. Collagen was stained with picrosirius red. Images were analysed using Image J software.

Results/Discussion

As previously reported, MRL/MpJ mice showed either improved healing or regeneration of the ear pinnae tissues over 42 days, whilst C57Bl/6 mice healing stalled after approximately 2 weeks. Neither mouse strain showed perfect regeneration of digit tissues at 42 days; however, MRL digits showed signs of ongoing tissue remodelling which is being probed further using immunohistochemical and proteomic analysis. Future investigation of the molecular differences between the two mouse groups may identify cues for better healing of such injuries in a clinical setting. Preliminary analysis suggests that MRL/MpJ eyes cultured *ex vivo* show improved healing compared to C57Bl/6 eyes over 7 days. Scrape wounds and partial thickness burn injuries in both strains heal within 3 days. However, C57Bl/6 eyes do not show healing of full thickness burns due to formation of thick eschar at the wound site which impedes cell migration, whereas in some MRL/MpJ eyes, this eschar appears not to form. This wounding system is a promising model for mechanistic interrogation of eschar formation and regenerative healing of epithelia in MRL mice.

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DISSOLVING MICRONEEDLE ARRAYS: EFFECT OF POLYMER COMPOSITION ON PHYSIOCHEMICAL PROPERTIES

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Introduction

Dissolving microneedle arrays (DMNs) combine the benefits of increased drug penetration of intravitreal injections (IVIs) with the user-friendly nature of topical dosing to provide an exciting alternative system for ophthalmic drug delivery.[1] DMNs require sufficient mechanical strength for application with minimal deformation, and a fast dissolution rate for maximum drug release and minimum patient discomfort. DMNs are formulated from water soluble, biodegradable polymers such as poly (vinyl pyrrolidone) (PVP).[2] Plasticisers, such as poly (ethylene glycol) (PEG) can be introduced to DMNs to alter their physiochemical properties; the additive disrupts interchain interactions and introduces flexibility and chain mobility between polymeric chains within a rigid structure.[3] The aim of this investigation is to determine the effects of increasing the concentration and varying the molecular weight (M_w) of PEG within PVP:PEG blends on both the mechanical strength and dissolution rate of baseplates (BPs) and DMNs to determine an optimum composition.

Materials and Methods

PVP (K30, 40 000 g mol^{-1}) and PEG (1 000, 2 000 and 5 000 g mol^{-1}, 5 – 40 wt. %) BPs and DMNs were fabricated *via* the solvent casting method. Mechanical strength of baseplates and DMNs were measured *via* compression testing at a rate of 500 µm s^{-1} and 50 µm s^{-1} respectively until a maximum load was reached. Dissolution rates (mg mL^{-1} s^{-1}) of BPs and DMNs were measured using refractive index (RI) measurements within an aqueous solution and *via* insertion into an *ex vivo* corneal model respectively.

Results

Incorporation of PEG (1 000 and 2 000 g mol^{-1}) within PVP:PEG BPs at increased concentrations led to a reduction in the Young's modulus relative to PEG (5 000 g mol^{-1}) which resulted in an increase in the Young's modulus (Figure 1A). Increasing concentrations and increased M_w of PEG within baseplates studies showed a faster dissolution rate (Figure 1B). Dissolution rates of DMNs showed the incorporation of PEG within the *ex vivo* porcine corneal model resulted in a faster dissolution compared to when no PEG was present.

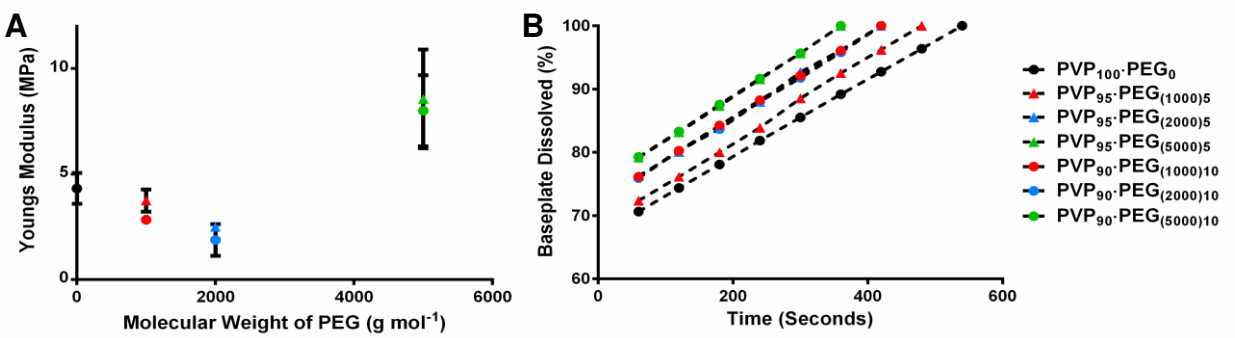

Figure 1: *Effect of PEG incorporation at varying concentration and M_w within $PVP_{(\%)}$·$PEG_{(Mw)(\%)}$ BPs and DMNs on **A)** mechanical strength and **B)** dissolution rate.*

Discussion and Conclusions

PVP:PEG BPs and DMNs were successfully fabricated to investigate the effect of plasticiser incorporation on mechanical strength and dissolution rate properties. Incorporation of lower M_w PEG (1 000 - 2 000 g mol^{-1}) highlighted the ability of PEG to disrupt intermolecular interactions between PVP chains, confirmed *via* a decreased mechanical strength and increased dissolution rate. The incorporation of PEG at a higher M_w also resulted in a faster dissolution rate, due to the increased concentration in hydrogen-bond donors, however a more rigid structure was seen *via* an increase in the Young's modulus. This increase in mechanical strength is due to the semi-crystalline nature of PEG with higher Mw being responsible for additional crystallinity.[4] Overall this study demonstrated physiochemical properties of materials used to fabricate DMNs can be tailored *via* plasticiser incorporation to provide bespoke properties for minimally invasive materials for ophthalmic drug delivery.

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INVESTIGATION OF *HNF1B*-ASSOCIATED RENAL DISEASE IN A HUMAN KIDNEY ORGANOID MODEL

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Abstract Body

Introduction: Heterozygous mutations in the *Hepatocyte nuclear factor 1B* (*HNF1B)* gene are the most prevalent monogenic cause of congenital kidney disease. Several mutations have been identified and display a phenotypic heterogeneity that does not correlate with the mutations. Renal cysts are the most common disease feature, among a wide range of renal and extra-renal abnormalities.

Materials and Methods: Our research uses a CRISPR-Cas9 gene-edited human pluripotent stem cell (hPSC)-kidney organoid model of *HNF1B*-associated renal disease to understand the disease mechanism.

Results and Discussion: Mutant organoids display histological disease features that include the presence of large multi-layered tubule structures and infrequent, enlarged glomeruli (as we have also shown in patient induced PSC-derived organoids). RNA-sequencing comparing the transcriptome of isogenic hPSC-organoids with and without an *HNF1B* exon1 frame-shift mutation and immunohistochemistry revealed that the endocytic receptors Megalin and Cubilin, expressed by proximal tubule cells, are misregulated in mutant organoids. In mutant organoids, Megalin was not detected by immunohistochemistry and Cubilin was not localised to the apical membrane. Both receptors are responsible for reabsorption of ultrafiltrate proteins and their misregulation may account for some of the renal dysfunction in *HNF1B*-associated renal disease, with proteinuria being a common feature.

Conclusion: This work demonstrates the utility of hPSC-kidney organoid disease models for investigating the molecular basis of human disease.

ROUND, RELIABLE CELL SPHEROIDS FOR REPRODUCIBLE CELL-BASED ASSAYS

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Introduction

3D in vitro model systems becoming more and more relevant in cancer and stem cell research as well as in pharmaceutical applications due to their potential to resemble natural microenvironment and human pharmacokinetics. Unnatural 2D cell cultures restricted to cell-cell contacts in one dimension and cells reveal an altered cell behavior influencing e.g. drug response. 3D cell spheroids – dense cell aggregates – allow cell-cell contacts in all dimensions for more precise validation of cell-based assays.

Materials and Methods

We developed a chemically defined and biologically inert coating solution which can be easily applied and prevents nonspecific binding of proteins and cells. In this environment cells start to aggregate and form cell spheroids by connecting with each other. We benchmarked our new technology using different cell lines.

Results and Discussion

The new technology is extremely robust and shows a stability over several washing and mechanical surface treatment steps. Our new technology shows a reproducible, rapid generation of round spheroids within 24 h outperforming current competitors. Furthermore, spheroids in coated 96-well plates are highly viable and reveal a regular 3D shape.

Conclusions

This new technology allows the development of spheroid-based model systems within a remarkable short time and outperforms current competitors. The reliable system allows the establishment of model-based assays for high throughput applications in pharmaceutical as well as medical research.

3D MODELLING SKIN WITH PODS® PARTICLES

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Introduction. In the field of skin and wound healing pre-clinical research, obtaining reliable results and safety data is essential for subsequent clinical trials with patients. This requires a full complement of skin cells and a mechanically equivalent microenvironment, ideally with an immune system. Currently, research investigation of trauma, chronic wound healing, and scarring necessitates the use of animal models, as available skin models lack the required complexity. This is costly, associated with an ethical burden, and the relation between the recorded results and the response in humans can be skewed by inter-species differences. Hence, there is a need for a functional skin model which recapitulates the tissue's native features. Such a model presents an opportunity to advance the reliability of pre-clinical skin research, as well as reduce its cost. Furthermore, it has the potential to better align the field with the principles of the three R's – to Replace, Reduce and Refine the use of animals in research. One of the main issues when modelling skin in 3D is mimicking the continual exposure of cells to biological cues in a controlled manner, throughout the construct. To address this, our project will employ PODS® particles. They comprise polyhedrin microcrystals produced in insect cells which can be loaded with different growth factors. Their small size and zero order-like kinetics allows for them to be incorporated in bioscaffolds, while preserving microstructure and providing prolonged local release of growth factors. In our project, we aim to utilise this potential by incorporating differentially loaded PODS® particles in a cell-seeded 3D environment of our own design which recreates native skin's mechanical properties.

Materials and Methods. The mechanical properties of the extra-cellular matrix (ECM) of skin play an important role in defining the fate and function of the resulting tissue. (1) A review of the literature was performed to identify the native stiffnesses of the epidermis (excluding the top cornified layer); the dermis – both papillary and reticular; and the hypodermis. Multiple hydrogel-based compositions are being evaluated for their potential to meet both the biological and the mechanical requirements. We are testing a natural and a synthetic hydrogel base: the former utilising Ca^{2+} crosslinking and the latter – UV cross-linking. Various techniques are explored for each, so as to achieve maximal batch-to-batch consistency, which is quantified by assessing homogeneity. Currently, measurements are performed on the natural-base hydrogel with X-Ray Fluorescence (XRF) scanning for Ca^{2+} distribution. Once satisfactory properties for both hydrogel bases are achieved, their effect on skin cells' response will be tested. Unless any undesirable effects are observed, we will continue by quantifying PODS® particles' properties when incorporated to our gels. This will be followed by introducing the PODS® particles in cell-seeded structures to modify skin cells' phenotypes and interactions. The latter will be further extrapolated by 3D bioprinting technologies which will allow the intricate engineering of the physical properties of the construct. These technologies will also facilitate the spatial distribution of differentially loaded PODS® particles.

Results and Discussion. Data from the literature suggests the stiffness of each of the human skin layers is as follows: Epidermis: E = 300 – 600 kPa (2); Papillary dermis: E = ~0.75 kPa (3); Reticular dermis: E = ~1.25 kPa (3); Hypodermis: E = ~1.6 kPa (4), where `E` denotes Young's modulus. These data define the variance in stiffness we aim to achieve with our UV-crosslinked synthetic hydrogel base, as well as the Ca^{2+}-cured natural hydrogel base. Initial results from the XRF scanning show a heterogenous distribution of calcium in the natural-base hydrogel, pointing to uneven cross-linking. This will be a potential inducer of variability, once cells are introduced. To address this, we are working on a system which slows down the Ca^{2+}-mediated cross-linking, and thus allows for more homogenous and consistent constructs.

Conclusions. A sufficient amount of data has been reported in the literature to allow recapitulating the mechanical properties of skin in a 3D model, given the scope of the project. Introducing the aspect of mechanical stiffness will give our model a dimension of representativity which is important but under-explored in the field of skin modelling. Further work is needed, as far as manufacturing techniques are concerned, to ensure the reliability of any cell-derived data which is to be generated in the next stage of the project.

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DEVELOPMENT OF A TISSUE-ENGINEERED CONSTRUCT USING POLY(GLYCEROL SEBACATE) METHACRYLATE AND UNCULTURED ADIPOSE-DERIVED CELLS FOR ADIPOSE TISSUE ENGINEERING.

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Introduction

Soft tissue repair is required following various traumas to the body, including damage from oncologic resections, such as breast cancer mastectomies or for facial reconstructions or congenital abnormalities. These soft tissue defects can not only affect patients cosmetically, emotionally and psychologically, but can also impair function. The current gold standard treatment for these conditions, autologous fat grafting, has limitations, including a requirement for repeat procedures, varying efficacy depending on the skills and techniques of the clinician, and a 40-60% reduction in graft volume following transplantation[1]. This loss of volume is thought to be associated with a lack of viable cells being grafted, poor survival of mature adipocytes once implanted, and poor revascularization at the donor site. Therefore, there is a need for a standardised procedure to repair soft tissue defects that can maintain volume following implantation.

Adipose tissue engineering is a field that aims to address the challenges presented by soft tissue replacement. Despite recent advances, a suitable strategy to replace autologous fat grafting that can be clinically translated has yet to be found. For most tissue engineering applications, scaffolds are required to provide mechanical support and promote cell survival and integration. These scaffolds require certain properties to make them fit for purpose, such as biocompatibility and biodegradability, as well as suitable mechanical properties. Interest in poly(glycerol sebacate) as a candidate scaffold biomaterial for adipose tissue engineering has increased in recent years as it has an assortment of properties that are better suited to match soft tissue than other commonly used biomaterials. Additionally, by introducing methacrylation to the polymer during synthesis, the mechanical and degradation time can be tailored to suit the requirements of the target tissue[2].

In addition to a scaffold, to encourage the growth of new adipose tissue, a source of regenerative cells may be required. Adipose tissue is an excellent source of autologous cells that can be easily harvested via liposuction procedure. Alongside mature adipocytes, adipose tissue contains a group of cells that are thought to have excellent regenerative potential, known as the stromal vascular fraction (SVF). The SVF contains extracellular matrix and cells such as pericytes, immune cells and a population of mesenchymal stem cell-like cells known as adipose-derived stromal cells. These adipose-derived stromal cells have shown good regenerative effects in tissue-engineered models, however, their requirement to be isolated and expanded in culture limits their clinical translation.

Researchers have developed a method to mechanically process lipoaspirate adipose tissue, removing mature adipocytes and creating a gel-like cell suspension of SVF cells and extracellular matrix[3]. This SVF gel can then be injected back into the patient directly after processing and has been shown to promote wound healing in animal models. The main advantage of this technique is that it is purely mechanical and does not require the addition of any exogenous material, or the *ex vivo* expansion of cells, factors that can often limit clinical translation through regulatory pathways.

Project Aims

This PhD project aims to develop a tissue-engineered device that can aid the regeneration of soft tissues. This device would incorporate a biodegradable poly(glycerol sebacate) methacrylate scaffold, to provide mechanical and structural support, with uncultured adipose cells (SVF gel) to provide a source of regenerative cells and stimuli. The project will explore how uncultured adipose cells can be incorporated into PGS scaffolds; how the properties of these scaffolds can be optimised to promote cell attachment and proliferation; and the ability of the construct to regenerate adipose tissue *in vitro*.

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IMPROVING EX VIVO BONE MARROW MODELS FOR STEM CELL MAINTENANCE

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Introduction

The therapeutic potential of hematopoietic stem cells (HSCs) in bone marrow transplants for blood diseases such as leukemia is well known. However, harnessing their full potential is limited since they quickly lose their stem cell properties in vitro. In the marrow niche, HSCs are supported to self-renew by mesenchymal stromal cells (MSCs). However, despite significant research, MSCs fail to adequately support HSCs ex vivo. These limitations in ex vivo culture of stem cells are believed to be due to lack of regulatory signals that are normally supplied to HSCs and MSCs within the bone marrow niche from the extracellular matrix. Biomaterials have been of great interest to mimic aspects of the 3D environment of tissues[1]. In our laboratories, recent work demonstrated that pericytes (MSC-like precursors) can be maintained and supported using low stiffness collagen hydrogels on PEA coated surfaces functionalised with fibronectin and the growth factor BMP-2 in vitro to mimic aspects of the bone marrow niche[2]. This 3D niche model could support MSC niche characteristics including nestin, SCF and CXCL12 expression and promote maintenance of long-term HSCs[2]. However, the use of collagen hydrogels brings limitations to this model since they are animal derived, difficult to modify and demonstrate batch-to-batch variability. Alternative hydrogels are therefore of interest to advance this model.

Materials and Methods

This new project will investigate the use of alginate gels in our PEA-collagen system to improve HSC maintenance. Alginate offers a highly tunable and modifiable alternative to collagen, offering flexibility in adjusting its properties and has the potential to preserve cells in a more quiescent state.
Current approaches to the project involve repeating the PEA-collagen niche system using bone marrow derived MSCs (BM-MSCs) as opposed to pericytes. MSC phenotype will then be investigated using several techniques such as in-cell western, immunofluorescence microscopy and ELISAs to monitor the expression of MSC niche characteristics such as nestin, CXCL12 and SCF expression.

Results and Discussion

Current data repeating the pericyte-HSC work in collagen gels with MSCs supports the concept that the addition of low stiffness collagen hydrogels promote an increase in nestin expression and supportive phenotype in MSCs. Next, HSCs will be added in co-culture to assess the ability of MSCs in this model system to support and maintain long term HSCs in culture. Finally, once the system with BM-MSCs fully supports previous findings using pericytes, we can look into further modifying the system by replacing low stiffness collagen hydrogels with a more highly tunable gel, alginate. Alginate gels have been used in previous studies to encapsulate MSCs whilst maintaining their viability, functionality and paracrine secretions to secondary cell populations[3] and therefore provides a hopeful alternative that will allow scale up and broad usefulness.

Conclusions

In conclusion, the use of biomaterials to mimic the 3D environment of tissues is promising to harness the full potential of stem cells in the bone marrow. This is important to investigate the physiology and pathology of haematopoiesis, develop drug screening tools and investigate cellular based therapies. Our current PEA-collagen system shows potential in maintaining MSC phenotype and maintaining long-term HSCs however, the replacement of pericytes with BM-MSCs and collagen hydrogels with alginate gels could further improve this model.

Acknowledgements

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BIOENGINEERING DUAL GRADIENT PLATFORMS FOR THE CONTROL OF CELL BEHAVIOUR AND DIFFERENTIATION

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Introduction

The development of neurons *in vivo* is dependent on the dynamic local environment, whereby tightly controlled signalling cues, specific morphogen gradients and the composition of the extracellular matrix enable precise control over cell fate. In particular, morphogen gradients such as that provided by Wnt, and proteins such as nerve growth factor (NGF) play key roles in the movement towards certain lineages, neurite extension and cellular migration. Harnessing these vital characteristics *in vitro* could provide an improved culture environment by mimicking the cues presented *in vivo.* Here, we report the development and use of dual gradient polymer surfaces, using surface initiated atom transfer radical polymerisation (SI-ATRP) of poly(potassium 3-sulfopropyl methacrylate) (pKSPMA). The gradient is provided by continuous and orthogonal changes in polymer chain length and density across the surface. Further functionalisation through the tethering of NGF is also reported. We demonstrate the differences in attachment, neurite outgrowth and differentiation dependent upon regional interaction with the dual gradient surface. Development of a platform that enables a stable, consistent, and cost-effective way to define cell characteristics is important for the potential use within a clinical setting, where specific neuron subsets could be developed for use as a therapy for disease.

Materials and methods

A dual gradient was synthesised by firstly applying a changing density of surface-bound SI-ATRP initiator 2-bromoisobutylryl bromide (BIBB), followed by sequential attachment and growth of polymer. BIBB density was reproducibly prepared by the backfilling of an inert silane with the pKSPMA then grown by dynamic filling of a chamber with the ATRP monomer solution. Chain density and length gradients were produced orthogonal to each other. NGF and FGF functionalisation was subsequently achieved via NHS/EDC covalent immobilisation to the ends of the pKSPMA polymer brush. Surface characterisation via XPS, WCA and fluorescence microscopy confirmed the surface chemistry at each stage. SH-SY5Y neuroblastoma cell line was used to assess cellular response to the surfaces.

Results and Discussion

pKSPMA surfaces were used as an anti-fouling control to prevent non-specific cell attachment; some cell attachment was observed in areas of low density and low chain length, but data was used as a background for comparison with growth factor-tethered surfaces. The covalent tethering of NGF and FGF respectively provides a specific protein gradient environment. On protein bound gradients, the level of cell attachment was improved in areas of higher density, where protein concentration was higher. Further, neurite outgrowth was enhanced on NGF bound surfaces compared to pKSPMA alone, with a movement towards higher neurite numbers per cell. This was found to be dependent upon NGF concentration with some interaction also with polymer density: mid-ranging density generally gave rise to longer neurites. FGF and NGF were also compared. Time lapse imaging enabled the dynamic nature of attachment, migration and neurite growth of SH-SY5Ys to be determined.

Conclusions

Biomaterials surfaces are well known to play a major role in determining cell fate, with cells often mediating their environment by deposition of growth factors and proteins to better support cellular characteristics. Here we demonstrated a biomimetic surface presenting dual gradient in polymer density and chain length, with additional binding of NGF presented as a gradient. We found that cell attachment was highly impaired by the underlying polymer at high density and longer chain length, with cells attaching readily to NGF and FGF surfaces. A general trend towards longer neurites was observed on mid-ranging NGF concentrations, indicating some control over cell parameters through the underlying surface conditions. This work could lead to a significant step-change in cell culture materials development for, e.g. the controlled differentiation of stems.

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DEVELOPMENT OF GRAPHENE-CONTAINING, 3D PRINTED SCAFFOLDS FOR ORTHOPAEDIC APPLICATIONS

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Introduction

There is a huge unmet need for millions of people worldwide who suffer bone loss due to injury, infection, disease or abnormal skeletal development for substitute bone that can seamlessly incorporate itself into the body [1, 2]. Synthetic materials that can mimic natural bone architecture at the micro- and macro-scale as alternative bone graft substitutes eliminate risks of donor site morbidity and infection, whilst being readily available [2]. Substrate intrinsic characteristics such as chemistry, nanotopography and mechanical properties can also induce adult stem cell differentiation towards specific lineages [1, 2].

For bone cell growth, stiff 3D porous scaffolds that mimic the bone tissue microenvironment and encourage vascularization and extracellular matrix deposition are needed, which traditional methods such as freeze-drying and salt-leaching alone cannot produce [1, 2]. Three-dimensional printing technologies are capable of seamlessly controlling scaffold size, density and pore interconnectivity due to uniform deposition of material according to a computer aided design, thereby producing patient tailored architectures.

Electrical stimulation (EStim) has been reported in many animal and clinical studies to enhance bone tissue regeneration. However, to date, scaffold designs that couple critical requirements of bone tissue engineering (BTE) and electrical compatibility are yet to be simultaneously achieved [3]. Graphene is an atom-thick material, which due to its remarkable electrical conductivity, tuneable surface chemistry, maximal surface-to-volume ratio, easy functionalisation capacity and mechanical properties may present an ideal solution [4, 5].

Materials and Methods

This project will focus on the development of alginate-graphene based inks for extrusion 3D printing of electrically conductive BTE scaffolds. Post-printing processing techniques shall also be investigated, such as those that may impart a higher degree of porosity and/or mechanical properties. Various imaging and characterisation techniques shall be used to establish scaffold biological, chemical, mechanical and electrical compatibility specifically for BTE.

Expected Results and Discussion

It is predicted that the optimal current for bone tissue regeneration ranges from 5 to 20μA as various studies have reported stimulation of bone repair through induction of alkaline phosphatase enzymatic activity, extracellular matrix deposition and mineralisation [6, 7]. It has also been observed that a current above 50μA induces cell death and bone necrosis, and so careful consideration of current strength as well as application duration, frequency and distribution will be required [6, 7]. Variations in graphene derivative, concentration, size and shape are expected to affect scaffold conductivity, mechanical properties and levels of toxicity to cells [4, 5]. It is also hypothesised that adjusting the molecular weights, solution concentrations and molar ratios of sodium alginate for various ink compositions shall affect ink rheology and therefore printability [8].

Conclusion

EStim in BTE has already proven its correlation with bone healing via clinical therapies and is now being translated into research environments that aim to investigate responses at a cellular level. However, the challenge remains in the construction and preparation of electrically conductive BTE scaffolds that allow controlled current strength, distribution and application duration, as well as upscaling [9, 10].

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MICROPHYSIOLOGICAL ENGINEERING OF INFLAMMATORY BOWEL DISEASE

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Introduction

IBD is a chronic inflammation of the gastro-intestinal tract which include Crohn's disease and ulcerative colitis. Contributors to the development of the disease are thought to include environmental factors (stress, diet, smoking) and genetic susceptibility [1]. Due it has not been possible to precisely manipulate simultaneous immunological, physiological, and molecular processes, IBD remains an idiopathic disorder [2]. Animal models have been extensively used to develop an understanding of the pathophysiology, however, numerous parameters in these systems and significant differences to humans, the results provided poor prediction and failed for the development of efficacious treatments [3]. Alternatively, static in vitro cell culture models have been used to model IBD most of which failed to mimic 3D morphogenesis, self-organization, and differentiation [4]. To overcome such limitations, microphysiological systems have emerged [5]. Here we aim to develop a close mimicry system that can be used for not only drug discovery and delivery studies but also to map the complex interactions occurring in IBD.

