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Cardiff Institute for Tissue Engineering and Repair

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Cardiff Institute for Tissue Engineering & Repair Annual Scientific Meeting (CITER ASM)

Proceedings

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#CITERASM21poster **13th to 14th of September, 2021** #CITERASM21

CITER ASM 2021

The abstracts appearing in this book comprise the proceedings of the CITER ASM 2021, held between the 13th and 14th of September 2021. The posters presented reflect the authors' opinions and are published as presented and without change (formatting and minor editing expected). Their inclusion in this publication does not necessarily constitute endorsement by the editors, CITER, or the Cardiff University.

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Introduction

A warm welcome to the 2021 virtual CITER Annual Scientific Meeting (ASM). This annual two-day scientific meeting is an opportunity for our members including biologists, engineers, material scientists, social and healthcare scientists, pharmacologists, and clinicians from across the network to discuss the latest research findings. All of the sessions in this year's virtual ASM are also open to the public. We would like to thank our four keynote speakers: Dr Tom Kean; Professor Rebecca Shipley; Professor Antonio Belli; and Professor Christoph Plass, for accepting our invitation to share their exciting data and research directions.

This meeting provides an opportunity for early career researchers (ECRs) to present their research under conference conditions, either as a short oral presentation or a poster. For several ECRs this experience will extend to Chairing sessions, with senior researchers as Co-Chair. Many thanks to all students and Postdocs who have embraced these opportunities. We would also like to thank the CITER Research Committee, for their commitment to the review process, as well as the authors of all the abstracts submitted to the CITER ASM 2021.

This year, the programme comprises seven sessions over two days and is built around four research themes across Cardiff University where CITER interest meets the theme, including Immunology, Infection and Inflammation, Cancer and Mind, Brain and Neurosciences, and Applied Healthcare Technology. The programme includes oral presentations from our members as well as short talks from colleagues in the University which are relevant to the interdisciplinary work of CITER. Additionally, after a peer review process, the CITER Research Committee selected 26 high-quality poster abstracts that are included in this proceeding (ISSN 2634-100X) and will also be presented as 10-minute invited talks or 2-minute fire-talks linked with the poster session.

We thank you in advance for completing the feedback form which should take just a few moments of your time (https://forms.office.com/r/CZQLYTW9pJ). We strive for improvements and such feedback is invaluable in helping deliver better events for the CITER members and our associates. Finally, thanks to all CITER committees and members not currently serving on committees for their valuable inputs.

We hope you will have a productive and enjoyable two days of talks and discussions at the 2022 CITER ASM; we are sure you will find it stimulating!

Mohammad Al-Amri & Rebecca Weiser

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THEMES OF CITER ASM 2021

THEME ONE: Immunology, Infection and Inflammation

Chair: Dr Elaine Ferguson

Co-Chair: Dr Sian Rizzo

KEYNOTE:

Dr Tom Kean, College of Medicine, University of Central Florida

Tissue Engineering of Cartilage for Drug Discovery

Osteoarthritis is a major healthcare burden both in terms of financial and quality of life costs amounting to over 10% of the US healthcare burden. There are currently no curative therapies for osteoarthritis. Drugs treat pain and inflammation before a total joint replacement is required. Further, adult cartilage has no innate repair mechanism. Part of the challenge is that current osteoarthritis drug discovery methods are inadequate. To overcome these barriers, I have developed in vitro models for cartilage engineering cells to contain a bioluminescent reporter tied to the crucial cartilage protein, type II collagen. We form cartilage spheroids in 96-well plates and have screened a natural product library and found several active compounds. This work is part of a broader overall focus on regenerative methods to improve tissue engineered cartilage and improve osteoarthritis treatment.

GUEST SPEAKERS:

Dr You Zhou, School of Medicine, Cardiff University

Informatics in Inflammation: Translating Big Data into Better Livers

The amount of data now being collected in a single day by a doctor can surpass what might have been generated over her/his career even a decade ago. This deluge of biomedical information requires innovative thinking about how to leverage the data for improving healthcare and saving patients' lives. Responding to this challenge, the long-term goal of my lab is to develop and apply innovative informatics approaches for disease diagnostics and therapeutics. We particularly focus on non-alcoholic fatty liver disease (NAFLD), the most common disease of body's engine (liver). As the leading cause of liver transplantation, the disease is rapidly becoming a major public health concern: affecting 20% to 25% of Europe's population, including up to 30% of the UK's population or 20 million people. Despite its soaring healthcare cost (estimated £6,040 annual cost per NAFLD patient care), there are no FDA approved NAFLD therapies; and current therapies only treat the co-morbidities associated with NAFLD. Through harnessing and integrating different types of omics, clinical and molecular data, we recently (i) found new indications of immunometabolism underlying NAFLD; (ii) developed computational pipelines for diagnosis and drug screening. Our methods could be further adapted for other diseases with unmet needs.

Dr James Pearson, School of Medicine, Cardiff University

Circadian Rhythm Modulation of Host Immunity in Type 1 Diabetes

Circadian rhythms, referring to time-of-day-dependent changes over a 24-hour period, influence many immune cell functions and can alter susceptibility to autoimmune diseases; however, nothing is known about time-of-day influences in altering susceptibility to Type 1 diabetes (T1D). T1D is increasing in frequency and a number of genetic and environmental factors, together alter the balance between the pathogenicity of the autoreactive T-cells causing disease, and the efficacy of regulatory T-cells (Tregs) in preventing disease. We hypothesise that circadian rhythms alter T cell functions at different times-of-day leading to changes that may help protect from, or promote, T1D development. Using the Non-obese diabetic mouse model of autoimmune diabetes, we have found time-of-day-dependent changes in both proinflammatory and anti-inflammatory markers suggesting circadian influences may contribute to disease susceptibility. By understanding the impact circadian rhythms can have on the immune response, we hope to develop ways to modulate these effects to enable better translational success in developing preventative therapies.

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THEME TWO: Applied Healthcare Technology

Chair: Dr Mohammad Al-Amri

Co-Chair: Mr Jake Willott

KEYNOTE:

Professor Rebecca Shipley, Faculty of Engineering Sciences, University College London

From Fast Cars to Breathing Aids: the UCL Ventura Non-Invasive Ventilator for COVID-19

In March 2020, as COVID-19 cases started to surge for the first time in the UK, a team spanning UCL engineers, University College London Hospital (UCLH) intensivists and Mercedes Formula 1 came together to design, manufacture and deploy non-invasive breathing aids for COVID-19 patients. We reverse engineered and an off-patent CPAP (continuous positive airways pressure) device, the Philips WhisperFlow, and redesigned it to minimise its oxygen utilisation (given that hospital oxygen supplies are under extreme demand). The UCL-Ventura received emergency regulatory approvals from the MHRA within 10 days, and Mercedes HPP manufactured 10,000 devices by mid-April. Working with SMEs we also developed associated technologies such as oxygen analysers, CPAP hoods and pressure monitors, receiving 6 emergency regulatory approvals in total. UCL-Ventura CPAP devices have been deployed to over 130 NHS hospitals.
In response to international need, the team released all blueprints open source to enable local manufacture in other countries, alongside a support package spanning technical, manufacturing, clinical and regulatory components. The designs have been downloaded 1900 times across 105 countries, and around 20 teams are now manufacturing at scale and deploying in local hospitals including Peru, Mexico, Paraguay, Pakistan. We have also worked closely with NGOs, on a non-profit basis, to deliver devices directly to countries with urgent need, including Palestine, Uganda and South Africa. The UK government have donated devices to India and Nepal.

GUEST SPEAKERS:

Professor Johannes Benedikt, School of Engineering, Cardiff University

Impact of Radio-Frequency Near-Fields on Cancer Cells and Their Potential Application for Tumour Treatment

Dr Yulia Hicks, School of Engineering, Cardiff University

AI-based Automatic Quality Feedback for Cardiac Doppler Ultrasound

Haemodynamic monitoring is an invaluable tool for diagnosing and treating cardiac health, and is an integral component of intensive care units, obstetrics wards and other medical units. Doppler ultrasound provides a non-invasive, cost-effective, and fast means of haemodynamic monitoring, which traditionally necessitates highly invasive methods such as Pulmonary artery catheter or transoesophageal echocardiography. However, Doppler ultrasound scan acquisition requires a highly experienced operator and can be very challenging. Machine learning solutions that quantify and guide the scanning process in an automatic and intelligent manner could overcome these limitations and lead to routine monitoring. Development of such methods is the primary goal of the presented work.

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THEME THREE: Mind, Brain and Neurosciences

Chair: Dr Malik Zaben

Co-Chair: Dr Ron Ved

KEYNOTE:

Professor Antonio Belli, Surgical Reconstruction and Microbiology Research Centre (SRMRC), University of Birmingham

Diagnosis and Management of Sport Concussion

GUEST SPEAKERS:

Dr James Galea, Cardiff University and C&VUHB

IL-1-Mediated Neuroinflammation in Cerebrovascular Disease

Inflammation is a key driver of acute neurodegeneration following acute cerebrovascular disease. Interleukin-1 (IL-1) is a pro- inflammatory cytokine that orchestrates a myriad of pathological processes that include cytotoxicity, vessel spasm, excitotoxicity, and blood-brain barrier dysfunction. There are various triggers for IL-1 upregulation and release following stroke and the resultant inflammatory burden may be exacerbated by a complex interaction between peripheral and central inflammation. Abrogation of the interleukin-1 inflammatory cascade can be safely achieved using naturally occurring antagonists of the cytokine. Equally important is the interruption of the upstream and downstream mediators of the cytokine such as the NLRP3 inflammasome and interleukin-6 as well as attenuation of the initial mechanisms of injury. Essential to development of these therapeutic modalities as clinical tools is rigorous bench-to-bedside-and-back science and a comprehensive understanding of pharmacology of drugs in spatial and temporal relation to their targets. The research history of IL-1 antagonism from cellular research, through animal trials and culmination in clinical trials in man is an illustration of this process and serves as a learning model for other disease states.

Dr Aminul Ahmed, School of Biosciences, Cardiff University

Stem Cell Therapy for Traumatic Brain Injury

Traumatic brain injury (TBI) is a leading cause of death and disability globally. No drug treatments are available so interest has turned to neural stem cells (NSCs) as alternative strategies for treatment. Approaches to harness stem cells include recruiting endogenous cells following injury or alternatively, transplantation of stem cells into the injured brain. Firstly, we demonstrate in a rodent in vivo head injury model, that endogenous cells within the injured cortex acquire NSC characteristics and can be altered by pharmacologically modulating specific signalling pathways. Next, we show that transplanted human NSCs survive, differentiate and improve outcome in a rodent penetrating head injury model. Finally, we demonstrate that biomaterials can help support transplanted human NSCs to improve outcome in a rodent head injury model. Thus, the use of NSCs have the potential to improve outcome following a TBI.

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THEME FOUR: Cancer

Chair: Dr Renata Jurkowska

Co-Chair: Stephanie Pohl

KEYNOTE:

Professor Christoph Plass, German Cancer Research Center (DKFZ), Heidelberg, Germany

Evolution and Cellular Origin of the Cancer Methylome

GUEST SPEAKERS:

Professor Alan Parker, School of Medicine, Cardiff University.

Taming the beast: refining pathogenic viruses into precision virotherapies for therapeutic applications

Dr Catherine Hogan, School of Biosciences, Cardiff University.

Competition shapes epithelial homoeostasis and early cancer

Epithelial homeostasis is fundamental to health and is maintained via processes that regulate the number and fitness of cells contributing to a tissue. Cell competition describes a 'quality control' process that requires neighbouring cells to compete for space and survival in tissues, with 'loser' cells triggered to die and eliminated. Interestingly, cells carrying cancer-causing genes are outcompeted from healthy tissues, suggesting competition is tumour preventative and initiation of tumorigenesis requires deregulation of competition mechanisms. How cells identify loser or winner populations remains poorly detailed and may be tissue dependent. We identified EphA2 (a receptor tyrosine kinase of the Eph-ephrin family of bidirectional signals) as an evolutionarily conserved signal that triggers the expulsion of RAS-transformed cells from simple epithelia. EphA2 is also essential to eliminate KRAS mutant cells from adult pancreas in vivo and prevent premalignant disease development in tissues. Our goal is to define what regulates cell competition in healthy epithelial tissues and determine how mutant cells override this process to form a tumour.

