EPSRC CDT IN ADVANCED BIOMEDICAL MATERIALS
PhD Projects

Projects based at the University of Manchester

ABM CDT Design and evaluation of a novel composite conduit for peripheral nerve repair

Supervisory Team: Prof Alberto Saiani, Dr Aravind Vijayaraghavan, Dr Adam Reid

Abstract

Peripheral nerve injuries are common, with approximately 9000 cases occurring each year in the UK in a predominantly young and working population. Despite microsurgical nerve repair techniques, normal restoration of function is unattainable, which results in impaired sensation, reduced motor function and frequent pain and cold intolerance. Such injuries have a profound and permanent impact on the patient and their ability to perform daily living activities with less than 60% returning to work.[1] The current method of repairing a large gap between nerve endings is to graft nerves taken from another area of the patient (autograft). This results in a further surgical procedure and morbidity at the harvesting site. In addition there is limited donor nerve availability. This state of affair has prompted researchers to focus on the design and use of bio-engineered nerve conduits (tubes), an artificial means of guiding nerve regeneration. However, to date, commercially available conduits have not been able to match the results of the current clinical state of the art technique (autograft). This is likely due to the nerve conduits in current use being empty tubes and failing to re-create the 3-dimentional environment required for optimal nerve regeneration.[2]

Main questions to be answered

The challenge we will tackle through this project is the design of a fully defined synthetic composite 3D-scaffold that promotes nerve regeneration to be used within nerve conduits. For this purpose the project aims to develop an amino acid based elastomeric tube and a graphene oxide functionalised peptide hydrogel scaffold to promote cell neuronal cell differentiation and proliferation. Graphene oxide nano-carriers will be used to immobilise and deliver growth factors within the hydrogel to promote re-innervation. One of the key novel approaches to be used is additive manufacturing (3D-bioprinting) to micro pattern the composite scaffold to promote directional growth of neurones. Graphene oxide will be deposited as thin filament / layer along the main axes of the conduit to drive and direct neuronal cells migration and nerve growth along the tube promoting innervation.[3-5]

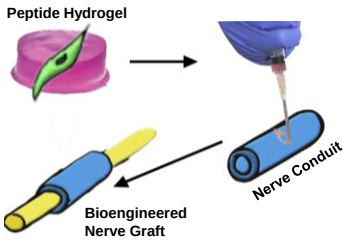


Fig. 1: Project aim and principle

ABM CDT Improving the longevity of oesophageal stents

Supervisory Team: Dr Andrew Thomas, Professor Julie Gough, Dr Hans-Ulrich Laasch, Dr Joao Quinta Da Fonseca

Abstract

Cancer Research UK describes 9,200 new cases of oesophageal cancer per year in 2017. 70% are diagnosed at a late stage, being incurable and causing 7,925 deaths / year.

Treatments using chemotherapy and radiotherapy – and more recently hormone therapy - have improved patient survival in recent years, extending survival of stent patients *after receiving a stent* from an average of 3months in 2004 to currently 15-18months. Most oesophageal cancers present late and are not curable and most patients eventually require insertion of a stent (most commonly a nitinol stent) to keep the lumen of the oesophagus and allow the patient to continue to eat.

These stents were originally designed for use in blood vessels but have been adapted for use in the gastro-intestinal tract and oesophagus therefore subjecting the stents to a different working environment especially chemical, due to exposure of the low pH of gastric acid, but also mechanical due to the movement and compression of oesophageal function (peristalsis). As a consequence an increasing number of patients experience device failure, requiring repeat procedures. The re-intervention rate at 6months reaches 60%, which is now resulting in increased stent failures, which necessitate further procedures, and puts the patient at additional risk.

By improving the properties of these nitinol stents, we can improve their working life and remove the need for removal and replacement. This will improve clinical outcome and patient experience and reduce the need for repeat procedures and the associated costs to the NHS.

Main questions to be answered

1. What are the failure mechanisms of currently used stents – does removal of the native oxide layer occur and is repassivation of the stent impeded in the oesophageal environment?
2. Can we increase time in service by changing the properties of the current stent composition by e.g. metal processing technologies affecting microstructure?
3. Can we alter the composition of the current stents to increase time in service without compromising the shape memory properties?
4. Are advanced coatings (including graphene) another option for increasing time in service?