Material and Methods

The initial aim is to understand the effect of geometry in the colon. Since the natural microenvironment consists of a tubular structure, understanding the effect of local and overall curvature on intestinal and immune cells will be crucial for the model. Using decellularized colon and photo-crosslinkable hydrogels, we aim to develop a 3D tubular cell structure using primary human colon cells and immune cells. Later, this structure will be cultured under dynamic conditions in a microfluidic device. Using proteomics and molecular biology techniques, complex interactions will be elucidated. Later, this data will be used to build a drug screening setup to identify novel drug candidates as well drug delivery methodologies.

Results and Discussion

Although, several studies reported partial recapitulation of IBD pathophysiology in microfluidic devices, a relevant model that can be used for pharmaceutical applications is lacking [6]. Moreover, many studies were reported on modelling gut microenvironment, however, the majority focuses on the small intestine. The colon on the other hand has been slow to progress. Therefore, there is a significant gap in terms of development of suitable biomaterials and microfluidic drug discovery setups for colon.

Conclusions

The human colon hosts a unique microenvironment consisting of epithelial cells, immune cells and microbiome. As this balance breaks, microbiome leaks into the tissue causing inflammatory responses. Coupling microfluidics with integrated electronics, this system will allow real-time observation of these interactions. Direct observation of this phenomena will allow understanding the key mechanisms in its pathology. Consequently, this approach is promising for novel methodologies and effective treatments for IBD.

Acknowledgements

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Closing Session

REPRODUCIBLE RESEARCH: WHY SHOULD WE CARE?

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Abstract Body

Owing to COVID-19, I would not be able to present much new data relative to last year's conference and so I thought it would be more interesting to discuss an important topic I feel often gets overlooked. I'd like to highlight the value of reproducible research methods on an individual level, as opposed to merely focusing on the broader philosophical level, and give a brief practical introduction to some of the tools and techniques that can be utilised to craft more reproducible workflows.

I feel there are a lot of resources for good practices around publication, particularly the value of open-access publishing, and additional funding that can be available to cover the extortionate fees, which I myself have taken advantage of.(Bernardo Harrington et al., 2020)

However, I feel there is much less appreciation for the value of using a reproducible methodology in research, particularly for data analysis.

The fact that so many colleagues still use software like Microsoft Excel, or SPSS which are not, and never will be reproducible, indicates to me that many colleagues don't appreciate that simply saying "we did a paired t-test in SPSS" in their methodology reporting is **not** reproducible. I also suspect there is a lack of understanding of the personal benefits of working reproducibly, as the philosophical benefits of open research tend to be more emphasised.

I therefore suggest it would be valuable to raise awareness of this topic, both by highlighting the issues that can arise from using non-reproducible methods and the benefits, both individually and philosophically that are presented by using more robust reproducible approaches.

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Poster Abstracts

Cell and Gene Therapies

EFFECT OF CRYOPRESERVATION ON POST-THAW CELL SURVIVAL AND FUNCTION OF MESENCHYMAL STEM CELLS (MSCS)

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Abstract

There is currently a growing interest in the use of MSCs in the development of cell therapies for the treatment of many common conditions, with trials now also being performed to treat patients with COVID-19. However, loss of cell viability and function following cryopreservation, a logistical necessity throughout the manufacturing process, remains an area of concern. The onset of delayed apoptosis (a form of programmed cell death), in which cells appearing viable straight after thaw undergo apoptosis 24hrs post-thaw, is a particular challenge with there being no rapid method to identify cell populations undergoing delayed apoptosis.

This work will use gas chromatography-mass spectrometry (GC-MS) as a novel technique for the early detection of the delayed apoptosis, post-thaw, by identifying biomarkers that might indicate the onset of apoptosis. Initially, a robust MSC culture protocol, and good/bad freezing protocols expected to have varying effects on post-thaw cell viability and function were developed. The protocols involved varying freezing rates and DMSO concentrations used during freezing. Viability, and the extent of apoptosis immediately post-thaw, 24hrs post-thaw, and at five days post-thaw, was determined using the NucleoCounter 3000 using standard viability and apoptosis assays. To test for changes in MSC functionality post-thaw, changes in the immunoregulatory properties of MSCs will be studied. This will include identifying how cryopreservation affects the ability of MSCs to suppress T cell proliferation, and the ability to induce the differentiation of CD4+ T cells into T regulatory cells (Tregs).

Before performing GC-MS, initial viability tests identified that increasing DMSO concentration to 20% had the greatest impact on immediate post-thaw viability (10% drop in viability in comparison to the control). Following 24hrs in culture, the viability of all the cell populations (all freezing processes and DMSO concentrations) recovered to >95%. Further work is however required to confirm these results. Additionally, the apoptosis assays which will identify early apoptotic cells that would not have been detected in the viability assay are yet to be performed.

DEVELOPING MODELS FOR ADVANCED GENETIC THERAPY TESTING

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Introduction

There is currently need for development of advanced genetic therapies; present animal based models do not provide accurate translation for human treatment, and expensive in vivo pre-clinical trials require thousands of animals. Precision cut lung slices (PCLS) offer a potential alternative, as they maintain the lung structure and cell distribution found in vivo and can employ any host tissue. Air liquid Interface culture (ALI) of differentiated human lung cells also present as a potential model for such studies. Despite lacking the structure and organisation found in ex vivo lung tissue, the utilisation of human cells and the presenting mucociliary phenotype once differentiated make ALI culture potentially more translational for genetic delivery. Here, we aim to develop and utilise these models to demonstrate non-viral peptide based delivery; our group previously demonstrates Glycosaminoglycan enhanced transduction (GET) peptide mediated delivery in a variety of cell types [1,2], and our work here with PCLS also shows peptide mediated delivery is possible within tissue ex vivo. Within this project, we aim to demonstrate peptide based delivery within both PCLS and a fully differentiated ALI model, alongside cell monolayers, with a final aim of focussing on a specific genetic disease, Cystic Fibrosis (CF) to demonstrate delivery of a deltaf508 correction to CF positive cells at both monolayer and ALI.

Materials and Methods

GET peptide was used for non-viral peptide mediated transfection to PCLS tissue, and immortalised human bronchial epithelial (iHBEC) lung cells in both monolayer culture and at the Air liquid interface (ALI). ALI culture was carried out using transwells to expose the apical surface of cultured cells. Transepithelial electrical resistance (TEER) measurements were taken of transwell cell layers to help determine differentiation, alongside Immunofluorescence (post fixing) with specific markers to help identify lung cell types and potential cell layer formation.

Results and Discussion

Transfection was demonstrated in PCLS and iHBEC monolayers with a variety of vectors, including Glux (Gaussia luciferase) and enhanced Glux forms to aid in improvement of sustained expression. Initial delivery to transwell ALI cultures showed minimal to no expression. Initial TEER measurements showed some increase from day 14 to day 28, potentially demonstrating layer formation, however readings were overall much lower than expected. Further immunofluorescence is required to determine any potential differentiation.

Conclusions

GET peptide mediated delivery is possible both in cell monolayers and ex vivo tissue, however further optimization of methods is needed for ALI culture. Full confirmation of differentiation of ALI cultures is key to successful model use and development.

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ASSESSMENT OF COMPUTATIONAL TOOLS FOR AUTOMATED FLOW CYTOMETRY DATA ANALYSIS WITHIN CELL AND GENE THERAPY MANUFACTURING

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Introduction

Flow cytometry is widely used within the manufacturing of cell and gene therapies to measure and characterise cells. However, conventional (manual) flow cytometry data analysis relies heavily on operator judgement and as a result, there are limitations in reproducibility and the process is liable to bias. This can adversely impact the quality and predictive potential of therapies given to patients.

Computational tools have the capacity to minimise operator variation in flow cytometry data analysis, with some 50 packages in current use [1]. In many cases confidence in these technologies have yet to be fully established mirrored by aspects of regulatory concern.

This research presented here aims to investigate the performance of a range automated cell population identification tools that utilise different classes of clustering algorithms, benchmarking for a range of cell analysis conditions, using novel synthetic toolsets for highly controlled numerical evaluation.

Materials and Methods

Synthetic flow cytometry datasets containing controlled population characteristics of separation, normal/skew distributions, rarity, and noise were designed and generated using R. Platforms investigated include: Flock2, FlowMeans, FlowSOM, PhenoGraph, SPADE1, SPADE3 and SWIFT (density-based, k-means, self-organising map, k-nearest neighbour, hierarchical, deterministic k-means, and model-based clustering, respectively). Performances were assessed based on absolute accuracy and repeatability.

Results and Discussion

Outputs from software analysing the same reference synthetic dataset vary considerably and accuracy deteriorates as the cluster separation index falls below 0. Consequently, as clusters begin to merge software platforms struggle to identify target clusters. For rare cell populations, different limits of detection as a function of total cell events were observed. Most software were unable to consistently identify 10^2 rare cells in 10^5 events. The presence of skewed cell populations negatively affected SWIFT more than other platforms. Finally, the addition of noise elements caused a significant loss of performance in FlowSOM.

Conclusions

Here we have presented the generation of novel synthetic flow cytometry data which overcome limitations of real biological datasets, and demonstrated their use in the unique validation of a range of automated cell identification methods. This work has revealed differing trends in performance between different software when analysing clusters with simulated distance, probability distribution, rare and noise properties.

Very careful consideration therefore needs to be given to derive a suitable range of metrics for judging the strengths and weaknesses of automated data analysis platforms, when selecting such platforms for off-line and in-process evaluation of cell metrics.

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LINKING MOLECULAR TARGETS TO PHARMACOLOGICAL MANAGEMENT OF HYPERTROPHIC CARDIOMYOPATHY USING GENOME EDITED HUMAN PLURIPOTENT STEM CELL CARDIOMYOCYTES

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Introduction: Hypertrophic cardiomyopathy (HCM) is the most common monogenic cardiovascular disease, estimated to affect 1 in 500 people, and is the most prevalent member of the group of cardiomyopathies. It is often characterised by left ventricular (LV) hypertrophy and is incredibly diverse in disease course, age of onset, severity of symptoms and LV outflow obstruction. The result of such a broad disease spectrum is due to the vast genetic heterogeneity and variable penetrance – over 1400 mutations have been identified in 11 sarcomeric genes. One of the most frequently mutated sarcomeric genes leading to some of the most severe cases of HCM is the *MYH7* gene.
Long non-coding RNAs (lncRNA) are a novel group of RNA (ribonuclease) molecules greater than 200 nucleotides in length, with little ability to code for proteins. lncRNAs have been implicated in HCM disease progression and although their functions and mechanisms are still relatively unknown. Previous work has identified four lncRNAs possibly involved in HCM disease progression or disease protection. These were validated using qPCR and this project investigates their potential roles in progression or prevention of HCM using various phenotypic techniques.

Materials and Methods: All experiments were conducted using induced pluripotent stem cell derived cardiomyocytes (iPSC-CM) which were differentiated in house. To investigate whether the lncRNAs may be involved in HCM progression or prevention, their expression was modulated by overexpressing them and then performing various phenotypic assays, presented here is the results from the BNP assay, measuring hypertrophy.

Results and Discussion: The BNP assay was performed to assess the levels of the hypertrophic marker BNP in our lncRNA-overexpressing iPSC-CMs. Expression of BNP was maximised with treatment of ET1 and the phenotype rescued with the antagonist BOS. As expected, in the healthy line there is not a significant effect of the lncRNAs on BNP expression levels. However, in the heterozygous and homozygote lines, the lncRNAs cause significant changes in expression levels, indicating that they may work as gene modifiers.

Conclusions: The lncRNAs we are studying may have cardioprotective and disease-causing roles through unknown mechanisms. Further experiments, including RNA Immunoprecipitation may shed some light on these mechanisms which can allow for specific pharmaceutical targeting.

REMOTE MAGNETIC NANOPARTICLE MEDIATED ORIENTATION OF NEURONAL LIKE CELLS

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Introduction:
The remote control of neuronal migration and their processes is a challenge for novel therapies for neuro-regenerative medicine. One novel therapy proposed, is the use of magnetic nanoparticles (MNPs) and magnetic fields. MNPs have shown to exert forces intracellular and extracellularly. The current study sets out to explore subcellular localisation of MNPs, and their effect on differentiated SH-SY5Y cell alignment across a static magnetic field

Materials and methods:
SH-SY5Y cells were expanded in basal media, and underwent differentiation with retinoic acid (RA) and brain derived neurotrophic growth factor (BDNF). Cells were incubated with MNPs and imaged using fluorescence and transmission electron microscopy. Cells were then placed on a 96 well magnetic array and orientation analysed using two-dimensional (2D) fast Fourier transform (FFT).

Results and discussion:
The current study finds that after 24hrs of co localisation, MNPs were retained within the cell lysosomes. Similarly, TEM images show that after 3hrs, MNPs are internalised within the cell. It also observes that after 24hrs, majority of MNPs are within endo-compartments.The current study has also observed that differientiated SH-SY5Y cells extend their neurites along the magnetic field when subjected to magnetic field stimulation. This suggests that it may be possible for MNPs direct the orientation and processes of differentiated SH-SY5Y cells in a spatial manner.

Conclusion:
The current study addresses the subcellular localisation of MNPs. It has also demonstrated that MNPs can induce a physical and mechanical effect within cells and direct their processes along a magnetic field. Future work well address extracellular effects of MNPs on neurite orientation as well as electrophysiological properties of aligned cells.

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DIFFERENTIATION OF DENTAL PULP STEM CELLS INTO ENDOTHELIAL-LIKE CELLS FOR TISSUE ENGINEERING

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Introduction: The inclusion of vasculature within engineered tissues is currently one of the greatest hurdles in tissue engineering that prevents the generation of large-scale functional tissues for clinical applications. The current lack of vasculature in engineered tissues means that it is difficult to deliver oxygen and nutrients to cells beyond a diffusion limit of approximately 250µm and in addition there are no sites for surgical integration of the engineered tissues into a patient's own vasculature leading to necrosis of the tissue after implantation. One of the key issues preventing the vascularisation of engineered tissues is the lack of a suitable cell source to create the vasculature as isolation of a sufficient number of endothelial cells to produce vasculature is invasive and difficult and the use of an allogeneic cell source comes with a high risk of immune rejection. A promising cell source candidate for vascularisation is the use of an adult stem cell population such as bone marrow mesenchymal stem cells (MSCs) and adipose derived MSCs, which are able to differentiate into endothelial-like cells and form vasculature-like structures *in vitro*. However, the isolation of these and other adult stem cells is invasive and may not be suitable for patients requiring treatment for some conditions. Dental pulp stem cells (DPSCs) are another adult stem cell population that isolated from both adult and exfoliated teeth and have been shown to be able to express essential vascular genes such as vascular endothelial growth factor receptor 2 (VEGFR2) and cluster of differentiation 31 (CD31). The main issue with the use of DPSCs to vascularise engineered tissues is the lack of a clearly defined method for their differentiation into an endothelial-like cell lineage. The aim of this research was to develop an easy and cost effective method of differentiating DPSCs into an endothelial-like cell lineage that are able to produce vasculature-like structures *in vitro*.

Materials and Methods: DPSCs (passage 4 or 5) from adult human teeth were used for all experiments. DPSCs were cultured in basal medium until 90% confluent and then the cells were cultured in endothelial differentiation media (PromoCell Endothelial Growth Medium 2 plus supplements) for 5 or 10 days with daily media changes. qPCR was used to monitor expression of endothelia markers (VEGFR2 and CD31) and confocal microscopy was used to monitor the expression of both proteins. 2D Matrigel tube formation assays were performed to determine the ability of differentiated cells to form vasculature-like structures.

Results and Discussion: qPCR results showed that after 5 days of treatment with endothelial differentiation media, the expression of VEGFR2 and CD31 was significantly upregulated in treated DPSCs compared to untreated cells which increased further after 10 days. Confocal laser scanning microscopy images confirmed that after receiving treatment for 5 days there were significantly more positively labelled cells for VEGFR2 and CD31 in comparison to cells grown in basal media which also increased after 10 days. Tube formation assays showed that under endothelial differentiation culture conditions, DPSCs were able to form vasculature-like structures after 5 days of treatment but failed to do so after 10 days.

These results indicate that endothelial differentiation medium can direct the differentiation of hDPSCs into an endothelial-like cell lineage *in vitro*, however it is unclear why differentiated DPSCs formed vasculature-like structures after 5 days of treatment but not 10. It may demonstrate that short term exposure to growth factors in the differentiation medium causes a fast short term response in DPSCs but a prolonged exposure leads to immature endothelial cells. There may be several explanations as to why this is the case which warrant further investigation of different treatment times to find the optimum conditions for the controlled differentiation of DPSCs into endothelial like cells for the vascularisation of engineered tissues.

Conclusions: These findings demonstrate the potential of using human primary DPSCs for vasculogenesis and that endothelial differentiation medium is capable of enhancing DPSCs expression of endothelia specific markers essential for the assembly of vascular structures. As such, DPSCs may be able to be used in vascularisation strategies in tissue engineering.

DIFFERENTIATION THERAPY IN OSTEOSARCOMA: A PROMISING ALTERNATIVE

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Around 160 people are diagnosed with osteosarcoma in the UK each year. Osteosarcoma is a malignant primary bone cancer, with highest incidence in young people between the ages of 10-24. Osteosarcoma treatment usually involves surgery, chemotherapy and/or radiotherapy, with a 5-year survival rate near 60%, and a high rate of pulmonary metastasis. A non-terminally differentiated phenotype is one of the hallmarks of osteosarcoma. Healthy osteogenic differentiation of mesenchymal stem cells is disrupted in osteosarcoma, leading to highly proliferative, immature cells, that express reduced levels of mature osteogenic markers. A potential strategy for osteosarcoma treatment is differentiation therapy, where instead of killing tumour cells, the aim is to “reprogramme” them to a healthy state, by driving them to maturation. Identifying small molecules that can selectively differentiate immature cells in osteosarcoma is especially of interest, with flavonoids, steroids and retinoids, having been used in literature, and showing potential.

3D SPHEROIDS – A VANTAGE POINT IN THE TREATMENT OF PROSTATE CANCER

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Introduction

Prostate Cancer (PCa) is one of the most common malignancies in the world[1]. An advanced stage of PCa is 'metastatic castration-resistant prostate cancer' (mCPRC) which identifies a heterogenous population of both symptomatic and asymptomatic patients[2]. Androgen Deprivation Therapy (ADT) is the mainstay in the treatment of mCPRC with improved clinical outcomes in recent years[3]. Although the current prognosis delivers a wealth of treatment options, it remains incurable[4]. In exploration to find cure to this disease, Ridge et.al identified the role of MSCs in the invasiveness of PCa cells[5]. It is evident that secreted cytokines and/or metabolites from tumour conditioned media stimulate MSCs to an 'activated' pro-tumorigenic state whereby assisting the tumour cells in migration and invasion[6]. An elaborate analysis of this phenomenon requires a 3D vantage point. Implications of MSCs in 3D tumour-stromal microenvironment can be predictive in recapitulating the real tumour microenvironment. Synthetic hydrogels allow mechanically tuneable in vivo conditions that mimic true physiological state and provide platforms to run extensive research on molecular level[7]. The aim of this study is to incorporate hydrogels in building 3D tumour-stromal microenvironment in vitro that will aid in the investigation of MSCs in the development of mCRPC.

Materials and Methods

Human PCa androgen dependent (LNCaP) and androgen independent cell lines DU145, PC3 with low, moderate, and high metastatic nature respectively were cultured for 48 hr at 37°C with 5% CO_2 until they were 60–70% confluent. Cells were trypsinised and the assays were performed with uniform seeding density of 5000 cells/well in comparison between 2D (flat plate) and 3D (ULA bottom plate) 96 well plates. Proliferation assay was carried out for 7 days to determine the formation of spheroids. Cytotoxicity assays include MTS and Acid Phosphatase (APH) was performed to analyse cell viability. Hydrogels with different stiffness parameters will be designed to optimise the spheroid formation in three different cell lines.

Results and Discussion

It is observed that LNCaP and DU145 form tight spheroids whereas PC3 form loose spheroids. In this scenario, we expect that addition of hydrogels will help in the tight construction of spheroids. The cytotoxicity assay revealed that 2D culture has higher viability compared to 3D spheroids. It is because 3D spheroids are comprised of cells include proliferating, quiescent, apoptotic, hypoxic, and necrotic stages. It is speculated that results will vary with the support of hydrogels.

Conclusion

3D spheroids are a promising advancement in designing strategies for the treatment of metastatic castration-resistant prostate cancer. The recapitulating nature of the 3D in vitro environment will pave a way for better understanding of molecular phenotypic changes with the rapid stages of cancer. It is also believed to be a platform for path breaking diagnostics in the future to treat various cancers. They aid in elaborate analysis of determining key factors in tumour-stromal interactions and a targeted approach for cancer therapeutics.

Acknowledgements

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DEVELOPMENT OF AN ON-DEMAND BIOSENSOR FOR MONITORING CELL THERAPY BIOMANUFACTURE

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Introduction

The field of cell therapies is currently experiencing a boom, bringing new hope to patients in a wide range of diseases. However, the manufacture of these therapies remains a major barrier to meeting increased market demand and patient access. New technologies are needed to implement integrated process analytics, enabling real-time monitoring of process parameters and critical quality attributes of cell therapies during biomanufacture. The incorporation of biosensors into bioreactor systems would allow for fast, non-destructive sensing to achieve in-process monitoring of cell products within closed systems, avoiding the need for multi-step, off-line assessments which are costly and time consuming. We therefore aim to develop an electrochemical nanobody-based biosensor capable of sensing cell-specific cytokines and proteins that will better inform cell culture processes. Furthermore, this sensing device will incorporate switchable surface technology, enabling ON/ OFF electrical control of antigen- nanobody binding to achieve real-time, on-demand sensing of analytes.

Materials and Methods

Preliminary work was conducted using anti-GFP nanobodies (NbGFP). Sensors were constructed from NbGFP immobilised on piranha cleaned Au electrodes via NHS/ EDC coupling to 11-mercaptoundecanoic acid (11-MUA) self-assembled monolayers. Advancing and receding water contact angles and thicknesses were measured for each sensor layer. Faradaic electrochemical impedance spectroscopy (EIS) and cyclic voltammetry using 10 mM $K_3Fe(CN)_6$/ $K_4Fe(CN)_6$ redox couple in PBS (0.1 M KCl) pH 7.4 was used to electrochemically interrogate each sensor layer.

Results and Discussion

Contact angles and measured thicknesses suggest formation of 11-MUA monolayer and successful NHS/ EDC activation. However, the data was not consistent for NbGFP coupling. Cyclic voltammetry and EIS data do however suggest successful NbGFP coupling, with coupling shown to be pH and time-dependent as expected.

Conclusions

Immobilisation of nanobodies to Au electrodes using NHS/ EDC coupling was found to be successful, and the electrochemical profile of these sensors was defined. Future work includes developing a method of detecting GFP binding to NbGFP functionalised electrodes using non-faradaic EIS, the electrochemical sensing method of choice. In addition, surface plasmon resonance analysis is ongoing in tandem to determine a fabrication method that will enable ON/ OFF switching of nanobody-antigen binding. Non-faradaic EIS detection of analyte binding in this system can then be developed, enabling investigation of sensor performance in media and cell culture samples. These methods of sensor fabrication and analyte detection can then be applied to nanobodies targeted towards cytokines and proteins relevant to cell therapy culture.

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THE REGENERATIVE POTENTIAL OF INJECTABLE PEPTIDE HYDROGELS WITH MESENCHYMAL STEM CELL SECRETOME FOR INTRACEREBRAL HAEMORRHAGE THERAPY

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Introduction

Intracerebral haemorrhage (ICH) is a subtype of stroke that makes up approximately 10-15% of cases. Although ICH is the deadliest form of stroke, current interventions are limited to best medical management and surgical intervention. ICH survivors often suffer life-changing motor and cognitive impairments. The significant unmet clinical need and socioeconomic burden of stroke means innovative tissue engineering approaches are gaining interest. To facilitate regeneration of the stroke cavity, injectable hydrogels are proposed, as they can be designed to mimic properties of healthy neural tissue, act as delivery platforms for therapeutics, and provide a scaffold for regeneration. Mesenchymal stem cell (MSC) secretome has been shown to have regenerative properties in a variety of disease and injury models including stroke models. Co-administration of hydrogel with MSC secretome at the stroke site is a potential therapeutic strategy, which provides a mechanical scaffold and a pro-regenerative stimulus, respectively.

Materials and Methods

In a rat model of ICH, self-assembling peptide hydrogels (SAPH) from Manchester BIOGEL® were injected intracerebrally, during the chronic phase of the disease (day 7) with or without concentrated conditioned medium (CM) from human MSCs. The aim was to determine whether SAPH +/- MSC CM injection would lead to functional and histological recovery and repair after moderate to severe ICH. To compare functional outcomes of the ICH+hydrogel+CM (n=12), ICH+hydrogel (n=12), ICH+CM (n=12) and vehicle-injected (n=12) groups, various measures of sensorimotor function were assessed over 8 weeks, including neurological scoring, novel object recognition, corner and cylinder tasks. To determine the effect of SAPH/CM injection on the adjacent injured and healthy brain tissue, immunohistochemical markers were chosen to study inflammation at the injection site, as well as regenerative markers of neurogenesis and angiogenesis. Three magnetic resonance imaging (MRI) sequences were studied to measure lesion and ventricle volume, cerebral blood flow and blood-brain-barrier integrity.

