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

Proceedings

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Introduction

A warm welcome to the 2020 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. Most of the sessions in this year’s virtual ASM are also open to the public. We would like to thank our four keynote speakers: Professor Yamni Nigam, Professor Philip Rowe, Dr Zameel Cader and Professor Xin Lu, 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 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 2020, and Sponsors.

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. New theme, Applied Healthcare Technology, has been included for the first time to represent the diversity of the CITER.

The programme includes oral presentations from our members and bursary awardees 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 22 high-quality poster abstracts that are included in this proceeding (ISSN 2634-100X) and will be presented as fire-talks in the poster session.

We thank you in advance for completing the feedback form which should take just a few moments of your time. 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 2020 CITER ASM; we are sure you will find it stimulating!

Mohammad Al-Amri & Renata Jurkowska
CITER ASM 2020 Organisation

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   Dr Beky Weiser, School of Biosciences, Cardiff University.
   Dr Malik Zaben, Neuroscience & Mental Health Research Institute, Cardiff University.
THEMES OF CITER ASM 2020

THEME ONE: Immunology, Infection and Inflammation

Chair: Dr Renata Jurkowska
Co-Chair: Nina Stöberl

KEYNOTE:

Professor Yamni Nigam, College of Human and Health Sciences, Swansea University

The Role of Maggots in Wound Disinfection and Healing

Maggot therapy is a revived clinical treatment for chronic wounds. Used primarily for wound debridement (removal of wound debris and dead, sloughy tissue), it has also been shown to play a role in the removal of bacterial infection from wounds and to accelerate the healing process. Here, we review the scientific and clinical evidence which supports the multi-faceted benefits of maggot therapy for chronic wounds.

GUEST SPEAKERS:

Dr Emma Tallantyre, School of Medicine, Cardiff University

Multiple Sclerosis: Current Landscape, Ongoing Challenges and Unmet Needs

Multiple sclerosis (MS) MS affects more than 120,000 people in the UK, over 2 million people worldwide and is one of the most common cause of disability in young adults. The diagnosis can usually be made with a high degree of accuracy, and immune-modulating disease-modifying therapies (DMTs) are now available that hold promise for improving long-term outcomes. However, there remain several areas of unmet need in MS. Clinical outcomes are highly varied; some people with MS accrue severe irreversible disability within years of onset, while others remain minimally disabled many years after onset, even in the absence of DMTs. Tissue and imaging biomarkers capable of robustly predicting these varied outcomes would allow more personalised management. There is also a need for effective neuroprotective or remyelinating strategies in MS, as adjuncts or alternatives to immune-modulating DMTs. The study of candidate compounds in early clinical trials will require reliable surrogate imaging markers of repair. In this session we will discuss key unmet needs in the field of MS, with examples of local research addressing these areas.

Dr Malik Zaben, Neuroscience & Mental Health Research Institute, Cardiff University

Targeting HMGB1 as a Master Switch of Neuroinflammation after Traumatic Brain Injury: A Novel Approach to Enhance Brain Repair

Traumatic brain injury (TBI) is a global public health problem; a significant number of survivors left with life changing neurological disabilities. Post TBI neuronal loss is associated with surprisingly high levels of neural stem cell (NSC) proliferation. Newly-born neurons however largely die before developing into mature, functioning neuron; an observation which may explain the lack of meaningful functional recovery. I will present key recent findings on High Mobility Group Box protein-1 (HMGB1) roles as a key driver of post-TBI neuroinflammation and share some of our novel findings on HMGB1 detrimental effects on the ability of the brain to repair itself. Immunohistochemistry, PCR and western blot analysis were utilised to examine HMGB1 activation and subsequent induction of
neuroinflammation using postnatal cortical mixed neuro-glial cell cultures subjected to needle scratch injury. We also performed genome-wide RNA-seq studies in cortical perilesional tissue samples at 24 hours post penetration needle injury in adult C57Bl6 mice to investigate level of expression of HMGB1 signalling related genes and networks. We then harvested adherent NSC cultures generated from post-natal rat cortex for 7 days in vitro (DIV) to quantify the effects of injury condition medium on neurogenesis and oligodendrogenesis in the presence or absence of HMGB1 antagonists.

We, herein, demonstrate that sterile needle cortical injury activates the canonical pathways of the pro-inflammatory cytokine HMGB1 at the level of the transcriptome in vivo, and enhances its sustained activation in microglia and neuronal cells in vitro. We further show that mixed-neuro-glia injury conditioned medium has a detrimental effect on cortical neurogenesis and oligodendrogenesis. These effects are pharmacologically reversible by the HMGB1 antagonist glycyrrhizin and BoxA, respectively. Our findings, for the first time, implicate HMGB1-RAGE-TLR2/4 axis in inhibiting post-injury cortical and white matter regeneration, and highlight HMGB1 antagonists as a potential drugs to enhance brain repair after TBI. For the first time, we have evaluated the effects of HMGB1 on cortical neurogenesis following mechanical injury. We demonstrate that canonical pathways of HMGB1 are activated following cortical injury in vivo; injury enhances microglial activation and neuronal loss HMGB1 exerts a detrimental but pharmacologically reversible effect on neurogenesis and oligodendrogenesis.

**THEME TWO: Applied Healthcare Technology**

Chair: Dr Mohammad Al-Amri
Co-Chair: Alhanouf Almutairi

**KEYNOTE:**

**Professor Philip Rowe**, Biomedical Engineering Department, University of Strathclyde, Glasgow

**Future biomechanics technology for rehabilitation and orthopaedic surgery**

Biomechanics technology used in clinical practice has changed little since its inception in the 1980s. This is in stark contrast to other movement science and motion capture areas such as sports biomechanics, robotic orthopaedic surgery, virtual reality, animation and gaming where the technology has developed remarkably in the last 20 years. Techniques for motion capture and real time visualisation of human movement are now available which could revolutionise the way in which rehabilitation is undertaken and also its outcome for the user. Over the last 10 years our research group has been exploring the potential of these techniques to contribute to clinical biomechanics and in particular to real time biomechanical feedback of movement performance during rehabilitation sessions. We have explored cluster based marker approaches, automatic labelling of markers, real time reconstruction of movement and visual feedback through augmented avatars and computer graphics.

We have found in a series of studies including stroke, CP, arthroplasty and amputees that these methods can be used and are valued by users and clinicians alike. Further there is growing evidence that they are clinically more effective than traditional rehabilitation approaches. The visual feedback enables users to correct their movement patterns and to improve function while the visualisation and gaming aspects increase cognitive engagement, motivation and adherence. However current technologies are not sufficiently user-friendly, robust or inexpensive to allow their wide deployment in society. We have concluded that if such technology is to reach the user and to be deployed in the community for user
self-management of rehabilitation these exciting developments need a further round of re-design and we intend to undertake this, initially focusing on stroke users, in our newly established centre for the co-creation of rehabilitation technology.

GUEST SPEAKERS:

Professor Monica Busse, School of Medicine, & Dr Philippa Morgan-Jones, School of Engineering, Cardiff University

DOMINO-HD: Exploring Multi-Domain Lifestyle Targets for Improving ProgNOsis in Huntington’s Disease

Technological advancements in wearable devices are beginning to change the face of clinical trials, allowing an unprecedented amount of clinically important information to be collected remotely and over long periods of time using digital devices. This type of digital health data drastically differs from standard clinical trial data in terms of format, size and data capture method, resulting in significant challenges when embedding into standard clinical trial processes and data management frameworks. As part of DOMINO-HD, we aimed to develop a digital data framework capable of capturing objective physical activity and sleep data in people with Huntington’s disease over a 12-month period across multiple European clinical sites. In close collaboration with members of the DOMINO-HD Public and Patient Involvement group, we selected an appropriate device capable of capturing longitudinal free-living physical activity and sleep data in a way most accepted by the cohort of interest. A GDPR compliant digital framework was then developed that addressed the wide-ranging aspects of managing the collection, transfer and storage of digital health data within a clinical trial setting using a consumer grade, wearable activity tracker.

A digital data framework was established for capturing digital health data from a Fitbit Charge activity tracker. Key processes detailed involve how to sign up devices to the research study, gaining access to the consumer data cloud, identifying a data flow from device to central data storage at the co-ordinating study site, monitoring data quality / drop out and scheduling prompts to participants to promote device compliance. In this presentation we will discuss the complexities and challenges faced when integrating consumer grade digital health technologies into a clinical trial setting and has developed a digital framework for the customised collection and management of Fitbit data. As part of DOMINO-HD, this framework will be implemented in i) a 12-month longitudinal clinical study seeking to establish the feasibility of linking lifestyle factors with genetic risk factors to explore their interplay with HD symptom severity and ii) a validation study evaluating how accurately a Fitbit tracker can measure physical activity and sleep in people with Huntington’s disease.