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Co-localisation of HMGB1 within Oligodendrocyte progenitor cells in a human tissue model of traumatic brain injury

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Background and Aims: There is an unmet clinical need to develop greater mechanistic understanding of white matter injury after traumatic brain injury1. High-mobility group box 1 protein (HMGB1) is implicated as a regulator of brain inflammation following neurotrauma, and elevated levels of it in the CSF and serum of TBI patients are associated with worse outcomes. This study utilises a novel human tissue experimental paradigm to assess for HMGB1 expression in oligodendrocyte progenitor cells (OPCs, NG2+ cells) following neurotrauma. Despite in vitro and in vivo research into the mechanisms underlying traumatic brain injury (TBI)1, few agents have successfully translated to clinical practise. We demonstrate a human tissue, explant model of TBI and identify co-localisation of the neuroinflammatory mediator, HMGB1 in oligodendroglial cells following injury [1].

Methods: Normal human cortical tissue samples were collected from appropriately consented patients undergoing resections of non-traumatic lesions, such as sclerosis or neoplasia. This tissue was plated as monolayers, and after seven days in vitro underwent scratch injury to simulate neurotrauma, as described previously [2]. Cells were fixed 24-hours post-injury and immunostained for DAPI (total cells), NG2 (oligodendrocyte precursors) and HMGB1.

Results and Discussion: Total cell counts and NG2 cell counts were not significantly different between control and injured cell cultures (Fig 1a). HMGB1 staining was significantly higher in injured cultures compared to controls (Fig 1b). Co-localisation of HMGB1 within NG2 cells was markedly greater following scratch injury compared to control (Fig. 1c). This supports recent animal tissue data suggesting that HMGB1 is upregulated following TBI, and that it may influence oligodendroglial cell responses to trauma [2].

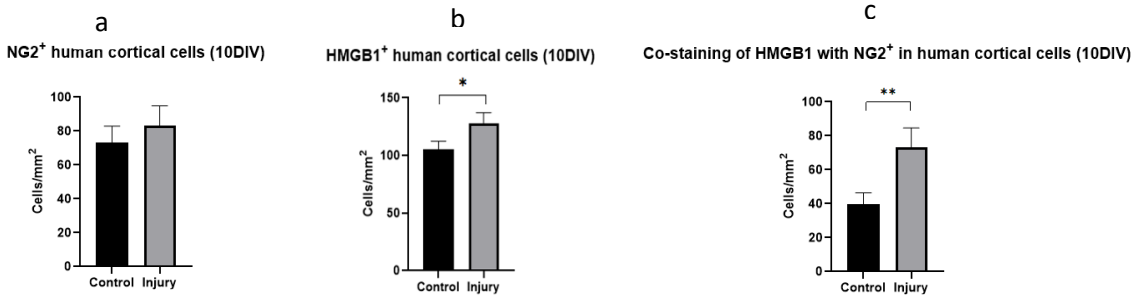

Fig 1: HMGB1 expression in human cortical cultures following neurotrama. NG2 cell (OPC) cell counts were not significantly affected after scratch injury (a). HMGB1 staining was significantly raised in traumatized cultures (b) and there was a marked increase in the proportion of OPCs (NG2+ cells) co-staining with HMGB1 following scratch injury to cultures (c).

Conclusions: We demonstrate use of a human tissue explant model of TBI to identify co-localisation of HMGB1 within NG2 cells following simulation of traumatic brain injury. We will next assess whether this co-localisation of HMGB1 in NG2 cells is caused by upregulation of HMGB1 expression, and how it may influence human NG2 cell behaviour in response to neurotrauma.

Keywords: *Neuroinflammation, white, matter, neurotrauma*

Acknowledgment: The authors acknowledge the support of the BRAIN unit at University Hospital of Wales for supporting this work.

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MCF-7 Cancer Spheroid Formation in Microfluidically Formed Microgels With 3D-Printed Devices

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Background and Aims: Droplet microfluidics offers a precision tool for cell encapsulation in hydrogels, in order to promote 3D cell culture systems for applications, such as regenerative medicine and drug screening [1]. While the fabrication of microfluidic devices may be challenging and time consuming, 3D printing offers a rapid prototyping method which reduces the time and effort associated with the manufacturing [2]. Therefore, more attention can be given to the application of the microfluidic devices; the formation of 3D tumour clusters for drug testing.

Methods: Cyclin olefin copolymer (COC) microfluidic devices (Figure 1A) were fabricated with fused filament 3D printings, to encapsulate MCF-7 cancer cells in an alginate-collagen (Type I, rat tail) matrix with a cell density around 1×10^6 cells/ ml. Our microfluidic device has a T-shaped droplet forming junction for the hydrogel droplet generation, and an extended, curved channel for on-chip hydrogel gelation with $CaCl_2$ infused mineral oil continuous phase. The cell-laden microgels were collected in a 0.2M $CaCl_2$ bath, washed thoroughly with phosphate buffer silane buffer (PBS) and cultured in RPMI cell culture medium.

Results & Discussion: The resultant microgels were highly monodispersed (PDI=0.007, n=35) with a mean diameter of 400um. Cell cluster formation was observed within the microgels over a period of 3 weeks, and the cell proliferation was indicated with live/dead cytotoxicity assay and imaged by confocal microscopy (Figure 1B). Tumour spheroid clusters were formed within 10 days, and their diameters ranged between 100-200 um.

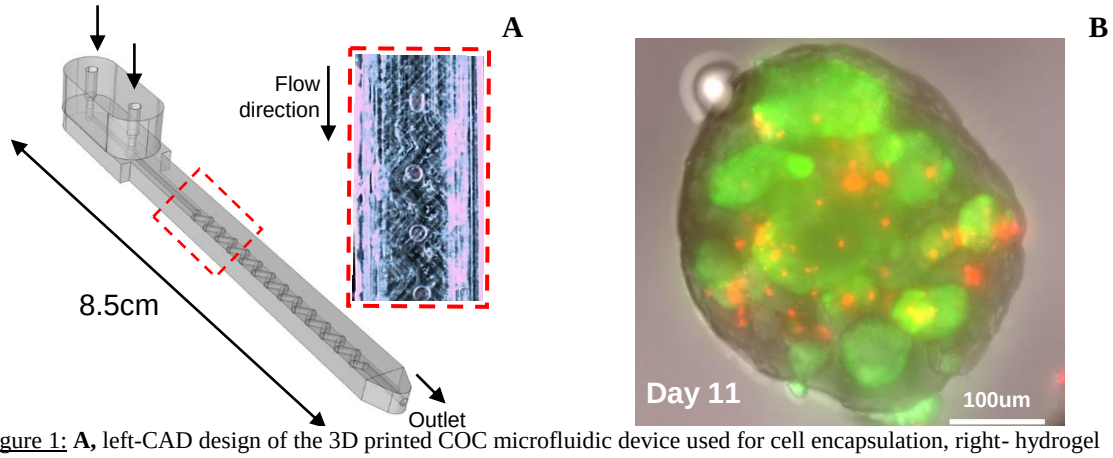

Figure 1: **A,** left-CAD design of the 3D printed COC microfluidic device used for cell encapsulation, right- hydrogel droplets flow in the extended microfluidic channel. **B,** confocal microscopy image at Day 11 of a single collagen-alginate capsule with live (green) and dead (red) cells.

Conclusions: The reported encapsulation technology and materials present a precise method of promoting the formation of tumour clusters, maintained over a long period of time. These 3D MCF-7 cancer spheroid models will be used to develop new drug delivery method in our following work.

Keywords: *droplet microfluidics, 3D printing, cell encapsulation, cancer spheroids*

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Polymer Enzyme Liposome Therapy (PELT) for targeted antibacterial delivery

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Background and Aims: "Polymer Enzyme Liposome Therapy" (PELT) is a novel, two-step approach that employs a polymer-phospholipase conjugate as a trigger for liposomal drug. Proof of concept has been shown in cancer [1, 2], but recent studies have shown that nanomedicines can also passively target to infected tissues [3]. Therefore, the aim of this study was to assess whether they could also be used to trigger antibiotic liberation from liposomes *in vitro*, thereby enhancing their antibacterial efficacy.

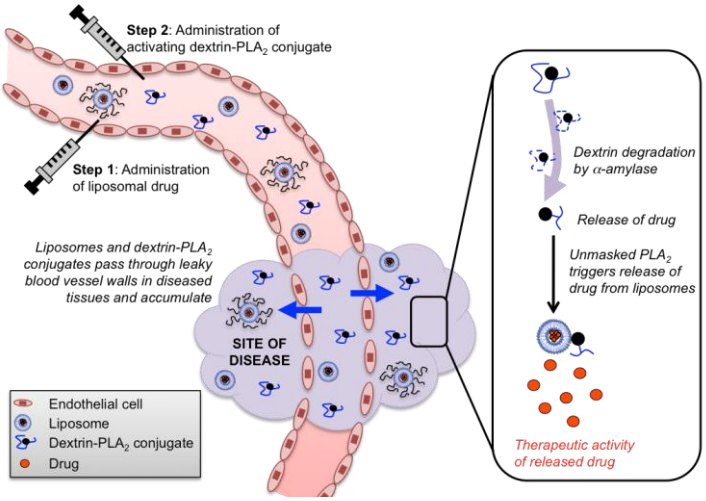

Figure 2: Schematic of PELT hypothesis.

Methods: Gentamicin-loaded small unilamellar liposomes were prepared using thin film hydration and extrusion methods. An *in vitro* pharmacokinetic-pharmacodynamic (PK-PD) model was used to quantify liposomal gentamicin release, while liposome stability was assessed using a Zetasizer Nano ZS to determine liposome diameter and zeta potential. Antibacterial activity of the PELT combinations was assessed using minimum inhibitory concentration (MIC) and checkerboard assays and growth curves in a range of susceptible and resistant Gram-negative and Gram-positive bacteria.

Results: Average liposome size was 113.7 nm ± 38.3 nm, with an encapsulation efficiency of 11.7 ± 2.3%. Neither free nor dextrin-bound phospholipase A2 (PLA2) affected liposome size, up to 24 h incubation, however PLA2 caused liposomal zeta potential to become more negative (-1.8 mV to -10.9 mV). Gentamicin release was also unaffected by addition of free or dextrin-bound PLA2. Antibiotic activity of the liposomal and free gentamicin were equivalent and unaffected by addition of free or dextrin-bound PLA2. No synergism was observed between gentamicin and PLA2.

Conclusion: These results indicate that phosphatidylcholine may not be a suitable substrate for PLA2 and there is no advantage of the PELT approach for antibiotic delivery.

Keywords: *Liposomes, gentamicin, polymer therapeutics, antimicrobial resistance, antibiotics, drug delivery*

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Validation of a novel in vitro model of traumatic brain injury- weight drop injury of human cortical 3D cultures

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Background and Aims: Traumatic brain injury (TBI) is amongst the leading causes of mortality and morbidity worldwide. Attempts at developing pharmacological treatments have been hindered by: (i) an incomplete understanding of the underlying pathophysiology; and (ii) inter-species differences necessitating injury models using human tissue. Herein, we describe and validate a novel *in vitro* model of TBI using human cortical brain tissue to study acute post-traumatic neuroinflammatory pathways.

Methods: Normal human cortical tissue was obtained from patients undergoing surgical resection of deeply seated brain lesions. Surgical samples were mechanically and chemically dissociated into single cell suspensions. Three dimensional (3D) cultures were generated by depositing 5µL of viable cell suspension onto semipermeable membranes on an air-liquid interface. Mechanical weight drop injury was performed at 10 days in vitro (DIV) and the following studies were performed: (i) ELISA & Western Blot to assess HMGB1 and NF-kB as markers of the acute post-traumatic neuroinflammatory response; (ii) propidium iodide (PI) staining to examine cell death at the site of injury; (iii) immunohistochemistry to identify expression of RAGE, TLR-2, and TLR-4 receptors (key HMGB1 target receptors).