Results and Discussion

Results from previous studies suggest that intracerebral SAPH injection after ICH is well tolerated. Previous data show there was no benefit of SAPH injection compared to the ICH only group. Hydrogel injection appeared to worsen post-stroke asymmetry in the cylinder task. Host cells were able to infiltrate the hydrogel, and there was no marked exacerbation of inflammatory response or glial scarring at the injection site. There was an increase in collagen-IV labelled vessel-like structures within the lesion of hydrogel injected group compared to ICH only group, indicative of an angiogenic response. Functional MRI was performed in this study to explore whether SAPH +/- CM led to a functional restoration of blood flow and BBB integrity at the affected brain region.

Previous work showed a lack of a beneficial effect of hydrogel injection alone, without encapsulation of a therapeutic entity such as stem cells. As such this study aimed to co-administer MSC CM with SAPH, to provide a mechanical scaffold and pro-regenerative stimulus to the damaged tissue. Due to unforeseen circumstances, the combined SAPH+CM group was removed during the study. As a result, results presented will show the effect of ICH and SAPH or CM alone, compared to a vehicle group on multiple MRI, histological and functional measures of brain recovery.

INVESTIGATING THE ANTI-INFLAMMATORY AND TISSUE-PROTECTIVE PROTIEN TSG-6 AS A REGULATOR OF INTERVERTEBRAL DISC DEGENERATION

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Introduction:

Degeneration of the intervertebral disc (IVD) is a leading cause of lower back pain, and a significant clinical problem. Inflammation mediated by Interleukin-1 beta (IL-1β) and tumour-necrosis factor alpha (TNF-α) drives IVD degeneration through promoting a phenotypic switch in the resident nucleus pulposus (NP) cells towards a more catabolic state, resulting in extracellular matrix degradation. TSG-6 (the protein product of tumour necrosis factor-stimulated gene 6) is expressed during inflammation and has been shown to have anti-inflammatory and tissue-protective effects in a broad range of disease models including osteoarthritis. However, there has been little exploration of TSG-6 in the IVD. A greater understanding of endogenous TSG-6 expression by NP cells in the healthy and degenerate disc will shed light on its role in IVD homeostasis and its potential as a therapeutic target for IVD degeneration. Informative studies of the effects of TSG-6 on NP cells requires the development of *in vitro* 3D culture systems where cells deposit an NP-like matrix.

Methods:

Human IVD samples were obtained from patients undergoing surgery for disc degeneration and associated back pain. NP cells were extracted from IVD samples and cultured in monolayer prior to RNA extraction for qPCR analysis. Formalin-fixed, paraffin-embedded IVD samples were sectioned for RNAscope analysis. NP cells were expanded in monolayer before encapsulation in alginate beads using the $CaCl_2$ gelation method; beads were cultured in chondrogenic media for up to 28 days prior to being fixed and sectioned for histological analysis.

Results:

Using qPCR, we generated a large gene expression dataset and demonstrated that TSG-6 expression is positively correlated with the expression of a range of catabolic factors, including IL-1β and TNF, and negatively correlated with expression of the NP matrix component aggrecan. Exploration of mRNA expression by single cells *in situ* using RNAscope, showed expression of TSG-6 by NP cells in disc tissues with both low and high degenerative grades. A method has been optimised for 3D *in vitro* culture of human NP cells in alginate beads; extensive staining for the matrix component hyaluronan after 21 and 28 days in culture suggested that an NP-like matrix is being deposited in this model.

Discussion & Conclusions:

These data offer an insight into the expression of TSG-6 in the human IVD, both on extracted cells and human IVD tissue, which will be of use to future investigations. The development of a 3D culture model will be a valuable tool for characterising the effects of exogenous TSG-6 on NP cells.

ENGINEERING POLY(HEMA-co-GMA) HYDROGEL PLATFORMS TO STUDY THE ROLE OF UNDIFFERENTIATED ADIPOSE STEM CELLS (uASCs) IN PERIPHERAL NERVE REGENERATION

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Introduction: Adipose stem cells constitute one of the most desirable cell therapies for tissue engineering applications due to their availability and high yield without major interventions needed to obtain them. While their application for peripheral nerve regeneration has been commonly due to their ability to differentiate in Schwann-like phenotypes; recent research has shown that undifferentiated cells could elicit positive effects in the area. 2-hydroxyethylmethacrylate (HEMA) and glycerol monomethacrylate (GMA) are commonly used monomers in the medical device sector and are capable to polymerize to form interesting cheap, easy to manufacture, tailorable (in terms of mechanical properties or surface properties) and controllable copolymers to be used for tissue engineering applications.
Materials and Methods: poly (HEMA-co-GMA) copolymers have been engineered via redox free-radical polymerization. The viability, metabolic activity, and morphological changes of uASCs were assessed when in contact with these copolymers using LIVE/DEAD analysis, metabolic activity studies and morphological changes, respectively. In addition, immunostaining techniques were used to detect common markers expressed by glial cells in the peripheral nerves.
Results and discussion: the mechanical properties of these engineered hydrogels vary in the 5-60 KPa range for shear storage modulus; with increased water uptake that peaked at 200 % in equilibrium with marginal dimensional changes. Fibronectin (FN) and laminin (LM) coated hydrogels show high uASCs viability; with increased metabolic activity for cells seeded on FN-coated materials. In addition, morphological changes occur. While uASCs appear to have spread cell bodies with a fibroblast-like structure on top of FN-coated surfaces; cells acquire bipolar and elongated shapes for LM-coated surfaces. Furthermore, cells have shown to be positive for S100, GFAP and GDNF, a great indication of their possible positive effect for peripheral nerve regeneration applications.
Conclusions: poly(HEMA-co-GMA) hydrogels are interesting platforms to study the role of stem cell therapies in tissue engineering applications due to their tailorability and ease to manufacture. ASCs are a promising cell source for stem cell therapies and tissue engineering applications. In particular, ASCs have shown to elicit positive effects in nerve regeneration.

INVESTIGATING THE MOLECULAR IDENTITY OF CARDIAC PROGENITOR CELLS FOR APPLICATIONS IN HEART DISEASE

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Introduction.

With an increasing incidence, compounded by the heart's inherent lack of regenerative capacity, heart disease is an increasing burden on health care and a significant cause of death. Human pluripotent stem cell (hPSC)-derived cardiac progenitor cells (CPCs) have therapeutic promise for myocardial regeneration, but their molecular identity remains ill-defined and the conditions required for CPC self-renewal and differentiation are poorly understood.

The drug regulatable transgene system and fluorescent reporters in the Tet-On-*MYC NKX2-5*^{eGFP} *SOX17*^{tdTomato} hPSC line enable expansion of CPCs which can be further differentiated to cardiomyocytes and endothelial cells and visualisation of endoderm and cardiac specification during differentiation. The differentiation potential of progenitor cells depends on their earlier lineage specification, however in heart development this relationship is poorly understood.

Materials and Methods.

To explore how developmental patterning impacts progenitor (including CPC) fate, I varied the signalling gradients of BMP4 and Activin A, mimicking gastrulation-like events that occur during development, to direct differentiation of hPSCs across a mesoderm-endoderm gradient. From this range of populations, progenitors have been expanded and further differentiated to determine their fate potential. I will be investigating the relationship between differentiation potential and genetic and epigenetic identity using RNA-seq and ATAC-seq.

Results and Discussion.

My data so have confirmed specification of populations across the mesoderm-endoderm gradient and their varying capability to expand and induce NKX2.5 in culture. Utilising the dual reporter line I have also uncovered a previously minimally described NKX2.5+ SOX17+ population. The specific identity of these cells, and their potential role in cardiac differentiation, is being examined using populations sorted based on NKX2.5 and SOX17 expression. Using these thoroughly characterised populations from across mesoderm and endoderm specification, I will determine how their genetic and epigenetic identity relates to their differentiation potential by RNA-seq and ATAC-seq analysis.

Conclusions.

By identifying the genes which functionally determine their fate potential, these data will greatly improve our understanding of the molecular identity of CPCs. These advances are an important step towards using CPCs in a therapeutic context.

INVESTIGATION OF *HNF1B*-ASSOCIATED RENAL DISEASE IN A HUMAN KIDNEY ORGANOID MODEL

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Abstract Body

Introduction: Heterozygous mutations in the *Hepatocyte nuclear factor 1B* (*HNF1B)* gene are the most prevalent monogenic cause of congenital kidney disease. Several mutations have been identified and display a phenotypic heterogeneity that does not correlate with the mutations. Renal cysts are the most common disease feature, among a wide range of renal and extra-renal abnormalities.
Materials and Methods: Our research uses a CRISPR-Cas9 gene-edited human pluripotent stem cell (hPSC)-kidney organoid model of *HNF1B*-associated renal disease to understand the disease mechanism.
Results and Discussion: Mutant organoids display histological disease features that include the presence of large multi-layered tubule structures and infrequent, enlarged glomeruli (as we have also shown in patient induced PSC-derived organoids). RNA-sequencing comparing the transcriptome of isogenic hPSC-organoids with and without an *HNF1B* exon1 frame-shift mutation and immunohistochemistry revealed that the endocytic receptors Megalin and Cubilin, expressed by proximal tubule cells, are misregulated in mutant organoids. In mutant organoids, Megalin was not detected by immunohistochemistry and Cubilin was not localised to the apical membrane. Both receptors are responsible for reabsorption of ultrafiltrate proteins and their misregulation may account for some of the renal dysfunction in *HNF1B*-associated renal disease, with proteinuria being a common feature.
Conclusion: This work demonstrates the utility of hPSC-kidney organoid disease models for investigating the molecular basis of human disease.

DEVELOPING A POTENTIAL COMBINATION THERAPY FOR ISCHAEMIC STROKE USING HYDROGEL-DELIVERED THERAPEUTIC PROTEINS

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Introduction. With no available treatments for brain tissue repair and functional recovery in patients with lost or impaired neurological functions, stroke therapy remains an important unmet clinical need. The lack of structural support in the stroke cavity resulting from dead tissue clearance by immune cells and insufficient endogenous tissue repair dramatically limit brain functional recovery after stroke. Repair processes, such as microglial switch from pro-inflammatory to pro-regenerative phenotype, angiogenesis and neurogenesis, are potential targets to be considered when designing novel regenerative therapies[1]. For example, soft hydrated biomaterials called hydrogels may offer the necessary structural support and deliver pro-regenerative therapeutic agents for improving their half-life[1,2]. To explore a novel hydrogel-based combination therapy, we tested the biocompatibility of two novel self-assembling peptide hydrogels (SAPHs) with the brain tissue, and their capacity to release the angiogenic vascular endothelial growth factor (VEGF). Furthermore, we have assessed the reparative properties of tumour necrosis factor-alpha stimulated protein-6 (TSG-6), a microglial modulator and multifunctional protein known to mediate anti-inflammatory effects of mesenchymal stem cells (MSCs)[3].

Materials and Methods. To study the biocompatibility of two novel SAPHs with the brain tissue, PeptiGel-Alpha2 and PeptiGel-Alpha4 hydrogels were injected intra-cerebrally in healthy mouse brains, and microglial activation as a measure of brain inflammation, as well as phagocytosis and neuronal apoptosis were assessed by immunohistochemistry (IHC). Levels of recombinant human VEGF(rhVEGF) released by the hydrogels following incubation in PBS at 37°C for six weeks were assessed by enzyme-linked immunosorbent assay. To study the effect of TSG-6 on brain repair and functional recovery in a mouse model of ischaemic stroke, rhTSG-6 was injected intra-cerebrally 5 days post-stroke, and wellbeing and motor asymmetry were assessed up to 28 days post-stroke. Levels of microglial activation as well as proliferating endothelial cells were assessed by IHC on brain sections.

Results. The PeptiGel-Alpha2 hydrogel, unlike PeptiGel-Alpha4, did not induce microglial activation nor neuronal apoptosis in vivo, therefore is biocompatible with the brain tissue. Both hydrogels induced the recruitment of phagocytic cells indicating a clearing mechanism. The hydrogels also released rhVEGF in a sustained manner *in vitro*, and ongoing experiments are testing the bioactivity of this angiogenic growth factor. These findings support the use of PeptiGel-Alpha2 as a temporary scaffold for brain repair and VEGF release system. In parallel, our *in vivo* study revealed a trend toward improved wellbeing as assessed by burrowing test and animal weight following rhTSG-6 administration into the stroke cavity. Furthermore, a trend toward decreased microglial activation and increased endothelial cell proliferation has been observed, which indicates that TSG-6 has beneficial tissue repair properties post-stroke.

Conclusion. Our work describes a novel brain biocompatible SAPH hydrogel with VEGF release capacity suitable for angiogenic applications following brain injury. In parallel, we have tested for the first time the reparative properties of TSG-6 administered sub-acutely in the stroke infarct, and the results have shown the potential this anti-inflammatory and pro-regenerative protein has as a therapeutic tool for the treatment of stroke. Ongoing work is testing the efficacy of TSG-6 and VEGF as a dual combination, assisted by a SAPH-based delivery system, which is also aimed to structurally support tissue repair post–stroke. VEGF and TSG-6 will be delivered by the PeptiGel-Alpha2 hydrogel into the stroke cavity 5 days post-stroke. The animal wellbeing, motor asymmetry, cerebral blood flow, microglial activation, angiogenesis, and neurogenesis will be assessed up to 28 days following stroke. The findings of this study will reveal the therapeutic potential of VEGF/TSG-6/PeptiGel-Alpha2 hydrogel combination for structurally supporting tissue repair and enhancing the endogenous brain regeneration processes following stroke.

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INVESTIGATING THE POTENTIAL OF THE MESENCHYMAL STEM CELL SECRETOME IN HAEMORRHAGIC STROKE

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Introduction

Intracerebral haemorrhage (ICH) is a deadly and debilitating disease, caused by the rupture of blood vessels in the brain. Blood leaks into the brain parenchyma, creating a haematoma, which expands and compresses adjacent brain tissue. In addition, immune cells infiltrate the brain and release pro-inflammatory cytokines, exacerbating brain injury. There are no regenerative therapies currently available for ICH patients, with present treatments aimed at reducing the damage caused by haematoma expansion and other secondary effects of ICH. Cell based approaches have been investigated for their potential in ICH, with many studies administering mesenchymal stem cells (MSCs) in rodent models. However, growing evidence suggests that it is the paracrine effects of the MSCs, and their conditioned medium (CM) containing cytokines, growth factors and extracellular vesicles that is responsible for the therapeutic effects observed. Previous work in our lab has modulated the MSC secretome by priming the cells with interleukin 1-α (IL-1α), driving them towards an anti-inflammatory phenotype. Therefore, the following study was designed to investigate the efficacy of the primed MSCs (αMSCs) and primed MSC CM (αCM) as potential therapies for ICH.

Materials and Methods

Adult male Sprague Dawley rats were subjected to striatal collagenase injection to induce ICH. T2 MRI scans of the brain were obtained and used to stratify animals to the following treatment groups (n=12 per group): vehicle, receiving 10µl cell culture medium; αMSC, receiving $2x10^5$ αMSCs in 10µl cell culture medium; αCM, receiving 10 µl conditioned medium derived from $2x10^5$ αMSCs. Treatments were delivered via intracerebral injection at 24 hours after ICH induction. Animals underwent behavioural testing to determine neurological, motor, and sensorimotor deficits, and blood samples were obtained to measure systemic inflammatory markers. Animals were culled at 14 days post-ICH and brains removed for further immunohistochemistry and proteomic investigation. All measurements were performed by an investigator blinded to the treatment of the animals.

Results and Discussion

Upon completion of the study, we anticipate that the behavioural tests will report differences in functional recovery between the treatment groups, based on our hypothesis that the αMSC and αCM treatments will reduce inflammation during acute brain injury and have possible neuroprotective effects. Haematoma volume and oedema at 7 days will be assessed with MRI and we expect that there will also be group effects for these outcomes. Histology will be used to confirm extent of damage in the brain at 14 days, where we expect to observe differential microglial activation across groups.

Conclusions

This study will shed light on the therapeutic potential of our two novel treatments, αMSCs and αCM. While cell therapies have already been widely investigated for ICH, there is a paucity of secretome-based therapies, and this study will allow a direct comparison between the cells and the secretome with a variety of outcome measures. We expect this study to inform future work in our lab using these therapies and allow us to further refine them for improved ICH recovery.

STUDY OF STEM CELLS-ENDOTHELIUM INTERACTION TO UNDERSTAND AND IMPLEMENT SYSTEMIC CELL TRANSPLANTATION FOR MUSCULAR DYSTROPHY

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Introduction: Many new therapies are being tested for MD but none has yet reached clinical efficacy. Our lab pioneered stem cell transplantation that led to a first in man trial in DMD patients. The first trial was safe but not efficacious[1]. This project aims at implementing critical steps of MABs transplantation. We want to study and positively modulate their diapedesis and extravasation.

Materials and Methods: An *in vitro* IBIDI microfluidic assay, mimicking blood stream dynamic, allows tracking in real time MABs adhesion to the endothelium. Through this system, TNF∝ inflammatory activity, and deflazacort (DFZ) or prednisone (PRED) anti-inflammatory effects were studied at the single cell level.

Results: When activated via TNF∝, HUVECs over-express VCAM-1, I-CAM1, and E-SEL adhesion molecules at both mRNA and protein level. MABs-adhesion ability increases about 84% after pre-activation of HUVECs with TNF∝ for 3 or 6 hours. As expected, adhesion events were reduced in TNF∝ treated HUVEC or other human microvasculature ECs, after exposure to DFZ or PRED. Surprisingly, despite these steroids did not reduce expression of adhesion molecules, but even increased it. Therefore, MABs ability to interact with HUVECs is specifically dependent on the endothelium status. Since protein expression is not reduced by steroids, we reasoned that post-translational modifications may be involved in reducing adhesion. HUVECs pre-treated with TNF∝ and DFZ over-express ST6GAL1 – a transferase involved in ∝2,6-linked sialic acid synthesis – at both mRNA and protein levels. MABs-adhesion ability was increased of 48% when HUVECs were pre-treated with TNF∝, DFZ, and SNA lectin – a carbohydrate binding protein specific for ∝2,6-linked sialic acid.

Conclusions: These results will be confirmed *in vivo*, through experiments conducted on dystrophic mice under steroid administration. Perivascular ECM reconstruction will elucidate the migration mechanism adopted by these cells to reach the dystrophic muscles. The results may have important implications for future protocols based upon intra-vascular cell delivery for DMD.

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Bioengineered Models

GRAPHENE BASED BIOINKS FOR 3D PRINTING BIOACTIVE ARTICULAR CARTILAGE IMPLANTS

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Articular cartilage (AC) has a poor capacity for self-repair, consequently defects are one of the major causes of immobility and poor quality of life for millions of individuals worldwide. Growth factors from the TGF-β and BMP signalling families are key regulators of the production and maintenance of the articular cartilage phenotype and consequently are a feature of many in vitro protocols for chondrogenesis. However, their use in regenerative therapies is limited by their short half-lives and low protein stability. In this project we aim to use Graphene Oxide (GO), a 2D carbon nanomaterial, to deliver a chondrogenic growth factor to human pluripotent stem cell derived chondroprogenitors within 3D printed hydrogel/scaffolds to generate articular cartilage tissue.

Methods: In order to determine an appropriate concentration range of GO to use throughout this project we began with investigating the cytotoxic effects of GO-cell exposure. A human chondrocyte cell line (TC28a2) was chosen as a model to assess the cytotoxicity of 3 sizes of Graphene oxide (large (2-8µm) small (25nm-1.5µm) and ultra-small (10nm-590nm)before moving on to human embryonic stem cell (hESC) derived chondroprogenitors.. For further characterisation of cell- material interactions, cell uptake and plasma membrane interactions were investigated via confocal microscopy with graphene oxide and live-cell imaging with Cell Mask green membrane stain. We also investigated the effects of GO on TGF-β signalling activity within the human chondrocyte cell line using a SMAD2/3 dependent reporter developed by Dr Steven Woods (Humphreys *et al*., 2020).

Results to date show no cytotoxicity of graphene oxide with Tc28a2 cells after 72 hours. Confocal microscopy provided novel insight into the interaction between GO and a chondrogenic cell line indicating uptake was size dependent with only large GO being taken up noticeably. Interestingly we have found that GO alone was able to significantly increase TGFβ signalling within the human chondrocyte cell line which could reduce the amount of growth factor required within our final GO construct. Further work is required to determine the mechanism behind this effect and whether or not it is sustained.

Conclusion, this project seeks to bring together established protocols to introduce GO into our biomaterials, capable of guiding hESC differentiation towards articular chondrocytes by the spatio-temporal delivery of a chondrogenic growth factor.

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DEVELOPMENT OF A TISSUE-ENGINEERED CONSTRUCT USING POLY(GLYCEROL SEBACATE) METHACRYLATE AND UNCULTURED ADIPOSE-DERIVED CELLS FOR ADIPOSE TISSUE ENGINEERING

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Introduction

Soft tissue repair is required following various traumas to the body, including damage from oncologic resections, such as breast cancer mastectomies or for facial reconstructions or congenital abnormalities. These soft tissue defects can not only affect patients cosmetically, emotionally and psychologically, but can also impair function. The current gold standard treatment for these conditions, autologous fat grafting, has limitations, including a requirement for repeat procedures, varying efficacy depending on the skills and techniques of the clinician, and a 40-60% reduction in graft volume following transplantation[1]. This loss of volume is thought to be associated with a lack of viable cells being grafted, poor survival of mature adipocytes once implanted, and poor revascularization at the donor site. Therefore, there is a need for a standardised procedure to repair soft tissue defects that can maintain volume following implantation.

Adipose tissue engineering is a field that aims to address the challenges presented by soft tissue replacement. Despite recent advances, a suitable strategy to replace autologous fat grafting that can be clinically translated has yet to be found. For most tissue engineering applications, scaffolds are required to provide mechanical support and promote cell survival and integration. These scaffolds require certain properties to make them fit for purpose, such as biocompatibility and biodegradability, as well as suitable mechanical properties. Interest in poly(glycerol sebacate) as a candidate scaffold biomaterial for adipose tissue engineering has increased in recent years as it has an assortment of properties that are better suited to match soft tissue than other commonly used biomaterials. Additionally, by introducing methacrylation to the polymer during synthesis, the mechanical and degradation time can be tailored to suit the requirements of the target tissue[2].

In addition to a scaffold, to encourage the growth of new adipose tissue, a source of regenerative cells may be required. Adipose tissue is an excellent source of autologous cells that can be easily harvested via liposuction procedure. Alongside mature adipocytes, adipose tissue contains a group of cells that are thought to have excellent regenerative potential, known as the stromal vascular fraction (SVF). The SVF contains extracellular matrix and cells such as pericytes, immune cells and a population of mesenchymal stem cell-like cells known as adipose-derived stromal cells. These adipose-derived stromal cells have shown good regenerative effects in tissue-engineered models, however, their requirement to be isolated and expanded in culture limits their clinical translation.

Researchers have developed a method to mechanically process lipoaspirate adipose tissue, removing mature adipocytes and creating a gel-like cell suspension of SVF cells and extracellular matrix[3]. This SVF gel can then be injected back into the patient directly after processing and has been shown to promote wound healing in animal models. The main advantage of this technique is that it is purely mechanical and does not require the addition of any exogenous material, or the *ex vivo* expansion of cells, factors that can often limit clinical translation through regulatory pathways.

Project Aims

This PhD project aims to develop a tissue-engineered device that can aid the regeneration of soft tissues. This device would incorporate a biodegradable poly(glycerol sebacate) methacrylate scaffold, to provide mechanical and structural support, with uncultured adipose cells (SVF gel) to provide a source of regenerative cells and stimuli. The project will explore how uncultured adipose cells can be incorporated into PGS scaffolds; how the properties of these scaffolds can be optimised to promote cell attachment and proliferation; and the ability of the construct to regenerate adipose tissue *in vitro*.

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LAB-BASED PRODUCED VASCULARISED TISSUE FOR IN VITRO LAB ON A CHIP MODELS OF METASTATIC CANCER

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Lab-on-a-chip devices are developing to closely mimic the structural and biological complexity of human tissue. Thus, becoming increasingly popular as an alternative to animal testing for pharmaceuticals. However, the lack of perfusion through vasculature within current models remains to be a challenge, reducing the physiological development capacity for tissue-on-a-chip models. This project utilises high internal phase emulsion templating combined with 3D bioprinting techniques to create an *in vitro* vascularised tissue via fabrication of a porous PCL PolyHIPE scaffold. Initial results demonstrate the fabrication of microporous structures (20-50 µm) using high internal phase emulsion templating. Via 3D printing the microporous PCL polyHIPE in lattice structures, macroporous structures (190-390 µm) were incorporated, critical for mature vascular formation. The use of such porous networks will allow for vascular outgrowth to occur in which cell, cell spheroids and small biopsies can be placed and resulting cell behaviour can be analysed. This will be further developed to overcome the short comings in current models and provide an *in vitro* alternative to *in vivo* testing platforms. Furthermore, this *in vitro* model will be used to explore cancer models. Cancer is a becoming a huge social and economic burden on society, being the most significant barrier to life expectancy in the 21st century. In particular, the model will be able to explore metastasis of cancer which is responsible for 90% of cancer related deaths.