Professor Derek Jones, Brain Research Imaging Centre (CUBRIC), School of Psychology, Cardiff University

Adventures with ultra-strong gradients in MRI: Towards in vivo histological quantification

In this talk, I will describe the work that CUBRIC’s microstructural imaging team (‘MicroTeam’) has been doing with the Siemens Connectom scanner. This Connectom is one of only four such MRI scanners in the world and features unusually strong magnetic field gradients (300 mT/m compared to the closest commercially-available strength of 80 mT/m). This forms the centrepiece of the EPSRC/Wolfson-funded ‘National Microstructural Imaging Facility’. By applying magnetic field gradients, we can sensitize the MRI signal to the diffusional displacement of water both inside and
outside of cells and, from this, infer size, shape and orientation of compartments within each imaging voxel. Critically, the scale that we are sensitive to with diffusion MRI (on the scale of microns) is about 1000 times smaller than the achievable image resolution.

The unprecedented gradient strength means that we can start to examine time-dependence of diffusion-displacements within tissue, and go to excessively large diffusion-weightings while retaining sufficient signal-to-noise ratio (SNR) to make these measurements robust. We will review applications both in neural white matter and grey matter. In the former, we are able to infer multiple orientations within a voxel (3D image pixel) corresponding to multiple axonal bundles, the density and, for the first time, the diameter of axons. By extending to N-dimensional correlation experiments, we are able to extract orientationally-specific measures of other biophysical properties such as relaxivity, with the promise of extracting myelination indices.

We have recently extended our research into the grey matter, both in the cerebellum and cortex, and exploring the unique signal from the soma, estimating volume fraction/density and even size. Critically, most of these measurements are directly translatable from the high-performance human Connectom MRI scanner to the Experimental MRI Centre (EMRIC) 9.4T animal system, with comparable gradient amplitude. In turn, this opens up collaborative opportunities with members of CITER to perform multi-modal validation of in vivo measurements, and to assess tissue properties longitudinally in vivo. The speaker would be super-keen to explore such collaborations with interested members of CITER. Finally, if time permits, we will discuss extensions of our methodologies to non-neural tissue “below the neck” including the prostate and heart.

**THEME THREE: Mind, Brain and Neurosciences**

Chair: Dr Yasir Syed  
Co-Chair: Gareth Chapman

**KEYNOTE:**  

**Dr Zameel Cader,** University of Oxford  

**Enabling stratified neurotherapeutics using stem cell disease models**

Drug discovery in neurological disorders remains challenging and many patient worldwide remain without meaningful therapies. We have developed an integrated cross-validating method to arrive at new drug discovery starting points and also opens the path to precision medicine. Hence through the use of real-world data from electronic health records we can identify drugs that might improve headache outcomes. Our analysis using Cox logistic regression, confirms the real-world effectiveness of known migraine preventative drugs such as topiramate and propranolol. Furthermore, it reveals drugs not presently used in migraine but having superior migraine treatment efficacy. Whilst valuable, this approach can be subject to confounds, so we provide mechanistic validation of the efficacy of these drugs using human cellular models of migraine. The latter include nociceptive neurons that secrete CGRP and respond to know migraine provocants such as prostaglandins and PACAP. We can then use these cellular models to understand the targets and that these drugs are exerting their effects. Through discovery of the molecular mechanism of action, novel targets for neurological conditions are revealed.
GUEST SPEAKERS:

Professor Lawrence Wilkinson, Neuroscience & Mental Health Research Institute, School of Medicine, Cardiff University

Mechanistic insights into genetic risk for psychiatric disorder

Recent years have seen tremendous strides in our understanding of the genetic risk for conditions such as schizophrenia, bipolar disorder and intellectual disability, with the MRC Centre for Neuropsychiatric Genetics and Genomics in Cardiff leading the world in this area. The emerging genetic findings have given us new insights into these hitherto biologically mysterious conditions and the challenge now is to use the leads provided by molecular genetics to drill down to the specific brain changes underlying maladaptive cognition, feelings and behaviour. In my talk I will focus on the work we have been doing in Cardiff at the Neuroscience and Mental Health Research Institute linking genetic risk for psychiatric disorder with altered glial cell function leading to abnormalities in myelination of axons and neuronal survival/death and migration.

Dr David Petrik, School of Biosciences, Cardiff University

Neural stem cells and adult neurogenesis in various neurogenic niches of the brain

During prenatal development, neurogenesis is responsible for the brain formation, but in the adult brain it becomes restricted to very discrete areas, the neurogenic niches, which host the adult neural stem cells (aNSCs). There are three adult neurogenic niches in the mammalian brain. The subgranular zone (SGZ) of the hippocampus generates newborn neurons critical for spatial memory and mood control and the subventricular zone (SVZ) in the walls of lateral ventricles gives rise to new olfactory interneurons. We have shown how different genetic, epigenetic, and pharmacological regulators influence adult neurogenesis in the SGZ and hippocampus-dependent learning. Also, we have demonstrated how mechanical forces influence the proliferation of aNSCs in the SVZ. Our latest work has focused on adult neurogenesis in the hypothalamus, where aNSCs generate newborn neurons involved in regulating appetite. We will present new data on stem cell heterogeneity in the hypothalamic neurogenic niche and on targeting the hypothalamic newborn neurons by novel anorexigenic peptides to limit weight gain in a model of diet-induced obesity.

THEME FOUR: Cancer

Chair: Prof Jean-Yves Maillard
Co-Chair: Dr Mike Pascoe

KEYNOTE:

Professor Xin Lu, Ludwig Institute for Cancer Research, University of Oxford

Tumour heterogeneity, Cellular plasticity and upper GI cancers

Cell plasticity – the ability of cells to change their characteristics and fate – is a key feature of development, regeneration and cancer. Intriguingly, many of the pathways that are dysregulated in cancer are also fundamental in stem cell biology. This is elegantly illustrated by the fact that the well-known tumour suppressor protein p53 puts a brake on somatic cell reprogramming, whereas oncogenes such as MYC accelerate the formation of induced pluripotent stem cells. Our research investigates the molecular switches that control cell plasticity, particularly in the initiation, progression and treatment...
of cancer. Our group has a long-standing interest in regulators of p53, including the ASPP family of proteins, which have essential roles in conferring transcriptional target selectivity. Upper gastrointestinal tract cancers and pre-malignant conditions provide an ideal human model system for studying cellular plasticity. For example, in a common pre-malignant condition called Barrett’s oesophagus, squamous epithelial cells lining the oesophagus are replaced by columnar epithelium in response to acid reflux, conferring increased risk of oesophageal adenocarcinoma. We recently used single cell RNA-seq to shed light on the potential cellular origins of Barrett’s, and discovered potential links to pathways involved in early development. We consider how cell plasticity is controlled at several levels, providing a basis for understanding cancer initiation and progression.

**GUEST SPEAKERS:**

**Professor Matt Smalley,** European Cancer Stem Cell Research Institute, School of Biosciences, Cardiff University.

**All Model Animals Great and Small: Working with Vets to Better Understand Cancer in Humans and Companion Animals**

Mice have proven incredibly important in developing our understanding of the biology of cancer, particularly the genetics of cancer formation, and also typically as the first animal in which an experimental therapy is tested. However, mice have many limitations as model systems, including their short lifespan, the rarity with which they naturally get cancer and often the lack of similarity between the tumours they do get (in genetic knock-out models) and human cancer. In contrast, companion animals – in particular dogs and cats – naturally get cancer and these cancers show many similarities to human disease, including benign and malignant forms, histological subtypes that are recognisably similar to human subtypes, hormone dependency (in mammary disease) and an approach to clinical management analogous to that seen in humans (diagnosis with imaging and histology, surgical intervention, systemic therapy, radiotherapy, long-term follow-up). Furthermore, at least in dogs, different breeds have different predispositions to different cancer types – meaning that the genetics of dog breeds can be used to understand the genetics of cancer predisposition. Collaborating with veterinary oncologists offers outstanding approaches for establishing common rules of cancer across species – ‘comparative oncology’ – which can lead to improved clinical outcomes for both humans and their companion animals.

**Professor Oliver Ottmann,** School of Medicine, Cardiff University.

**Advanced Therapies in Haematology and Oncology**

In recent years, advanced therapy medicinal products (ATMPs) have come to the forefront of cancer therapy and now offer groundbreaking new opportunities for the treatment of numerous types of malignancies. By definition, ATMPs are medicines that are based on genes, tissues or cells and can be classified into three main types, i.e. a) somatic-cell therapy, b) gene therapy medicines or c) tissue-engineered medicines. In the realm of cancer, cell and - as yet to a lesser extent - gene therapies are starting to make an impact on cancer treatment.