Results: At 10DIV, aggregates of 3D cortical tissue were observed; cell characterization revealed the presence of Sox2-expressing neural stem cells alongside other key stem cell niche cell components such as astrocytes ($GFAP^+$), microglia ($IB\text{-}4^+$), and neurons ($TuJ1^+$). At 24hr post weight-drop injury, we observed significant increases in HMGB1 expression within the tissue as well as release into the cell culture supernatant, as quantified by western and ELISA respectively. HMGB1 release was associated with the activation of downstream pro-inflammatory pathways as indicated by a significant increase in the expression of transcription factor NF-kB. This inflammatory reaction was associated with remarkable cell death as quantified by the number of PI^+ cells in the injured tissue compared to controls. Using immunohistochemistry, we further observed a wide expression of the HMGB1 key receptors RAGE, TLR-2, and TLR-4 receptors in our cultures under control conditions and prior to injury.

Conclusions: Weight-drop injury on human cortical 3D cultures offers a viable opportunity for studying acute post-traumatic neuro-inflammatory pathways in vitro. This will provide an effective platform to study both pre-existing and future pharmacological strategies to limit secondary brain damage after trauma and enhance repair. The use of human tissue not only contributes to scientific advances in the field, but also facilitates translation of significant findings from these models to effective therapies that can lead to improved clinical outcomes.

Keywords: *Traumatic brain injury, HMGB1, neuro-inflammation, in vitro model*

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Injury Condition Medium Interferes with the Expression of the Human Cortical Neural Stem Cell Fate-Determining Transcriptional Factors Pax6 and Tbr2

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Background and Aims: Traumatic brain injury (TBI), a rising global health concern, is characterised by primary sheer force brain injury followed by ongoing neuroinflammation. We and others have recently demonstrated significant release of the pro-inflammatory protein high mobility group box protein-1 (HMGB1) and its neuroinflammatory cascades immediately post-TBI (1). Although stem cells respond by proliferation and generation of neurons in an attempt to repair the damaged brain, these newly born cells do not mature and die shortly after birth. Generation of cortical neurons under physiological conditions is tightly controlled by the sequential expression of a number of transcription factors (TF) such as Pax6, Tbr2 and Tbr1 (2). Pax6 maintains stemness of the cells whereas Tbr2 and Tbr1 enhance a neural fate. We hypothesise that the neuroinflammatory microenvironment interferes with the expression of cortical cell fate and therefore designed this study to examine the effects of injury condition medium on the expression of these TFs in vitro.

Methods: Neural stem cells dissociated from neurospheres were either maintained under control or injury condition medium for 7days in vitro (DIV). The control condition medium (CCM) was collected from 3D human cortical cultures generated from normal human cortex at 10DIV. At the same time point, injury condition medium (ICM) was collected from other cultures generated from the same patient but exposed to weight drop injury 24hr earlier. Immunocytochemical analysis was utilised to quantify the number of cells expressing Pax6, Tbr2 and Tbr1 with respect to total number of DAPI expressing cells.

Results and Discussion: Under ICM the number of cells that were $Pax6^+$ was significantly higher than those maintained under CCM ($p=0.00318$). Interestingly, the number of $Tbr2^+$ cells was significantly lower under ICM treatment compared to CCM ($p=0.0388$). There was however no significant change in the number of cells expressing Tbr1 between the two conditions.

Conclusions: Our preliminary data clearly implicated ICM with a cell-fate determining effect in the adult human brain for the first time ever. These novel findings demonstrate that ICM enhances and supresses the expressions of Pax6 and Tbr2 respectively. This may explain why the dividing stem cell after trauma fails to differentiate into mature neurons and subsequently dies.

Keywords: *TBI, neurogenesis, Pax6, Tbr2, Tbr1, neuroinflammation*

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Use of a Dissection Depth Model to learn good dissection technique

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Background and aims: Traditionally, anatomy has been taught through cadaveric dissection. The benefits of this include gaining practical skills such as: appreciation of the human body, first-hand understanding of anatomical variability, learning teamwork and peer interaction[1]. But with COVID-19 restrictions, it has become a challenge to schedule face to face dissection whilst maintaining a safe distance. So that the students do not have any disadvantages in their training as doctors, we have designed a gadget that enables them to learn the dissection skills such as precision and scalpel grip without the use of cadavers while giving the students a similar experience. The aims of this project are: to create a model which will be cost-effective, reusable and sustainable and will enhance learning dissection skills in the absence of dissection; and to create a model that can help with acquiring skills needed for conducting anatomical dissection

Methods: The project was started by determining which variable was to be measured. Force that was acting on the material will be measured to reach the threshold at which the magnets will attract. A CAD (computer automated design) software was used to create a virtual model of what the prototype will look like as shown in Figure 1. As part of the student's preliminary dissection, they dissect on an orange with a surgical scalpel. Any incision deeper than 2mm (normal skin thickness) is considered too deep and is a dangerous dissection practice. Our gadget replaces the need for surgical scalpel for unsupervised practice sessions while maintain the depth perception required for dissection. The way that this prototype would work is when a force large enough to cut through the compressed wood on the top, the magnets would attract each other and pull the copper strips together. This would complete a circuit which can be used to power a buzzer and small light which will alert the student that they are going too deep when a force of around 5N is applied.

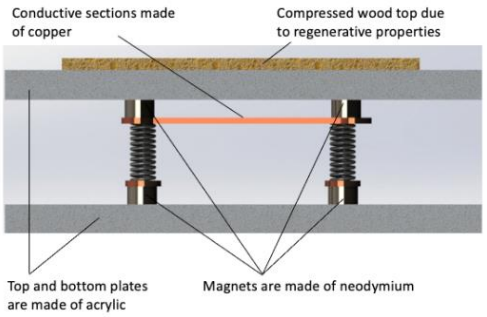

Figure 3: The materials of each component

Results: Early feedback from friends and family suggest that the gadget is user friendly irrespective of the subject's dissection skills. We will be conducting a feedback and comparative test when the dissection resumes in the next academic year.

Conclusion: There are no universities in the UK employ a similar dissection technique which allows students to practice their dissection skills using such gadgets. As this would be a pioneering experience, the following is being proposed: use the gadget in conjunction or as stand-alone to train undergraduate and early clinical year students the necessary dissection skills; and as it is thought that this gadget will improve student confidence in dissection and reduce surgical scalpel injuries, it is suggested the prototype can be integrated as part of regular undergraduate training.

Keywords: *Computer automated design, Dissection, Model, Prototype, Skin thickness*

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A System Designed to Maintain the Position of the TMS Coil while Walking

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Background and Aims: Transcranial magnetic stimulation (TMS) is a non-invasive tool that can be used to investigate the role of the motor cortex in human motor function. The position of the TMS coil has to be precisely maintained during TMS stimulation sessions. This presents a challenge for researchers wishing to study the role of the motor cortex in dynamic movement. A system called COMSTIG (Motor **Co**rtex **M**agnetic **Sti**mulation during **G**ait) was designed to keep the location of the TMS coil constant during dynamic movement. The aim of this study was to optimise COMSTIG's ability to maintain the TMS coil in the desired position on the head while volunteers walked on a treadmill at a speed of their choice.

Methods: Sixteen healthy volunteers participated across three experiments designed to iteratively test the accuracy of the COMSTIG system and make appropriate design adjustments. The TMS coil was placed on the head over the motor cortex [1] and secured in place using the COMSTIG system. Heel strike was identified using a force sensor placed on the heel of the right shoe. The position of the TMS coil on the head was monitored throughout the gait cycle using a Brainsight neuronavigation system and recorded at the instant of heel strike and 20%, 40%, 60% and 80% of the gait cycle. Coil position was considered acceptable if it was within 3 mm of the target position. The target was to achieve an acceptable coil position for >70% of stimuli in a testing session.

Results: In Experiment 1, nine participants (one male, eight female; age range: 25.1–38.4 years; height range: 1.58–1.90 m) walked on a treadmill at a self-selected gait speed (range 0.6–1.6 m/s) for >5 min. Across all participants, coil position was acceptable (within 3 mm of target) for 19– 99% of stimuli, and for four of nine participants it was acceptable in >70% stimuli. In Experiment 2, performed after design adjustments to COMSTIG, seven participants (one male, six female; age range: 24.2–38.4 years; height range: 1.53–1.73 m) walked at a self-selected gait speed (range 0.4–1.3 m/s) for >5 min. Coil position was acceptable in >70% stimuli for six of seven participants (range, 96–100%). In the seventh participant coil position was only acceptable in 22% of stimuli. In Experiment 3, performed after further design adjustments, six participants (one male, five female; age range 20.8–38.4 years; height range 1.55–1.90 m) walked at a self-selected gait speed (range 0.5–1.3 m/s). Coil position was acceptable in >70% stimuli for four of six participants (range, 99–100%). In the other two participants coil position was only acceptable in 53 and 56% of stimuli.

Discussion Conclusion: The final two COMSTIG designs successfully maintained a stable TMS coil position in ≥96% of stimuli during treadmill walking in > 75% of participants. Future design iterations will focus on improving the performance across all participants. Future experiments using COMSTIG will involve use of surface electromyography to obtain a measure of the motor response to stimulation by TMS while a person is walking on a treadmill.

Keywords: *Transcranial magnetic stimulation, motor cortex, walking*

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Modification of the antibiotic, colistin, with dextrin alters uptake, enhances cytotoxicity and triggers apoptosis in leukaemia cell lines

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Background and Aims: Bacterial infections affect >80% of patients receiving chemotherapy for acute leukaemia, with a 30% mortality rate, necessitating the use of prophylactic antibiotics. The antibiotic colistin can kill certain cancer cells *in vitro* [1], but routine use of colistin is limited due to nephrotoxicity and neurotoxicity. The aim of this study was to evaluate whether dextrin-colistin conjugates, which retain antibacterial activity, reduce *in vivo* toxicity and prolong plasma half-life [2,3], could be used to simultaneously act as antibacterial and anticancer agents.

Methods: Dextrin-colistin conjugates containing 8 and 51 kDa dextrin with 1 and 10 mol% succinoylation were synthesised using previous methods [2]. *In vitro* cytotoxicity (24 & 72 h incubation) was assessed in leukaemia cell lines (THP-1, TF1, MV411) using assays measuring metabolic activity, necrosis and apoptosis (caspase 3/7 activity, annexin V binding) and nuclear morphology/ DNA content (Hoechst 33342). Internalisation and cellular localisation of AlexaFluor 594 (AF594)-labelled colistin and dextrin-colistin conjugate were assessed by flow cytometry (1 h incubation), and confocal microscopy (2 h incubation, 2 & 16 h chase), respectively.

Results and Discussion: As observed in human kidney cells and fibroblasts [2], dextrin conjugation abrogated colistin's toxicity towards leukaemia cell lines, especially in conjugates containing 10 mol% dextrin. However, unlike kidney and fibroblast cells, attachment of 8 kDa, 1 mol% dextrin led to increased toxicity in leukaemia cell lines, compared to free colistin (IC_{50} in TF1 cells = 0.28 and 0.67 mg/mL, respectively). Cell death in response to this lead conjugate occurred via phosphotidylserine exposure followed by caspase 3/7-dependent apoptosis (0.25 mg/mL colistin equiv. caused 3.75-fold increase in caspase activity), while treatment with colistin caused necrosis (0.5 mg/mL caused 100% necrosis within 48 h), but not apoptosis (no increased caspase activity). Confocal microscopy showed both, colistin and dextrin-colistin conjugate, were endocytosed and co-localised with lysosomal markers, however, dextrin conjugation significantly altered colistin internalisation.

Conclusions: The lead dextrin-colistin conjugate (8 kDa, 1 mol%) is a promising candidate for simultaneous targeting of leukaemia and bacterial infections. Mechanistic studies are ongoing.