DEVELOPMENT OF GRAPHENE-CONTAINING, 3D PRINTED SCAFFOLDS FOR ORTHOPAEDIC APPLICATIONS

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Introduction

There is a huge unmet need for millions of people worldwide who suffer bone loss due to injury, infection, disease or abnormal skeletal development for substitute bone that can seamlessly incorporate itself into the body [1, 2]. Synthetic materials that can mimic natural bone architecture at the micro- and macro-scale as alternative bone graft substitutes eliminate risks of donor site morbidity and infection, whilst being readily available [2]. Substrate intrinsic characteristics such as chemistry, nanotopography and mechanical properties can also induce adult stem cell differentiation towards specific lineages [1, 2].

For bone cell growth, stiff 3D porous scaffolds that mimic the bone tissue microenvironment and encourage vascularization and extracellular matrix deposition are needed, which traditional methods such as freeze-drying and salt-leaching alone cannot produce [1, 2]. Three-dimensional printing technologies are capable of seamlessly controlling scaffold size, density and pore interconnectivity due to uniform deposition of material according to a computer aided design, thereby producing patient tailored architectures.

Electrical stimulation (EStim) has been reported in many animal and clinical studies to enhance bone tissue regeneration. However, to date, scaffold designs that couple critical requirements of bone tissue engineering (BTE) and electrical compatibility are yet to be simultaneously achieved [3]. Graphene is an atom-thick material, which due to its remarkable electrical conductivity, tuneable surface chemistry, maximal surface-to-volume ratio, easy functionalisation capacity and mechanical properties may present an ideal solution [4, 5].

Materials and Methods

This project will focus on the development of alginate-graphene based inks for extrusion 3D printing of electrically conductive BTE scaffolds. Post-printing processing techniques shall also be investigated, such as those that may impart a higher degree of porosity and/or mechanical properties. Various imaging and characterisation techniques shall be used to establish scaffold biological, chemical, mechanical and electrical compatibility specifically for BTE.

Expected Results and Discussion

It is predicted that the optimal current for bone tissue regeneration ranges from 5 to 20μA as various studies have reported stimulation of bone repair through induction of alkaline phosphatase enzymatic activity, extracellular matrix deposition and mineralisation [6, 7]. It has also been observed that a current above 50μA induces cell death and bone necrosis, and so careful consideration of current strength as well as application duration, frequency and distribution will be required [6, 7]. Variations in graphene derivative, concentration, size and shape are expected to affect scaffold conductivity, mechanical properties and levels of toxicity to cells [4, 5]. It is also hypothesised that adjusting the molecular weights, solution concentrations and molar ratios of sodium alginate for various ink compositions shall affect ink rheology and therefore printability [8].

Conclusion

EStim in BTE has already proven its correlation with bone healing via clinical therapies and is now being translated into research environments that aim to investigate responses at a cellular level. However, the challenge remains in the construction and preparation of electrically conductive BTE scaffolds that allow controlled current strength, distribution and application duration, as well as upscaling [9, 10].

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BIOPRINTING THE HIERARCHICAL EXTRACELLULAR MATRIX ENVIRONMENT FOR ARTICULAR CARTILAGE REPAIR

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Introduction

Articular cartilage (AC) damage is one of the leading causes of immobility worldwide and can ultimately progress into osteoarthritis. Therapies used presently in clinics are showing to be insufficient in regenerating AC with the required properties and structure for long-term success. Tissue engineering (TE) and three-dimensional (3D) bioprinting technologies can together offer an innovative approach in constructing complex tissues. By directing the positioning of specific biomaterials, cells and bimolecular cues within a scaffold, it is possible to mirror the functional framework of the native tissue. However, the challenge still remains in recapitulating the intricate collagen fibre network that provides the AC its depth- and directional-dependent biomechanical properties. Cellulose, the plant analogue of collagen, has shown promise in improving the structural integrity and modulating the rheological properties of biomaterials for TE. Interestingly, cellulose nanocrystals (CNCs) have the ability to self-assemble into hierarchical phases similar to that seen with collagen architectures. It is anticipated that CNCs can interact with collagen, thus could act as structural directing agents within a 3D cell-laden scaffold to guide the alignment of newly synthesised collagen fibrils so as to mimic that of AC.

Objectives

To exploit the self-assembling nature of CNCs by controlling their directing axis to mimic that of the collagen fibres in AC within a 3D bioprinted hydrogel scaffold to improve the chondrogenesis of encapsulated human pluripotent stem cells (hPSCs) and the mechanical and structural properties of the scaffold and neotissue.

Materials and Methods

CNCs were made using a sulphuric acid hydrolysis and purification method described by Dumanli *et al.*.[1] The concentrated CNCs (ca. 5% (w/v)) were combined with alginate in various ratios between 0.5-2% (w/v) to form different hydrogel formulations, which were ionically crosslinked using $CaCl_2$. The rheological properties of the formulations have been evaluated and they were imaged using SEM. An immortalised chondrogenic cell line is currently being used to assess the biocompatibility using a LIVE/DEAD assay and the influence on phenotype using qRT-PCR when encapsulated within the CNC-based hydrogels.

Future Work and Conclusions

Following validation from these experiments, hPSCs will be encapsulated in the CNC-based hydrogels to study their impact on chondrogenesis using a differentiation protocol based on that described by Wang *et al.*.[2] After rheological optimisation and biological validation, the hydrogel system will be used to bioprint AC models. As a result, CNCs may provide an alternative method to produce complicated collagen organisations for tissue repair and disease modelling applications.

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THERAPEUTIC CYTOKINE RELEASE TO TARGET MACROPHAGE POLARIZATION IN OSTEOARTHRITIS

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Introduction

Osteoarthritis is a debilitating condition characterised by the erosion of articular cartilage, remodelling of the subchondral bone and inflammation of the joint synovium. Patients often rely on pain medication to alleviate the sensation of both physical and neuropathic pain. Currently, there is no long-term regenerative therapy available due to the many challenges experienced in osteochondral tissue engineering. The macrophage is a promising therapeutic target due to its ability to orchestrate the immune cascade[1]. Previously, the cytokine combination of IL-4, IL-10 and TGF-beta1 gave an M2 phenotype that was significantly more potent in reducing inflammation in vitro [2]. In osteoarthritis, the long-term release of these cytokines could reduce inflammation, alleviate neuropathic pain, stimulate osteochondral differentiation of mesenchymal stromal cells and may decelerate the progression of the disease pathobiology.

Materials and Methods

UV photocrosslinking, Mammalian cell culture, Enzyme linked immunosorbent assay, High performance liquid chromatography

Results and Discussion

At the current stage, only a low cumulative release of TGF-beta1 has been observed from PEG based hydrogels. Parameters such as PEG molecular weight, crosslinking methodology, protein aggregation, protein adsorption and protein-gel interactions will be explored to increase the cumulative release of TGF-beta and other cytokines. Gel chemistry will then be modified to control the release rate so that therapeutic doses are released for immunomodulation.

Conclusion

While it is challenging, the long-term release of cytokines may be a valuable tool for generating improved and long lasting therapeutics for osteoarthritis in the future.

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COMBINATION OF MICROFLUIDICS AND RETINAL ORGANOID TECHNOLOGIES TO DEVELOP A *RETINA-ON-A-CHIP*

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Introduction: Retinal degenerative diseases are a leading cause of vision impairment worldwide. Regardless of the initial cause of degeneration, the majority of diseases result in a loss of photoreceptor cells, often resulting in vision loss. Due to the inherent lack of regenerative capacity of the mammalian retina, diverse therapeutic concepts are currently being investigated. Cell transplantation approaches for the replacement of lost photoreceptors is a major goal in the research community and has been investigated for the last 3 decades. Recent advantages in 3D culture technology have enabled the generation of *in vitro* stratified retinal organoids (RO) in an endogenous manner that mimics the native tissue spatiotemporal development. RO technology has revolutionized the field, not only in providing advanced *in vitro* research models but also, enabling the generation of clinically relevant numbers of retinal cells for transplant therapies for the first time. Here, we report optimised culture of RO.

Materials and Methods: Murine ESC E14TG2a cells were maintained as previously described (Homma et al., 2013) and differentiated into 3D RO based in Eiraku and Sasai's (2012) protocol with minor modifications. Briefly ESC E14TG2a growing colonies were dissociated with 0.25% trypsin-EDTA and resuspended in retinal differentiation medium (GMEM, 1.5% KSR, 0.1mM nonessential amino acids, 1 mM pyruvate, and 0.1 mM 2-mercaptoethanol) supplemented with 0.1 µM AGN193109 in Sigmacote® coated 24-well plates incubated at 37ºC under 5% CO_2, 20% O_2. Matrigel, 2% (vol/vol) was added at day 5. On day 10, the aggregates were transferred into retinal maturation medium 1 (DMEM/F12 medium supplemented with 1% N2 and 1% penicillin/streptomycin). On day 14, media was changed to retinal maturation medium 2 (DMEM/F12 medium,1% N2, 10% FBS and 1%penicillin/streptomycin), supplemented with 1 mM L-taurine and 0.5 µM all-trans retinoic acid (media changed on day 16). From day 18 media is supplemented with 1 mM L-taurine only. Phase contrast images were taken throughout culture period and analysed using ImageJ and OriginLab.

Results: Growth from individual isolated cells was observed after seeding, producing hundreds of small cell clusters during the first 5 days of the process (~ 0.005 mm^2), which resulted in 1-2 organoids of sizes up to 2 mm^2 by day 17. In the absence of Matrigel, the cells were not able to fuse and grow into developed organoids.

Discussion: The preliminary results indicate that the customised protocol was successful in generating RO from murine ESC by day 17. Furthermore, we have demonstrated how the addition of Matrigel into the culture is crucial for the optimal development of the organoids, as equivalent protocols without Matrigel fail to generate the desired large, clearly defined cell aggregates. Despite the revolutionary role that 3D RO technologies have in the field of ocular sciences, they still present some limitations. These include the lack of a vascular system or incomplete cell maturation. It is foreseen that these can be addressed using *lab-on-a-chip* emerging technologies to create an *in vitro* retina. Such systems have the potential of quantifying biological processes at a single cell level and with high temporal resolution (Neto *et al.* 2016). Future work will require immunohistochemical analysis, to determine the degree of development of the organoids and the retinal lineage of the cells; we will also investigate the spatio-temporal development and organisation of these cells. After design and construction of a microfluidic chip, RO-derived cells will be sorted by MACS and cultured in the device.

Conclusions: In this study we demonstrate that our customised protocol has worked successfully for the generation of 3D RO. The study has also highlighted the crucial role that Matrigel plays in the success of organoid development. Importantly, *retina-on-a-chip* platforms could address if RO-derived cells have the capacity to connect with each other and form functional networks, which has not been yet irrefutably proven with other methods. *Retina-on-a-chip* devices will serve as a reliable, controllable, reproducible and inexpensive research model. From the best of our knowledge, this is the first time the combination of RO and microfluidics technologies have been researched into the generation of a functional *retina-on-a-chip* device which would serve as the next generation diagnosis and research platform.

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INVESTIGATING AND REPLICATING THE HUMAN LIMBAL EPITHELIAL STEM CELL NICHE

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Introduction

In the adult eye the cornea is the outermost surface of the eye and is subject to a variety of insults including tissue damage, physical and chemical stimuli throughout a person's life. In order to maintain the cornea's vital role of providing a physical barrier to the sensitive ocular environment and enable vision through this uniquely transparent epithelial surface, the cornea must constantly renew and if necessary regenerate. To facilitate these processes an adult stem cell niche – the limbal epithelial stem cell (LESC) niche works to provide a constant source of corneal epithelial progenitors. This niche is found within the limbus, an anatomic feature which presents as an undulating crypt structure, visually located as the border between the corneal surface and the conjunctiva [1]. The undulating structure of the limbus is proposed to be key in stem cell fate regulation. The LESC's reside in the basal apex of the limbal crypt which is interspersed by undulations called the Palisades of Vogt[2]. Disruption of this structure has correlated to a loss of limbal function attributable to limbal stem cell deficiency (LSCD)[3]. LSCD is a degenerative disease which can lead to loss of sight by corneal opacification, which has multiple proposed origins including chronic limbal inflammation, injury and limbal stem cell loss / limbal niche degradation due to aging[4]. The purpose of current proof-of-concept work is to generate an anatomic and disease model using material topographies, moving towards a dynamized bioreactor system and model, whereby the clear correlation of whether changes in limbal topography can control stem cell fate is drawn. Wrinkling of polymer surfaces has provided the means to create such a model[5], as the undulating surface of a wrinkled polymer surface closely matches to undulating structure of the limbus[6]. The current study has optimised the fabrication techniques for wrinkle formation. HEK 293 cells have been used to assess the developed model. In addition, the acquisition and isolation of limbal cells from human cadaveric tissue has been undertaken for use in the topographical substrate system.

Materials and Methods

Cells: Human corneoscleral tissue was acquired from the NHSBT Ocular department via an MTA. Limbal epithelial cells were obtained from this tissue by dissection of the limbus from the corneoscleral rim and digested in Collagenase IV, followed by serial passage to obtain limbal stem cells. HEK 293 cells were thawed from an in-house cell bank.

Substrate preparation: Polydimethylsiloxane (PDMS) was mixed as 5% curing agent to 95% elastomer and cast to produce 2mm thick chips in custom moulds or cast as a very fine film on a polished steel plate, both cured at 85°C for 2 hours.

Wrinkling generation: The PDMS substrates were deformed by uniaxial stretching, followed by one of two surface treatment methods to oxidise and stiffen the uppermost surface. The first method is the exposure of the strained substrate surface to a mixture of sulfuric and nitric acid mixed in a 3:1 ratio, followed by quenching in distilled water. Early implementations of this method use the acid mixture as-mixed, however in later experiments surface biocompatibility was improved by weakening the acid by thermal decomposition (80°C for 30min) and surface neutralisation in 0.1M sodium bicarbonate solution. The second method used a low temperature oxygen plasma, generated at 50W with an exposure time of 10min, to treat the stretched PDMS substrates. After these treatments, relaxation of the applied strain caused the PDMS surface to wrinkle.

Results

Initial implementations of the topographies showed that the wet chemical oxidised surfaces generated topographies most closely relatable to the limbal POV structure. Culturing of the HEK293 model cells shows that these surfaces were able to segregate cell populations and change HEK293 cell morphology. Plasma treated surfaces, on the other hand, produced a very fine wrinkled structure which had little effect on HEK cell distribution, but did influence limbal cell behaviour. Using the HEK 293 cells highlighted that improvements to surface biocompatibility of the chemically oxidised substrates were essential to enable cell growth. Early experiments demonstrated low cell proliferation after seeding, showing barren substrates. Weaking the acid aided in improving biocompatibility of the surface in addition to the implementation of sodium bicarbonate neutralisation. Isolation of limbal epithelial cells has been demonstrated with positive ABCG2 (a limbal stem cell marker) expression which were then cultured on the topographical substrates.

Conclusions

The presented topographies of substrates have been demonstrated to be suitable for the construction of a limbal epithelial stem cell niche model, wherein a degree of cell behaviour control has been demonstrated and the biocompatibility of these surfaces has been sufficiently optimised to enable the culture of limbal epithelial cells on these topographical substrates.

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ENGINEERING HUMAN BRONCHIAL TISSUE

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Introduction

This project will establish a novel *in vitro* model of the human airway. Such a model would provide valuable insight into both the native biology and pathophysiology of the human airway and, as such, this project will focus on asthma.

Human airway smooth muscle cells (hASMCs) and human airway epithelial cells (hAECs) will be co-cultured on either side of a gelatin-methacrylate (Gel-MA) scaffold. This scaffold will be formed into an airway-like tube exposing the hAECs to an air-liquid interface (ALI) at the lumen whilst maintaining contact with the hASMCs. A bespoke bioreactor will be utilized to culture the 'engineered airway' for several weeks until it reaches a few centimeters in size. This system will provide a tool to study drug delivery across the membrane and altered air composition within the lumen.

Results and Discussion

Initially this project will focus on hASMCs and hAECs interactions with the Gel-MA scaffold. Previous work within this group has generated Gel-MA hydrogels with stiffnesses closely resembling that of non-asthmatic and asthmatic airways. Further to this, the stiffer (asthmatic-like) gels can cause primary non-asthmatic hASMCs to exhibit an asthma-like phenotype. Building on this work hASMC and hAEC interactions with different scaffold preparation methods (including hydrogel formation, electrospinning and 3D printing) will be assessed for native-like histology. This work is helped by our ability to produce Gel-MA cheaper and in greater quantities than is commercially available.

Next a bi-layer co-culture of the hASMCs and hAECs upon the selected scaffold (or composite scaffold) in ALI conditions will be optimized for both 'asthmatic' and 'non-asthmatic' conditions. Key features of these 'engineered airway tissues' will be tuned to resemble the native tissue. Cell morphology and orientation as well as expression of markers such as epithelial cell adhesion molecules, smooth muscle alpha actin and MUC5AC will be taken into consideration.

Later stages of this project will develop a robust protocol to roll the 'engineered airway tissue' on an automated mandrel to produce a 3D tubular engineered airway. Finally, this engineered airway will be mounted within a bespoke bioreactor. The bioreactor, designed by Dr Ramis, will be optimized using pig bronchial collected from abattoirs. Protocols will be developed to maintain the engineered airway housed within the bioreactor for up to two weeks.

Conclusions

This project will develop a novel, clinically relevant model of the human airway housed within a bespoke bioreactor able to be maintained in culture for up to two weeks. The scaffolds within this model may be 'tuned' for non-/asthmatic-like phenotypes. A key feature of this model is the exposure of hAECs to an ALI at the lumen. This allows for the investigation of both drug uptake across the membrane and effects of altered air composition at the lumen.

THE EFFECT OF MOCROMOLECULAR CROWDERS ON DEPOSITION OF EXTRACELLULAR MATRIX IN ASTROCYTES

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Introduction: Spinal cord injury leads to grave disablement and dramatic loss of quality of life [1]. The mechanism of spinal cord injury, usually a blunt trauma, results in the severing of neuronal tracts. The subsequent wound healing steps aggravate the situation by resulting in a glial scar that represents a permanent obstacle to the re-connection of neurons [2]. Frantic efforts to tackle glial scarring in animal experimental models focus on the gene and cell therapy and small chemicals. However, there is currently no in vitro platform that would allow fuller characterization of the deposited scar matrix before therapeutic modalities are applied to a challenging animal model. In this study Neu7 cells are cultured in the presence of macromolecular crowders (MMC) and ascorbic acid (AA). The macromolecular crowding can dramatically enhance the deposition of a variety of extracellular matrix (ECM) molecules, notably collagen I, IV and VII [3]. Crowding of cell microenvironment can be emulated experimentally by the addition of MMCs to culture medium. It is hypothesised that ECM deposition can be modulated by the biophysical properties of the crowder. Herein, we assessed the influence of dextran sulphate 500 (DxS 500) on collagen type I deposition by Neu7 cells.

Materials and Methods: Neu7 cells were cultured in DMEM-Low glucose supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% penicillin and streptomycin. They were maintained at 37℃ in a 5% humidified CO_2 atmosphere. The cells were seeded at a seeding density of 25000 cells/cm^2 in conventional medium. After 24 hours, medium was removed and replaced with the same conventional medium and medium with 100 μM AA and 100 μg/ml DxS 500. Cells were grown for 5 days in each of these types of media. Phase contrast images were captured using an inverted microscope at different time points (2,3,5 days) to evaluate the influence of DxS 500 on cell morphology. And also, the alamarBlue@ assay was used to evaluate the effect of DxS 500 on cell metabolic activity. At day 5, culture media were aspirated, and cell layers were briefly washed and then digested with pepsin in acetic acid at 37℃ for 2 hr under agitation. Cell layers were then scraped and neutralised with 1 M sodium hydroxide. Cell layer samples were analysed by SDS-PAGE. Bovine collagen type I was used as standard for all gels. Staining of the protein bands was performed with SilverQuest™ kit following manufacturer's instruction.

Results and Discussion: Phase contrast images showed that cell morphology was not affected by DxS 500 and cells maintained their spindle shaped morphology throughout the time points. Also, metabolic activity of cells cultured in medium with DxS 500 was identical to those cultured in conventional medium. In addition, SDS-PAGE analysis made apparent that 100 μg/ml DxS 500 significantly increased collagen I deposition. DxS 500 enhance the accumulation of locally secreted molecules and hence the development of a secreted matrix and may also promote positive feedback mechanisms.

Conclusion: This study employed MMCs in Neu7 cell culture in an effort to emulate environment in which cells naturally dwell. The dramatic changes in ECM pattern and deposition in this initial undertaking gives better heart that better cell specific ECM characterisation can continue from this study.

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TUNING MACROPHAGE POLARIZATION TO MODEL MYOCARDIAL INFARCTION IN THE GENERATION OF FUNCTIONAL CARDIAC ORGANOIDS

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INTRODUCTION: Myocardial infarction (MI) is an ischemic and inflammatory event majorly orchestrated by macrophages from infiltrating monocytes[1]. These macrophages play a critical role in deciding the fate of the heart post-MI[2]. However, there is no cardiac disease model in existence that incorporates and immune response. Hence, the aim of this project is to develop a humanized model of MI, using induced pluripotent stem cell (iPSC) derived cardiomyocytes together with inflammatory cytokine stimulation, to model the disease environment.

METHODS: The first objective of this project is to obtain conditioned media obtained from immune cells for stimulating cardiomyocytes. In order to achieve this, iPSCs were differentiated to obtain macrophages (iMacs). Their expression of general macrophage (CD14, CD11b) and resident macrophage (CX3CR1, CCR2) markers were assessed, in addition to their phagocytic potential.

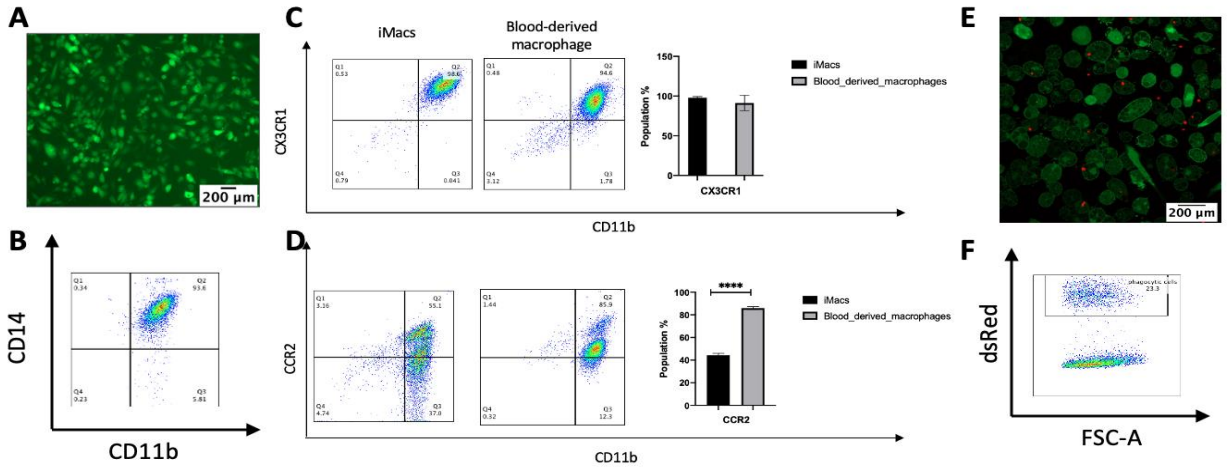

Figure 1 Characterization of iMacs. **A)** iMacs after 7 days of MCSF treatment, **B)** CD14 and CD11b expression of iMacs using flow cytometry, **C)** CX3CR1 expression of iMacs and blood-derived macrophages, **D)** CCR2 expression of iMacs and blood-derived macrophages, **E)** iMacs phagocytosing fluorescent red zymosan particles (40X), **F)** iMacs phagocytosis assay using flow cytometry. **** denotes $p<0.0001$, performed using t-test.

RESULTS: iMacs matured for 7 days with MCSF (Fig.1 A) were found to be 93.6% $CD14^{high}CD11b^{high}$ (Fig.1 B). Compared to blood-derived macrophages, CX3CR1 was upregulated (Fig.1 C) and CCR2 (Fig.1 D) was downregulated in iMacs showing a resident phenotype. Additionally, iMacs also showed phagocytic potential (23.3%) (Fig.1 E, F).

DISCUSSION & CONCLUSIONS: Comprehensively, according to the preliminary data, iPSCs have been successfully differentiated into macrophages. However, they need to be characterized further to confirm their functional ability.

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Keywords: iPSC, cardiomyocytes, macrophages, resident macrophages, engineered heart tissue

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LIVER METASTASIS IN A DISH

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer, typically found in 85% cases, and continues to have a poor 5-year survival rate of 10%. PDAC is a particularly aggressive cancer and metastasis at the point of diagnosis is the primary cause of poor survival rate. During metastasis, cancer cells disseminate from the primary tumour to other areas of the body and form a metastatic niche that promotes there survival and proliferation. An important aspect of the metastatic niche is the extracellular matrix (ECM) and further understanding of how disseminated cancer cells interact with the ECM is vital to aid the development of effective therapies. *In vivo* models have been used to study these interactions, however, these models are complex and have ethical limitations. To overcome these limitaitons, simplier and more accessible *in vitro* models are often used and there is an unmet need to develop more physiologically relevant *in vitro* models. We aim to address this unmet need by developing a 3D *in vitro* model of PDAC metastasis in the liver.