The paradigm of targeted cellular therapy was introduced by CD19-targeted chimeric antigen receptor (CAR) T cell therapy, which has demonstrated remarkable anti-tumour efficacy in paediatric and young adult relapsed/refractory B cell acute lymphoblastic leukaemia (B-ALL) and in patients with B-cell lymphomas. Many new CAR constructs are currently under development and/or under clinical
investigation that differ not only in details of their design but also the molecules that they target. While these CARs are autologous products, i.e. they use the patient’s own T cells, development of allogeneic “off the shelf” CARs is an exciting area of research. Notably, these T cell-based immunotherapies are associated with a unique range of toxicities of which cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) are the most common. Optimal management of these at times severe toxicities will be required before these therapies can be rolled out in large scale outside of specialized centres. Current developments will be discussed, together with gene therapy and oncolytic virotherapy approaches that are another area of great promise for cancer therapy.
Invited Presentations: Recipient of the CITER Awards

Chair: Dr Malik Zaben
Co-Chair: Ronak Ved

CITER Young Investigator Award 2020

Dr Rob Knight, School of Medicine, Cardiff University

Oral Progenitor Extracellular Vesicles – New options for wound healing

Rob Knight1,2,6, Emma Board-Davies1,2, Aled Clayton1,3, Terence Davis1, Ben Karatas2,4, James Burston1,4,5, Zsuzsanna Tabi1, Juan M. Falcon-Perez4,5, Stephen Paisey2,6, Phil Stephens1,2

1 Regenerative Biology Group, School of Dentistry, Cardiff University; 2 CITER, Cardiff University; 3 Division of Cancer and Genetics, School of Medicine, Cardiff University; 4 Division of Infection and Immunity, School of Medicine, Cardiff University; 5 Systems Immunity Research Institute, School of Medicine, Cardiff University; 6 PETIC, School of Medicine, Cardiff University; 

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Background and Aims: Scar tissue formation during wound repair can be devastating for affected individuals and can lead to reduced tissue function or whole organ failure. However, wound healing within the buccal mucosa, occurs in a scarless manner, which is related to the intrinsic properties of the resident mesenchymal (fibroblast/progenitor cell) populations. Our group has previously isolated and characterised a novel, patent protected progenitor cell population from the buccal mucosa. These Oral Mucosa Lamina Propria Progenitor Cells (OMLP-PCs) are multipotent, highly immunosuppressive and anti-bacterial. Small extracellular vesicles (sEVs) are largely of endosomal origin and range in size between 30-130nm. They have recently been demonstrated to not only play a role in cell to cell communication but to also play an important role in stem cell mediated repair and regeneration. The aims of the work were to determine if sEVs isolated from an immortalised OMLP-PC line stimulated a wound healing response.

Methods: Three OMLP-PC cell strains were hTERT immortalised to produce three OMLP-PC cell lines (OMLP-PCL). The line which demonstrated the greatest level of plasticity was used for sEV isolation from the OMLP-PCL conditioned medium. sEVs where characterised based upon the Journal of Extracellular Vesicles minimal essential criteria to characterise extracellular vesicles. The effects of OMLP-PCL sEVs were assessed in a number of in vitro wound healing assays (proliferation, migration, myofibroblast formation) as well as an in vivo murine model.

Results: An immortalised OMLP-PC line demonstrated a normal fibroblast like morphology, expressed the expected progenitor cell surface markers and retained its functional capabilities. sEVs isolated from this immortal OMLP-PC were confirmed by Nanoparticle Tracking Analysis, Cryo-EM, floatation density and Flow Cytometry. sEVs significantly increased both skin fibroblast proliferation and wound repopulation/migration in vitro. sEVs also demonstrated to significantly inhibit myofibroblast formation in vitro and in vivo demonstrating potential as an anti-scarring therapeutic.

Conclusion: OMLP-PC derived sEVs demonstrate significant potential for development as anti-scarring agents.

Keywords: Stem cells, oral mucosa, immortalisation, small extracellular vesicles, regenerative medicine, scarless wound healing
Dr Rachael Moses, School of Dentistry, Cardiff University

Epoxy-Tiglanes Alter Chronic Wound Fibroblast Responses

Rachael L. Moses*, Glen M. Boyle†, Jenny P. Johns†, Victoria Gordon†, Paul W. Reddell†, Robert Steadman†, Ryan Moseley*.

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Background and Aims: Non-healing chronic wounds represent significant causes of patient morbidity and financial burden to Healthcare Services, with incidence estimated to rise with ever-increasing ageing demographics worldwide. Whilst many treatment options are available, chronic wound prevalence is confounded by acceptance that existing therapeutic approaches offer limited benefit to healing outcomes. Consequently, there is a significant clinical need for novel therapies capable of restoring normal healing in chronic wound patients. Our previous studies have shown epoxy-tiglianes, developed by our industrial partner, QBiotics Ltd., stimulate epidermal keratinocyte proliferation and wound repopulation, corroborating in vivo veterinary case studies, leading to a potential pharmaceutical therapy for impaired re-epithelialisation [1,2].

Methods: This project aims to evaluate the wound healing efficacy of these novel epoxy-tiglianes on chronic wound fibroblasts (CFs). We screened a selection of epoxy-tiglianes through conducting numerous wound healing assays on CFs to determine the optimal analogue for impaired healing scenarios. Expression profiling was performed to determine the effects of these novel epoxy-tiglianes on gene expression. 3D spheroid cultures were established to observe the epoxy-tigliane induced effects on CFs in a more complex 3D structure.

Results: Our studies have demonstrated delayed senescence of epoxy-tigliane-treated-CFs compared to untreated controls. Epoxy-tiglianes also inhibited TGF-β1-driven fibroblast-myofibroblast differentiation, along with a decreased α-SMA expression. Expression profiling studies demonstrated a number of key differential gene changes, including an increased expression of a number of MMPs, in particular MMP-1, -3, -11. This increased MMP activity was observed through increased collagen degradation in the epoxy-tigliane-treated spheroid cultures.

Conclusion: This project obtained novel proof-of-concept data of epoxy-tigliane efficacy against chronic wound fibroblasts, corroborating the beneficial responses observed in the in vivo veterinary chronic wound case studies. This supports their development as chronic wound therapies, through promoting repair in these clinically challenging wounds.

Keywords: Chronic wounds, Epoxy-tiglianes, Spheroids, Expression profiling

References:


Evidence-Based Model of Using Immersive Virtual Reality for Managing People with Chronic Low Back Pain

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Background and Aims: Low back pain is one of the leading cause of disability worldwide and 23% develop chronic symptoms CLBP [1]. Current intervention involved psychological and exercise programs but these are associated with lack of adherence due to low motivation [2,3,4]. Immersive Virtual Reality (IVR) is a computing technology generate 3D environment via headset and users interacting through devices and sensory display system [5]. Despite IVR was suggested to reduce acute pain, understanding its mechanism to work with chronic pain is still unknown [6]. Therefore, the aim of the study is to map the present ‘state of play’ and provide an evidence based model for future development of IVR supporting the management of CLBP.

Methods: In accordance with the Medical Research Council Framework [7], scoping review of using IVR in management of adults with chronic pain was conducted at the first stage to summarise key components in the current evidence. At the second stage, mixed-method sequential study design was utilised in two parts: Part 1 was an online survey for international VR special interest group with experience in using IVR in healthcare including practitioners, researchers and IVR technology developers. Part 2 involved online interviews of subset the above individuals to explore their in-depth experiences, views and attitudes towards using IVR for chronic pain management including content, dosage and practicalities (in progress).

Results: In scoping review, 26 studies were identified in using IVR in management of disorders with chronic pain. Thirty-one international responses were received in the online survey from practitioners, researchers and IVR developers. High quality headsets were highly preferred and used primarily in hospitals (48%). Top ranked facilitators to IVR use were user’s motivation and bespoke games, whereas highest-rated barriers included limited game’s availability, lack of practitioners acceptance and lack of funding. Customised games (>50%) were used more highly than “Off the shelf” ones. Most (58%) respondents used IVR for pain management, commonly employing relaxing content (72%) and cognitive/active content (33%) for 15-20 minute and targeting pain (88%), emotional state (77%) and function (50%) outcomes.

Conclusion: Understanding the current use of IVR in healthcare, specifically in pain management is essential and the results of the online survey will inform the online interview (part 2). Mapping the existing evidence in the scoping review combined with the results from the online survey and online interview will assist building evidence-based model for future development of IVR for managing people with CLBP.

Keywords (6 max): Virtual reality, Chronic Pain, Immersion, Survey

References
“One Size Fits All” 3D Organotypic Cell Culture Possible?

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Background and Aims: Dysfunction of the well-orchestrated process of wound healing can lead to chronic wounds, through prolonging the inflammatory phase. Chronic wounds have both patient and economical burdens. Globally, chronic wounds cost $2.8 billion, the wound product market is expected to gross US$15 billion by 2022 [1]. The aim of this project was to evaluate the current in vitro, in vivo and ex vivo models for chronic wounds, and analyse which model best represents the in vivo mimic for human skin. Through this analysis the work then evaluated whether a 3D model would be a better in vivo mimic than current models available. Finally, a conclusion was drawn on whether it would be possible to manufacture a “one size fits all” 3D chronic wound model, which could be used within industry and research to benefit clinical outcomes.

Methods: A literature review was carried out using articles sourced from both PubMed and Google Scholar. Initial searches were carried out using the general search term “chronic wounds”, this was to gain basic understanding around the topic. Generalised searches then changed to “chronic wound burdens”, gaining understanding and why this is an area of interest. After this searches terms changed to ‘Biofilms in chronic wounds”, “Chronic wound biofilms” gaining insight to the link between biofilms and chronic wounds, as well as identifying with microorganisms appear more frequently. The rest of the searches were formulated around 3D chronic wounds and different models currently used: “chronic wounds” + “in vivo”, “in vitro”, “ex vivo” and “2D models”. Lastly to look at the future direction the search term “novel chronic wound models” was used to identify any new research.