Keywords: *Acute myeloid leukaemia, colistin, dextrin, toxicity, nanomedicine*

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Evaluation of genipin corneal crosslinking in an ex vivo corneal model Of infectious keratitis

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Background and aims: Corneal infectious keratitis is a sight-threatening ocular condition, causing visual impairment worldwide. Infectious keratitis can be caused by various pathogens including bacteria, fungi, protozoa and viruses and the primary risk factors include contact lens wear, ocular trauma and prior eye surgery. [1] Epidemiological data suggest that infectious keratitis may exceed 2 million new cases per year worldwide. [2] In spite of advances in clinical diagnosis, conventional treatment requires long-term, costly antimicrobial therapy and if the infection is poorly controlled, additional surgical management is required to preserve vision. Increased antimicrobial resistance is associated with poorer clinical outcomes and a decrease in the effectiveness of antibiotic therapy, emphasizing the need for new therapeutics for the treatment and management of infectious keratitis. We previously demonstrated the efficacy of corneal crosslinking using genipin, which besides its potential as a natural crosslinker [3, 4], has been also shown to possess antimicrobial and anti-inflammatory activity [5]. The aim of this study is to investigate the efficacy of genipin in reducing microbial colonization and proliferation in an ex vivo model of corneal infection.

Methods: The antimicrobial activity of genipin against *Staphylococcus aureus* (*S. aureus*; ATCC 25923), *Pseudomonas aeruginosa* (*P. aeruginosa*; ATCC 27853) and *Candida albicans* (*C. albicans*; ATCC 90028) was investigated using the broth dilution method and according to the Clinical and Laboratory Standards Institute guidelines for *in vitro* susceptibility testing of bacterial/fungal pathogens. In this study, an ex vivo porcine model of infectious keratitis has been employed, as described previously. Briefly, after sterilization, excised porcine corneoscleral buttons were infected with either *S. aureus*, *P. aeruginosa* or *C. albicans*. Eyes were kept in pairs and post microbial inoculation, corneas were treated with genipin at the determined minimum bactericidal /fungicidal concentration or with saline solution (control) and maintained for 24 hours at 37°C. After incubation, the corneas were homogenized, serially diluted and plated onto agar plates and incubated at 37°C for 24 and 48 hours. The number of viable colonies was calculated and the results were expressed as the log10 number of colony-forming units (CFU)/cornea. Histologic examination using routine staining methods was also carried out.

Results and Discussion: Genipin exerts antimicrobial action against *S. aureus*, *P. aeruginosa* and *C. albicans*. With regards to corneal infection by *S. aureus* and *P. aeruginosa*, treatment with genipin resulted in a significant reduction in the number of CFU/cornea. Genipin treatment reduced the *C. albicans*-CFU/cornea compared to control, however, the difference was not significant.

Conclusion: Future studies must elucidate the mechanism of action of genipin against microbes in order to unravel its potential for the management of infectious keratitis.

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Investigating microglia phenotypes in an induced pluripotent stem cell model of Huntington's Disease

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Background and Aims: Huntington's disease (HD) is a severe neurodegenerative disorder caused by a dominantly inherited CAG trinucleotide repeat expansion in the huntingtin gene (*HTT*). As in other neurodegenerative diseases, neuroinflammation is a prominent sign of HD pathology. Microglia are the principal resident immune cells of the CNS. Several positron emission tomography (PET) studies have demonstrated that microglial activation correlates with disease severity in HD patients. Nonetheless, an open question is whether mutant *HTT* (*mHTT*) expression leads to cell-autonomous phenotypes in microglia, which potentially contribute to disease onset and progression. We therefore aim to investigate the effect of *mHTT* on microglia morphology and function.

Methods: We used patient-derived induced pluripotent stem cell (iPSC) models of HD microglia with 109 CAG repeats and isogenic controls with a corrected wild-type length of 22 repeats. Differentiated iPSC-derived myeloid precursors and microglia were characterized by flow cytometry and immunohistochemistry for the expression of cell-specific markers. Microglia morphology, phagocytic function as well as lysosomal and mitochondrial numbers and morphology were determined using the Opera Phenix High-Content Screening System and Harmony software to analyse the data. Secreted cytokine levels were measured by a cytometric based bead array. Calcium imaging with Flu8 dye was conducted on the FLIPR Penta High-Throughput Cellular Screening System.

Results and Discussion: We found that HD microglia exhibit a less ramified morphology, with significant decreased cell area and complexity, compared to isogenic controls. These HD microglia showed an increased secretion of inflammatory-related cytokines as well as an impairment in phagocytosis, one of microglia's key functions. The assessment of calcium responses to purinergic signalling revealed that HD microglia were significantly less responsive to an ATP stimulus, however there was no difference in the responses to ADP and UDP compared to isogenic controls. No effect of *mHTT* expression on mitochondrial and lysosomal numbers and morphology were detected.

Conclusion: These observations suggest a cell-autonomous effect of *mHTT* on microglia status and activity and are consistent with rodent data, thus supporting the use of iPSC-derived microglia as a model to study neuroinflammation in HD. This model moreover provides the great opportunity to better understand cell-autonomous effects of microglia isolated from the impact of the brain environment.

Keywords: *Microglia, Huntington's disease, Neuroinflammation, iPSCs*

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Polycaprolactone Methacrylate (PCLMA) & Poly(glycerol) Sebacate Methacrylate (PGSM) Poly-High Internal Phase Emulsions (PolyHIPE) for Osteochondral Regeneration

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Background and Aims: Osteochondral (OC) defects are one of the most common musculoskeletal conditions in the UK. They are known for their low rate of healing and concomitant pathologies; they affect 3 out of 10 citizens over the age of 45. Every year, the NHS performs 100,000 hip & knee replacements; 90% of the patients are osteoarthritic [1]. We propose an integrated biomaterial and tissue engineering (TE) approach as an early corrective solution. To propose an alternative strategy for osteochondral defect treatments through an integrated biomaterial and TE approach [2]. Two synthetic functionalised polymers will be manufactured into 3D porous scaffold that promote cell attachment, proliferation, and migration of cells for tissue regeneration.

Methods: PCL and PGS prepolymer solutions were synthesised through ROP and condensation reactions. Further methacrylation was developed through methacrylic anhydride. Prepolymer solutions were purified; H+NMR and GPC were performed as characterisation. PolyHIPE emulsions were manufactured photocuring under UV light. Moulding was used to create mono disks. SEM imaging, water contact angle and mechanical testing were performed. Early cell work was done on bulk and porous materials. BACs and hES-MPs were seeded on PGSM and PCLMA scaffolds respectively to assess cytotoxicity, cell attachment, proliferation, early migration, and ECM production; the latter two reviewed through fluorescent imaging and histology assays.

Results and Discussion: High yield methacrylation was achieved by the control of number of free hydroxyl groups. Results showed photo and thermo-responsive polymers. DM was corroborated through NMR peaks on LM-PCL (35%) and HM-PCL (50%). PolyHIPEs were successfully manufactured through W/O emulsions. Emulsion stability was determined by experimenting with the type of solvent, speed & time of mixing, temperature of emulsion and volume of internal phase. Porosity and pore sizes have been tailored for each TE application. Production of collagen and proteoglycans in chondral tissues, and collagen and calcium sulphates in bone, was observed. Material shrinkage, swelling-deswelling and degradation assays were developed for both PCLMA and PGSM HIPEs. Cell migration was measured with viability assays; an increase in proliferation reflected an increase in migration (Alamar Blue residues). Scaffolds were imaged through SEM, confocal and LightSheet; cellular-like bodies were identified. Qualitative measures for collagen, calcium sulphates, proteoglycans, and alkaline phosphatase were developed in 2D and 3D cultures. Finally, the use of alginate hydrogels for cell encapsulation was successful for hES-MPs after 7 days with no reported natural polysaccharide degradation.

Conclusions: PCLMA and PGSM have mechanical properties suitable for OC applications [3]. Both polyHIPEs possess porous structures that allow cells to attach, proliferate and to slowly migrate through the scaffold. PCLMA- LM and PGSM 80% have shown better results for early cell attachment and proliferation.

Keywords: *HIPEs, Polycaprolactone, Poly(glycerol sebacate), osteochondral, biomaterials, tissue engineering*

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Heterogeneity in response of ankle and knee articular cartilage to pro-inflammatory cytokine stimulation

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Background and Aims The prevalence of idiopathic (primary) osteoarthritis (OA) in the knee is <44% compared to only ~4% in the ankle. It has previously been hypothesised that inherent differences in the biomechanics or biochemical composition of these two joints might explain this increased resilience to ankle OA [1]. Interestingly, it has been reported that ankle cartilage might be more resistant to the pro-inflammatory cytokine interleukin-1 (IL-1) [2]. Therefore, this study aimed to investigate whether there is a differential response to pro-inflammatory cytokines in ankle compared with knee articular cartilage.

Methods Full-depth articular cartilage explants were removed from the talar domes and femoral condyles of patients undergoing lower limb amputations (REC 15/NE/0337). Cartilage explants were cultured in the absence or presence of either '*low*' or '*high*' cytokine concentrations to represent physiological or pathological levels respectively, including IL-1α and oncostatin M (OSM), tumour necrosis factor alpha (TNFα) or IL-1α and TNFα in combination. Explants were stimulated for 28 days with media harvested and replenished over this period. Media was assessed for cytotoxicity, sulphated glycosaminoglycan (sGAG) loss, nitric oxide (NO) and prostaglandin E_2 (PGE_2) synthesis.

Results and Discussion Significantly more sGAG was lost from knee cartilage in response to cytokine stimulation, concomitant with increased synthesis of the pro-inflammatory molecules NO and PGE_2; significant differences were not observed in the ankle cartilage. Interestingly, the effects observed in the knee were most pronounced following '*high*' TNFα stimulation. Furthermore, patient-specific heterogeneity in response to cytokine stimulation was observed across all of the outcome measurements supporting the rationale of a specific 'inflammatory OA phenotype'.

Conclusions Ankle cartilage is less responsive to cytokine stimulation compared to the knee. Furthermore, TNFα elicited a consistent and pronounced inflammatory response in knee cartilage, therefore targeting this pathway may provide therapeutic approaches for treatment of primary knee OA. However, further studies are required to elucidate the underlying mechanism(s) that confer protection against inflammatory OA in the ankle.

Keywords: *Articular cartilage, ankle, knee, osteoarthritis, cytokines*

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Quality of online resources for traumatic brain injury patients and caregivers: time for quality improvement

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Background/ Aims: Traumatic brain injury (TBI) is a global public health problem, causing long-term burden to both patients and caregivers [1]. Consequently, patients often resort to online information-seeking whilst awaiting formal healthcare consultations [2]. Although online resources exist, there is a paucity of evidence regarding their quality, which may have deleterious effects on patients' expectations and understanding of their condition. Therefore, we evaluated the accessibility, quality, and readability of information regarding TBI from major online search engines.

Methods: Seven TBI-related search terms were entered into two online search engines (Google™, Yahoo™). The top 30 search results were assessed for eligibility with duplicates excluded. Quality (DISCERN score, JAMA Benchmark score), readability (Flesch-Kincaid Grade Level (FKGL), Flesch Reading Ease Score (FRES)), and the source of information were evaluated and to provide a holistic assessment of the webpages.

Results: 202 websites were evaluated with a mean DISCERN score (36.5±9.9/80) and a mean JAMA Benchmark score (2.8±1.1/4) signifying poor global quality. The majority required 9 – 12 years of formal education for readability according to FKGL (113/202; 55.9%) and were categorised as 'Difficult' on FRES (94/202; 46.5%); which exceeds the average reading level of developed countries. Website quality was not associated with search ranking or readability (R^2=0.004), indicating that patients did not have immediate access to high quality online resources.

Conclusions: Few high-quality online resources exist for TBI patients and are not sufficiently accessible nor readable. Healthcare providers must take a greater initiative to recommend suitable websites to patients based on the study's results and invest time in developing resources that are of superior quality, accessibility, and readability.