Materials and Methods

Human pluripotent stem cells were differentiated into hepatoblasts (HB), hepatic stellate cells (HSC) and endothelial cells (EC) by Prof. David Hay's lab at the University of Edinburgh [1]. Liver spheroids containing HB only, HB+HSC or HB+EC were formed by seeding cells in a 256-well microplate agarose mold and were then maintained in polyHEMA coated 12-well plates. PDAC A (Mouse KPC cell line) and MIA PaCa2 cells were labelled with CellTracker Red dye and added to the liver spheroid cultures, respectively. After 24hrs, the cells were fixed, embedded in 1% agarose and stained with Alexa Fluor-488 Phalloidin. Tiled Z-stack images were taken using an Opera Phenix™ High-Content Screening System. The interactions between the liver spheroids and PDAC A or MIA PaCa 2 cells were characterised as having no interaction, touching or invading.

Results

Both MIA PaCa 2 and PDAC A cell co-cultures had touching and invading interactions with the liver spheroids. PDAC A cells showed the highest percentage of interactions, either touching and invading, and the highest number of invading interactions occurred in the co-culture containing HB+HSC liver spheroids.

Discussion

These results show PDAC A and MIA PaCa 2 cells can be co-cultured with the liver spheroids and may have cell-cell interactions. PDAC A cells had a higher percentage of invading interactions with all types of liver spheroids compared to MIA PaCa 2 cells, which is consistent with a more aggressive phenotype. In addition, PDAC A cells had a higher invading interaction with liver spheroids containing HSC, which are the main cell type involved in liver fibrosis and promotion of cancer cell proliferation.

Conclusion

In conclusion, we have shown a promising PDAC cell and liver spheroid co-colutre system that can be used to develop a physiologically relevant 3D *in vitro* model. Future work will focus on incorporating this co-culture system into synthetic hydrogels which can be modified to recapitulate the physical, chemical and biological properties of the ECM and allow a reductionist approach to study the role of the ECM in metastasis. The use of synthetic hydrogels such as Biogelx hydrogels and PEG hydrogels will be explored.

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Acknowledgements

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INCREASED MCP-1 LEVELS IN FIBROBLAST-DOMINATED CO-CULTURE WITH MACROPHAGES

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Introduction

Diabetic foot ulcer(DFU) is characterized by unresolved chronic inflammation, which impedes healing. In bioengineering patho-physiologically relevant DFU models, recapitulating intercellular communication during the innate immune response in wound healing is necessary. MCP-1, a pro-inflammatory chemokine, favours healing when expressed during the earlier phases of wound repair by timely recruitment of macrophages to the wound site for bacterial clearance. However, wounds in diabetic mice are characterized by insufficient MCP-1 expression, which causes delayed macrophage response, resulting in impaired healing[1].
In this study, we checked how the presence of fibroblasts and macrophages affects MCP-1 production following a scratch assay and, after 15 days, subsequent treatment with an anti-inflammatory agent, dexamethasone (Dex).

Materials and Methods

RAW264.7 murine macrophages(MC) and human dermal fibroblasts(HDF) were used. Two distinct co-culture populations were cultured under high glucose conditions (45mM – mimicking hyperglycaemia in diabetes): i) 80%MC+20% HDF; ii) 20%MC+80%HDF. Upon confluence, a scratch was generated manually (to mimic a wound). At different time-points, supernatants were collected (days 1, 3 and 16) for MCP-1 quantification using ELISA. Following the scratch assay, treatment with Dex followed and supernatants were collected likewise(days 0, 4, 7 and 15) to determine MCP-1 levels.

Results and Discussion

Cell morphology remained unchanged throughout the study. Before Dex treatment, with increased HDF concentration and longer co-culture time, MCP-1 concentration levels increased significantly.
20%MC+80%HDF released increasing MCP-1 levels with increased period for both treatment groups. Furthermore, 20%MC+80%HDF released less MCP-1 when treated with Dex versus Non Dex-treated counterpart – except on day 4. It was observed particularly on day 4 in both groups that, only 20%MC+80%HDF produced MCP-1 – wherein Dex treated group had less MCP-1 compared to Non-Dex treated group. This observation may suggest that fibroblasts are key producers of MCP-1, which is needed for macrophage recruitment to wound site.
The arising clinical hypothesis regarding DFU is, fibroblasts in DFU produce less MCP-1 levels, resulting in reduced macrophage response and, subsequently, impaired wound healing.

Conclusions

Based on the obtained data, our key finding is higher HDF presence favours increased MCP-1 expression. Therefore, for future studies (in addition to running more controlled studies), we will use fibroblasts derived from DFU human tissues to verify whether they produce less MCP-1 in the presence of macrophages - hence accounting for the poor macrophage response at the earlier phase of DFU healing.

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OPTIMISING MYCOBACTERIAL DRUG DISCOVERY USING PICODROPLET TECHNOLOGY

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Introduction

Tuberculosis (TB) remains a global healthcare crisis with an estimated 10 million new cases and 1.4 million deaths in 2019 alone. The causative agent of TB is the major human pathogen *Mycobacterium tuberculosis*, which is approximated to infect up to a quarter of the worldwide population, in a 'latent' state known as non-replicating persistence (NRP). These infections are significant as they can relapse into active disease in immunodeficient individuals. Currently, there are no sufficient *in vitro* models which capably mimic all physiological conditions of NRP infections. There is an urgent need to design drug screening platforms which combine high-throughput technologies and replicate the human environment of the infection. The practises of microfluidics have promising future applications for bioengineered models and the miniaturisation of drug screening.

Aims of Project

The aims of this project are to engineer an *in vitro* model of non-replicating persistent mycobacteria using picodroplet microfluidics.

1. Design and fabricate a microfluidic chip
2. To encapsulate and incubate mycobacteria in picodroplets
3. To mimic the NRP state of the mycobacteria including hypoxia and nutrient deprivation
4. To screen new potential therapeutic agents against mycobacteria in the picodroplets as well as establishing their mechanism of action.

Materials and Methods

The model organism *Mycobacterium smegmatis* MC² 155 was grown in Middlebrook 7H9 broth at 37 °C before being encapsulated in polydisperse water-in-oil emulsion droplets (Novec™-7500 with 5% PicoSurf™ and Middlebrook 7H9 broth) by shaking. Stability of droplets containing mycobacteria were visualised by phase contrast microscopy.

Results

Droplets were stable over the course of 96 hours and with an increased growth of encapsulated mycobacteria.

Discussion

The preliminary exploration before moving onto microfluidics has shown that the surfactant used is compatible with Middlebrook 7H9 broth for creating droplets. Further research needs to be conducted to determine long term stability of droplets for the slower growing model organism of *Mycobacterium tuberculosis* - *Mycobacterium bovis* BCG. Physiological conditions such as hypoxia and nutrient deprivation need to be explored in the droplet 'bioreactors'. The application of droplet fluidics for optimisation of high-throughput screening will have important and significant impact upon mycobacterial drug discovery in the future.

NEW HYDROGELS FOR ENCAPSULATION AND BIOMEDICAL APPLICATIONS

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Over the past two decades hydrogels have become the most common scaffolds for tissue engineering. This is largely due to their high-water content and flexible 3D structure which mimics the extracellular matrix.[1] Supramolecular hydrogels are particularly of interest as they are formed by small molecules which assemble into a network to immobilize the solvent.[2] The reversibility of their gelation could be potentially beneficial by allowing the release cells while avoiding cell death. As well as this, these gels have a high drug loading capacity and the ability to carry out drug release in a controlled manner. These features point towards their immense potential in the field of bioengineering.[3]

In the past, natural or animal derived hydrogels, such as collagen, were commonly used for tissue engineering applications.[4] However, synthetic hydrogels possess a host of advantages compared to natural hydrogels. Firstly, natural hydrogels have a high variability and are chemically inconsistent, while synthetic peptide-based hydrogels have a defined chemical composition, reducing the likelihood of any batch-to-batch variation. Furthermore, peptide hydrogels have the potential to be highly biocompatible, as biological cues can be readily incorporated.[5] Finally, the tuneable mechanical properties of these gels make them particularly suited to tissue engineering applications as the stiffness of the gel drives cell differentiation.[6]

Here, I will describe our initial work towards forming peptides to form new hydrogels to 3D culture.

This work was supported by the EPSRC and SFI Centre for Doctoral Training in Engineered Tissues for Discovery, Industry and Medicine, Grant Number EP/S02347X/1.

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HUMAN CARTILAGE DEVELOPMENTAL MODEL USING EMBRYONIC STEM CELLS CULTURED WITHIN 3D HYDROGEL SYSTEMS

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Introduction

During development, articular cartilage is generated from the lateral plate mesoderm (LPM), an embryonic structure that gives rise to the limb bud (LB) and later forms the appendicular skeleton. Strategies to recapitulate cartilage development using human embryonic stem cells (hESCs) have been established [1-4]. However, current methods are complex and expensive [1-3], offer reduced control over the expression of key developmental markers [4], and lack translation into 3D culture using cell-laden hydrogel systems [1-4], a critical step towards applications in articular cartilage tissue engineering. In order to address these limitations, this work is aimed towards the establishment of a simple and cost-efficient strategy to guide hESC differentiation through the developmental route towards the generation of chondroprogenitor cells, and use them to generate a model of LB chondrogenesis in 3D.

Methods

hESCs were differentiated using a multi-step protocol with time-dependent modulation of WNT signaling using CHIR (WNT activator) and C59 (WNT inhibitor) and activation of retinoic acid (RA) signaling using TTNPB for 9 days. Then, hESC-derived chondroprogenitor cells were allowed to form aggregates for 72h. Finally, cell aggregates were encapsulated in alginate or alginate/type-I collagen hydrogels and cultured for a further 14 days. Cell viability was evaluated using live/dead staining and cell phenotype was characterised over the course of differentiation by qRT-PCR.

Results and Discussion

Treatment of hESCs with CHIR for 48h followed by C59 under RA activation led to the acquisition of a LPM-like phenotype with increased expression of markers FOXF1, HAND1, HAND2 on days 3-4. Then, the application of a second pulse of CHIR resulted in the controlled expression of limb bud mesoderm marker PRRX1 and forelimb marker TBX4 on day 6. Further treatment with TTNPB led to the acquisition of a chondrogenic phenotype on day 9, with upregulation of key transcription factors SOX5 and SOX9, and cartilage extracellular matrix components COL2A1 and ACAN. Further culture in 3D alginate-based hydrogels resulted in high levels of cell viability and maintained expression of key chondrogenic makers for up to 14 days.

Conclusions

These findings suggest that the modulation of WNT and RA signaling alone may be sufficient to guide hESCs towards LPM and LB-like stages towards chondrogenesis in a simple and cost-efficient manner. The use of alginate-based hydrogels may serve as a suitable platform to translate differentiation from 2D into a 3D culture system towards the development of new articular cartilage tissue engineering strategies for regenerative medicine and *in vitro* tissue modelling applications.

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3D BIOPRINTING WHOLE INTERVERTEBRAL DISCS

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Introduction

Back pain costs the UK economy an estimated £12bn per year and is driven largely by the degeneration of the intervertebral discs (IVDs). A lack of effective clinical interventions for back pain, coupled with an ageing population, means that novel regenerative therapies are essential. 3D bioprinting can be used to accurately model the composition, structure, and stiffness of the IVD and disc cell microenvironment, including the central, gelatinous nucleus pulposus (NP) and peripheral, fibrous annulus fibrosus (AF) regions. 3D bioprinted IVD models will therefore enable research into the processes driving IVD development and degeneration within these two regions and the IVD as a whole, the results of which will help inform novel regenerative therapies in future.

Methodology

Fresh samples of human IVD tissue were mechanically tested to determine their compressive and tensile stiffness (ElectroForce 3310, TA Instruments). Two immortalised NP cell subpopulations, one of suspected notochordal origin (CD24-positive) and one of unknown ontogeny (CD24-negative), were subsequently cultured on polyacrylamide gels of physiologically relevant stiffnesses (1kPa, 12kPa) for seventy two hours. The gels were functionalized with laminin-521, since the extracellular matrix of the developmental notochord and immature IVD are especially laminin-rich. qPCR analysis was conducted to assess the effect of these stiffnesses on the expression of phenotypic markers (COL, ACAN, KRT). Nuclear (DAPI) and F-actin (phalloidin) stains were additionally performed and image analysis software (CellProfiler, v3.1.8) was used to assess stiffness-induced morphological change (cell/nuclear area and shape), following 2D fluorescence microscopy. NP cells were then encapsulated and cultured in 3D within printable alginate-collagen and alginate-collagen-laminin blended hydrogels for fourteen days; cellular viability was assessed using a live/dead assay on days three and fourteen (Leica, SP8 Inverted Confocal Laser Scanning Microscope). qPCR data was generated to investigate expression of key NP cell markers. Rheology was also conducted to understand gelation kinetics, printability and stiffness of different gel formulations over time, whilst a BCA assay detected potential leaching of matrix components out of cell-free gels.

Results

Increased substrate stiffness in 2D resulted in both NP cell lines occupying a greater area and forming actin protrusions. These effects were more marked in the CD24-negative NP cell line. In contrast, the nuclei of the putative notochordally-derived NP cell subpopulation (CD24-positive) displayed greater sensitivity to changes in stiffness. Both NP subpopulations demonstrated good survivability in the 3D alginate-collagen and alginate-collagen-laminin hydrogels. Extensive clustering behavior was observed in the notochordally-derived NP cell line when cultured in the alginate-collagen-laminin gels, in both 2D and 3D. After twenty one days in 3D culture, collagen expression was significantly upregulated in the CD24-negative cell line, whilst other NP cell markers remained stable across time periods. Rheological data revealed that the alginate-collagen-laminin gels were of slightly lower stiffnesses than the laminin-free gels, although the explanation for this has not yet been determined. It has also been shown possible that a stiffness gradient can be created within the printed gels.

Discussion & Conclusions

Morphological analysis of the two NP cell subpopulations has indicated their response to stiffness differs, and this provides a useful tool when modelling healthy and degenerate discs using bioprinted cell-laden gels in future. The clustering behaviour observed in the laminin blended hydrogel strengthens the body of evidence that clustering behaviour in immature NP cells is both a function of matrix stiffness and composition; further experiments using a wider range of gel stiffnesses and a variety of matrix components can now be employed to examine this phenomenon and address important questions with regards to notochordal and immature NP tissue development. Cell viability, rheology and BCA assay data indicate that the gels are suitable for bioprinting and, after a full rheological characterization has been conducted, attempts will be made to model the interface between the NP and AF regions using bioprinting. Optimized qPCR and IHC protocols can then be applied to cell-laden 3D printed IVD constructs of varying stiffnesses and stiffness gradients.

NOVEL MODELS OF MAMMALIAN WOUND HEALING AND REGENERATION

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Introduction

It has long been of clinical interest to stimulate scarless wound healing after traumatic injuries. Healing of composite tissue injuries in complex structures such as the hands is particularly difficult to coordinate. The resultant scarring can be debilitating, as fibrosis of the tendon and skin layers leads to loss of digit functionality. No animal models of hand injury including tendon currently exist in the literature. We describe a new model of composite tissue injury, including tendon, in the mouse digit. We assess its utility in comparing healing phenotypes between wild type mice and MRL/MpJ mice, which show regenerative ear healing[1]. Additionally, we have developed an *ex vivo* culture system of mouse eyes to examine three corneal injury types varying in severity, which demonstrate both scar-free healing and eschar formation. This system will facilitate further examination of the underlying causes of epithelial scarring in mammals.

Materials and Methods

In vivo digit and ear punch: An observational study of a microsurgical murine composite tissue injury of the digit in C57/Bl6 (wildtype) and MRL/MpJ (regenerative healer) mice was performed and assessed for healing over 42 days. The injury involved excision of the neurovascular bundle, tendon and skin between the distal interphalangeal joint and the proximal digital crease. Concordant ear punches were made to assess the regenerative capacity of the mice during their healing. Tissue was harvested at day 7, 14, and 42, along with unwounded contralateral controls. Digits were decalcified in EDTA, and both tissue types were processed into wax. *Ex vivo* eye injuries: C57Bl/6 and MRL/MpJ mice were enucleated. The eyes were injured using three methods: epithelial scraping using Vannus scissors, and partial thickness and full thickness burns using a Bovie cauteriser. The eyes were stained for LIVE/DEAD (Invitrogen, cat no. L3224) and imaged on a stereo microscope over a period of 7 days. Healing of each injury model was assessed both from wholemount examination and from sectioning and staining with haematoxylin and eosin. Collagen was stained with picrosirius red. Images were analysed using Image J software.

Results/Discussion

As previously reported, MRL/MpJ mice showed either improved healing or regeneration of the ear pinnae tissues over 42 days, whilst C57Bl/6 mice healing stalled after approximately 2 weeks. Neither mouse strain showed perfect regeneration of digit tissues at 42 days; however, MRL digits showed signs of ongoing tissue remodelling which is being probed further using immunohistochemical and proteomic analysis. Future investigation of the molecular differences between the two mouse groups may identify cues for better healing of such injuries in a clinical setting. Preliminary analysis suggests that MRL/MpJ eyes cultured *ex vivo* show improved healing compared to C57Bl/6 eyes over 7 days. Scrape wounds and partial thickness burn injuries in both strains heal within 3 days. However, C57Bl/6 eyes do not show healing of full thickness burns due to formation of thick eschar at the wound site which impedes cell migration, whereas in some MRL/MpJ eyes, this eschar appears not to form. This wounding system is a promising model for mechanistic interrogation of eschar formation and regenerative healing of epithelia in MRL mice.

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VASCULAR REGENERATION WITH FUNCTIONALISED BIODEGRADABLE SCAFFOLD

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Introduction

The development of small-diameter vasculature remains a key challenge in Tissue Engineering. Over the last two decades, strides have been made on the biofabrication of vessel mimics with similar biophysical characteristics to those of mammalian vasculature. However, with only modest success, these vessels lack remodelling/regenerative capacity. This study aims to use induced pluripotent stem cells (iPSCs) and functionalised biomaterials to produce patient-specific bioengineered vessels.

Materials and Methods

Human iPSCs were maintained in Essential 8 medium. SB431542, a TGF-β1 inhibitor, was used for mesenchymal stem cell (MSC) differentiation from iPSCs. Human bone marrow MSCs (hBM-MSCs) were differentiated into vascular smooth muscle cells (VSMCs) via platelet-derived growth factor (PDGF)-BB and transforming growth factor beta 1 (TGF-β1) supplementation. Vascular scaffold was produced using electrospinning of poly-L-lactide (PLLA) and coated with silk fibroin. MSCs were grown on the scaffold to evaluate the biocompatibility of the materials. Gene expression was determined using qRT-PCR, and cell-specific marker proteins were detected using immunofluorescent staining and flow cytometry.

Results

During the 34-36 days differentiation, the human iPSCs from three different cell lines gradually gained a fibroblast-like morphology, had increased expressions of MSC marker genes CD73, CD90 and CD105. The decrease of pluripotent markers OCT4, SOX2 and TRA-1-81 was also observed. Further characterisations of the iPSC-MSCs are underway. Meanwhile, hBM-MSCs and iMSCs were successfully differentiating into VSMCs during a 9-day culture in PGDF-BB and TGF-β1 supplemented medium. The MSC-VSMCs express SMC marker genes of α-SMA and CNN1, SM22 and MYH-11. The PLLA-silk fibroin scaffold produced via electrospinning was compatible for MSCs adhesion and supports cell proliferation during the 10 day culture period. Ongoing work is to functionalise the scaffold with the specific growth factors to support in-situ vascular cell differentiation.

Discussion/Conclusion

An optimized protocol for MSC to SMC differentiation was established using PDGF-BB and TGF-β1 as stimulants. Also, the SB431542 supplementation protocol demonstrated to be efficient at deriving MSCs from hiPSCs. Work has been conducted on electrospun silk fibroin coated scaffold strategies with promising results assessing their biocompatibility after 10 days in culture. Future work will concentrate on the conjugating growth factors to the silk fibroin coating and evaluating the optimal release from these scaffolds to promote SMC differentiation *in situ* on the human iPSC derived MSCs.

A RETINOIC ACID ANALOG IMPROVES THE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO ARTICULAR CARTILAGE

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Introduction

Osteoarthritis is the most widespread degenerative condition of joint cartilage leading to chronic pain and immobility. Drug therapies are incapable of providing tissue restoration, and joint replacements are required in the most severe cases, indicating the need for an articular cartilage regeneration strategy in vitro. Articular cartilage has a crucial role in joints, which are derived from lateral plate mesoderm and limb bud during development. Our group established protocols for the differentiation of pluripotent human embryonic stem cells (hESCs) to osteochondral limb bud derived progenitors [1]. TTNPB 4-[(E)-2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid, a retinoic acid receptor agonist, has been reported to induce chondrogenesis in stem cells [2]. The addition of TTNPB to our hESC showed to improve expression of articular cartilage related genes while downregulating hypertrophic genes. Further optimisation of the protocol and addition of TTNPB significantly improved the differentiation quality outcomes, allowing the development of a robust protocol for the generation of articular cartilage.

Materials and Methods

Our previously established protocol [1] was further optimised by testing different concentrations and times of addition for the growth factors and small molecules involved, especially for Bone Morphogenetic Protein-2 (BMP2). Titration of TTNPB was performed and its addition in 2D and 3D stages of the protocol was assessed. Protocols for optimisation were run in parallel and chondrogenic quality output was assessed by qRT-PCR, Safranin O and Alcian blue staining. Histology was performed on 3D pellets after 40 days of differentiation. Flow cytometry was performed to quantify quality of differentiation testing for pluripotent and chondrogenic specific markers.

Results and Discussion

The addition of TTNPB significantly improved the expression of SOX9 and COL2A1 while reducing expression of hypertrophic markers such as COL1A1 and RUNX2, when compared with the previous protocol, suggesting enrichment for an articular cartilage population. Markers for Lateral Plate mesoderm (FOXF1, HAND1) and limb bud (PPRX1, HOXB5, HAND2) were significantly expressed at the respective stage of the protocol. Expression of Paraxial mesoderm markers (TBX6 and PAX1) was lower than undifferentiated hESC, suggesting that cells were efficiently driven into lateral mesoderm and limb bud populations. Cells stained for Safranin O and Alcian Blue in 2D and 3D phases. Histology of the 3D phase showed characteristic articular cartilage structure.

Conclusions

TTNPB significantly improves the differentiation of hESCs into articular cartilage progenitors allowing the reported protocol to provide a promising and robust cell source for better understanding the mechanism of articular joint development in humans. However off-target subpopulations may form during the process of differentiation, hindering further application for this protocol. The implementation of a cell-sorting strategy based on surface markers for the discrimination of chondro and osteo progenitors would improve the potential and safety of our protocol. We are currently assessing the expression of chondroprogenitors markers (such as PDGFRβ, CD146, CD166) and evaluating their use in combination with other cell surface markers of limb bud progenitors. When refined by cell-sorting and fully characterised, this protocol could lay the foundations for possible cell therapy for osteoarthritis.

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A NEUROVASCULAR 3D CELL MODEL TO INVESTIGATE THE ROLE OF PERICYTES IN DEMENTIA

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Introduction:

Pericytes, brain microvascular endothelial cells (BMECs), astrocytes, and neurons form the neurovascular unit (NVU). NVU dysfunction is commonly seen in vascular dementia and Alzheimer's disease[1]. Pericytes have been shown to contract capillaries and thus reduce cerebral blood flow, in a downstream response to amyloid-β, a hallmark protein of Alzheimer's disease[2]. However, the full role of pericytes in health and disease remain poorly understood, partially due to a lack of adequate models that can recapitulate the complexity of the multi-cellular NVU. The aim of this study is to incorporate pericytes into a 3D *in vitro* model to study the function, and dysfunction, of the NVU and the pericytes within it.

Materials and Methods:

Brain pericyte-like cells and BMECs were differentiated from human induced pluripotent stem cells (iPSCs)[3,4] and co-cultured in a transwell-type NVU model. Integrity of the endothelial barrier function was evaluated by transendothelial electrical resistance (TEER) measurements. Hydrogel encapsulation was used as a contraction indicator.

Results and Discussion:

Brain pericyte-like cells stained positively for pericyte markers NG2, PDGFR-β, CD13, and CD146. Co-culture of pericyte-like cells with BMECs resulted in a significantly increased TEER compared with mono-cultured BMECs. Furthermore, these brain pericyte-like cells significantly decreased the hydrogel surface area by 40% showing their potential ability to contract.

Conclusion:

These results suggest that functional brain pericyte-like cells were obtained and can be incorporated into a NVU model. We will build on these findings to develop a robust model to study pericytes within the NVU to investigate their function and dysfunction in health and disease.

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BIOENGINEERING A MODEL OF THE OUTER BLOOD-RETINAL BARRIER TO TEST TREATMENTS FOR DIABETIC RETINOPATHY

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Introduction. Diabetes represents a big burden for society and among many complications, diabetic retinopathy (DR) is one of the major leading causes of sight loss, significantly reducing the quality of life of those affected. Nowadays, the gold standard treatment for DR consists of intravitreal drug injections. However, this treatment is costly to the NHS and relies on recurrence. Nanomedicine could transform the management of diabetic eye diseases through gradual-release-drug nanoparticles to improve the action of already used drugs and to deliver those characterized by poor solubility.
Modelling a diabetic *in vitro* model of the outer blood-retinal barrier (BRB) may help in understanding pathological changes that may occur in DR progression and be useful to test drug permeability and efficacy. Even though outer BRB is not as well studied as the inner BRB, a growing number of works have emerged outlining its importance in DR pathogenesis[1]. Various *in vitro* and on-a-chip co-culture models of the outer BRB have been reported, including mixed human/animal and primary/cell line co-cultures[2,3]. However, to the best of our knowledge, none of them specifically use ARPE-19/hREC combination in diabetic-like conditions with an optimised substrate to allow a proper barrier formation.