Results: Review of the wound model literature shows that over the last 16 years there has been a shift away from in vivo animal models to more cellular in vitro models that pose better mimics for native human skin. Most commonly used in vivo animal models currently are mice and pigs. The literature also shows that Human Skin Equivalents have been one of the most successful 3D models as they provide clinical use as skin substitutes, as well as being used as models within research. From reading, ex vivo models look to be a more regularly used method, as they are more complex than 2D models, as well as being more biologically similar to human than animals.

Conclusion: As chronic wounds are a diverse condition, creation of a “one size fits all’ model is not possible. However, it may be possible to create a quality controlled 3D chronic wound model, that can then be altered to represent specific research and conditions being investigated. A “basic” model that could be developed would use a scaffold system, as this provides a better chance at replicating the model by giving direction to the cellular material. Possible scaffolds could be collagen based hydrogel in a fixed model under mechanical stress. Common adaptions would be changed for wound complexity, severity and infections. An important adaption would be creating more chronic wound models with biofilm infections.

Keywords: Chronic wounds, Biofilms, in vivo, in vitro, ex vivo, in silico, 2D models

Reference:
Investigation of the Human Glaucomatous Lamina Cribrosa in Vivo.

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**Background and Aims:** Glaucoma remains a leading cause of irreversible blindness worldwide. The disease is characterised by the death of retinal ganglion cells (RGCs) and loss of RGC axons. RGC axons leave the eye via the optic nerve head (ONH) and pass through a multi-layered connective tissue sieve-like structure: the lamina cribrosa (LC), which is proposed as the site of axonal injury in glaucoma. Connective tissue changes within the LC has been shown to occur in primary open angle glaucoma (POAG); this in vivo study aimed to identify if LC microstructural changes could be detected in early disease prior to permanent vision loss.

**Methods:** Enhanced depth imaging by 1040nm spectral domain optical coherence tomography was performed on the ONHs of 30 controls (age: 65.6 ± 6.4 years) and 64 glaucoma participants (age: 72.55 ± 8.41 years). Regional measures of anterior and posterior LC surface depths and LC thickness, and additionally total LC volume, were quantified in 3D-OCT image datasets, using ImageJ (v1.52a) and Amira software (v6.0), respectively. LC connective tissue coherence (regionally and depth-wise) was analysed using ImageJ OrientationJ. Statistical analyses were performed to assess change in ONH parameters as a function of glaucoma disease stage and visual field sensitivity (VF Mean Deviation [VFMD]).

**Results:** Lamina cribrosa were thinner in preperimetric glaucoma (PG) compared to controls in the inferior, superior-nasal, and inferior-nasal ONH regions (P<0.05), whilst central LC was thinner in moderate-advanced glaucoma (MAG), compared to PG (P=0.013). LC volume was lower in early glaucoma (EG; P=0.015) and MAG (P=0.002), compared to control eyes. LC surface depths, anterior and posterior, did not differ between glaucoma disease stages (P>0.05). Regional LC coherence varied depth-wise; PG LC coherence was higher in mid-to-posterior superior LC compared to controls (P<0.05), and EG (P=0.040) and MAG (P=0.035) LC coherence was higher in the anterior inferior-temporal LC compared to PG.

**Conclusion:** Significant differences in LC thickness, volume and coherence were observed as a function of glaucoma disease stage indicating significant LC structural alterations in early disease stages. Increased LC coherence was significantly associated with VFMD in regions known to undergo LC deformation in early glaucoma. These findings support LC microstructure as a biomarker for early detection of glaucoma onset.

**Keywords:** ganglion cells, lamina cribrosa, in vivo
Cutibacterium Acnes Modifies the Formation and Antibiotic Susceptability of Staphylococcus Aureus Biofilms

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Background and Aims: Cutibacterium acnes (formally Propionibacterium acnes) is co-isolated with other opportunistic pathogens within deep tissue infections. They are particularly problematic during upper torso surgical procedures; such as shoulder arthroplasty. Studies have demonstrated that C. acnes is able to form biofilms and when co-cultured with Staphylococcus sp. a number of studies have reported both inhibitory and stimulatory effects. We hypothesized that the presence of C. acnes and S. aureus together within biofilms may lead to modifications in behaviour by one or both bacteria. The aim of this research, in part supported by a CITER summer studentship, was to investigate these modifications.

Methods: S. aureus biofilm cultures were supplemented with sterile filtered supernatant from C. acnes planktonic cultures and the response of the S. aureus biofilms measured. Crystal violet staining was used to measure gross differences in biofilm biomass with fluorescent and scanning electron microscopy providing a more detailed view of how the presence of C. acnes supernatants altered the biofilm structure. Finally, microbroth dilution assays were carried out, in the presence or absence of C. acnes supernatant, to determine if its presence altered the susceptibility of S. aureus planktonic and biofilm cultures to antibiotics.

Results: All C. acnes isolates were able to form biofilms with no correlation observed between isolation site and biofilm biomass. All C. acnes supernatants reduced biofilm biomass of S. aureus NCTC 6571 biofilms, with the reduction linked to the ability of the C. acnes supernatants to suppress biofilm maturation, rather than attachment. The antibiotic susceptibility of S. aureus planktonic cultures was decreased in the presence of C. acnes supernatant, although biofilm susceptibility was increased.

Conclusion: This study suggests that complex interactions between C. acnes and other opportunistic pathogens are likely to exist during colonisation and infection events. Further investigation of these interactions may lead to increased treatment options and a better prognosis for patients.

Keywords: Cutibacterium acnes, Propionibacterium acnes, Staphylococcus aureus, biofilm, mixed species interactions, antibiotic resistance
Nanoparticle and Nanotopography-Induced Activation of the Wnt Signalling Pathway in Bone Regeneration: A Review of the Literature

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Background and Aims: Recent research has focused on the development of nanoparticle and nanotopography-based technologies for bone regeneration. The Wnt signalling pathway has been shown to play a vital role in this process, in particular in osteogenic differentiation and proliferation [1]. The exact mechanisms by which nanoparticles and nanotopographies activate this signalling pathway, however, are not well understood. This review aimed to elucidate the mechanism by which nanoparticles and nanotopographies activate the Wnt signalling pathway in bone regeneration.

Methods: The terms “Wnt”, “bone” and “nano*” were searched on PubMed and Ovid with no date limit. A total of 551 articles were identified from the searches, and after removing the duplicates and screening based on the inclusion criteria, a total of 30 articles were included in the review. The inclusion criteria were original research articles; articles related to Wnt signalling and bone regeneration; articles related to nanotopographies, nanoparticles or scaffolds with nano topographies or nanoparticles; and in vitro, in vivo and ex vivo studies. Review articles, non-English articles, conference abstracts and articles not relevant to bone were excluded.

Results: The main mechanism by which nanoparticles activated Wnt was through internalisation via the endocytic pathway or by diffusion through the cell membrane. The internalisation caused mechanical stimuli, increasing accumulation of non-phosphorylated β-catenin in the cytoplasm and subsequently downstream osteogenic signalling (e.g. upregulation of Runx2). Nanotopographies were shown to directly activate the frizzled receptors, activating the Wnt/B-catenin pathway. Additional studies showed nanotopographies to cause changes in the conformation of the primary cilia, inducing Wnt signalling via calcium channels. Finally, scaffolds containing nano topographies/nanoparticles were found to induce Wnt signalling via a combination of the release of ions, which inhibit GSK3β (e.g. lithium, boron, lanthanum and icariin), and via nano topographical mechanisms.

Conclusion: This review concludes that nanoparticles and nano topographies cause Wnt activation via several different mechanisms, specific to the size, shape and structure of the nanoparticles or nano topographies. Mechanical stimulation was found to be the main initiator of the Wnt cascade for the majority of studies, with few studies linking signalling to the release of ions. Knowledge of these mechanisms will help develop more effective targeted nano-scale technologies for bone regeneration.

Keywords: Wnt, nanoparticles, nano topographies, scaffolds, β-catenin, frizzled receptors.

References:
The role of HMGB1 in mediating neuronal injury and repair following traumatic brain injury

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Background and Aims: Traumatic brain injury (TBI) is a major cause of morbidity and mortality. Following the initial impact, an inflammatory process occurs within brain tissue. High Mobility Group Box 1 (HMGB1), is a protein recognised to propagate the inflammatory signalling [1]. Cellular stress triggers HMGB1 release and downstream interaction with receptors TLR2, TLR4 and RAGE [2]. Despite the increasing evidence of HMGB1’s role in TBI inflammation, robust studies using human tissue remains limited. In this study, we sought to investigate if human derived neural stem cells co-express HMGB1 receptors and whether HMGB1 affects their cell survival and differentiation.

Methods: Using a developed human cortical 3D cell culture system (Hispots®) generated from cerebral cortical tissue donated by patients undergoing therapeutic epilepsy surgery, we performed immunohistochemistry to test for the expression of SOX2 (a neuronal stem cell marker) and HMGB1 receptors (RAGE, TLR2, TLR4). Secondly, we generated Hispots® in the presence of HMGB1 (10ng/ml and 100ng/ml). These underwent immunohistochemistry testing for the expression of TUJ1 (a neuronal cell marker) and Caspase3+ (a cell death marker). Imaging undertaken with an upright timelapse microscope. Raw data counted and analysed with Prism software.