Keywords: Traumatic brain injury, internet, health information, information seeking

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The role of complement factor C3 in lipid accumulation

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease, an estimated 1 in 3 of the UK population are thought to be affected. It is often referred to as a "silent disease" due to its absence of symptoms resulting in the majority of cases remaining undiagnosed. NAFLD is a progressive disease that follows a distinct pattern of events. Chronic elevation of free fatty acids (FFAs) can lead to excess lipid uptake by hepatocytes that result in inflammation leading to fibrotic scarring (Non-alcoholic Steatohepatitis NASH) this can develop into irreversible cirrhosis. Often this will further develop into hepatocellular carcinoma (HCC). NAFLD is linked to obesity and metabolic disorders such as Type 2 diabetes, especially in the western world, often associated with a high fat western diet. This increased prevalence, presents both a clinical and financial burden on healthcare. Without any pharmacological treatments available, the current challenge in preventing disease progression is for improved early diagnostics that could enable disease reversal, also the development of better treatment options. The complement system is a pivotal part of the innate immune system, that may have a potential role in fatty liver disease. Therefore, an important target for investigation. The aim of this project is to uncover the involvement of complement protein C3, one of the key drivers of inflammation in hepatic cells that have been treated to mimic a NAFLD diseased state.

Methods: To investigate the effect increased intracellular lipid uptake has on complement C3 levels, cultured human hepatoma cells (Hu7 cells) were plated onto a 12 well chamber slide and treated with a high concentration lipid solution containing oleic acid and palmitic to mimic a high fat diet and produce a disease model. Cells were stained with 'Oil red O' a dye specifically for lipids then imaged. Hu7 cells were then plated onto a 12 well plate, 6 wells were treated with a lipid solution, whilst 6 non-treated wells acted as controls. RNA was extracted using RT-qPCR, a technique to measure RNA levels. This process includes RNA extraction, cDNA synthesis and qPCR. Nanodrop was used to assess the quality of the RNA, then cDNA synthesis using QPCR to measure expression levels of C3.

Results: Huh7 cells that had been treated in high concentration lipid solution showed a significant uptake of lipids compared to controls, staining displayed increased fat droplets within the cells. C3 expression is significantly higher in fatty liver compared to control with log2 fold changed of 0.171 ($p<0.05$). These results suggest hepatic cells that are exposed to elevated free fatty acids (FFAs) undergo lipotoxicity, leading to increased complement C3 expression and inflammation.

Conclusion: Uncovering the role of complement C3 and inflammation during the early NAFLD and NASH stages of the disease could enable possible targets to be developed for improved disease diagnosis as well as development of new pharmacological treatments.

Keywords: *Complement system, Non-alcoholic Fatty liver Disease, Non-alcoholic Steatohepatitis, Huh7, Inflammation, Hepatoma*

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Adipose stem cell-derived exosomes for the treatment of central nervous system trauma

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Background and Aims: Damage to the Central Nervous System (CNS) occurs in many neurodegenerative disorders like Glaucoma, Parkinson's and Alzheimer's Disease and it is also found in traumas like Spinal Cord Injury and Traumatic Brain Injury. The damage inflicted on adult neurons is irreversible due to irreplaceable neuronal cell death and the inability of neurons to regenerate their axons. It has been demonstrated that bone marrow-derived stem cell (BMSC) exosomes exhibit neuroprotection and axon regeneration of retinal ganglion cells (RGCs) after damage both *in vivo* and *in vitro*. To this effect, this study aimed to determine whether adipose-derived stem cell (ADSC) exosomes could stimulate axon regeneration and protect RGCs from cell death after trauma *in vitro*.

Methods: To achieve this, RGCs were harvested from the eyes of rats and co-cultured in the presence of ADSC exosomes, Dulbecco's phosphate buffered saline (DPBS), or ciliary neurotrophic factor (CNTF). Immunocytochemistry was performed on the cultures, after which they were viewed under the microscope to determine the average number of RGCs present, the average number of RGCs with neurites and the average length of the longest neurite.

Results: Treatment of the RGC cultures with ADSC exosomes caused a significant increase in RGC survival when compared to treatment with DPBS and CNTF. Neither treatment of the RGC cultures with ADSC exosomes, DPBS nor CNTF stimulated significant axon regeneration. However, ADSC exosomes did produce on average the longest neurite when compared to DPBS and CNTF.

Conclusion: ADSC exosomes do elicit significant neuroprotective effects on RGC cultures *in vitro* but they do not significantly stimulate axon regeneration.

Keywords: *CNS, ADSC exosomes, axon regeneration, neuroprotection*

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Novel approaches for enhanced drug delivery

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Background and Aims: The rise in the use of antibiotics is fuelling an increase in antibiotic resistance and new ones are not being developed to replace them [1]. This project investigates new ways of enhanced drug delivery through exploring the potential of exopolysaccharides (EPS) from probiotic bacteria as water-soluble, bi-functional polymers for antibiotic encapsulation, and their controlled release via microwave-assisted drug delivery.

Methods: *EPS extraction. Lactobacillus salivarius* (CUL61) *Lactobacillus paracasei* (CUL08), *Lactobacillus acidophilus* (CUL21/60), *Lactobacillus rhamnosus* (CUL63), *Lactobacillus plantarum* (CUL66), *Lactobacillus reuteri* (CUL55), *Lactobacillus fermentum* (CUL67) and *Bifidobacterium lactis* (CUL34) probiotics were cultured in MRS containing excess glucose or sucrose (100g/L) for 24, 48 or 72 hours at 30 or 37°C to explore the optimal conditions for EPS production. The cell-free supernatant was subject to EPS extraction [2]. Fourier Transform Infrared (FTIR) spectroscopy and bicinchoninic acid (BCA) assay, was then performed to identify putative carbohydrate functional groups and protein quantification, respectively. *Microwave excitation.* Gold (100nm spherical- or urchin-shaped) and magnetic (1µm) nanoparticles (NP) were encapsulated in alginate microgels along with an antibacterial dye using 3D-printed microfluidic chips. The resulting microgels were exposed to microwave electric (E) or magnetic (H)-fields within a TM010 mode resonator at 2.5GHz and 1.2 W rms at varying time periods and duty cycles and resulting temperature increases recorded. The absorbance (at 650 nm) of the surrounding solution was measured spectrophotometrically to determine the degree of microwave-assisted dye release from the microgels.

Results: Of the eight probiotic strains explored *L. reuteri* cultured in 100g/L of sucrose at 37°C consistently produced high quantities of dry weight EPS of, 23.3mg at 24h, 33.7mg at 48h and 50.8mg at 72h from 10ml cultures. Cell counts increased in line with EPS production. The FTIR spectra all follow similar patterns, however the *L. reuteri samples* have a sharper absorbance in areas of the spectrum corresponding to aldehyde (-CHO, 1008cm^{-1}) and ketone (C=O, 1733cm^{-1}) functional groups, present in carbohydrates. The BCA assay revealed low levels of protein content (ca. 19% on average). During microwave excitation free nanoparticles in buffer reacted very differently to those encapsulated within microgels. In the E-field, the nanoparticles' temperature was increased by 9°C, whereas when encapsulated increased by 5°C. On the other hand, in the H-field the free nanoparticles' temperature was increased by average of 4.5°C but when encapsulated increased by 10°C. This could be due to the E-field influencing the H-field, but not vice versa.

Conclusion: EPS yield from lactobacilli is strain-specific. L. reuteri was identified as the highest EPS-yielding strain and is therefore a good candidate for further physical and biological characterization for drug delivery applications. For the first time, the feasibility of microfluidics for the construction of NP-drug microgels was demonstrated and their behaviour in microwave fields was assessed. Future work will look at replacing alginate with probiotic-derived EPS for microgel formation. This could lead to new and improved routes to administer last resort antibiotics.

Key Words: *Probiotics*, *Nanoparticles*, *Microwaves*, *Exopolysaccharides*, *Drug Delivery*

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Characterisation of immortalized human cartilage progenitor cells

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Background and Aims: Osteoarthritis (OA) is an increasing clinical problem in the UK with 9 million sufferers in an ageing population [1]. Long waiting lists have only worsened since the COVID-19 pandemic. Despite this, there are no effective clinical treatments available, and our knowledge of disease onset is still lacking. A population of multipotent progenitor cells (chondroprogenitors) have been identified and isolated from human-cartilage tissue, and have proven to have potential for de novo development of cartilage tissue [2, 3]. There is an urgent need for a consistent supply of engineered human articular-like cartilage to model human disease (as opposed to an animal model), allowing us to screen potential drug candidates and develop biomarker assays for early diagnosis. Chondroprogenitors (CPs) isolated from cartilage via differential adhesion to fibronectin were immortalised by infection with retrovirus carrying hTERT [4]. Subsequent single cell cloning produced 52 different clones. 3D culture using a permeable trans-well demonstrated differences in the ability of these clones to form cartilage (AC et al., unpublished results). Histology showed that some clones produced fibrous tissue, whereas others produced a tissue akin of articular cartilage (full-depth differentiation and matrix synthesis over a 35-day period). This study aimed to characterise these clones in terms of their cell surface markers and adhesion to fibronectin in order to explain the differences in their cartilage-generating capacity.

Methods: Flow cytometry was employed to analyse a panel of cell surface markers and MTT assays were undertaken to quantify adhesion to fibronectin. Seven different chondroprogenitor clones were analysed and compared to each other, but also to mesenchymal stem cells (Y201) and chondrocytes (TCs28A2).

Results: Results of these FACS analyses showed that CD49e and CD29 (α5 β1 integrin) cell surface markers were expressed at a high level in all clones at cell surface, but also in Y201 and TCs28A2 cell lines. This supports results from the MTT assays suggesting that the clones and cell lines were strongly adherent to fibronectin. Furthermore, all cells were >99% positive for CD44, a major receptor for hyaluronic acid. Conversely, all clones were negative for CD317 (4), a marker of inflammation.

Conclusion: The results suggest that the cell surface markers analysed are not indicative of the capacity of the clone to differentiate into articular cartilage, neither are their adherence to fibronectin. Flow cytometry analysis was contrary to some current literature suggesting that CD49e can act as a biomarker for chondroprogenitors. Though all chondroprogenitor clones are positive for CD49e, there was no correlation to the ability of the clones for chondrogenic differentiation. Neither was there a difference seen between chondroprogenitor clones and the Y201 and TCs28A2 cell lines. The absence of CD317 in all clones confirmed the absence of inflammatory conditions within the culture. Given that established markers proved uninformative RNA-Seq and qPCR analysis is currently being undertaken to uncover any genetic differences that could explain the differences in ability to undergo chondrogenesis by the different clones.

Keywords: *Chondroprogenitor, fibronectin, cartilage, differentiation, model, osteoarthritis*

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Morphological profiling cells with genetic variants associated with neurodevelopmental and neurodegenerative disease

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Background and Aims: Neuropsychiatric disorders are a broad range of debilitating diseases. The biological mechanisms and neuropathology are poorly understood though a degree of heritability is conserved. These disorders involve (but not limited to) Schizophrenia, Bipolar disorder, Major depressive disorder and attention deficit disorder [1]. Neurodegenerative disorders too are heritable diseases but also can occur sporadically. Neurodegenerative disorders include (but not limited to) Alzheimer's, Parkinson's and amyotrophic lateral sclerosis [2]. Cell painting is a morphological profiling assay that stains cells to allow for imaging and quantification of data in a high throughput manner, it can acquire ~1500 morphological properties of an individual cell whilst screening 100,000s of cells. It allows for phenotyping small molecules and their mechanisms of action, characterise cellular heterogeneity and relationships between genes and cell morphology [3]. The aims were (i) to profile morphological changes in cell lines with genetic mutations associated with neuropsychiatric and neurodegenerative disorders via cell painting, and (ii) to observe changes in morphology after known cell lines are treated with varying drugs and concentrations.

Methods: To determine effects of drugs on cells, cell lines consisting Induced Human Pluripotent Stem cell-derived neural progenitor cells (NPCs) carrying genetic variants associated with neurodegenerative and neurodevelopmental disorders (namely Huntington's, Schizophrenia, Autism Spectrum Disorder) and patient derived NPC's carrying associated mutations were incubated and treated with varying concentrations; 1µM, 10µM and 100µM. The Table contains cell lines and associated genetic perturbations. Cultured onto 384 well plates with controls and 4 replicates. The cells were then fixed and stained as described previously [3]. Phalloidin-568, Concanavalin A-488, Hoescht 33342, Wheat Germ Agglutinin- 488 and SYTO 14 dyes were used to stain. Plates were then imaged via the Opera Phenix. After imaging by the data was analysed and extracted via Cell profiler and R-studio with a pipeline and custom scripts, respectively. The custom scripts were used to clean up the data, normalise and extract features of value so as to be represented descriptively.