Aim. To establish an *in vitro* co-culture, diabetic model of the outer BRB for pharmacologic studies.

Materials and methods. ARPE-19 (p27-p32) and primary human endothelial cells (hREC, p4-p9) were cultured and characterised by analysing their growth rate (cell count), marker expression (ICC), and phenotype (phase-contrast microscopy) over time. Cells were subjected to diabetic-like conditions (33 mM glucose, 2% oxygen)[4] for 14 days and cell phenotype, metabolism (resazurin sodium salt), and oxidative stress (CellROX) were evaluated. Additionally, commercially available porous inserts - expanded polytetrafluoroethylene (ePTFE), polycarbonate (PC), and polyester (PET) - were selected to mimic the Bruch's membrane and tested to determine the optimum substrate for cell adhesion and growth to be used in the model. TEER measurements and FITC-labelled dextran particles were used to evaluate proper barrier formation.

Results. ARPE-19 and hREC showed different growth rates and ARPE-19 exhibited various phenotypes in long-term culture (3 months). Cells cultured on different materials, in both mono- and co-culture, formed tighter barriers on both PC and PET. Additionally, ARPE-19 cultured on substrates expressed both CRALBP and ATPase markers, compared to plastic. Furthermore, diabetic-like conditions had a great impact on cell behaviour, influencing cell metabolism and causing a reduction in barrier formation indicated by the ZO-1 signal.

Discussion and conclusions. With access to human samples limited and the need for reproducibility in screening models, the use of commercially available cell lines is useful when developing an *in vitro* model of the outer BRB. These cells behave differently under various culture conditions that need to be optimised. All presented materials have shown the ability to support cell growth and adhesion, while potentially allowing the cells to maintain a physiological phenotype. Furthermore, while DR is characterised by a hyperglycaemic and hypoxic environment, the application of diabetic-like conditions *in vitro* demonstrated the impact of such insults also on cultured cells (increased oxidative stress and leaking tight junctions). The step-by-step characterisation process described here offers a diabetic outer BRB model suitable for pharmaceutical studies aimed at novel DR therapy assessment.

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Clinical Advances and Patient Benefits of Applications

UNDERSTANDING THE MECHANISMS OF ACTION OF COLLAGEN-BASED WOUND DRESSINGS

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Introduction

Wound healing is a complex process that involves numerous cell types, cytokines, chemokines, growth factors and extracellular matrix (ECM) components, which work synergistically to achieve healing[1, 2]. It consists in four overlapping phases that occur smoothly to achieve healing[1–3]. When the healing process fails to proceed through these physiological phases, the wound is referred to as a chronic wound[1,2,4,5]. Chronic wounds are a significant global problem, causing patient morbidity and a substantial financial burden on health services worldwide. The rising prevalence of chronic wounds puts increasing pressure on global health services, calling for the development of therapies that can relieve both patients and healthcare systems of this economic and societal burden [6,7].

There are a vast variety of commercially available dressings aimed at managing chronic wounds, making it difficult for healthcare professionals to choose the most appropriate therapy[2]. Collagen-based dressings are a large class of dressings which offer numerous beneficial properties for the treatment of recalcitrant wounds[8-11]. Commercially available collagen-based wound dressings differ in composition but present similar claims. There is a lack of comparative data to differentiate between these dressings and understand their mode of action. This project is set to compare the effects of collagen-based wound dressings using a diabetic murine full-thickness wound model.

Materials and Methods

The dressings chosen for analysis were a control non-woven dressing (3M, Saint Paul, MN, US), 3M™ Promogran™ Protease Modulating Matrix and 3M™ Promogran Prisma™ Wound Balancing Matrix (3M, Saint Paul, MN, US), Puracol® (Medline Industries Inc., Northfield, IL, US), ColActive® Plus (Covalon Technologies Ltd., Mississauga, Ontario, Canada) and UrgoStart® (UrgoMedical, Chenôve, France), a synthetic dressing with similar claims to collagen-based dressings. Thirty-six diabetic (*db/db*) female mice received two full-thickness excisional wounds. Wounds were treated with a pre-moistened control or collagen dressings (12 wounds per group). Dressings were changed or re-applied after 3 days according to the products' IFU. After 7 days, macroscopic images were taken, and wounds harvested. Wounds were bisected and processed for histological and biochemical analysis. Wound width, wound area, and re-epithelialisation were quantified from haematoxylin and eosin stained sections using ImagePro software.

Results and Discussion

Wound area calculated from the macroscopic images for all wounds (12 per group) showed that 3 of the 4 collagen dressings and UrgoStart promote healing compared to the control ($p=<0.05$), achieving 60-70% closure within 7 days. Analysis of wound parameters from histological sections of 4 of the 12 wounds for each dressing revealed greater re-epithelialisation compared to the control, albeit owing to the limited numbers of wounds analysed so far this increase in re-epithelialisation was not significant. Granulation tissue area, wound length and re-epithelialisation varied amongst the collagen dressings but no significant differences were observed. Full analysis of all 12 wounds for each dressing is underway.

Conclusion

Our preliminary results show that collagen-based dressings promote healing of diabetic murine wounds to a greater extent than a non-collagen control dressing. The rate at which these dressings promoted healing differed between dressings. Full analysis of all 12 wounds for each treatment group may provide differentiation between the type of collagen dressing and effect on healing. Future work will determine if the composition of these different collagen-based dressings differentially impact specific cell types within the wound including keratinocytes, fibroblasts, and immune cells. These results may help healthcare professionals with a greater understanding of how collagen-based dressing can modulate healing of chronic wounds.

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3D BIOPRINTING TISSUE ENGINEERED MENISCAL CONSTRUCTS

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Meniscal injuries are the most commonly recorded knee injury by orthopaedic clinicians, affecting over 1.5 million people across Europe and the USA. Injury can greatly reduce knee joint mobility and quality of life, and often leads to osteoarthritis. Tissue engineering (TE) strategies have emerged in response to a lack of viable treatments for meniscal pathologies. This promising avenue involves the combination of an engineered supporting scaffold with the incorporation of cells and growth factors to stimulate repair and regeneration of damaged meniscal tissue. To date, TE constructs fail to mimic both the structural and functional organisation of the native healthy meniscus whilst promoting the deposition of new extracellular matrix for regeneration.

This project aims to develop a truly biomimetic meniscal substitute. Operating in a layer-by-layer fashion, 3D Bioprinting allows for precision deposition and patterning of biological materials with high spatial resolution. By exploiting various design/process parameters in combination with the right biomaterials, cells and bioactive molecules, 3D bioprinting can eventually be used to create biomimetic human tissue equivalents.

Initial experimentation involves characterisation of meniscal tissue to effectively inform the design of biomaterials for bioprinting meniscal implants with adequate structural and functional properties. Histological and cell culture analysis revealed regionally distinct variations in tissue architecture, matrix compositions and cell populations. 3D laser scanning was employed to acquire external geometrical information and a 3D printed meniscus was fabricated, highlighting the potential to print patient-customised implants. 3D bioprinting allows for the precise spatial deposition of biological materials to generate complex multifunctional structures. A blended alginate and collagen hydrogel has been investigated for its use as a bioink. A Live/Dead Assay™ reveals the hydrogel supports meniscal cell viability, supporting its use for development in future studies.

3D Bioprinting is a novel technology that is attractive to recapitulate the intricate meniscal architecture for regeneration of damaged meniscal tissue. Future work within this project will involve further characterisation of meniscal tissue and cellular phenotype. Co-printing technologies will be employed to fabricate a structure containing materials, cells and bioactive molecules. With the aim to produce a biomimetic meniscal tissue with its regionally distinct variations, capable of restoring knee articulation.

DEVELOPMENT OF A NOVEL, SMART, AND DISPOSABLE TEAR COLLECTION DEVICE FOR POINT-OF-CARE OCULAR AND SYSTEMIC DISEASE DIAGNOSIS

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Introduction

The most recent World Health Organisation (WHO) report estimates globally that 2.2 billion people from preadolescence suffer from vision impairment, and over half of these suffer eye conditions such as dry eye disease; glaucoma; and diabetic retinopathy[1]. The outer eye surface is populated by a precorneal tear film layer (7-10 µL) composed of tears that contains thousands of biomarkers with correlating concentrations to a myriad of ocular diseases. Moreover, exploitation of the blood-ocular barriers has enabled in recent years the diagnosis of systemic diseases including cancers and motor pathologies. In order to analyse these film biomarkers for diagnosis, tears are collected conventionally by methods such as Schirmer test filter strips; microcapillary glass tubes; and cellulose absorbent sponges, which remain suboptimal. High variability between achievable sample volumes; types of tears collected; tear biochemistry; tear proteome; and the necessity for post-processing to extract the tear sample, justify the lack of a standard protocol for tear collection and analysis for accurate disease diagnosis and staging. Therefore, there is a need to develop next-generation tear collection devices that are reliable and indicative of diseases to enhance the diagnostic potential of tears.

Project Overview

In partnership with Menicon Co., Ltd, a global contact lens manufacturer, this research sets out to develop and test a novel tear collection device composed of biomaterial polymers that is able to remain under the lower eyelid while simultaneously capturing a low volume of tear fluid. Establishing requirements of a new collection device gears the research direction towards the use of contact lens substrates, and stimuli-responsive biomaterials which have shown microfluidic potential for capturing and storing biological fluids. The project scope can be broken down into four key milestones: (1) development and characterisation of a prototype smart-based collection device with microfluidic components in wet and dry-lab environments; (2) integration of the device into industry partners analysing system by a microfluidic channel for investigating the sample; (3) establishment of an interface permitting fluid transfer between the device and analysis system; and (4) clinical studies on human participants for device efficacy. Findings from such work will allow for tear film biomarkers derived from the ocular system and body to be correlated with baseline values for point-of-care diagnosis.

Expected Results

It is expected that a prototype collection device without electronics will be developed inexpensively using microfabrication techniques, and it is capable of collecting and storing between up to 500 nL of basal tears under non-turbulent flow conditions. Furthermore, incorporating a stimuli-responsive biomaterial, while being comfortable to the wearer and non-obtrusive to vision, will prevent sample evaporation and ensure the device is safe to use. After extensive *in vivo*, *in vitro*, and *in silico* experiments it is hypothesised that the miniature device will remain mechanically stable during wear in the lower eyelid and not cause ocular irritation or corneal damage, ultimately leading to transfer of the collected sample volume to the analysis system following device removal. Analysis will result in the identification of numerous biomarkers and their concentrations for correlating to a variety of ocular and systemic diseases for diagnosing, monitoring, and staging clinical conditions. When compared against conventional collection methods, the device will collect tear samples that better represent the biomarkers present day-to-day as it will not require post-processing or reflex tear sampling.

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THE DEVELOPMENT OF DRUG-ELUTING ELECTROSPUN DEVICES FOR CLINICAL APPLICATIONS IN GYNECOLOGY

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Introduction

Gynaecological conditions are extremely common, affecting up to an estimated 20% of people with a uterus (1); they can be at best irritating and at worst life limiting. Whilst pessaries are effective for the delivery of drugs in some conditions, they are not applicable for all and have known drawbacks. The multi-disciplinary team supervising this project have already successfully developed Electrospun polymer devices that have proved effective in the delivery of drugs to the mouth (2), and it is believed that this technology can be adapted to address unmet needs in gynaecology as well. Topical drug delivery is preferable to systemic treatments in many gynaecological interventions, however given the nature of gynaecology this can be exceptionally challenging. Development of a drug-eluting, smart patch that can respond to local conditions within the uterus and release drugs topically could help solve these issues. Gynaecological conditions that would benefit from direct and sustained drug delivery include infections (e.g Candida albicans) and lichenoid reactions in the vagina, endometriosis in the uterus, and various gynecological cancers. The aim of this research is therefore to design, fabricate, characterise and evaluate an electrospun patch to help meet this need in gynaecology.

Materials and Methods

The delivery of therapeutic nucleic acids (TNA) has already proved effective in treating a range of conditions, including various different cancers (3), but as yet there is limited research that focuses on utilising these treatments for gynecology. Given the potential for TNA, both realised and not, this project's initial aim is to develop an electrospinning technique that can successfully produce TNA-containing fibre patches without reducing the efficacy of the TNA. Related research has already gone some way to demonstrating the protective capabilities of coaxially electrospinning but as yet there is no reliable or simple way of producing TNA-loaded electrospun patches. Initial trials will utilise poly[2-(dimethylamino)ethyl methacrylate-*block*-di(ethyleneglycol) methyl ether methacrylate (poly-DMAEMA) as the carrier for the TNA, given it have proven use in gene delivery in a vesicular structure. Analysis of these data will then be used to determine the next steps to be conducted in the research.

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CREATING A MULTISCALE, HIERARCHICAL MIMIC OF THE ACL USING CELLULOSE NANOSTRUCTURES

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Introduction

The Anterior Cruciate Ligament (ACL) is a fibrous connective tissue that plays an integral role in stabilizing the knee joint. It consists of a hierarchy of aligned collagen fibres that endow the tissue with remarkable mechanical properties: fibrils (tens of nm in diameter) bundle into fibres (1 – 20 µm in diameter), which assemble into fascicles (hundreds of µm in diameter). Each level of the hierarchy contributes to the mechanical properties of the tissue in a discrete and individual manner, whilst also interacting with one another to form a continuous tissue with an improved mechanical performance than any level would in isolation [1]. Injury to the ACL destroys this intricate structure, leading to a rapid deterioration in function which can seriously impact quality of life. ACL ruptures carry a significant socioeconomic burden: in the US alone, approximately 350,000 ACL reconstructions are performed each year, at a cost of around $6 billion [2],[3]. This issue is exacerbated by a dearth of effective treatments: the current 'gold standard' is an autologous tendon graft, despite the associated donor site morbidity and disappointing long term results [4]. Attempts to construct synthetic grafts have been hindered by insufficient mechanical properties and poor integration with the host tissue [5]. It is hypothesised that any synthetic graft must mimic the hierarchical structure seen within native ligament tissue. Not only does this allow for the efficient transfer of stress between different layers of the hierarchy, but also presents the opportunity to engineer a nanoscale environment that mimics the natural cellular milieu. Cellulose is emerging as a promising biomaterial of virtually limitless resource, readily available from natural sources including tunicates and bacteria [6]. Analogous to ligament tissue, it also exists in nature as a hierarchical structure. The cellulose nanostructures that exist within tunicates – termed cellulose nanofibrils (CNF) – can be extracted through acid hydrolysis. Bacterial cellulose (BC) is synthesised by Gluconacetobacter xylinusin. Both BC and CNF present with remarkable mechanical properties and dimensions that closely mimic the collagen fibrils seen within ligament tissue. Moreover, an abundance of hydroxyl groups on their surface allows for simple functionalisation that can improve integration with the host tissue. However, harnessing these nanoscale properties and transferring them into a high-performance ligament graft depends upon their alignment across multiple length scales.

Project Overview and Expected Results

This project will aim to engineer a synthetic acellular ACL graft that mimics the hierarchy seen within the natural tissue. The project will use a bottom-up approach, beginning with fibrilar cellulose nanostructures including CNF and BC (isolated from tunicates and bacteria respectively). The gelation of these structures at different wt % will be investigated, and the most appropriate will be processed into fibre form on a larger scale using advanced manufacturing techniques such as flow assisted assembly, electrospinning and direct ink writing. It is hypothesised that the shear forces acting upon the nanostructures will cause them to align as a nematic liquid crystal phase within the larger fibre, mimicking the alignment of fibrils within collagen fibres as seen in native ligament tissue. Different cross-linking agents will be incorporated into the system, to provide the graft with improved extensibility. Moreover, controlling the amount of cross-linker within the structure offers an opportunity to modulate stiffness as a control over cell response. We will then investigate how these fibres can be assembled into higher order structures using simple textile techniques including bundling and weaving; the impact of these different techniques on mechanical properties will be assessed. At each stage of the project, the cell response to the material will be investigated.

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OPTICAL DETECTION OF AIRBORNE FUNGAL PATHOGENS

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Introduction: *Aspergillus spp*. are asexual, saprophytic fungi that are ubiquitous in the environment and are more commonly known for their persistence in agricultural crops. From a clinical perspective, species such as *Aspergillus fumigatus* are responsible for triggering a range of conditions known as aspergillosis, predominantly affecting immunocompromised patients where invasive aspergillosis (IA) can lead to 40–50% mortality in leukaemia patients. Even in immunocompetent individuals, chronic pulmonary aspergillosis (CPA) causes a serious clinical challenge (80% 5-year mortality) with both CPA and IA requiring the use of long-term azole anti-fungal treatment. The overuse of these treatments has promoted an increase in resistant *A. fumigatus* isolates. As a result, oral therapies such as triazoles, which are the only class of oral antifungal with activity against *Aspergillus spp.*, are no longer effective. To effectively manage and reduce infection by *A. fumigatus* suitable monitoring and detection techniques are required to prompt decontamination protocols and preventative measures. The Sentinel system is a series of networked environmental monitoring systems featuring an interchangeable, selective biomimetic growth surface to identify *A. fumigatus* by its growth characteristics. The film will be imaged by a commodity camera, the images processed by machine-learning algorithms, and diagnostic information shared wirelessly. The focus of the CDT research is to create this selective element, making a thin film growth niche to germinate *A. fumigatus*, which can be mass produced.

Materials and methods: The organism will be grown in media of varying composition to investigate the boundaries of growth that impose a stress response in a form of phenotypical mutation. Stress response will be explored by changing temperature, nutrients, trace elements, pH in combination with polymer membranes of varying structure, type, colour/opacity, pore size, surface charge, topography and thickness. Morphological changes should be visible at 20x magnification and lower with optical microscopy. To increase specificity to resistant strains, anti-fungal compounds will be incorporated into the niche and sensitivity will be explored alongside stress response to identify the lowest concentration of spores in the shortest period of time.

Results, Discussion and Conclusion: The adaptability of *Aspergillus* species allows it to grow on an array of surfaces and environments, therefore by changing its growth conditions stress responses are initiated causing morphological changes in cell colour, structure and shape. By exploring its natural response to varying environmental conditions and nutrient sources a sensitive and specific niche can be developed, allowing faster growth and the developed of an identification criteria. In this way, decontamination protocols can be initiated to prevent infection outbreaks of population at risk, whilst the presence of *A. fumigatus* is confirmed much earlier than conventional microbiological techniques.

A NOVEL 3D OSTEOCHONDRAL SCAFFOLD WITH MECHANO-IDENTICAL PROPERTIES OF THE NATIVE TISSUE FOR *IN SITU* TISSUE REGENERATION

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Osteoarthritis (OA) is a chronic, global, degenerative disease of synovial joints. Most prevalent in load-bearing joints, OA is the most common musculoskeletal condition, form of arthritis and cause of pain and disability. The increasing prevalence of obesity, sedentary lifestyles, OA in younger populations and an ageing population will increase OA incidence and the psycho-socioeconomical burdens associated. Osteochondral (OC) tissue is a composite system comprising of articular cartilage (AC), underlying subchondral bone and a complex interfacial zone between them. Grafting of OC tissue poses challenges including limited supply, immune rejection and ethical considerations. Surgical treatments (e.g., autologous OC transplantation) can only be used in non-weight bearing joints and normal cartilage function is rarely restored. Traditionally, health-care providers passively await the final 'joint death' necessitating an irreversible total joint arthroplasty (TJA) procedure, the clinical gold standard treatment, however full joint function isn't always restored and presents long-term complications including aseptic loosening, infection, morbidity rates following surgery and functional failure. Revision surgery is complex due to the weakened surrounding tissue and association with additional short- and long-term complications. Revision surgery rates are increasing and there are significantly greater risks that individuals under 60 will require revision surgery within 10 years resulting in younger patients living with a lower quality of life for several decades. This project aims to develop and produce an "off-the shelf" scaffold to provide a novel means of preventing TJAs through effective and economical tissue regeneration, treating OC defects and OA and restoring lost joint function. A biomimetic multiphasic scaffold with a gradual interface will provide necessary mechanical support and ensure successful integration for long-term functionality by permitting separate osteogenic and chondrogenic factors, simultaneously. This "smart" tissue engineering (TE) scaffold will contain a trabecular bone-regenerating region (BRR), a subchondral plate region (SPR) and an articular cartilage-regenerating region (ARR).

Polyhydroxyalkanoates (PHAs) are a relatively novel family of biopolyesters produced by bacteria under various environmental and often unbalanced growth conditions, offering fantastic potential as a sustainable, environmentally friendly, renewable resource for the successful fabrication of TE scaffolds. The structural, mechanical, physical, biocompatibility and biodegradability properties of PHAs can be optimised by altering biosynthesis conditions, blending with other PHAs and surface modifications to support cell adhesion and ultimately tissue regeneration. The scaffold will be manufactured from blends of Poly(3-hydroxybutyrate) and poly(3-hydroxyoctanoate-*co*-3-hydroxydecanoate) utilising 3D printing for the BRR phase and electrospinning for the ARR phase. Investigation of varying PHA blends will determine the optimal composition for the desired mechanical and degradation properties. These fabrication methods demonstrate the potential to produced functionalised scaffolds with controlled micro- and nanoscale biomimetic features. Recent bioprinting techniques enable fabrication of scaffolds with more personalised biomimetic multifunctional architectures by converting clinical imaging data into computer-aided design (CAD) files and designing anatomically accurate scaffolds. Cold plasma deposition of allylamine and binding of cell homing and chondrogenic factors will further enhance the scaffold surface to promote regeneration of articular cartilage through recruiting, binding and promoting chondrogenesis of stem cells. Scanning Electron Microscopy will be used to examine morphological characteristics and microstructure of the scaffold and to study the morphology and distribution of cells growing on the substrate as well as quantifying osteoblasts and chondrocytes at suitable time periods (e.g., 1, 4, 7, 14 days) to examine cellular adhesion, formation, division and proliferation.

Biocompatibility will be assessed *in vitro* utilising Live/Dead cell viability assays histological and immunohistochemical analyses. RT-PCR will be used for analysis of gene expression of major osteogenic and chondrogenic proteins of mesenchymal stem cells (MSCs) and MG62 chondrocytes for the BRR and MSCs for the ARR, evaluating suitability for cell growth and appropriate tissue formation. Chondrogenic and osteogenic differentiation can be enhanced through a complex interplay of biochemical cues. Osteocalcin, alkaline phosphate and collagen type I will be used as osteogenic biomarkers, while aggrecan and collagen type II will be chondrogenic biomarkers. *In vivo* animal models will be crucial to evaluate the biocompatibility of the scaffolds and chosen bioactive factors for clinically relevant feasibility and safety evaluations. Mechanical characterisation is required on a macro-, micro- and nanoscale to evaluate scaffold suitability for OC TE: macroscopical three-point bending will determine Young's Modulus (YM) during 'whole joint loading' of the scaffold; Vickers microhardness indentation performed at different locations will determine YM of the BRR, SPR and ARR individually; nanoindentation utilising Scanning Probe Microscopy will determine the strain elastic modulus at varying planes and create topographical images of the fibre surfaces. Confined/unconfined static compression testing will enable direct measurement of YM and yield strength and observation of deformation behaviour which should demonstrate three distinct regions: a linear elastic region; the offset yield strength; the densification and crushing of the scaffold. Fatigue performance will be evaluated by dynamic compression testing to determine scaffold lifetime under physiological conditions.

DEVELOPMENT OF A NAPPY FOR THE DETECTION OF CYTOMEGALOVIRUS IN THE URINE OF NEONATES

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Introduction

Human Cytomegalovirus (HCMV) is the most common congenital infection, affecting around one in every 200 new-borns.[1] About 90% of cases are asymptomatic.[2] Undiagnosed infants may develop symptoms such as sensorineural hearing loss and organ failure. Currently, the favoured detection method is polymerase chain reaction of amniotic fluid. This complex procedure requires primers specific to the virus genome that become useless upon mutation. Additionally, sample extraction by amniocentesis endangers the mother and foetus. A virus detection technique that is rapid, non-specific, easy to use, works independently of symptoms and safe for the baby is necessary. This projects aims to create a test strip that inserts into a nappy to detect HCMV infection.

Materials and Methods

The experimental work performed thus far relates to the first of four project phases, namely the synthesis of virucidal sulphonated polymers. The chosen synthetic route is reversible addition fragmentation chain transfer (RAFT) polymerisation for its controllability and theoretical 100% degree of sulphonation. A RAFT agent is necessary to facilitate RAFT conditions. If it has at least two reaction sites, “starred” polymers with increased sulphonate density are achievable. The synthesis of a four-armed trithiocarbonate RAFT agent was attempted. This included an overnight reaction under basic conditions and purification *via* silica gel column chromatography.

Results and Discussion

^{1}H-NMR spectra revealed the presence of multi-armed trithiocarbonate chain transfer agent. Integral peak ratios indicated a high but not complete functionalisation, suggesting the product was a mixture of three and four-armed compounds. The next step is to convert the terminating groups from hydrophobic to hydrophilic. This would match the solubility of the RAFT agent with sulphonated monomers for future polymerisations. Deprotection reactions will be attempted to achieve this.

Conclusion

HCMV-detecting nappies have the potential to improve the lives of thousands of infants. Future work includes finalising the multi-armed RAFT agent and synthesising derivatives of the fluorescent DNA dye Thiazole Orange.