Results: On immunohistochemistry with primary antibodies for SOX2 and either TLR2, TLR4 or RAGE, we have demonstrated co-expression of these receptors in SOX2 positive cells. Secondly, we investigated the effect of HMGB1 on neuronal progenitor cell survival and differentiation. The effect of HMGB1 on both total cell death and neuronal cell death was variable. Hispots® treated with 10ng/ml and 100ng/ml of HMGB1 indicated an increase in neuronal cells 11.5% and 35.7% respectively (p-value 0.1387).

Conclusion: We have demonstrated the expression of HMGB1 receptors (TLR2, TLR4 and RAGE) in neuronal stem cells in adult cortical tissue. This suggests that HMGB1 plays a vital role in the response of stem cells to inflammatory conditions. Our preliminary data demonstrated a trend towards an increase in neurogenesis under HMGB1 treatment; however, this requires further validation.

Keywords: Trauma, Brain Injury, HMGB1, Neurogenesis.

References


Damaging effects of HMGB1 on Cortical Neurogenesis Post-Traumatic Brain Injury

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Background and Aims: Recent medical advances have improved mortality after severe traumatic brain injury (TBI) but neurological recovery remains poor. Despite post-injury cortical progenitor cell proliferation, new-born neurons fail to survive long term. Optimising the post-injury microenvironment for neurogenesis may improve neurological outcomes. Elevated levels of the pro-inflammatory cytokine High Mobility Group Box protein-1 (HMGB1) in TBI patients is associated with poor outcomes; likely via interaction with receptor for advanced glycation end-products (RAGE). We examined the hypothesis that HMGB1 released post-TBI is anti-neurogenic and whether this effect can be pharmacologically manipulated.

Methods: Immunohistochemistry, PCR, and Western Blot on postnatal rat cortical mixed neuro-glial cell cultures subjected to needle scratch injury were used to examine HMGB1 activation and neuroinflammation. HMGB1 signalling-related genes/ networks were examined using genome-wide RNA-seq studies in cortical perilesional tissue samples at 24h post penetration needle injury in adult C57Bl6 mice. Neural stem/progenitor cell cultures were generated from post-natal rat cortex for 7 days-in-vitro to quantify effects of injury condition medium (ICM) on neurogenesis with/without RAGE antagonist glycyrrhizin.

Results: Injury of mixed glial cultures upregulated TNF-α and NOS-2 mRNA expressions at 6h, increased proportions of activated microglia and caused significant neuronal loss (62.8 ± 11.6 vs 17.4 ± 4.0 cells/mm²) at 24h. Transcriptome analysis revealed activation of HMGB1 pathway genes in vivo at 24h post-cortical-needle-injury. Consistently, HMGB1 gene expression was upregulated at 2h post-injury in vitro. This resulted in enhanced HMGB1 protein expression, and HMGB1 nuclear-to-cytoplasmic translocation in neurons and microglia at 24h. Whilst ICM reduced numbers (160.6 vs. 93.3 Tuj1+ cells/mm²; p=0.013) and proportions (20.2% vs. 13.4 %; p=0.013) of neurons, these effects were reversed by 0.5μM glycyrrhizin.

Conclusion: HMGB1 is activated following in vivo post mechanical injury, and associated with microglial activation and neuronal loss. The HMGB1 antagonist glycyrrhizin alleviates the detrimental effects of ICM on cortical neurogenesis. From therapeutic standpoint, our findings highlight HMGB1 and its receptors as potential targets to enhance brain repair post-TBI.

Keywords: traumatic brain injury, neurogenesis, HMGB1, neuroinflammation
Transcriptomic and Epigenetic Profiling Identifies Novel Regulators of COPD in Lung Fibroblasts

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**Background and Aims:** Chronic obstructive pulmonary disease (COPD), representing the third leading cause of death worldwide, is a progressive lung disease inducing life-threatening breathlessness and predisposing to exacerbations (sustained worsening of the patient's condition) [1,2]. The currently available treatment options for COPD do not stop the disease progression, nor revert the disease phenotypes. Therefore, novel therapeutic strategies are urgently needed. In this project, we aimed to perform first unbiased genome-wide profiling of primary human lung fibroblasts isolated from controls and COPD patients with different disease stages. The knowledge obtained from this approach was used to identify novel regulators of COPD which could in future be developed as drug targets.

**Methods:** Fibroblasts were isolated by explant outgrowth from human distal lung tissue obtained from ex-smoker controls and COPD patients. Employing whole-genome bisulfite sequencing (WGBS) and RNA-sequencing (RNA-seq) of the isolated cells enabled us to gain a deep insight into the DNA methylation and transcriptome changes between controls and COPD patients and with that to identify potential disease regulators. Numerous differentially expressed genes (DEGs) and differentially methylated regions (DMRs) were identified and validated in a phenotypic siRNA-based screen in commercially available healthy and COPD diseased fibroblasts. The siRNA-screen results were further validated with the orthogonal technique CRISPR/Cas9 for constitutive knockout in fibroblasts.

**Results:** We obtained genome-wide DNA methylation and gene expression signatures of COPD across disease stages and identified genes that are dysregulated already in early disease stage. Many dysregulated genes also showed changes in the methylation state of their promoter, suggesting their regulation by epigenetic enzymes in the disease. Integration of our profiling data, together with upstream regulator analysis identified numerous candidate regulators of COPD phenotypes. Using siRNA-screens in primary HLF, we linked the function of several of these genes to key fibroblastic processes in COPD (for instance collagen deposition, which is important in wound healing) establishing them as potential novel targets for COPD therapy.

**Conclusion:** The cell-based assays confirmed the functional role of multiple candidates identified from profiling data, indicating that integrative epigenetic and transcriptomic profiling of normal and diseased human samples is a powerful approach for the identification of novel disease regulators. The representation of epigenetic factors among positive hits demonstrates that epigenetics in COPD is an exciting research filed, which holds promise for novel therapeutic avenues for COPD patients.

**Keywords:** COPD, fibroblast, WGBS, RNA-seq

**References:**
[1] World Health Organisation (WHO), [www.who.int](http://www.who.int);
Evaluation of The Molecular Mechanism of Transport of Dextrin-COLISTIN Conjugates Across the Cell Membrane

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Background and Aims: Bacterial infections affect > 80% of patients receiving chemotherapy for acute myeloid leukaemia (AML), and about one third of them will die. Antibiotics are used to prevent infections during chemotherapy treatment. Polymyxins, such as colistin, are antibiotics with anticancer activity [1], but their use is limited by nephro- and neurotoxicity. Modification of colistin by conjugation to the polysaccharide, dextrin, can lower toxicity to normal cells [2], but, despite reduced cellular uptake, certain modifications can enhance toxicity towards AML cells in vitro. This study aimed to review the mechanisms used by polymer therapeutics to access the intracellular compartments of cells and examine potential reasons for the reduced uptake of dextrin-colistin conjugates by AML cell lines.

Methods: Visualisation of the cellular localisation of dextrin-colistin conjugates using flow cytometry, subcellular fractionation or fluorescence microscopy requires fluorescent labelling with an appropriate probe (e.g. AlexaFluor dyes). A comprehensive literature review (using PubMed) was performed to describe the i) processes of endocytosis and intracellular trafficking, ii) mechanism of colistin internalisation and intracellular fate, and iii) factors affecting cellular uptake of polymer therapeutics. mRNA expression (RNAseq) of membrane proteins associated with endocytosis (megalin) and facilitative transport (carnitine/organic cation transporter 2 (OCTN2), human peptide transporter 2 (PEPT2)) of colistin, and its proposed intracellular target (Lysinespecific histone demethylase 1A (LSD1)) in the 5 AML cell lines showing differential toxicity and uptake of colistin in our previous studies was retrieved from the Cancer Cell Line Encyclopedia.

Results: Cellular uptake of colistin involves binding to megalin, PEPT2 and/or OCTN2. Cellular uptake is enhanced when polymer therapeutics have a positive charge, small size and by attachment of endocytic targeting residues (e.g. folic acid). Polysaccharides, such as dextrin, are negatively charged, and modification by succinylation (to incorporate carboxylic acid groups to allow binding to colistin) increases their negative charge. The use of an amide linker means residual sugar molecules remain attached to colistin, even after enzyme degradation of dextrin. mRNA expression of endocytic and facilitative transport proteins varied among AML cell lines, but showed no correlation with toxicity of free or dextrin-bound colistin.

Conclusion: The amount of drug reaching its intracellular target could be increased by i) using an alternative linker (e.g. ester) that can be fully cleaved to release intact colistin, ii) attachment of a targeting residue (e.g. CD33, found on the surface of some leukaemia cells) and iii) using minimally modified dextrin, to reduce the conjugate’s negative charge.