Cell lines	Description
iCas 9	Control HESC
4KO	*CTIP2* knockout derived from iCas9
33KO	*CTIP2* knockout derived from iCas9
44KO	*CTIP2* knockout derived from iCas9
3H2	IPSC Control HESC 21 CAG repeats
11B11	IPSC 109 CAG repeats *HTT* gene
I202	Patient derived IPSC Control
KOLF2	Patient derived IPSC Control
008.4	IPSC 15q11.2 deletion
030	IPSC 16p11.2 duplication
083	IPSC 16p11.2 deletion
091	IPSC 16p11.2 deletion
CKO	iCas9 derived Cyfip1 knockout
H7	HESC Control
CTG	*CYFIP1* overexpression

Results: The data gathered so far is undergoing analysis via R. The analysis could be sped up with improvements in the software and accessibility i.e Cell Profiler and Phenix system.

Conclusions: Cell painting offers a broad range of information on the morphology of a cell and subsequently data on cell cycle, abnormal development and small molecule perturbations thus allowing a wealth of data to be extracted and analysed.

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The effects of Levodopa on the survival of human embryonic stem cell (hESC) grafts in a rat model of Parkinson's Disease

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Background and Aims: Parkinson's Disease (PD) is a progressive neurodegenerative condition characterized by the loss of dopaminergic neurons. Cardinal features include resting tremor, bradykinesia, and postural instability [1]. Current treatments such as Levodopa (L-DOPA), are effective initially, but lose their effectiveness over time [2]. Cell replacement therapies have the potential as a therapeutic strategy for PD by incorporating differentiated dopaminergic cells into the dopamine-depleted caudate/putamen to restore dopamine supply. Clinically, patients are still administered L-DOPA post- graft transplantation, as it takes time for cells in the graft to differentiate [2]. However, the effects between L-DOPA and the transplanted cell graft and whether it impacted the body's systemic immune response remain unclear. We therefore aimed to investigate cell survival in grafted 6-OHDA-lesioned rats when exposed to L-DOPA while immunosuppressed and following withdrawal of immunosuppression. Additionally, we investigated whether a systemic immune response could be detected and if too was impacted by L-DOPA treatment.

Methods: Neonatally tolerized Sprague-Dawley rat pups were housed and injected with 6-OHDA at 6 weeks to induce parkinsonism. They were immunosuppressed and then split into different treatment groups, either receiving a sham/hESC cell graft and an L-DOPA/saline treatment. The rats underwent Magnetic Resonance Imaging (MRI) at 20 weeks. Immunosuppression was removed post-MRI analysis and at 25 weeks, peripheral blood mononuclear cells (PBMCs) were extracted from peripheral blood samples at the time of perfusion. Quantitative PCR (qPCR) was conducted on PBMCs, allowing quantification and assessment of immune markers during L-DOPA treatment post-immunosuppression. Analysis of graft volume in the histological samples was also conducted post-perfusion and was detected by the presence of human nuclei.

Results: The results showed a greater graft volume in the non-L-DOPA treated, cell grafted rat groups in comparison to its L-DOPA treated, cell grafted counterpart when assessing the MRI images. This was not consistent with the histological analysis, where the L-DOPA/cell grafted groups produced a higher mean graft volume when immunosuppression was withdrawn. However, it was observed that there was a statistically significant difference between the saline/cell graft group; this was not the case for the L-DOPA/cell grafted rats. Furthermore, qPCR results showed great variation in immunological marker expression between L-DOPA (non-) treated and sham/cell grafted rat groups, inferring that L-DOPA interacts with various immune pathways.

Conclusion: From the data in this project, we can conclude that L-DOPA is associated with immunomodulation of the immune response and can potentially aid in the protection of the cell graft, although further studies are warranted. Future directions can focus on identifying the pathways each marker is involved during L-DOPA treatment; these can then be up/downregulated to investigate whether cell viability improves. Furthermore, this could be translated to human models, and potentially novel treatments to not only maintain cell graft viability but also help combat L-DOPA progression.

Keywords: *Parkinson's Disease, L-DOPA, Cell replacement therapies*

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Phosphatidylserine Receptor Localisation, Expression and Activation in Human Bone Marrow Stem Cells Treated with Liposomes

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Background and Aims: Fracture non-union is exceedingly becoming one of the most debilitating medical conditions affecting patient quality of life, creating a significant economic burden on health care systems. Approximately 850,000 new adult fractures occur each year in the UK [1], with each non-union case estimated to cost between £7,000 and £79,000 to treat [2]. Although several treatments have been developed to manage non-unions, success rates are low. Therefore, a better understanding of the mechanisms controlling bone repair is required to develop more effective treatments. Phosphatidylserine (PS), a naturally occurring phospholipid, has been implicated in regulating bone formation, showing promise as a functional molecule in osteogenic biomaterials and liposomal drug delivery systems [3]. However, the biological mechanism of action of PS on bone cells, specifically human bone marrow stem cells (hBMSCs), remains unclear. This project investigated whether hBMSCs express PS receptors and whether these receptors are activated when treated with PS-containing liposomes.

Methods: RNA from hBMSCs were extracted and RT-PCR was performed to establish the presence or absence of PS receptors using total human RNA as a positive control. hBMSCs were treated with 10 and 100 ug/mL PS liposomes or PC liposomes and the response measured using a TGF-ß1 ELISA. Changes in the expression of PS receptors were also examined using qRT-PCR and confirmed at a protein level using immunocytochemistry. Downstream activation of the PS receptor was examined using western blots to detect phosphorylation of extracellular signal-regulated kinase (ERK).

Results: hBMSCs expressed mRNA for TYRO3, AXL, B2GPI and RAGE PS receptors and were negative for MERTK,TIM-1 and CD300A PS receptors. The presence of TYRO3 receptors in hBMSCs was confirmed by immunocytochemistry. Treating hBMSCs with PS liposomes resulted in significant increases in TGF-ß1 production, but not when treated with PC liposomes. qRT-PCR demonstrated an increase in TYRO3 expression after treatment with 100ug/mL PS liposomes for 120 minutes. Furthermore, western blots demonstrated PS treatment to result in phosphorylation of ERK, suggesting PS may act through TAM family tyrosine kinase (TYRO3) activation and subsequently the mitogen-activated protein kinase (MAPK)/ERK pathway.

Conclusion: This research shows for the first time that the osteogenic mechanism of action of PS is through activation of PS receptors, such as TYRO3, present on hBMSCs and subsequent downstream activation of the ERK 1/2 signalling pathway. The study also demonstrated PS treatment to result in increased TYRO3 expression, however the biological function of this response still needs to be elucidated.

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Evaluation of the impact of colistin and a dextrin-colistin conjugate on differentiation and *in vitro* survival of acute myeloid leukaemia (AML) cell lines

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Background and Aims: Acute Myeloid Leukaemia (AML) is currently a difficult to treat disease with a five-year survival rate of only 20% [1]. The identification of new treatment types is crucial. Colistin is an antibiotic used prophylactically in AML patients to reduce infection during chemotherapy and has also been proven to kill certain cancer cells *in vitro* [2]. However, its use is limited due to severe side effects such as nephrotoxicity. The conjugation of dextrin to colistin has been shown to reduce the toxicity of colistin to normal kidney cells whilst retaining antibacterial ability [3]. Initial results identified a lead dextrin-colistin conjugate with enhanced cytotoxicity in AML cell lines with some evidence of increased apoptosis. Morphological changes were observed that may indicate differentiation following treatment (also observed with some current AML therapies). The aim of this study was to identify whether colistin and the lead dextrin-colistin conjugate activated differentiation pathways within the AML cell lines THP1 and MV411, to determine the temporal induction of apoptosis and necrosis and to investigate impact of treatment on proliferative capacity.

Methods: Real-time-Glo™ annexin V apoptosis and necrosis assays were carried out on THP1, MV411 and TF1 cell lines with repeated measurements taken up to 72 hours after treatment. Differentiation was assessed by flow cytometry. Cells were treated with colistin, 8,000 g/mol dextrin with 1 mol % succinoylation and 8,000 g/mol dextrin with 1 mol % succinoylation conjugated to colistin for 48 hours followed by a 24 hour recovery period [4]. Cells were then assayed for CD86 and CD11b expression using fluorescent antibodies and flow cytometry to determine the MFI of live cells. The proliferative capacity of these treated cells was assessed by semi-solid methyl cellulose colony assays.

Results: Annexin V and necrosis measurement showed that treatment with colistin caused primary necrosis whereas conjugation to dextrin triggered primary apoptosis followed by secondary necrosis. The cells that survived treatment with colistin and conjugates showed no evidence of differentiation: the MFI of surface markers CD86 and CD11b was not significantly altered by these treatments in comparison to the vehicle only control. Colistin reduced colony formation to 54-69 % of vehicle control. However, the conjugate reduced colony formation to a greater degree (4.7-23% of vehicle control). Although differentiation was not induced, the conjugate had an impact on the functionality of AML cells.

Conclusions The dextrin-colistin conjugate enhanced cell death by activating primary apoptosis followed by secondary necrosis. In the surviving cells differentiation was not induced, however the ability to proliferative and form colonies appears to be impaired.

Keywords: *Acute Myeloid Leukaemia, Dextrin, Colistin, Differentiation*

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Investigation of a hydrogel-based *in vitro* model of vasculogenesis

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Background and Aims: The study of the interactions between tissues, the vasculature and the immune system are essential for understanding organ function, dysfunction, many diseases and drug responses. Modelling these complex dynamic interactions in a human-specific context *in vitro* remains a challenge in research [1]. Currently we are missing the ability to mimic the intricate structure of blood vessels and replicate the two-way communication between immune cells and tissues. With the development of an *in vitro* model of vasculogenesis, the opportunity for studying and understanding these interactions is opened up and will hopefully lead to advances in the medical field. As a step in this direction, we explored here whether hyaluronic acid hydrogels developed by the research group can support de novo vessel formation. Human umbilical vein endothelial cells undergo tube formation in a 3D environment with appropriate biological cues [2,3]. This report details our investigation of a modified hyaluronic acid (HAED) hydrogel seeded with endothelial cells (EA.hy926) to promote blood vessel formation.

Methods: Hydrogels were made by chemical modification and subsequent crosslinking of hyaluronic acid. Seeding of cells onto HAED hydrogels followed by live/dead staining or immunolabeling for CD31, a marker for vascular differentiation, confirmed that the hydrogels supported cell spreading / migration and viability. Tube formation supported by hydrogels was compared to fibroblast extracellular matrix (ECM) or Matrigel (currently accepted standard). VEGF and other supplements were introduced to the hydrogel to determine whether or not the hydrogel material could sufficiently stimulate tube formation in the presence of these cues. Fluorescently labelled endothelial cells were seeded and tube formation observed for up to 48 hours to optimize assay conditions. Images taken at peak formation were used to quantify tube formation (number of junctions, number of branches, tube length, area of vessels). Immunocytochemistry was used to detect VE-cadherin.

Results: The HAED hydrogel supported viable endothelial cells. These cells were able to form tubes with Matrigel when seeded after 24hrs of serum starvation. We found that peak neo-vessel arrangement was observed between 20-30 hours. EA.hy926 cells grown with HAED and with Matrigel expressed VE-cadherin, demonstrating adhesion between endothelial cells essential for angiogenesis. VE-cadherin also modulates the VEGF receptor, so is crucial for the formation of vessels [4]. It remains to be determined whether the presence of VEGF with the hydrogel is a sufficient factor for inducing tube formation.

Conclusion: To address the need for a microvascular network to maintain cells in a 3D tissue environment when forming micro-physiological systems, this work on a novel hydrogel presents an initial step towards developing such an integrated microvascular network in that the we demonstrate that the hydrogel and supplements support vessel formation. In the future, the system needs to be fine-tuned to sustain vessel formation in a specific pre-determined location only. Combining this material with a perfusing recirculating pumping system in future projects will pave the way for further advances in this field of engineering of micro-tissues and organs.