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3D PRINTING ANTHROPOMORPHIC PHANTOMS FOR MRI GUIDED RADIOTHERAPY

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Introduction

Anthropomorphic Phantoms are 3D models of human structures that can be used as Magnetic Resonance Imaging (MRI) calibration aids, training tools for MRI scanning and they can assist in treatment planning for MRI guided radiotherapy. In order for a phantom to be effective, it must represent the tissue or organ of interest in an anatomical, geometric and radiological sense. Therefore the need for more realistic phantoms continues to grow to in tandem with the on-going development of MRI scanning systems. Currently, commercially available MRI phantoms are simple geometric structures filled with a gel –paramagnetic ion solution. While these phantoms can successfully replicate T_1 and T_2 relaxation times of many organ systems, they do not capture the heterogeneous nature of human organs. Within the field of soft robotics, polydimethylsiloxane (PDMS) has been successfully 3D printed to produce soft human- like structures. Therefore this research aims to assess the rheology and printability of a silicone blend widely applied in the field of soft robotics, while also assessing the radiological relevance of this material within the context of MRI.

Materials and methods

A PDMS ink made by mixing SE1700 (high viscosity) and Sylgard184 (low viscosity) in ratio of 8:2 where each silicone base material was mixed with its catalyst in a 10:1 ratio. SE1700, Sylgard184 and the blend then underwent rheology testing where the linear viscoelastic region (LVR), storage modulus (G') and the loss modulus (G'') were derived from an amplitude sweep, frequency sweep and viscosity curve. The same PDMS blend was then 3D printed and subsequently undertook MRI scanning to obtain T_1 and T_2 relaxation decay values.

Results

Sylgard184 exhibited no LVR with Newtonian behaviour throughout the amplitude test. Both SE1700 and the blend of both silicones exhibit a change from dominant G' at lower strains and a dominant loss modulus G'' after the yield point. The frequency sweep for Sylgard184, SE1700 and the blend of the two materials shows that as the frequency is increased, G' was dominant. The rotational flow curve for Sylgard184 shows an initial decrease in viscosity as shear rate is increased, followed by Newtonian behaviour where the shear rate has no effect on the viscosity. SE1700 and the blend of the two materials observe shear thinning behaviour where the viscosity decreases as the shear rate is increased. Following rheological testing, the blend of the two material was printed to produce two 3D parts. The printed part observed T_1 and T_2 relaxation decay values of 1702.10 $\pm$209.94 and 16.54$\pm$1.33 ms respectively.

Discussion

The Rheology tests confirm that blending a higher viscosity PDMS with a smaller amount of lower viscosity silicone produces a blend that exhibits Herschel-Buckley behaviour; making it suitable for 3D printing. The crossover point between G' and G'' at higher strains is desirable for extrusion based 3D printing since the printing material must be able to flow when subjected to pressure, in order to be deposited, but also present gel like behaviour to maintain its shape once it has been extruded to keep print fidelity. Following printing, the parts were successfully scanned and showed enough contrast to be MRI visible. The relaxation decay values observed could be tuned by altering the formulation to include the paramagnetic ions and gels used in more traditional phantoms. A change in formulations must ensure that the material is still printable.

Conclusion

These findings provide a starting point for producing MRI visible soft elastomeric anthropomorphic phantoms. Future work would require formulation optimization to select a tissue of interest and aim to simulate accurate relaxation decay values.

Acknowledgements

The impact of COVID- 19 meant that much of this work was run on a service basis. I would like to thank of Andy Wallwork, Jasmine Fernley, Benjamin Rowland and Ben Dickie for ensuring that I was still able to continue with my research despite the restrictions.

PREDICTING PATHOLOGY: PROGRESS TOWARDS USING SIFT-MS TO FACILITATE EARLY INTERVENTION FOR AGE RELATED HEARING LOSS

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Introduction

Age related hearing loss (ARHL) affects the majority of those over 65 years old as the third most prevalent condition affecting the elderly worldwide, with treatments (restricted to hearing aids and cochlear implants) unable to offer continued improvement as the illness progresses. However, research suggests that where degradation of cochlear fibrocytes is the leading pathology (i.e. metabolic ARHL), biological interventions may be possible. In order to facilitate this, an early detection strategy for fibrocyte damage is required to enable timely intervention. This research focuses on the development of such a strategy, wherein ear wax samples may be used to ascertain the health of cochlear fibrocytes.

Materials and Methods

In preliminary steps towards development of an early detection method, this research examined levels (parts per billion) of volatile organic compounds (VOCs) in the headspace of murine cochlear fibrocyte cultures and human ear wax samples in real time using selected ion flow tube mass spectrometry (SIFT-MS). Murine cochlear fibrocyte cultures were isolated from tissue explants, with cell type characterised via morphological assessment and immunocytochemistry.

Results and Discussion

SIFT-MS measurement of murine cochlear fibrocytes demonstrated a visible difference between cellular samples and media controls. Some overlap of results is seen in places. Such overlaps may occur due to variations in cell health (indicated by varying DMSO levels), sample temperatures, media age, and dilution of samples via air purge. This sensitivity in detection, in fact, bodes well for the next stage of research- distinction of healthy and unhealthy fibrocytes. SIFT-MS measurement of ear wax samples yielded a detectable VOC profile of wax, distinguishable from both air controls and blank swabs. The strength of this profile appeared to diminish with time as, when ear wax samples were subjected to SIFT-MS several times (with new air purge and incubation period before each measurement), fewer distinct features could be seen with later profiles. This would appear to suggest an element of time sensitivity in sampling, particularly where a diagnostic goal is concerned.

Conclusions

The suggested methods for SIFT-MS based measurement of cochlear fibrocyte cultures and ear wax samples appear to be successful thus far, though refinement may still be required in order to optimise the procedures. The results seen when detecting cell signals show promise when moving towards the next stage of research- distinction of healthy and unhealthy fibrocytes, with the variation in cellular samples seen possibly already indicative of capacity to detect such variations. Overall, the research to date suggests excellent progress towards the use of ear wax samples to ascertain the health of cochlear fibrocytes.

CHARACTERISING THE EXPRESSION OF MUSCULAR DYSTROPHY ASSOCIATED NUCLEAR ENVELOPE PROTEINS DURING MYOBLAST DIFFERENTIATION

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Introduction

Mutations in nuclear envelope (NE) proteins, emerin, lamin A/C, nesprin-1/2, SUN1/SUN2, and FHL1 have each been identified in the causation and development of Emery-Dreifuss muscular dystrophy (EDMD). EDMD is a rare genetic disorder characterised by a triad of symptoms, the early development of muscle contractures, progressive muscle weakness, and heart abnormalities. Some extremely rare forms of congenital muscular dystrophy (CMD) have also been linked to mutations in lamin A/C and nesprin-1. Currently, very few treatment options exist for these two conditions. Despite knowledge of which genes cause these diseases, it is not yet known why these proteins are so important for growing and maintaining healthy muscle. The aim of this study, therefore, was to characterise the expression patterns of muscular dystrophy-associated NE proteins during differentiation in myoblasts from both healthy donors and CMD patients. From this, the intrinsic expression of these proteins, and any abnormalities in NE protein expression in the mutant cell lines were determined. Through investigating the molecular mechanisms behind muscle cell development defects in inherited muscular dystrophies, therapeutic targets for the repair of dystrophic muscle can be identified.

Materials and Methods

A seven day time-course experiment was established in which immortalized myoblast cells from six healthy subjects and from four CMD patients harbouring *LMNA* (p.Leu380Ser, p.Lys32del, p.Arg249Trp) and *SYNE1* (p.Glu7854*) mutations were cultured in skeletal muscle cell growth medium supplemented with fetal bovine serum for three days. Differentiation was induced at 80-90% confluency by culturing in serum-free growth medium, and after a further four days of culture, the majority of cells had formed myotubes.

Results and Discussion

Using quantitative western blotting and immunofluorescence microscopy, we show that emerin is consistently expressed in myoblasts but decreases in myotubes from all donors and that potential SUN2 isoforms are differentially expressed during differentiation. Lamin A/C expression levels are highly variable but do appear to be considerably reduced in older cell lines.

Emerin is known to be involved in activities that precede myogenic differentiation, having key roles in the regulation of myogenic signaling pathways that initiate myogenesis or that are important for proper differentiation. This may be why the expression of the protein is elevated in myoblasts, and then reduced in myotubes when it may no longer be required. Changes in the expression of SUN2 could be associated with its roles in nuclear positioning and movement. The decrease in lamin A/C expression in older cell lines suggests that lamin A/C expression is affected by age. In EDMD patients with already reduced levels of functional lamin A/C, this could cause symptoms to be exacerbated as the patient ages.

Conclusions

Together, these findings provide some insight into the roles of muscular dystrophy-associated NE proteins during differentiation and should be considered when investigating NE-protein-associated diseases. Furthermore, investigating the defective molecular mechanisms that occur during muscle cell development and differentiation in muscular dystrophy will reveal novel insights into the requirements for attaining functional muscle from myoblast stem cells, and may identify potential therapeutic targets.

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A WORKFLOW FOR CREATING A LARGE COHORT OF SUBJECT-SPECIFIC L4/L5 FINITE ELEMENT MODELS

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Introduction

It has been shown that geometry strongly influences the outcomes of spinal finite element models. Niemeyer [1] showed in a finite element study that specific spinal geometries strongly influenced the intradiscal pressure, range of motion and facet joint forces in around 400 fully parameterized functional lumbar spinal units under different loading conditions. This shows that accurate geometries are required to obtain accurate, and more relevant, finite element outcomes. Although realistic parameters' limits and distributions were used by Niemeyer, using fully parameterized synthetic models may have produced anatomically unrealistic or unlikely geometric combinations. Furthermore, fully parameterizing the models required using extremely simplified geometries. On the other hand, studies that use patient specific models are usually limited in terms of sample size. The aim of this work is to create a large cohort of finite element models from patient-specific lumbar spinal segments (L4/L5). This cohort will be the basis for a multitude of finite element studies on the lumber spine. This includes creating even larger cohorts of realistic virtual cohorts for population-based studies.

Materials and Methods

Model generation: The finite element models were built in Abaqus CAE 2017 (Dassault Systèmes, USA). Using ParaView 5.8.0 (Kitware Inc., USA), a Python script was developed to extract 200 subject-specific meshes of the L4/L5 segment from the MySpine database [2]. MATLAB 2019a (Mathworks Inc., USA) was used to build Abaqus-compatible meshes. Then, several Python scripts were developed to assign appropriate material properties for the different anatomical structures, apply load and boundary conditions, and acquire several outputs. Materials: The cortical bone, cancellous bone, cartilaginous endplates, and facets cartilage were modelled as isotropic linear elastic materials. The nucleus pulposus was modelled as a nearly incompressible isotropic hyperelastic material. A method was developed for consistently modelling the anisotropic hyperelastic material of the annulus fibrosus. Load and boundary conditions: A 400 N axial load was applied as a uniformly distributed pressure applied to the upper boney endplate of the L4. The lower boney endplate of the L5 was fully fixed. A frictionless hard contact was defined between the articulation surfaces of the facet joints. Interim outputs: Nodal displacements, Nodal von Mises stress, maximum facets contact pressure, maximum facets normal force and maximum nucleus pressure (intradiscal pressure) were acquired from each model. Indirect validation: The models were indirectly validated by comparing intradiscal pressure results with values from literature.

Results and Discussion

After excluding clearly duplicated or inconsistent meshes, 168 models were built, out of which 159 models converged successfully, with computational time of around 20 minutes per model. Successfully acquired all outputs. More duplicates were identified and eliminated based on their matching results and a subsequent visual inspection. This resulted in a cohort of 152 unique models. Indirect validation of the intradiscal pressure results show good agreement with previous studies.

Conclusion

The workflow works successfully. Initial results show good accuracy and consistency. The models can be subsequently used for a variety of population-based studies. Nevertheless, the workflow is limited to the meshes sourced from the MySpine database [2]. Therefore, further work is required to generalize it.

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IDENTIFICATION OF LABEL-FREE BIOMARKERS FOR VISCERAL MYOPATHY

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Abstract Body
Visceral myopathy (VSCM) is a rare gastrointestinal motility disorder which is characterised by impaired intestinal function and motility in the absence of mechanical obstruction. It is a severe disease which mainly affects infants and children. Although, VSCM can be seen in adults as well. The main symptoms include abnormal intestinal activity, malnutrition and abdominal pain.

VSCM is a smooth muscle disorder where *ACTG2* gene has been identified as the genetic cause in 40% to 50% of cases. However, there is currently no clear genetic driver which has made early diagnosis for VSCM difficult. This has led to unwanted surgical intervention used to treat patients once the disease has progressed. Thus, it is vital to have methods of early diagnosis and since a clear genetic driver is unknown there is a need for a label-free biomarker for VSCM.

We are investigating the potential descriptors of the cell as potential label-free biomarkers. The initial activity has concentrated on morphology (cell height, shape and perimeter), mechanical properties and ability to generate traction forces on the substrate. Currently, I am carrying out cell morphology experiments based on the dye exclusion microscopy method. This technique is based on the direct application of the Beer-Lambert Law (Schonbrun *et al*., 2013), where the absorption of a biocompatible dye mixed in the medium is measured, and the cellular volume is reconstructed based on two images obtained while illuminating with different colours. This simple technique provides a map of the cells which is simple to segment using standard image processing algorithms, and so ideal to perform morphometric quantification.

This work will be performed on primary skin fibroblasts from VSCM patients and from control patients who are affected by other diseases, not related with intestinal pseudo-obstruction. All cells are supplied by a paediatric hospital in Genova,Italy called Istituto Giannina Gaslini.

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TOWARDS HIGH THROUGHPUT CELL MECHANOSENSITIVITY ASSAYS

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Introduction

Cell mechanosensitivity is the ability of cells to sense mechanical stimuli arising from their surrounding environment or from within the body [1]. It is inevitably followed by mechanotransduction, the translation of the mechanical stimulus into biochemical signals [2]. Both processes play a crucial role in cells' fate and allow them to adapt to their physical surroundings by remodeling their cytoskeleton, activating different signaling pathways and changing their gene expression, ultimately controlling physiological processes such as proliferation, differentiation, migration, and apoptosis [3].

Dysregulation of cell mechanosensitivity results in cell dysfunctions and eventually various pathologies, such as cardiovascular diseases [4], osteoporosis [5], intestinal problems [6]. Currently, cellular mechanosensitivity is assessed mainly using single cell biophysical methods. While this approach allows us to characterise the mechanical response of single cells with very high resolution, it offers a very limited throughput. The measure of mechanosensitivity has the expectation to provide physiologically-relevant information, but a paradigm change is required to translate this concept towards biomedical applications, moving to high throughput single cell analysis.

Materials and Methods

Here we propose a concept design of a high throughput mechanosensitivity assay based on the integration of microfluidic and fluorescent microscopy. Floating cells will be aligned within the microfluidic channel and mechanically stimulated while passing through a neck in the channel. In order to track cells' response, we will use fluorescent ion sensitive dyes and a fluorescent inverted microscope. Studies on cell mechanosensitivity could contribute to the discovery of novel therapeutic strategies for treating diseases with mechanobiological elements.

Acknowledgements

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IMMUNOBIOENGINEERING: THE NEXT FRONTIER IN REGENERATIVE MEDICINE

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Introduction: The primary challenge for reconstructive surgeons is that of tissue loss and the desire to restore both form and function. Regenerative medicine was born out of the need to combat this problem, aiming to improve the body's inherent regenerative capacity after an injury or to replace damaged tissues with fully functional, engineered ones(1). Modern biomaterials hoping to achieve this purpose, have included the use of 3D scaffolds with the application of polymers as hydrogels, metals and ceramics to augment, repair or replace damaged, diseased or lost tissues. Due to the ever-increasing demands for smarter and more creative healthcare solutions, the potential clinical applicability of modern biomaterials has been propelled into the more recent research space(2). Current limitations with progress in the use of biomaterials, are centralised on vascularisation and host tissue integration(3). A cornerstone for the applicability and clinical translatability of bioengineered tissues will be developing a thorough understanding of the interplay between the biomaterial and the host immune system. **The challenge**: Implanted biomaterials will interact with both the innate and adaptive arms of the host immune system in ways that determine the biological functionality and performance of the implant over time(5,6). The properties of the biomaterial constructs, its degradation products over time and of the local tissue microenvironment, are therefore crucial to understanding and favourably tuning this immune interplay. Additionally, an important concept is the interconnected nature of biomaterial properties. Changes to any component will alter the immune responses, for example, changing the shape of the particle may also change the size, whilst altering a functional group on the surface has an impact on the surface charge and hydrophobicity. Future biomaterials will need to understand the relative contributions of the different properties in order to modulate the immune function and interactions with them. **The proposed solution**: Using anti-inflammatory factors to immunomodulate biomaterials could represent a new avenue to explore ways to control the local microenvironment and favourably tune interactions with the host immune system. The next frontier for regenerative medicine is the design of biomaterials capable of tuning the local immune response at the implantation site. A control of the local immune response tailored to a specific biomaterial, in a specific tissue, for a specific local environment offers the potential to engineer biocompatible biomaterials that will integrate with the host. **Conclusion**: This project aims to modulate biomaterial immunogenicity through physicochemical modification and immunobioengineering, in the quest for clinical translatability of advanced tissue engineering for reconstructive and regenerative medicine.

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MICROFLUIDICS FOR PROBING MESENCHYMAL STEM CELLS

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Introduction

Mesenchymal stem cells (MSCs) have great potential in regenerative therapies for their ability to differentiate into osteocytes, chondrocytes, and adipocytes, and their preferred cell lineage can be predicted from the expressed surface markers and mechanical properties. But surface markers are not optimal lineage predictors and probing mechanical properties has yet to be comprehensively utilised. The properties of stem cell populations suffer from intrinsic heterogeneity on a single-cell level, and it is considered to be a contributing factor towards therapeutic inconsistencies and inefficiencies. There is a demand for exploiting the mechanical properties of MSCs in order to sort them into groups with discrete differential potential.

Materials and Methods

In this project, we will use the power of microfluidics to probe the mechanical properties of MSCs and sort them into subpopulations. A sample of MSCs will initially be sorted into subsamples based on size using deterministic lateral displacement, followed by using a secondary microfluidic device to probe cell stiffness using micro-constrictions. The cells will then be encapsulated in droplets for sorting into different channels using acoustic waves based on a decision tree of their properties. The work will contribute towards improving stem cell regenerative therapies by sorting MSCs into subpopulations that prefer differentiation into osteocytes, chondrocytes, and adipocytes in the hope of providing more targeted treatments.

Acknowledgements

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KIDNEY ORGANOIDS AS AN *IN VITRO* MODEL FOR ALPORT SYNDROME

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Introduction Alport syndrome is an inherited disease characterised by mutation in COL4A3, COL4A4 or COL4A5 [1]. The predominant location of disease manifestation is the kidney due to the presence of the trimerized α3α4α5 network in the glomerular basement membrane (GBM) required for structural integrity of the filter. Kidney organoids present a unique opportunity to model inherited disease in a 3-D culture model using patient-derived pluripotent stem cells [2].

Methods Using a serial fractionation protocol we enriched Alport kidney organoid glomeruli for extracellular matrix proteins before performing LC MS-MS mass spectroscopy. **Results** Kidney organoids have thus far shown the ability to express the necessary type IV collagen genes and proteins as part of the GBM making them a possible model for Alport syndrome. Additionally, a pilot study of mass spectrometry analysis of glomeruli isolated from Alport and control kidney organoids shows a potential shift in the composition of the glomerular matrix with downregulation of key basement membrane proteins and upregulation of interstitial matrix genes. **Discussion** Some of these genes including LAMB1 and LAMB2 form part of the GBM that remodels and matures during development and could provide insight into early developmental changes that occur during the disease.

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UNRAVELLING CHITINASE-LIKE PROTEINS ROLE IN TISSUE REMODELING AND REGENERATION

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Introduction: Chitinase-like proteins (CLPs) are strongly associated with a wide range of inflammatory diseases, and are particularly implicated in tissue repair and remodelling in the lung during type 2 immune responses [1]. The molecular mechanisms involved in these CLP functions are not fully understood. We have hypothesised that CLP function will be determined by the specific interactions between CLPs and glycosaminoglycans (GAGs), heterogeneous polysaccharides that are components of the extracellular matrix that have well documented roles in the regulation of protein localisation and function. The aim of this project is to determine whether GAGs regulate CLP function during tissue repair.

Materials and Methods: Recombinant CLPs Ym1 and Ym2 (both murine proteins) have been made in bacteria using different methodologies. Ym2 was expressed using BL21 *E. coli* cells and purified using ion exchange and size exclusion chromatography; whilst Ym1 was expressed using SHuffle cells and purified using a nickel resin followed by His-trap, ion exchange and size exclusion chromatography.

Results: SDS PAGE analysis and electrospray ionisation mass spectrometry has shown that the purified Ym1 and Ym2 proteins have the expected molecular weights consistent with the presence of three disulphide bonds. Heparin affinity chromatography has indicated that both Ym1 and Ym2 bind to heparin (a type of highly sulphated GAG made only by mast cells) and do so in a pH dependent manner (with no binding at neutral pH and increasing binding as the pH is lowered). Further biochemical interaction analysis using biolayer interferometry (BLI) has allowed the screening of Ym2 binding to multiple GAG preparations. This has shown for the first time that Ym2 interacts with heparan sulphate (HS), binding with high affinity to this ubiquitous component of the extracellular matrix. Preliminary experiments with Ym1 have also indicated that this CLP is likely to interact with HS. Further BLI studies are ongoing to determine the sulphate specificity of the Ym2-HS interaction and investigate binding to other types of GAG (e.g. chondroitin sulphate and dermatan sulphate).

Discussion & Conclusions: Ym1 and Ym2 have been shown to bind to GAGs (e.g. HS), which are present in lung tissues. The unusual pH dependency of their interactions indicates that binding only occurs under acidic conditions and thus will likely increase during inflammation, perhaps regulating their localization in particular lung compartments. Work is in progress using site-directed mutagenesis of Ym2 to identify the GAG-binding site and generate mutant proteins with altered GAG-binding properties for analysis in *in vivo* models of helminth infection; here we have targeted histidine residues we predict will mediate Ym2's pH-dependent binding to heparin/HS. These studies will further our knowledge of the role that CLPs have in type 2 immune responses.

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DEVELOPING NOVEL OUTCOME MEASURES FOR PERIPHERAL NERVE REGENERATION

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Introduction:

In order to target peripheral nerve injury (PNI) treatment to individuals and patient groups it is important to understand who PNIs effect and how. We currently have no national-level data on the basic epidemiology of this devastating type of injury in order to focus treatments and services to those patient groups in most need.

In those patients with the most significant nerve injuries, where a gap exists between the two nerve ends, an unmet clinical need has been identified in order to replace peripheral nerve autograft repair with bioengineered nerve conduits. Modification and refinement of the currently available nerve conduits are required for the initial phase of this process.

Even in those with less severe injuries, clinical treatments have not progressed for several decades. This is in part, due to the lack of standardised outcome measures that would allow us to effectively compare outcomes between PNI centres. In addition, current clinical outcome measures lack the sensitivity to detect early biological changes after PNI therefore restricting the ability to demonstrate the clinical effectiveness of novel nerve repair solutions. New clinical outcome measures are needed that can identify and quantify early, microbiological changes of nerve regeneration.

Materials and Methods:

5. Nationwide retrospective cohort study analysing trends in PNIs over the past 15-years (2005-2019).
6. Phase I clinical trial of novel, microgrooved synthetic nerve conduit (1).
7. Systematic review of clinical outcome measure use in clinical peripheral nerve regeneration (2).
8. Prospective cohort study comparing end-organ epidermal thickness and sweat duct density changes after hand sensory PNI and repair.

Results:

5. The median, non-birth related incidence of PNI in England is 11.3 (IQR 0.89) persons per 100,000 population. There were a median of 5,401 (IQR 418.5) primary procedures for PNI per year. There is an increasing trend of birth-related facial-nerve injury across the study period. Male-to-female incidence remained constant at 2.2 - 2.9 : 1. The commonest age group for PNI were patients in their third decade (incidence 2.95/100,000, IQR 0.29). The upper limb was the most common site of injury with a median of 5788 (IQR 369.5) FCEs per year.

6. 17 patients had a Polynerve device fitted with 14 patients completing the study follow-up. No increased risk of infection or abnormal wound healing was reported.

7. A total of 96 studies were included (15 RCTs, 8 case-control studies, 18 cohort studies, 5 observational studies, and the remainder were case series or retrospective reviews). A total of 56 individual outcome measures were identified, utilized across 28 different countries and 7097 patients. Ten core domains were defined: sensory subjective, sensory objective, motor subjective, motor objective, sensorimotor function, psychology and well-being, disability, quality of life, pain and discomfort, and neurotrophic measures.

8. A total of 28 patients have been recruited thus far (recruitment ends May 2021) with a target of 37 patients to be recruited. Patients are followed for a 6-month period after sensory nerve repair in the hand. Figure 4 is an image of a patient's hand skin demonstrating the end-organ structures (sweat ducts and epidermal thickness) measured in hand sensory nerve injury.

Conclusions:

Peripheral nerve injury is common and most often affects the upper limb of young men (20 – 40 years). Polynerve, our topographically enhanced nerve conduit, provides a cost effective, efficient and scalable approach to nerve gap management that enhances peripheral nerve regeneration through effective neurobiological integration in sensory nerve gap injuries <20mm. Lack of consensus on outcome measure use hinders comparison of outcomes between nerve injury centres and the development of novel treatments. Development of a core outcome set will help standardize outcome reporting, improve translation of novel treatments from lab to clinical practice, and ensure future research in PNI is more amenable to systematic review and meta-analysis. Epidermal thickness and sweat duct density changes after PNI may be a more accurate measure of peripheral nerve regeneration in humans than current clinical outcome measures.