Keywords: Drug delivery, endocytosis, colistin (polymyxin E), polymer therapeutics, leukaemia

References

1q21.1 CNV Diminishes Neurotrophic Function of Human iPSC-Derived Astrocytes

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Background and Aims: 1q21.1 CNV has been associated with a range of neurodevelopmental (NDD) and neuropsychiatric disorders (NPD) such as autism and schizophrenia. Among 9 critical genes present on the distal region of 1q21.1 locus, astrocyte specific genes GJA5 and GJA8 have been found to hold a significant association with schizophrenia. Emerging studies in patients with NDD and NPD suggest morphological and functional alterations in astrocytes. However, the effect of 1q21.1 deletion and duplication on astrocyte physiology still remains unknown. Hence, modelling astrocyte dysfunction in these disorders using patient-derived induced pluripotent stem cells (iPSCs) provides a critical paradigm for investigation.

Methods: Here we have established an efficient and rapid protocol for modelling astrocyte pathology. Mature and functional astrocytes were generated successfully in 30 days using iPSCs carrying 1q21.1 deletion or duplication. The astrocytes were characterized using immunofluorescence, flow cytometry and qRT PCR at day 30. The physiological assays were performed between day 30 and day 45 to study phagocytosis, ATP production, glutamate uptake and calcium signalling in the astrocytes carrying 1q21.1 CNV (CNV-Astro). Furthermore, in order to study the impact of CNV-Astro on neuronal health we developed co-culture system where CNV-Astro were cultured with healthy neurons followed by functional tests.

Results: 1q21.1 CNV was not only associated with altered astrocyte morphology but also demonstrated compromised functional abilities. Further, RNA-seq data from CNV-Astro had very distinct PCA and hierarchical clustering from the control astrocytes and had highly significant changes in genes linked to autism and schizophrenia. The co-culture of neurons with CNV-Astro resulted in differential expression of synapse associated genes suggesting negative impact on synapse formation in healthy neurons. The multi-electrode array (MEA) performed in the co-culture system also demonstrated a deficit in healthy neuronal network activity as quantified by network firing rate and synchronization.

Conclusion: Our results have demonstrated that the CNV-Astro have morphological as well as functional deformities and hence the importance of astrocytes as a novel medication for the neurodevelopmental and neuropsychiatric disorders. Further work remains necessary to understand the molecular mechanisms driving the astrocyte dysfunction.

Keywords: 1q21.1 CNV, astrocytes, neurodevelopmental disorders, co-culture, multi-electrode array (MEA)
Identification of Cell-Autonomous 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 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-like cells were characterized by flow cytometry, immunohistochemistry and qPCR for the expression of cell-specific markers. Both HD and isogenic control cells express desired microglia markers. Microglia morphology as well as phagocytic function was analysed using the IncuCyte S3 live-cell imaging system and IncuCyte S3 software (2017A).

Results: We found that HD microglia exhibit a less ramified phenotype, with significant decreased cell area and complexity, compared to isogenic controls. The phagocytosis of pathogens, apoptotic cells and debris is a key feature of microglia function. Phagocytosis of E.coli beads was significantly decreased in HD microglia compared to controls.

Conclusion: These initial observations suggest a cell-autonomous effect of mutant HTT on microglia status and activity and are consistent with previous data on microglial activation, thus supporting the use of iPSC-derived microglia as a model to study neuroinflammation in HD.

Keywords: Microglia, Neuroinflammation, Huntington’s disease, iPSCs
Recreational Runners in Wales and their Views on the Use of Digital Technologies in Training, Running Injury Prevention and Self-Management

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Background and Aims: Running is an increasingly popular activity but many runners stop running due to running-related injury (RRI) (1). Injured runners are turning to digital platforms to help them prevent and self-manage their injuries. The aims of this study are to 1) map the use of digital platforms and smartphone apps by recreational runners in Wales and their views on running injury prevention, self-management and a proposed ‘Ideal RRI prevention and self-management app and 2) map characteristics of recreational runners in Wales, identify the most common injuries and identify patterns of injury among sub-groups of runners in Wales.

Methods: An online survey was developed which was distributed to recreational runners in Wales. The survey was designed to gain demographic information from runners in Wales, their training habits, injury history, how they prevented and managed RRI and what their views were on an RRI prevention and self-management digital platform. Participants were recreational runners in Wales over the age of 18. Elite runners and those under 18 were excluded from this study. Analysis of results was performed using SPSS v25 to produce descriptive results and sub-group analysis of groups of runners.

Results: 233 runners completed the survey. 87% of runners survey reported having sustained an RRI. Achilles tendon injury was the most common injury. 97% of runners used a digital platform to monitor their training. GPS watches and smartphone apps were the most popular monitoring methods. Runners managed injury through rest, seeing a health professional or self-managed their injury. Only 8.6% used online advice to prevent or manage RRI. 85% of runners were interested in an evidence-based smartphone app to help prevent and manage RRI. Desired features including information self-diagnosing RRI, exercises and how to run safely and avoid RRI.

Conclusion: There is a high prevalence of RRI among recreational runners in Wales. Runners are using digital devices to monitor and programme their training but not to prevent or manage running injuries. Results from this survey show that runners are interested in self-managing and preventing their own RRI and reacted positively to the proposal of an injury prevention and self-management app.

Keywords: running, running-related injury, injury prevention

References

Nonlinear Elastic Properties of Crosslinked Actin Filament Networks

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Background and Aims: Living cells can bear large deformations to support different cell functions. In large deformation situations, the crosslinked actin filament networks (CAFNs) always show strong nonlinear elasticity to maintain the cell shape and integrity, which is known as strain stiffening. Thus, to investigate the nonlinear elastic properties of CAFNs becomes more and more pressing. Although in vivo and in vitro experiments, theoretical analysis as well as numerical simulations have been conducted, the nonlinear elastic properties of CAFNs are still not known very well. This research aims to investigate the nonlinear elastic properties of CAFNs by conducting numerical simulations.

Methods: A three-dimensional network model is developed in finite element method software (ABAQUS) to mimic the architecture of the CAFNs. The length distribution of actin filaments, physiological contour of crosslinkers and crosslinking principles are taken into consideration when constructing the model. To study the nonlinear elastic properties of CAFNs, many groups of numerical simulations are conducted on this model, and the stress strain curves as well as tangent moduli of CAFNs are obtained. Different levels of actin filament volume fraction, crosslinking density and material properties are applied to study their effects on the nonlinear elastic properties of CAFNs.

Results: Simulation results show that both the actin filament volume fraction and the crosslinking density can greatly influence the stress strain curves and tangent moduli of the CAFNs. Larger actin filament volume fraction and crosslinking density can result in earlier appearance of the stiffening and stronger stiffness, which indicates that the actin filament volume fraction and crosslinking density greatly enhance the stiffening behaviour of CAFNs. The Young’s moduli of actin filaments and crosslinkers are proved to have great effects on the nonlinear elastic properties of CAFNs, however, the Poisson’s ratios of actin filaments and crosslinkers just have slight influences.

Conclusion: The effects of the contents and physical properties of actin filaments and crosslinkers on the nonlinear elastic properties of the CAFNs are obtained. The nonlinear elastic properties of CAFNs are proved to be greatly affected by the contents and Young’s moduli of actin filaments and crosslinkers. These results provide valuable reference for studying other biopolymer networks and designing biological materials.

Keywords: Nonlinear elasticity, Modelling, Actin filament network, Crosslinking
LtaS Inhibitors as Novel Antibiotic Compounds

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Background and Aims: Recent decades has seen a decrease in antibiotic development, despite greater their demand because of antibiotic resistance. Targeting of lipoteichoic acid synthase (LtaS), an enzyme responsible of synthesising lipoteichoic acid (LTA) in Gram positive bacterial cell walls, could help address this problem. Compound 1771 has been proposed as an LtaS inhibitor, although its activity remains unclear. In this study, we report the first synthetic strategy for compound 1771, evaluate its activity as an inhibitor of bacterial growth, and evaluate its affinity through in silico investigation.

Methods: The synthetic strategy for compound 1771 was developed through bibliography research. Different compounds were synthesised and characterised using NMR and MS. The inhibitory activity of compound 1771 and its putative metabolites was investigated by standard broth microdilution measurement of minimum inhibitory concentration (MIC) against strains of Staphylococcus aureus and Enterococcus faecium. To ascertain if compound 1771 could bind LtaS in silico evaluation was performed using AutoDock software.

Results: Compound 1771 was successfully synthesised with a 50% yield. MIC testing resulted in compound 1771 equivalent antimicrobial effects or better compared with Vancomycin, which is used as last resort drug to treat several Gram-positive bacterial infections. Testing putative metabolites derived from compound 1771 revealed that these fragments lacked inhibitory activity at the investigated concentrations. In silico analysis identified that a conformation of compound 1771 that could occupy the LtaS binding site and facilitated via H-bonds and pi-interactions. The outcome of this would be prevention of 1-glycerol phosphate, the natural substrate of LtaS, entering the active site.

Conclusion: Achieving a synthetic strategy to obtain compound 1771 could allow the development of compound 1771 and its derivatives with enhanced activity and stability. The results demonstrated that antimicrobial effects of 1771 was dependent on the intact molecule and not any of its putative metabolites.