Keywords: *Vasculogenesis, hydrogels, tube formation, adhesion, modeling*

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Healthy and chronic wound fibroblasts exhibit antimicrobial effects on *Staphylococcus aureus* NCTC 6571

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Background and aims: Keratinocytes in the epidermis have been shown to inhibit the growth of *S. aureus* through antimicrobial peptides (AMPs) and several types of fibroblast have also demonstrated the ability to produce AMPs and complement proteins [2-4]. However, this has not been shown for dermal fibroblasts originating from chronic wounds. This project aimed to test whether fibroblast conditioned media (CM) from healthy and chronic wound fibroblasts was able to inhibit the growth of *S. aureus.*

Methods: Immortalised patient fibroblasts isolated from healthy or chronic wound sites were cultured and CM was collected. Three *S. aureus* strains (one type strain: *S. aureus* NCTC 6571 and two clinical chronic wound isolates: *S. aureus* 1004a and 1061a) were then supplemented with fibroblast CM and survival and biofilm formation was measured. Biofilm formation was evaluated using crystal violet staining and visualised with scanning electron microscopy. The CM was then heat inactivated or filtered to determine if the components responsible for changes in survival could be identified. Attachment assays were performed to evaluate if the attachment of *S. aureus* differed between healthy and wound fibroblasts.

Results: The results show that both chronic wound and healthy fibroblast CM inhibit the growth of *S. aureus* NCTC 6571 in a dose-dependent manner, but do not inhibit the growth of clinical isolates *S. aureus* 1004a and *S. aureus* 1061a. The same trends were seen in biofilm formation, with both healthy and chronic wound CM able to inhibit biofilm formation of *S. aureus* NCTC 6571, but not biofilm formation of the clinical isolates. When the CM was heat inactivated or filtered using a >30kDa size exclusion filtration unit the potency of both healthy and chronic wound CM decreased. However, the pattern between healthy and chronic wound CM differed. When inactivated the chronic wound CM lost the ability to suppress bacterial growth, but healthy CM potency was only reduced and could still suppress bacterial growth. This suggests healthy CM contains factors smaller than 30kDa which are responsible for the inhibition of bacterial growth. It also suggests these factors are heat resistant or not proteinaceous as they are not denatured after heat inactivation. Conversely, the chronic wound CM is likely to contain factors larger than 30kDa which inhibit bacterial growth as after filtration the CM can no longer inhibit growth. Additionally, these factors are likely to be non-heat resistant or proteinaceous as they denature after heat inactivation.

Conclusions: This research demonstrates that healthy and chronic wound dermal fibroblasts possess the ability to inhibit growth and biofilm formation of *S. aureus* NCTC 6571 but not clinical isolates of *S. aureus.* This may suggest clinical isolates have evolved resistant to fibroblast activity.

Keywords: *Chronic wounds, Staphylococcus aureus, fibroblasts, antimicrobial peptides, conditioned media*

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The role of CTIP2 in calcium signalling and handling within Medium Spiny neurons and cortical neurons– Implications for Huntington Disease

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Background and Aims: The pathological mechanisms underlying Huntington disease (HD) are still under investigation. CTIP2 has a suggested role in the pathogenesis of HD, it is highly expressed in medium spiny neuron (MSN) the primary cell type lost in HD. Recent research has identified the role of CTIP2 in calcium signalling [1], disruption of which has been widely reported in HD models [2]. The aim of this study is to further elucidate the impact CTIP2 has on Ca^{2+} regulation in neurons.

Methods: CTIP2 knock-out ($CTIP2^{KO}$) MSN, cortical (CTX) and dopaminergic (DA) cells were generated to investigate the impact CTIP2 has on Ca^{2+} signalling. Cells underwent Ca^{2+} imaging using Fluo-4 to investigate the impact $CTIP2^{KO}$ had on spontaneous Ca^{2+} activity, as well as resting levels in response to induced Ca^{2+} influx. The response to pulsatile glutamate stimulation was investigated in both $CTIP2^{KO}$ and WT, additionally the response of WT MSN to glutamate after treatment with glutamate receptor inhibitors was reported.

Results: The spontaneous Ca^{2+} activity of $CTIP2^{KO}$ MSN was observed to be significantly lower in frequency and spike amplitude Increased Ca^{2+} resting levels were observed in $CTIP2^{KO}$ MSN and CTX cells in response to induced Ca^{2+} influx. Impaired response to glutamate was observed in $CTIP2^{KO}$ MSN and CTX cells, as the glutamate pulses increased, the capability of the cell to buffer Ca2+ declined leading to Ca^{2+} saturation. This resulted in the decreased ability of the cell to respond to stimulation. $CTIP2^{KO}$ MSN also exhibited a significantly higher level of intracellular Ca^{2+} at the end of the glutamate stimulation.

Conclusion: We have demonstrated that $CTIP2^{KO}$ leads to dysregulated Ca^{2+} signalling, which suggests that the disruption of Ca^{2+} signalling observed in HD may be linked to the deficiency of CTIP2. This may also cause the early vulnerability of MSN to degeneration in HD.

Keywords: *CTIP2, Huntington disease, calcium signalling, medium spiny neurons*

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Nutraceuticals in the prevention and treatment of non-alcoholic fatty liver disease and atherosclerosis: the beneficial actions of the ellagitannin-derived metabolite Urolithin A.

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Background and Aims: Atherosclerosis is a major underlying cause of cardiovascular disease, and is often associated with accumulation of fatty acids in the liver, also known as hepatic steatosis or non-alcoholic fatty liver disease (NAFLD). Nutraceuticals have generated substantial interest in recent years as a means of prevention and treatment of atherosclerosis and NAFLD owing to their lipid-lowering, antioxidant and anti-inflammatory properties. However, the underlying mechanisms of these protective effects are poorly understood. The aim of this project therefore was to investigate the effect of ellagitannin-derived metabolites, such as Urolithins, on parameters associated with NAFLD and atherosclerosis *in vitro*.

Methods: HepG2 cells are extensively used as a model for NAFLD, a condition closely related to atherosclerosis, thus making them an ideal candidate for the experiments. The effects of ellagitannin-derived metabolites on lipid accumulation was assessed using Oil Red O staining. The antioxidant properties were analysed by reactive oxygen species (ROS) and mitochondrial (Mito)-ROS assays, and an Amplex Red cholesterol assay kit was used to test the effects on cholesterol levels. The Triglyceride level was measured using a triglyceride kit, and the anti-inflammatory effect was assessed via enzyme-linked immunosorbent assay (ELISA) for IL-beta, IL-6 and TNF-alpha, three key cytokines in the inflammatory process.

Results: Urolithins had no ill effects on cell viability and proliferation of HepG2 cells. Urolithin A (UroA) had the most potent and significant lipid-lowering effect of all ellagitannin-derived metabolites. UroA also displayed significant reduction in ROS production and a slight but insignificant decrease in Mito-ROS levels in HepG2 cells. A slight, but insignificant decrease was seen in Triglyceride, IL-B, and TNF-a levels after treatment with UroA, but no difference was seen in the IL-6 or cholesterol levels.

Conclusion: Overall the data strongly suggests that UroA holds a promise in limiting NAFLD development development and provides justification into further research of ellagitannin-derived metabolites as therapeutic agents for both NAFLD and atherosclerosis.

Keywords: *non-alcoholic fatty liver disease (NAFD)*; *atherosclerosis*; *nutraceuticals*; *ellagitannin*; *urolithin*

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Evaluation of novel anti-cancer agent in 2D and 3D models of breast cancer

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Background and Aims: Triple-negative breast cancer (TNBC) is an aggressive type of cancer that lacks receptors for oestrogen, progesterone and does not overexpress Her2 protein. This makes it difficult for targeted treatment, leaving systemic chemotherapy and radiation as the only choice of treatment. NF-κB signalling pathway has previously been linked to tumorigenic processes, breast cancer progression, apoptotic evasion, and treatment resistance. Bcl3 activates the NF-κB pathway by binding to p50/p52 homodimers. Recently, a novel inhibitor of this Bcl3:p50/p52 interaction, JS6, was discovered at Cardiff University by the Clarkson group [1], which was shown to significantly suppress metastasis. A refined version of JS6, termed CB1, was developed with better potency at inhibiting the Bcl3:p50 interaction. Based on the key role of Bcl3 in the activation of the broad survival NF-κB pathway, we aimed to investigate the role of CB1 as a potential sensitizer to the standard of care chemotherapy in 2D and 3D models. Drug screening has previously been reported to yield different responses depending on whether the testing platform was in 2D or 3D [2,3]. This encouraged us to utilise a simple 3D model to compare the effect of CB1.

Methods and Results: In this study, we show in a 2D culture that CB1 monotherapy has a moderate, yet significant effect on the viability of two TNBC cell lines, MDA-MB-231 and SUM149. Moreover, we show that CB1 in combination therapy with either paclitaxel, cisplatin, or 5FU reduced the IC50s in both TNBC cell lines, based on Cell-titre blue viability assay. We also show that combining CB1 with a PARP inhibitor, olaparib, improves the response to such an agent in both cell lines, regardless of the BRCA status. To investigate whether the response is caused by disrupting homologous recombination by inhibition of Bcl3 by CB1, we employed an I-SceI-based DR-GFP assay. We observe that CB1 indeed hampers the repair efficacy of dsDNA breaks through homologous recombination. However, we were unable to determine whether the reduction of viability after CB1 treatment is entirely dependent on homologous recombination and further research needs to be done to elucidate the mechanism of action of CB1 and the consequences of Bcl3 inhibition in TNBC. We also optimised a promising physiologically relevant 3D model using Matrigel and type I collagen to enable the simultaneous study of cancer growth and invasion. However, the comparison of the CB1 effect in 2D and 3D remains to be explored in the future.

Conclusion: We conclude that our findings support the use of CB1 as an anti-cancer agent with an additive cytotoxic effect to the standard of care chemotherapy for TNBC patients in the clinic. Furthermore, we propose that the combination of CB1 with olaparib potentially expands the spectrum of patients that would not normally be eligible for PARP inhibitor treatment.

Keywords: *Triple-negative breast cancer, chemotherapy, combination therapy, CB1, 3D model*

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Dental Pulp Stem Cell-Derived Exosomes for the Treatment of Central Nervous System Trauma

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Background and Aims: Degenerative eye diseases such as glaucoma is a major cause of permanent vision loss globally. It generates unusually high intraocular pressure by build-up of aqueous humour, which can damage the RGC axons at the optic nerve head, eventually destroying retinal neurons which are critical in allowing organisms to perceive light, and thereby, their surroundings. Treatments are limited, making cell based neuroprotective/neuroregenerative therapies a huge research interest. Mesenchymal stem cells have been utilised extensively for the treatment of neuro-diseases, and dental pulp stem cells (DPSCs) have sparked interest due to their neural crest origin. The therapeutic benefits of stem cells were hypothesised to be paracrine mediated as opposed to differentiation, and current literature suggests that the underlying prevailing mechanism mediating this effect are exosomes derived from stem cells. The aim of this research project was to investigate the therapeutic application of DPSC derived exosomes and to determine if it is a more applicable cell type in comparison to other stem cell derived exosomes as a treatment for traumatic and degenerative eye diseases.

Methods: To determine the feasibility of DPSC-exosome as a treatment for retinal ganglion cell (RGC) loss, exosomes from DPSC was cultured with RGCs in an in vitro rodent model of optic nerve crush (ONC). Total surviving RGC count, and regenerated axons were quantified in 8 chamber wells and compared to RGCs cultured with ciliary-derived neurotrophic factor (CNTF; positive control), and fibroblast exosome cultured alone (negative control).

Results: DPSC-exosomes promoted significant neuroprotection and neuritogenesis of RGC from ONC induced death, quantified by increased RGC survival and axon length. The mechanisms of action for this paracrine-mediated therapeutic benefit are hypothesised to be miRNAs contained within exosomes as supported by presented literature.

Conclusion: In conclusion, DPSC-exosome is neuroprotective within our in vitro model of retinal nerve injury, and its effects are most possibly acting through paracrine-mediated mechanisms involving miRNAs, thus making them a potentially useful treatment for preventing degenerative eye diseases while avoiding the disadvantages of traditional cellular transplantation.