Enabling Technologies

GRAPHENE FOR ENHANCED BONE TISSUE REGENERATION

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Graphene is an allotrope of carbon in which the atoms are arranged in a 2D hexagonal lattice (honeycomb) structure, forming a one-atom-thick sheet. The structure of the material has endowed it with many powerful properties that span the electrical, chemical and mechanical. Variants of graphene including graphene oxide (GO) and reduced GO, which are more efficient to produce, are also being investigated as they offer a route to mass-production. Graphene has thus become of interest in many fields including medicine. There is a growing body of evidence which shows how the material is able to facilitate cell adhesion, proliferation and even direct differentiation to some extent in a variety of cell types meaning it may be used in the production of functional biomaterials. In the first instance, we seek to coat titanium and stainless steel with graphene and assess bone cell compatibility, with a view to the long-term development of graphene-enhanced implants to improve bone fracture healing. Currently, fractures are a major socioeconomic burden due to their widespread, debilitating nature and lengthy healing time. Therefore, a device that improves the regenerative capacity of bone and significantly reduces healing time would be a major benefit to medicine and society.

INVESTIGATION OF THE VOLATILOME OF HUMAN EMBRYONIC STEM CELLS USING SELECTED ION FLOW TUBE MASS SPECTROMETRY

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Introduction

Human pluripotent stem cells (hPSCs), such as embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs), have gathered tremendous attention as they have multiple applications in regenerative medicine. Therefore, is not surprising that their cellular mechanisms, including metabolism, are being intensely studied. However, cellular metabolomics is challenging since cells' metabolomes are incredibly complex, being composed of many different metabolites (e.g., lipids, peptides, organic acids, thiols, and carbohydrates)[1]. Measuring such great diversity relies on large-scale qualitative and quantitative analytical platforms, like mass spectrometry, which are often destructive (i.e., involve destructive metabolite extraction) and need complicated sample preparation procedures that, in turn, prevent real-time analysis of samples[1,2].

Furthermore, most of the current knowledge regarding hPSC metabolism results from assumptions based on cancer studies[3]. As a result, more research focusing specifically on the metabolism of hPSCs is needed to provide more tangible data on how metabolism/metabolites influence cellular processes responsible for establishing and maintaining stem cell self-renewal, pluripotency, and differentiation.

Analysing the volatile metabolites that stem cells release in culture could give invaluable information regarding their metabolic processes. A strategy to efficiently detect such volatiles from cell samples is the use of selected ion flow tube-mass spectrometry (SIFT-MS). SIFT-MS allows for accurate analyses of humid gaseous samples for several compounds simultaneously in real-time, without the need for sample preparation and collection that can compromise the sample[2].

The aim of this study is to identify a Volatile Organic Compound (VOC) profile of hESCs in 21% oxygen using SIFT-MS.

Materials and Methods

Media from SHEF-1 hESC line samples will be cultured at 21% oxygen and then transferred into glass bottles for SIFT-MS measurements. The headspace of the bottles will be purged with dry and sterile air (20% oxygen and 80% nitrogen mixture) to strengthen the quality and reproducibility of the VOC data produced. Finally, the bottles will then be incubated for 16 hours at 37°C to allow the accumulation of VOCs before performing the measurements. Following incubation, the gaseous headspace above the cells will be measured using SIF-MS.

Results and Discussion

We will utilise a kinetic library (a list of compounds and their known mass-to-charge ratios) to look at 10 expected VOCs (acetone, acetaldehyde, ethanol, butanol, pentanol, DMS/ethanethiol, hexanal, butyric acid, pentene, putrescine) in the headspace of SHEF-1, which have been identified in a previous study[4].

This study's results will be further compared to other hPSC lines and oxygen conditions (hypoxia and physioxia) to investigate differences in VOC profiles.

Conclusions

SIFT-MS could potentially be employed as an additional non-invasive technique to identify and characterise stem cells in culture. Furthermore, we hope that this study will contribute to the establishment of a VOC blueprint for pluripotency and self-renewal, which, in turn, will enhance the understanding of metabolome dynamics of stem cells.

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FIBRONECTIN FUNCTIONALISATION THROUGH THE EXPLOITATION OF PEPTIDE HYDROGEL SELF-ASSEMBLY PROFFERS A FACILE AND VERSATILE PLATFORM FOR GROWTH FACTOR RETENTION AND PRESENTATION

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Introduction

In the last decade, hydrogels have become a staple biomaterial in tissue engineering, from synthetically produced to naturally-derived, many systems have been developed and documented; yet, the unwavering ambition of engineers remains the functionalisation of natural proteins to promote hydrogel-cell interactions. However, protein functionalisation is an intrinsically challenging process that can be costly and lengthy to accomplish for classic hydrogels; on the other hand, the emergence of the new self-assembling peptide hydrogels provides a promising alternative to this process, one that does not require post-production modifications or lengthy cross-linking reactions.
One of our focus at the Salmeron-Sanchez lab is the functionalisation of fibronectin, namely its growth factor binding domains (FNIII12-14), to the fiber network, thus proffering the nascent hydrogel construct with promiscuous growth factor anchor points that can be used to significantly stimulate cellular activity. In this project, we are exploiting the self-assembly system of peptide hydrogels to propose a novel method to the functionalisation of FNIII12-14, one that instructs the protein to tether to the fiber by itself, allowing its self-functionalisation.

Methodology

Peptide hydrogels consist of oligopeptides that self-assemble into a fiber network following a gradual structural hierarchy. As such, we are exploiting the assembly system by recombinantly expressing the FNIII12-14 regions with that oligopeptide sequence at its terminal so that it would incorporate itself in the fiber construct as is it being formed. Thus, the incorporation of the oligopeptide would lead to the spontaneous tethering of the protein to the fiber network as the hydrogel is produced, exempting the need for post-production reactions. Upon reaching proof-of-concept, the construct will be complimented with a library of growth factors, shaped in different growth factor combinations, to induce the proliferation or differentiation of numerous cell lineages, thus proving the versatility of the construct for multiple tissues.

Impact

Outside the scope of FNIII12-14, this method can be expanded for numerous proteins; from proteins acting as integrin/cadherin or drug binding domains to proteins acting as chemotaxis inducers, protein self-functionalisation could suit a large scope of *in vitro* bioengineering applications, provided that the chemical parameters of the peptide and the protein are conscientiously designed.

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THE EFFECT OF ELECTRICAL STIMULATION AND HYDROGEN PEROXIDE ON GENE EXPRESSION IN AN IMMORTALISED HUMAN MESENCHYMAL STEM CELL LINE

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Hydrogen peroxide (H_2O_2) is a by-product at the cathode during direct current electrical stimulation (DC ES), and it is now well accepted that H_2O_2 is an essential molecule within the cell for the regulation of processes such as proliferation, differentiation, and migration [1]. DC ES is currently under investigation as a tool to accelerate differentiation in human mesenchymal stem cells; several research groups have proof of its efficacy, but few truly understand the cellular mechanism underpinning the response. On stimulation, there is a marked release of intracellular calcium, changes occur to membrane ion channels, and there are alterations in the cytoskeleton, among other effects [2]. What is not understood is how much of a role the extracellular H_2O_2 is playing on the cellular effects that occur both during and after DC ES. Long term, it is hoped that DC ES could have the potential to accelerate differentiation in the mass production of stem cells, rendering the process more rapid and cost-effective.

Immediate responses to DC ES in hMSCs have not been studied in detail. The gene expression and metabolic activity of hMSCs from an immortalised hMSC cell line, Y201, will be studied after 1 stimulation period of 30 or 60 mins of electrical stimulation in the presence and absence of catalase, an enzyme that breaks down H_2O_2. In previous experiments, hMSCs from 2 donors (Donor A = M, 19Y, unknown race; Donor B = M, 24Y, Hispanic) were used, both with highly contrasting results. Donor A's bone marrow-derived hMSCs showed consistent and significant increases in metabolic activity after 4 days of DC ES, whereas Donor B showed either no change in metabolic activity, or significant decreases after 60 minutes of stimulation. Studies on gene expression also gave contrasting results. In the use of the new cell line, it is hoped that some consistency can be achieved, and that the role of H_2O_2 will become clear.
It is hoped that this work can help inform researchers working in the field of electrical stimulation the extent to which H_2O_2 contributes to the observed response. Furthermore, it may allow the fine-tuning of the extracellular environment to maximise the potential of DC ES as a tool in tissue engineering.

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Declaration: I took a six month interruption between July 2020 and January 2021. I have also moved to a new building which has presented challenges in the ability for progression with my work. I no longer am able to work with the cells I was previously, and so have begun working with a new cell line. I cannot predict the response to DC ES until I am able to carry out the experiment, which I will be doing the week commencing 12th of April 2021.

A SOFT ROBOTIC GLOVE FOR POST-STROKE REHABILITATION

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Introduction

A common after-effect of stroke is hemiparesis: the weakness, and in some cases paralysis, of one side of the body, with the arm and hand particularly affected. Rehabilitation requires intensive therapy and professional attention; even with treatment many do not ever recover the full use they had before. Additionally, many patients who do undergo treatment will have limited time with therapists, typically far less than would be ideal.

Soft rehabilitative orthotics, i.e. devices embedding flexible parts, have already been considered as a promising solution as they can be used when a therapist is not present, and also have potential to assist with daily tasks by providing strength to a weakened limb. The compliance of soft technology means designs can be safer and more suitable for human use.
However, the same compliance can also make it difficult to achieve an equivalent level of controlled precision compared to traditional hard robotics, which limits the ability of soft glove orthotics to perform complex movements. Some current compliant mechanisms can also struggle to provide adequate torque, particularly in certain directions. In addition to these practical challenges, there is no standardised definition of soft robotics or soft gloves.

This project overall aims to create a compliant rehabilitative glove that utilises both soft and rigid mechanisms to perform more precise actuation than possible using standard soft actuators, whilst simultaneously maintaining the safety benefits of compliance. In order to achieve this, not only must a glove be designed, but a provable relative standard of softness must be found and applied.
A universally mathematically applicable definition of softness has been found, and from simulations information on the physical parameters of a soft system has been found. An early prototype hinge system has been designed based on these results.

A VIRTUAL ASSISTANT FOR PROMOTING ACTIVE AND HEALTHY LIFESTYLES IN OLDER FRAIL PEOPLE

Nikhil Reji, Sebastiano Fichera, Kris D'Aout, Paolo Paoletti

With a growing older population comes the question of how to promote healthy ageing effectively, whilst ensuring scalability with the use of less human resources. Specifically, the project targets the physical strength and functionality aspects of healthy ageing. Frailty is a state of vulnerability of a person's health stability due to stressor events. An important characteristic of frailty is that it is dynamic, there is a risk that recovered frail older people can relapse. Hence, independent and non-specialised long-term rehabilitation methods are key in ensuring stable health in recovered frail older people. However, in the scope of the United Kingdom's health sector, there are not many packages of care for recovered frailty patients once they leave the short-term specialised rehabilitation environments in the hospitals. This gap in long-term outpatient care is what the project aims to fill: creating a platform to help monitor health and promote active lifestyles and healthy ageing, following the frail older person's rehabilitation journey, from hospital to home.

The project aims to create a virtual assistant platform to allow semi-independent rehabilitation and to promote active lifestyles in older people. Firstly, the virtual assistant will be able to detect body movement and assess the quality of movement using low-cost hardware and software. Being portable will allow the platform to be used both in hospitals and in patient's homes.

Skeletal motion is captured using infrared depth cameras and skeletal tracking libraries. The skeletal motion is a time series that contains a collection of body poses, where each pose is described by an array of linked joints holding both three-dimensional rotational and positional information. Hence, the skeletal motion data contains a large amount of raw information on the position and orientation of every limb in the body. More descriptive information can then be extracted to target specific features of motion, such as the range of motion of a specific joint. The goal is to create a motion feature set that allows the platform to evaluate the user's performance in a set of exercises and estimate the state of frailty overall.

To evaluate the user performance using these feature sets, first the platform must understand what type of motion is occurring (e.g. running, sitting, standing, etc.) within an exercise routine. This is called motion labelling. Once a motion is labelled, its extracted features can be evaluated against the set parameters of that motion in the exercise routine. A solution to motion labelling has been achieved using the Dynamic Time Warping (DTW) technique, which finds the optimal alignment between two time series sequences of varying lengths This concept has then been used to label motions by trying to match predefined motion sequences (shorter sequence) to the longer exercise sequence.

The subsequence DTW algorithm was tested on individual joint motion from a human motion dataset (CMU Mocap), where the predefined motion sequences were generated from the longer exercise sequence using random sampling with added gaussian noise and down sampling. The results showed the algorithm was robust to large noise variations, precise, and accurate within a margin of error of ten percent. This algorithm is now being extended to match full body motion, which will be tested on both the human motion dataset and real-time skeletal motion data. This will give the platform the capability to understand types of motions; giving context to the extracted feature sets.

This solution to motion labelling meets the first milestone towards enabling the virtual assistant to assess the quality of movement. Thus, laying the foundations to fulfil the objectives of monitoring health and providing semi-independent rehabilitation to promote healthy ageing.

DEVELOPING DATA-DRIVEN TOOLS TO SUPPORT CLINICAL DECISION MAKING: REFLECTIONS FROM A STAKEHOLDER WORKSHOP ON PERSON-CENTRED DEMENTIA CARE

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Introduction

Social inequalities in dementia outcomes and care have been widely documented. To address this, we are developing a data-driven tool that can support clinical decision-making for the care of people living with dementia (PLWD). To help the design and success of this tool, we sought stakeholder views on how useful decision-making tools are and key areas of focus for understanding and tackling inequalities in care pathways.

Methods

In March 2021, we hosted an online workshop targeting key stakeholders with vested interest in care for PLWD. Attendees included clinicians, service providers, researchers, data scientists and lay carers for PLWD. We explored perceptions of inequalities in care pathways, the importance of tackling differences in experience, the potential enablers and constraints on clinicians when making care decisions for PLWD, and stakeholder's ideas for a tool aimed to support care discussions.

Results

Overarching themes were revealed in relation to both enablers and constraints within the wider system in providing positive health and social care to PLWD. For PLWD, receiving a diagnosis and adequate care and support is dependent on clinician experience (i.e., GPs with sufficient experience diagnosing dementia), local service availability, access to care pathway navigators, and holistic care systems. A key theme emerged emphasising the need for decisions on care for PLWD needs to be made in the context of the individual and their changing care needs. Discussions demonstrated that how a data-driven tool is presented to clinicians in particular is vital for its successful uptake. The tool should be an aid in decision-making to improve care and outcomes, but done so alongside existing clinician knowledge, consideration of the individual needs of PLWD and their carer(s), and service availability.

Discussion

Clinicians' understanding of dementia, of the PLWD as an individual, of local service availability, and facilitating PLWD/their carer(s) in care discussions, are necessary to enhancing care choice and positive outcomes. Further, this fosters a care system for PLWD which is inclusive and pragmatic while also harnessing the needs and wants of the individual.

Conclusion

Success of a data-driven tool to support clinical decision making within person-centred care discussions depends on how it is presented to clinicians, PLWD and their carer(s). An accompanying narrative should recognise the importance of individual experience and the wider context of that experience. Crucially, if we can find patterns to the way in which the wider context of shapes individual outcomes, the tool can meaningfully support critical person-centred care discussions.

Material Nanopatterning and Properties

SYNTHESIS OF NANOPARTICLES OF POLY (9, 9' - DIOCTYLFLUORENE) FOR POTENTIAL APPLICATION AS MATERIALS FOR USE IN RETINAL PHOTORECEPTORS

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Introduction

The increased prevalence of different types of eye retinal diseases such as retinal pigmentosis and age-related macular degeneration (AMD) has had a large impact on the growth in blindness cases in the global population. Therefore, one of the key challenges in regenerative medicine is to develop a novel approach for functional eye retinal prostheses. Applications of current systems based on photoactive inorganic semiconductors such as silicon photodiodes (SPD) have many limitations regarding requirements for complex electrical circuits, and external power supply and use of rigid inelastic structures with low biocompatibility. [1] Moreover, the generated electric signals from SPD are insufficient to efficiently transmit and trigger the neuron retinal ganglion cells (RGC). An alternative approach is to use consolidated films of conjugated polymer nanoparticles (CNPs) such as derivatives of poly 9, 9' - dioctylfluorene (PFO), that have maximum light absorption in the range of the natural blue S-cones photoreceptors. Light absorption by the CNPs leads to excitations of the CNP that are capable of triggering the retinal ganglion cells (RGC) and improve the visual perception. PFO CNPs are semiconducting organic materials with unique required properties such as photosensitivity, mechanical, optoelectronic properties, high light absorption, extinction coefficient, and are capable of electrical stimulation of the RGC cells. [2] In this study, we report a research strategy to synthesise and characterise the PFO CPNs for potential future application in the retina implant system.

Materials and Methods

The PFO CNPs were synthesised through the Suzuki-Miyaura cross-coupling reaction of the an aryl-boronate ester with aryl-dibromide monomers using a palladium catalyst ($Pd(PPh_3)_4$) and dispersed in miniemulsion. [3] The addition of the sodium dodecyl sulfate (SDS) as a surfactant stabilizes the CNP dispersion. The reaction mixtures were emulsified with three different total times of ultrasonication (2, 4 and 8 minutes). The process of the polymerization was conducted overnight at 72°C. The obtained CNPs dispersions were characterised using various techniques such as UV/Vis Spectroscopy, Dynamic Light Scattering (DLS), Gel Permeation Chromatography (GPC), to investigate the influence of the different applied sonication times on selected physical properties and morphology.

Results and Discussion

The evaluation of the optical properties using UV/Vis spectroscopy of all analysed PFO CNPs dispersions revealed the maximum absorbance peak at 376 nm. Additionally, it was noted the appearance of a peak at 440 nm with lower intensity, this peak corresponded to the presence of the β-phase ordered morphology of the backbone PFO polymer. The results of the hydrodynamic diameter and polydispersity index (PI) obtained from DLS measurements show the tendency to decreasing the average particle diameter and polydispersity towards at longer time of the performed ultrasonication. The size of the PFO CNPs was significantly reduced from 177 nm for 2 minutes ultrasonication to 92 nm for the longest sonication time (8 minutes). In addition, the extended sonication time considerably narrowed the normal distribution of the diameter of the particles and consequently, it ensures the formation of smaller nanoparticles with a low polydispersity (PI = 0.16). Determination of the polymer molecular weight was performed by the GPC, it was observed the variations of the number average molecular weight (M_n) from 3200 to 5300 g/ml, which were increased with longer sonication time. The values of the polydispersity index (PDI) represented as a ratio between the weight average molecular weight (M_w) and M_n were in the range from 1.9 to 2.9.

Conclusions

We have demonstrated a successful strategy to synthesise the polyfluorene nanoparticles in the range of diameter below 100 nm using the Suzuki-Miyaura cross-coupling reaction in the miniemulsion stabilized by SDS surfactant. It was confirmed that longer sonication times lead to a significant reduction in the hydrodynamic diameter of the formed nanoparticles. Overall, these results provide a solid foundation to obtain uniform CNPs dispersion with appropriate optical properties for future application as the photosensitive platform to imitate the function of the blue S-cones retinal photoreceptors.

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PRODUCTION OF A PHOTOCURABLE POLYHYDROXYALKANOATES FOR LIVER TISSUE SCAFFOLDING IN AN ORGAN-ON-CHIP DEVICE

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Perhaps more than ever, there is a great impetus for science, and biomaterials in general to strive for a greater sense of sustainability in research. My PhD project, which involves the production of Poly (3-hydroxybutyrate-co-3-hydrovyvalerate) or PHBV, and its subsequent use as a cell supporting scaffold, will come from a renewable, bacterial source. After transesterification with a further chemical agent to allow it to be 3D printed into a cell-supporting scaffold, this complex can ultimately be incorporated into a three-dimensional latent flow Organ-on-chip device, for drug testing on the cell/tissue sample.

Further to the sustainable production aspect, the potential that this concept has, in the field of drug testing, proposes a solution to the age old issue of animal and tissue testing on new chemical compounds, by providing a small-scale, harm-free and more accurate model.

In order to make the polymer suitable for stereolithography, based upon a previously conducted regime (Hirt et al. 1996), transesterification with ethylene glycol, will produce photocurability in the PHBV. Without such reaction, the polymer be unable to be printed otherwise, as its glass transition temperature is far too low. Following production of the scaffold, and prior to cell seeding, the printed polymer will be subject to a multitude of tests, including mechanical analysis and thermogravimetric analysis, to uncover information regarding its tensile properties and transition temperature points, and overall suitability as a cell-supporting scaffold.

The scaffold is intended to be seeded with a soon to be chosen human liver cell line, due to the liver's importance in the metabolic pathway. Its response and integration and therefore biocompatibility of the scaffold will be measured in time using a Live/Dead assay.

The physical properties of this scaffold are intended to be similar to those of other polyhydroalkanoate, polycaprolactone, and hydrogel scaffolds, i.e. malleable, yet supportive, with a structure as hollow as possible, and of course biodegradable.
In all, this project aims to adapt the existing yet new technology of Organ-on-chip, whilst advancing on the little explored area of photo curing PHBV.

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CONDUCTIVE/ELECTROACTIVE HYBRID DRESSINGS TO PROMOTE CHRONIC WOUND REHABILITATION AND *IN SITU* TISSUE REGENERATION, RE-INNERVATION AND MATURATION

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Chronic Wounds

Cutaneous wound healing is a complex, highly regulated process, requiring a multitude of factors working in unison to restore the skins barrier function.[1] For most these events progress unnoticed, but in an increasingly large number of cases healing becomes stagnated, most often stuck in the inflammatory phase,[2] creating a chronic, non-healing wound. It is estimated that 2-6% of individuals in developed countries suffer from chronic wounds,[3,4] resulting in reduced quality of quality of life for patients[5] and far reaching socio-economic consequences. The dressing market alone is expected to reach $8.46 billion USD by 2021,[6] yet there is still no curative treatment available for chronic wounds. It is evident there is a gap in the wound care market for a truly advanced, affordable wound dressing, capable of actively promoting and accelerating healing.

Dressing Materials

Polyaniline, a conductive polymer, combines excellent electrical and optical properties, features normally seen in metals, with the desirable processing characteristics of polymers.[7] The inclusion of polyaniline in wound dressings offers an opportunity to confer many beneficial features including tuneability, antibacterial properties, antioxidant properties, drug release, sensing and the ability to apply electrical stimulation. Chitosan, sourced from the exoskeletons of shellfish, also displays antibacterial abilities and is already well established in the wound care sector, with regulatory approval for use in dressings and hemostatic agents.[9]

Project Outline

Develop advanced, electrically conductive, hybrid, polyaniline/chitosan wound dressings, that combine the delivery of electrical stimulation treatment, controlled, stimuli responsive transdermal release of therapeutic substances and inherent antibacterial and antioxidant properties, to actively promote and accelerate chronic and diabetic wound healing.

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PHOTOPATTERNABLE SOLUTION BLOW SPUN FIBRES FOR PERIPHERAL NERVE REGENERATION

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Introduction,

Peripheral nerve injury (PNI) affects 13.9 per 100,000 people annually.[1] PNI can cause temporary loss of motor function and sensation, with the more severe cases developing permanent loss of function and chronic pain due to the formation of neuromas. Autologous grafting, which utilises healthy sensory nerves, has long been considered as the gold standard treatment for severe PNIs. Current research is being directed towards finding alternatives to this procedure, with a significant effort being put towards fabricating artificial nerve guidance conduits (NGC) that facilitate repair.[2] This project aims to develop a material which combines the stimulatory effects derived from the topography of aligned fibres with chemotactic guidance cues from localised chemical functionalisation of the fibres via photo-click chemistry, for PNI.

Materials and Methods

1. Synthesis and characterisation of norbornene-modified chitosan.

Norbornene-modified chitosan (CTSNB) was synthesised according to a protocol modified from literature[3] by reacting chitosan dissolved in acetic acid with carbic anhydride in dimethylformamide (DMF). The degree of modification was determined utilising ^{1}H NMR.

2. Solution blow spinning of norbornene-modified chitosan and fibre characterisation.

CTSNB in dH_2O/acetic acid solution, with poly(ethylene oxide) as a spinning aid, was spun into sub-micrometric fibres utilising a coaxial solution blow-spinning (SBS) setup. Fibre diameters and orientations were measured utilising scanning electron microscopy (SEM).

3. Photopatterning of fibre mats with fluorescent markers and functional moieties.

CTSNB mats were photo-patterned with the fluorescent molecule dansyl-cysteine via thiol-ene click chemistry. Additionally, the photopatterning was also carried out utilising a trifunctional peptide containing a fluorescent moiety (Rhodamine B), a biotin moiety (which allows further functionalisation), and a thiol moiety.

Results and Discussion

The modification degree of norbornene-modified chitosan was determined to be equal to 16 ± 3%. Varying the length of the reaction steps allows fine-tuning of the modification degree. Aligned fibre mats with fibre diameters down to 428 ± 149 nm were produced utilising SBS. Photopatterning experiments produced chemical gradients and features with a resolution of ~70 µm on the surface of the fibre mats. Further modification of the DLP system and potential use of guided laser systems could improve pattern resolution.

Conclusions

Norbornene-modified chitosan combined with photo-initiated click functionalisation presents itself as a versatile system with potential biomedical applications, as well as a platform for studying cell-matrix interactions. Future steps will involve patterning with bioactive molecules (RGD/IKVAV)and investigating cell response.

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