Keywords: lipoteichoic acid synthase, in silico, Antibiotic

References
Neurotrauma and COVID-19

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Background and Aims: The COVID-19 pandemic caused unprecedented changes to healthcare worldwide. Apart from direct health-related effects, its indirect effects on healthcare infrastructure and logistics are particularly problematic. This is reflected by reports of decreasing emergency and elective neurosurgical procedures being performed globally. Traumatic brain injury (TBI) is a leading cause of morbidity and mortality, and time-critical management is paramount for life-saving intervention. Understanding the effects of the global pandemic on management of TBI is vital for service planning in the future. In this study, we report differences in TBI demographics and management pre- and post-onset of COVID-19 in our local Neurosurgery unit.

Methods: Retrospective search of our neurosurgical database was performed to identify adult TBI patients that were referred to our unit for two 3-month time periods before and after the onset of COVID-19 (01/10/19 – 31/12/19 and 01/04/20 – 30/06/20 respectively). The following variables were extracted: patient demographics, mechanism of injury, alcohol use, Glasgow Coma Score (GCS), pupillary response, radiological findings and management strategy (no input required, advice for local management, transfer to Neurosurgical unit).

Results: There was a significant change in the percentage of referrals regarding TBI patients that were managed locally (55% to 46%, p=0.01) and with a significant reduction in the percentage of TBI patients transferred (18% to 11%, p=0.047). Subgroup analysis of moderate-severe TBI revealed a 41% and 50% reduction in transfers respectively but was not statistically significant. There was an increase in RTAs (10% to 6%), reductions in alcohol (12% to 17%) and falls (69% to 76%) as mechanisms of injury, but none reached statistical significance. There were no significant differences in the demographics, severity of TBI, pupillary or radiological findings.

Conclusion: Although the demographics of TBI remain unchanged following the onset of COVID-19, there has been a significant shift in management strategies. This is demonstrated by an increase in proportion of patients that are being treated locally. Given the chronic morbidity associated with TBI, any diversions from a normal service will be of detriment to patients.

Keywords: COVID-19, Neurotrauma, Traumatic Brain Injury.
Can Manipulation of Neuroinflammation Modulate Oligodendrogenesis and White Matter Repair After Traumatic Brain Injury?

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Background and Aims: Whilst traumatic axonal injury (TAI) is a key hallmark of traumatic brain injury (TBI), there is an unmet clinical need to develop greater mechanistic understanding of white matter injury after TBI1. High-mobility group box 1 protein is implicated as a regulator of brain inflammation following neurotrauma, and its elevated levels in the CSF and serum of patients with TBI is associated with worse outcomes. The aim of this project is to investigate the effect of HMGB1 upon oligodendrocyte progenitor cells (OPCs) in an in vitro model of TBI.

Methods: We subjected rat cortical mixed neuro-glial cell cultures (7DIV) to standard scratch injury as described elsewhere2. Our previous data has identified that HMGB1 is released in these cultures following scratch injury. Media from these cultures, or control cultures, was collected and applied as a 24-hour pulse to rat cortical neural stem/progenitor cells after six days in vitro, (CCM and ICM respectively) with or without the presence of an antagonist of HMGB1 (BoxA; 100ng/ml). Immunofluorescence microscopy for NG2+ (OPCs) was performed to ascertain cell counts.

Results: Treatment of cells with ICM resulted in a significant decrease in the numbers of NG2+ cells compared to standard control conditions (59cells/mm2 ± 5.8 SE vs.16cells/mm2 ± 1.9; p < 0.0001). Co-treatment with BoxA did not affect NG2+ cell counts in the control, (59cells/mm2 ± 5.7 vs. 71cells/mm2 ± 2.8 ; p = 0.511) or CCM (78.6 cells/mm2 ± 12.9 vs. 66cells/mm2 ± 7.9; p = 0.061) conditions, but completely abolished ICM-induced NG2+ cell loss (16cells/mm2 ± 1.8 SE vs. 85cells/mm2 ± 14.0 ; p < 0.0001; Figure 1).

Conclusion:

Identification of neuronal, microglial and astrocyte secretion of HMGB1 following severe TBI in humans, and OPC-specific toxicity of HMGB1 in vitro, suggests that HMGB1 may be a potential therapeutic target for improving morbidity associated with TAI.

Keywords: white matter trauma oligodendrocyte progenitors HMGB1

References

A Review of *in Vitro* Models to Study the Host Immune Response to Biomaterial Infections

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**Background and Aims:** Biomaterials provide a scaffold to repair tissues and organs for a variety of diseases. After implantation, biomaterials undergo the process of serum protein adsorption, neutrophil and macrophage infiltration, foreign body giant cell formation, fibrous encapsulation and remodelling. Immune cells trigger the healing process, however, the scale of the inflammatory response may impair this process and lead to implant failure, as observed during implant infections [1]. The models to understand this process play a vital role in enhancing the success of biomaterial technologies. This review focussed on the models which replicate the *in vitro* immune host response to biomaterial infections.

**Methods:** PubMed and Ovid databases were search with the keywords “biomaterial” AND “macrophage” AND “infect*”. 181 results were retrieved from PubMed, with 131 results from Ovid and 3 additional records through publication references until the 30th May of 2020. Following screening according to the inclusion and exclusion criteria, 17 articles were included in the final review. Inclusion criteris included original *in vitro* research articles and conference abstracts related to the review topic and exclusion criteria consisted of *in vivo* studies, non-English articles and review papers.

**Results:** Four types of models were found during the review, lipopolysaccharide (LPS) induced inflammation, bacterial conditioned media, flow-chamber and co-culture models. LPS induced macrophages into an M1 phenotype in a dose and time-dependent manner, suitable for studying the acute phase of biomaterial host inflammation. Conditioned media models exposed immune cells to a wide range of virulence factors on biomaterials without direct contact and demonstrated protein factors from biofilms to inhibit phagocytosis. Flow chamber experiments showed the generated shear stress compromised neutrophil and macrophage phagocytosis. Co-culture models demonstrated bacterial cell wall proteins to modulate inflammation by direct contact.

**Conclusion:** *In vitro* models of biomaterial infections elucidated some of the mechanisms by which bacteria modulate the *in vivo* host response to biomaterials. The LPS model provided a simple system for studying the acute phase of inflammation; conditioned media models and co-culture models allowed interactions between immune cells and bacteria under indirect or direct contact to be studied, whilst flow chamber experiments demonstrated the influence of fluid flow on infection progression. Each model replicated a unique characteristic of the host response to biomaterial infections and elucidated potential therapeutic targets to prevent biomaterial failure.

**Keywords:** Host immune response; *in vitro* model; LPS; conditioned media; co-culture; shear stress

**References**

Evaluating the Performance of Cell Delivery Devices Using a Bioluminescence Model

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Background and Aims: Cell replacement therapy has the potential to treat multiple neurodegenerative conditions such as Huntington’s disease1. Despite the vast research invested in developing cell therapies, there has been scant research into developing devices for delivering these cell therapies. Better devices are needed, as the prior system of a simple cannula and syringe has shown inconsistent performance for successful graft delivery and survival in patients with Huntington’s disease2. We aim to instigate cell delivery strategies into brain including optimal velocity of delivery to minimise cell reflux using gel phantom and bioluminescence model.

Methods: A novel cell delivery cannula was manufactured (long; 10mm and short: 1mm distal to outer needle) and compared with the RENISHAW drug delivery catheter. 0.6% agarose gel was utilised as brain phantom. Using a third generation lentiviral vector and S2-luciferase plasmid; HEK 293 cells were transfected, and successfully transfected cells were sorted using fluorescent activated cell sorting. Initially Kinetics of the luciferase reaction were tested in triplicates. Two methods of delivery were tested, a withdrawal model: multiple deposits alongside the delivery track and a preformed tract model: depositing cells along a pre-formed track with no needle withdrawal between deposits.

Results: Luciferase signal decays rapidly to plateau for at least 30 minutes. Using 0.6% agarose phantom, transduced HEK cells were deposited using the withdrawal and the preformed tract method, long and short needle Reflux was defined as percentage of cells proximal to the distal tip of the catheter/needle. The withdrawal method produced significantly less reflux compared to the preformed tract method in both long and short needle. No significant change was observed between the 2 delivery methods using the RENISHAW catheter. The preformed method produced a tracking effect, where cells did not reach the original target compared to the withdrawal method.

Conclusion: No device is commercially available for cell delivery into brain. A novel cell delivery cannula was designed and validated to produced minimal cell reflux. Using a bioluminescence model - at optimal delivery speed- the withdrawal method via the long needle, produced the least cell reflux with no tracking effect. This method will be utilised in future in vivo models.

Keywords: Cell delivery, bioluminescence, gel phantom, Huntington’s disease

References

Liposomal Delivery of Growth Factors for Enhanced Mineralised Tissue Repair

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Background and Aims: Fractures and bone defects are a significant source of medical morbidity and often require substantial healthcare resources to treat. Despite technological advancements and modern treatments for bone fractures, critical size defects remain a challenge limiting bone healing and resulting in reduced quality of life. Liposomes are lipid vesicles that have been used for controlled and sustained delivery of molecules in tissue engineering, however the mechanisms by which liposomes produce a more beneficial response when used for delivering growth factors for bone regeneration is unclear. This review investigated the mechanisms by which growth factors in liposomes induce a superior repair response to the free growth factors.