Keywords: *CNTF, ciliary-derived neurotrophic factor*; *DPSC, Dental pulp stem cell*; *ONC, optic nerve crush*; *RGC, retinal ganglion cell*

References:

[1] Quigley, H. A. and Broman, A. T. 2006. British journal of ophthalmology 90(3), pp. 262-267.
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Who are we?

Cardiff Institute for Tissue Engineering and Repair was established in 2003 as a network of scientists principally within Cardiff University. CITER interests and expertise include basic, translational and clinical research in stem cell science, tissue engineering and repair, and disease translation. Within these broader remits, the research interest of individual colleagues is diverse. CITER embraces translational research that is conducive to commercial exploitation and encourages partnership with the industry. One of the core strengths of CITER is its network expertise enabling to address complex problems, accessing skill from different disciplines.

To support the network, CITER is organising several workshops, seminars and conferences throughout the year, encouraging and fostering new research collaborations, and promoting CITER expertise to external researchers and stakeholders. Industrial partners are encouraged to attend and showcase collaborative works and solutions tailored for researchers. CITER is particularly supportive of early career scientists with financial packages and organisational experience.

CITER recognises the importance of communication with the public, and is positively supporting public events, and engaging with primary and secondary school children with a number of different activities. The use of academic knowledge, technology, skills and innovation by industrial partners has been highly successful for improving competitiveness and productivity in Wales and in the UK. CITER is fully supportive of such partnership and aims in promoting academic-industry networking though its activities. Below are examples of projects among many carried out by CITER members.

Free Membership

- There are currently, 340 members across two colleges and ten academic schools within Cardiff University.
- Membership is open to academics, post-doctorate research associates, PhD, MSc and undergraduate students.

Member benefits

- Access to an exciting programme of CITER workshops & conferences
- Enhanced networking opportunities, including the Early Career Scientist Network
- Research and travel bursaries and awards for all career stages (£27,800 annually)
- Opportunity to develop career enhancing public engagement skills, through the delivery of activities at primary and secondary schools, public events and Cardiff University events.

Study

Tissue Engineering (MSc) Programme that is Led by Dr Wayne Ayre and run through the School of Dentistry, with seminars, lectures taught by colleagues across the CITER network.

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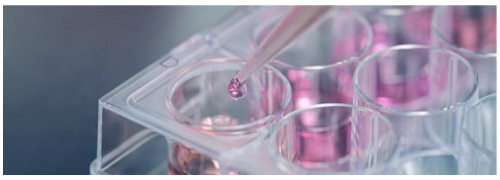
CITER Tissue Engineering (MSc)

Established in 2006, the Tissue Engineering MSc course was the first of its kind in the UK, building on Cardiff University's research excellence and critical mass of tissue engineering researchers across CITER. The course is a one-year, full-time MSc that aims to provide graduates with advanced knowledge, understanding and skills in the science and practice of tissue engineering and regenerative medicine: from theoretical science, through to research and clinical translation. It is split into a taught (Stage 1) and a research component (Stage 2), with teaching delivered by academics across the interdisciplinary CITER network. It also benefits from visits to clinics and industry to see how tissue engineering products are being developed and used. Modules in Stage 1 include: Research Methods; Cellular and Molecular Biology; Stem Cells and Regenerative Medicine; and Tissue Engineering from Concept to Clinical Practice. Stage 2 consists of a 5-month, laboratory-based research project chosen by students from topics supplied by academic supervisors across CITER. Previous projects have been in research areas such as stem cell biology, cartilage, bone, skin, oral tissues, fibrosis, biomaterials and drug delivery. Stage 2 culminates in the submission of a dissertation and a poster presentation at the CITER annual conference. This year's MSc student abstracts can be found in the conference proceedings. A high percentage of our graduates progress onto career paths highly relevant to tissue engineering and regenerative medicine including: PhD programmes within Cardiff and other UK and overseas Universities; Graduate-Entry Medicine; Specialist Registrar Training; teaching positions; and positions in industry or clinical laboratories. More information on the course and how to apply can be found on the course website:



https://www.cardiff.ac.uk/study/postgraduate/taught/courses/course/tissue-engineering-and-regenerative-medicine-msc.

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CITER Engagement

A key part of the CITER remit is public engagement and we are regularly engaged in delivering our portfolio of activities in an increasing number of outreach events each year. We support a vibrant culture of science communication and public engagement, and have developed our programme in line with The Way Forward, the University's strategy 2018-2023. Our outreach events include Cardiff University events, public events, primary school workshops as well as secondary school visits across Cardiff and the wider convergence area. More recently we have had to change the way we interact with the public and have been delivering our engagement activities virtually. We are always thinking of new ways to reach the wider community and welcome fresh ideas from our members.

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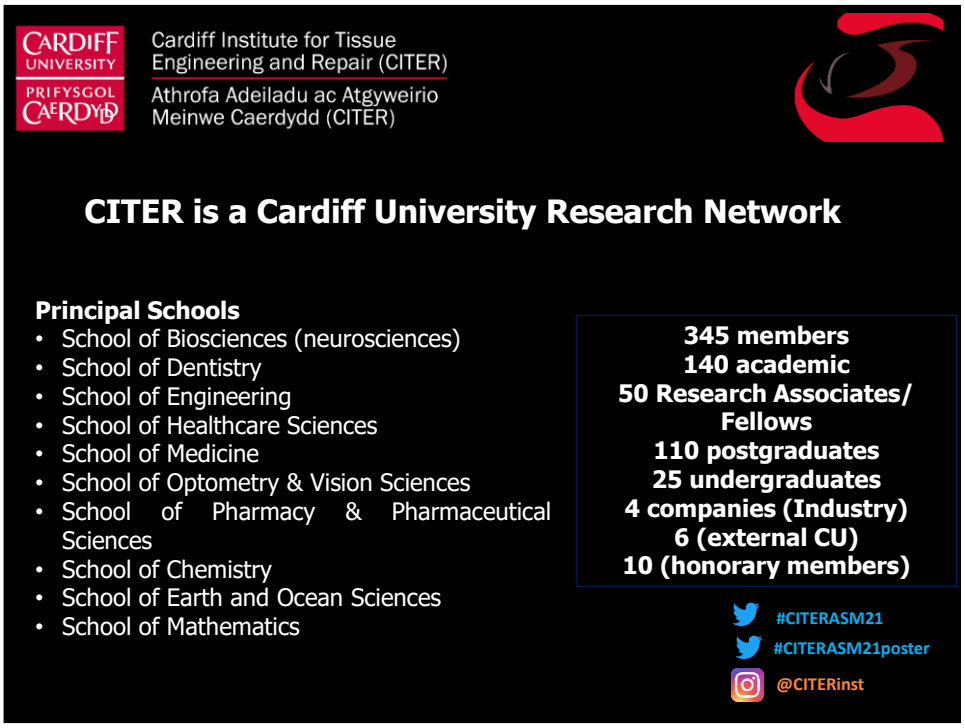

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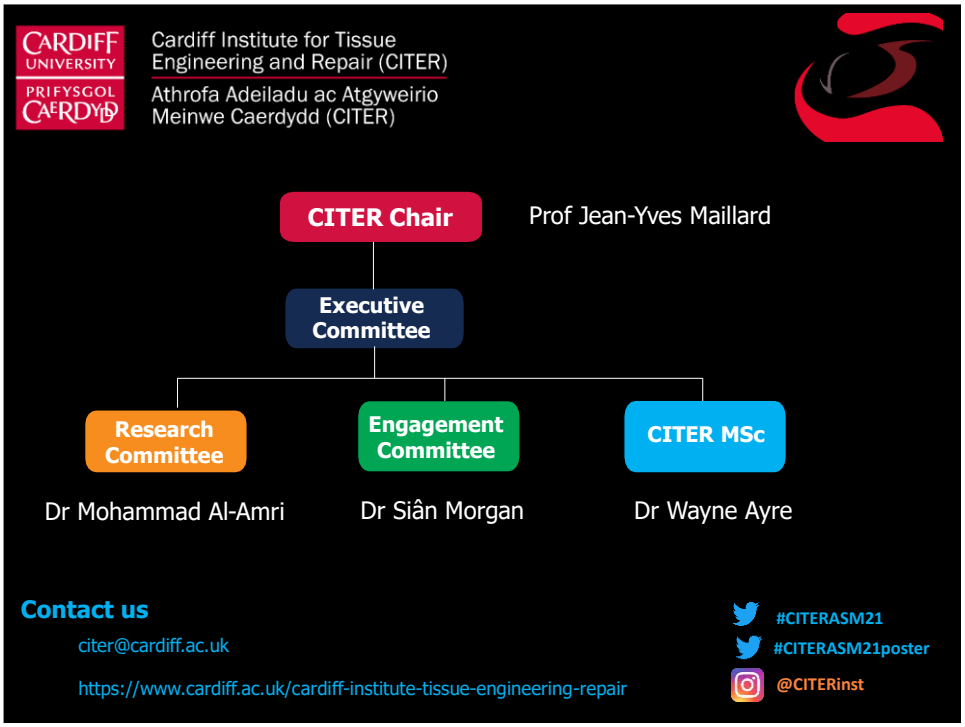

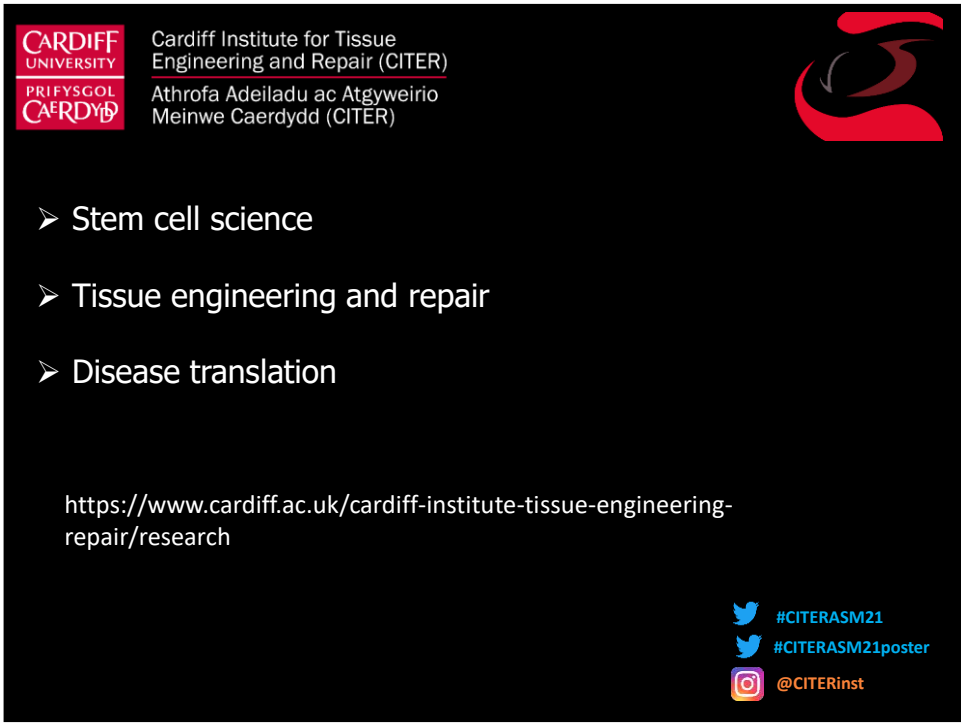

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➢ Stem cell science

- Manipulation of stem cell process for regenerative medicine
- Ethical considerations and regulatory aspects
- Stem cells and tissue engineering

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➢ Tissue engineering and repair

- Brain disease/repair
- Kidney repair
- Vision loss, bio-simulation and tissue repair
- Tissue repair and wound healing

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Cardiff Institute for Tissue Engineering and Repair (CITER)
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➢ Disease translation

- Musculoskeletal injuries
- Degenerative disease management
- Bone disease
- Remote detection systems
- Therapeutic design
- Microbial control and prevention (including biofims)
- Rehabilitation
- Photobiology

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