Methods: Articles were retrieved from searching the PubMed, ScienceDirect, Scopus, and Google scholar databases. The search scope was limited to English language articles and was filtered to include laboratory-based studies between the years 2000 and 2020. The initial search identified 2474 publications that were screened by title and abstract to include those investigating liposomal delivery of growth factors for mineralised tissue applications. Studies using recombinant plasmids or DNA loaded liposomes were excluded. A total of 21 studies were included in the review.

Results: In vitro studies showed that liposomes were able to stabilise the growth factors and significantly increase their biological activity compared to the free growth factors. Liposomal Wnt3a was found to significantly reduce apoptosis, promote osteoprogenitor cell proliferation, and differentiation into osteoblasts compared to the free Wnt3a. Similar results were reported in vivo with liposomal BMP-2/4, IGF-I, and PDGF-BB, which significantly increased the percentage of bone volume, calcium mineral content, and blood vessels compared to the free growth factors. This is due to the liposomes being able to retain the growth factors for a period sufficient to activate continuous bone formation. This could also be induced by liposomal lipids such as phosphatidylserine, which can bind calcium ions and promote mineralisation.

Conclusion: This review demonstrated that using liposomes as carriers to deliver growth factors can efficiently maintain the biological activity of the factor and protect it from degradation. Liposomes can also enhance bone regeneration and may promote a superior healing response compared to free growth factors, possibly due to the liposomal lipid composition. This could have therapeutic potential for mineralised tissue repair and other tissue engineering applications.

Keywords: Liposomes, growth factors, bone repair, osteogenesis, dentinogenesis, tissue engineering
Hydroxytyrosol and its metabolites in atherosclerosis

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Background and Aims: Atherosclerosis, an inflammatory disorder of medium and large arteries, is the main cause of cardiovascular disease (CVD). It is a disease in which plaque builds up inside the arteries which causes narrowing and hardening of the arteries and restricting blood flow. Hydroxytyrosol (HT) is a polyphenol found in olives and olive oil which has proven to have a significant role in CVD protection. The aim of my project is to critically analyse the roles of HT and its metabolites in CVD together with its potential in the prevention and treatment of atherosclerosis. Pharmacotherapy is still the gold standard treatment for controlling CVD, however they have significant adverse-effects and are not applicable or effective for everyone. Therefore, it is essential to carry out further research on safe, long term, non-pharmaceutical treatment.

Methods: The search strategy and selection criteria consisted of references between January 2015 to July 2020 using PubMed and Ovid Medline by combining the search term “HT” with the search term “atherosclerosis,” “cardiovascular disease”, “statins”, “Endothelial dysfunction”, “nitric oxide”, “atherosclerosis pathology”, “olive oil”, “inflammation”, “low-density lipoprotein”, “cholesterol” and “risk profile”. The search was restricted to publications in English. A total of 1,926 records were collected after duplicates were removed. Six full text with abstract mentioning HT and atherosclerosis and olive oil (or antioxidant/ cardio) were chosen for further evaluation. These consisted of two clinical trials, two in vitro studies and two pre-clinical in vivo studies.

Results: HT plays a significant role in CVD protection and together with its metabolites protects from endothelial dysfunction commonly associated with atherosclerosis. Both HT and its metabolites decreased the expression of markers associated with endothelial dysfunction and atherosclerosis such as E-selectin, P-selectin, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 [1]. HT also improved antioxidant status and reduced the size of atherosclerotic lesions measured as intimal layer areas of the aortic arch when compared with control groups in rabbits. HT also decreased LDL oxidation (by copper ions) and prevented platelet aggregation leading to the formation of a thrombus (clot) [2].

Conclusion: These studies demonstrate the ability of HT and its metabolites to prevent endothelial dysfunction which occurs early in atherosclerosis. Early prevention of atherosclerosis is necessary to improve the size of atherosclerosis lesions in the intima layer of arteries.

Keywords: Cardiovascular disease (CVD); Hydroxytyrosol (HT); intercellular adhesion molecule-1 (ICAM-1); vascular cell adhesion molecule-1 (VCAM-1); Low-density lipoprotein (LDL)

References:
TCF21 as a Candidate Deactivator of Lung Fibrosis

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Background and Aims: Patients with pulmonary fibrosis have a life expectancy ranging between 2 to 5 years post diagnosis [1]. Fibrosis is the formation of a thick scar tissue that impairs the normal functioning of organs. A central mechanism in fibrosis development is the aberrant activation of fibroblasts and their differentiation into myofibroblasts. The transcription factor 21 (TCF21) is a regulator known for its role in fibroblast cell specification during embryogenesis [2]. Notably, fibroblast populations originating from the TCF21-expressing lineages lose TCF21 expression upon differentiating into myofibroblasts, suggesting that TCF21 might regulate the fibroblast fate decisions. Despite this link, the role of TCF21 in organ fibrosis is yet to be discovered. The aim of this project was to systematically examine the published role of TCF21 in fibrosis across organs and provide grounds for further studies of TCF21 in the lungs.

Methods: Here, we critically reviewed the primary research about the role of TCF21 in fibrosis across organs. We provide the histological and molecular context necessary to understand how TCF21 is involved in the fibrotic pathways. We critically appraised the available experimental evidence and recognise common misconceptions and knowledge gaps. Overall, we build a comprehensive view on TCF21 regulation of organ fibrosis, and its potential involvement in lung fibrosis.

Results: In the coronary arteries and endometrium, expression of TCF21 acts as a switch for smooth muscle cells to differentiate into fibroblast-like cells. Similarly, liver myofibroblasts overexpressing TCF21 revert to fibroblasts. These results suggest that TCF21 is a key regulator promoting cell transitions into fibroblast cell states. Moreover, TCF21 inhibits a range of pro-fibrotic molecules by regulating their expression and has opposite effects to the known pro-fibrotic transcription factor (SMAD3). Overall, our results indicate a conserved mechanism for TCF21 across organs and establish TCF21 as a safeguard promoting a normal fibroblast state.

Conclusion: Our review of the published evidence indicates that TCF21 is a key transcription factor with the potential to resolve fibrosis, by maintaining a fibroblast phenotype. However, this potential might depend on the molecular context of tissues. Due to the lack of studies focusing on TCF21 in the lung fibrosis, experimental confirmation of its role is required.

Keywords: TCF21, Lungs, Fibrosis, Myofibroblast, Fibroblast

References
Modelling the Effect of 1q21.1 Deletion on Brain Development using Dorsal Forebrain Organoids

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Background and Aims: 1q21.1 syndrome is a copy number variant that increases the risk of certain neurodevelopmental disorders. Microdeletions within the distal region of the 1q21.1 loci are commonly associated with microcephaly and schizophrenia, however, the underlying biology that leads to these clinical phenotypes is not understood. Emerging studies have demonstrated that cerebral organoids are a useful, clinically relevant model when recapitulating brain size abnormalities, a clinical phenotype which cannot be observed in 2D culture. The aim of this research is to use cerebral organoid models of 1q21.1 distal deletion to identify and detail the mechanisms at work creating the disrupted neurodevelopment in the 1q21.1 patients.

Methods: In order to identify the causative differences between control and the microdeletion of 1q21.1 syndrome, we have generated dorsal forebrain organoids from patient-derived iPS cells carrying microdeletions at the 1q21.1 locus. Sequential brightfield imaging is used to monitor organoid growth. Real time qPCR is used to document gene expression across biologically-relevant modules of genes. Immunofluorescence analysis is used to understand internal morphology difference and cellular characteristics.

Results: Morphometric analysis of early organoid development reflects the microcephaly phenotypes seen in human patients, as well as clear disruption in neuroepithelial folding and resulting loss of cortical layer formation. Gene and protein expression analysis of day 30 organoids with 1q21.1 deletion show deteriorated neuroepithelia, dispersed proliferative radial glia and universal downregulation of dorsal forebrain lineage transcription factors. By day 60, gene and protein expression analysis show that these organoids alter their development towards increased heterogeneity of neuronal sub-type populations.

Conclusion: Cerebral organoids of 1q21.1 distal deletion accurately model the microcephaly seen in patients. The cause of which is currently being investigated. These organoids elicit an abnormal transition of cell fate during late development, a phenotype that has only been seen in one cerebral organoid model of idiopathic autism1. Further investigation will include characterisation of progenitor populations suspected to be involved.

Keywords: 1q21.1, schizophrenia, microcephaly, organoids, neurodevelopment

References:
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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.
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.
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.
CITER is a Cross College Research Network associated with the MSc Tissue Engineering and Regenerative Medicine

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- School of Dentistry
- School of Engineering
- School of Healthcare Sciences
- School of Medicine
- School of Optometry & Vision Sciences
- School of Pharmacy & Pharmaceutical Sciences
- School of Chemistry
- School of Earth and Ocean Sciences
- School of Mathematics

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140 academic
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120 postgraduates
25 undergraduates
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10 (honorary members)
Stem cell science

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

Tissue engineering and repair

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  • Kidney repair
  • Vision loss, bio-simulation and tissue repair
  • Tissue repair and wound healing

➢ Disease translation
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