ABM CDT Engineered cancer in vitro model to demultiplex biophysical cues in metastasis

Supervisory Team: Dr Annalisa Tirella, Dr Olga Tsigkou, Prof. Kaye Williams

Abstract

Breast cancer is the most common cancer in women in the UK. Whilst prognosis for patients with low grade disease continually improves, 70% of patients with advanced breast cancer develop incurable bone metastases. The mechanisms driving targeted metastases are complex, requiring changes in both the primary tumour and the metastatic niche. A significant risk factor for breast cancer incidence and prognosis is breast density. How this links with biological cascade that underpins the ability of breast cancer cells metastasise and colonise the bone is unknown.

We have developed alginate-based biomaterials, functionalised to mimic the stiffness of distinct stages of breast cancer progression, and bioactive hydrogels that mimic the bone microenvironment. Encapsulation of breast cancer/bone cell populations offers the possibility of generating physiologically relevant 3D tissue engineered models through the combination of materials and cells. Further, the inclusion of 3D models in a microfluidic system will mimic the blood vessels that cancer cells use as passage way for metastasis, hence enable the interconnection and modelling of the breast-to-bone dissemination.

This project aims to develop 3D systems with tuneable biophysical properties and cellular composition mimicking breast cancer and bone tissues. These will be established within a perfusion system with modifiable flow, nutrient and oxygen delivery to mimic systemic circulation. Response of encapsulated breast cancer cells to different biophysical stimuli, their extravasation and metastasis to bone-like recipient will be investigated to fill current knowledge-gaps. This will inform further model optimisation giving rise to realistic tissue models with direct pre-clinical application in early detection, drug screening and therapy intervention.

Main questions to be answered

The overall **aim** of the project is to optimise a library of hydrogels and to fabricate 3D *in vitro* models of breast cancer at late stages of tumour progression and co-culture with endothelial and pericyte cells. Engineered models will allow us to study the changes occurring in the extracellular components (aka matrix) during tumour progression, as well as phenotypic changes in breast cancer cells and their interaction with bone associated osteoblasts and osteoclasts. Bioactive components (e.g. hydroxyapatite) will be included to engineer hydrogels towards osteogenic properties.

The breast-to-bone engineered model will use biomaterials matching properties of target tissues (mechanical, chemical) and will capture physiologically relevant tumour features (shear stresses, pH, oxygen-levels). The integration of the vascular component (endothelial with/out pericyte cells) in a perfusion system will enable to study changes at the metastatic site, e.g. co-culture with osteoblasts and osteoclasts or engineered-bone.

The four project **objectives** are to: (1) optimise hydrogels in composition and stiffness to mimic late-stage breast tumour and bone; (2) manufacture and integrate 3D models in a perfusion system; (3) assess the remodelling of cells and production of extracellular matrix components at distinctive physical stimuli; (4) assess the extravasation of cancer cells and adhesion to bone-engineered tissue.

ABM CDT Advanced silk-based scaffolds with photo-clicked cell guidance cues for peripheral nerve regeneration

Supervisory Team: Dr Jonny Blaker Dr Frederik Claeyssens, Dr Chris Holland

Abstract

Promoting nerve regeneration following trauma is a major surgical challenge. There is an estimated incidence of 1 million peripheral nerve injuries annually world-wide, with huge socio-economic cost. Current standard practice for nerve gap repair is autologous nerve autografting despite deficiencies such as limited availability and harvesting of functioning nerve, donor site morbidity, time-consuming surgeries, or incomplete recovery. There are commercially available nerve guidance conduits, however none of these are better than the gold standard and there is unmet clinical need for improved scaffolds with guidance cues. Biomaterials are being developed to manipulate cellular interactions with their environment. The majority of engineered systems present signals (mechanics, topography, adhesion) in a spatially uniform manner. Biochemical patterns are more indicative of natural extra cellular matrix (ECM) signalling that occurs through gradients and spatial localisation of biomolecules, and their use in combination with topographical features is a powerful tool for guiding axons to their synaptic targets.

This project seeks to develop advanced silk-based nerve guidance conduits (NGCs) using additive manufacture and photo-click technology to provide stimulatory cues to guide neuronal cells to their synaptic targets. We will combine 3D additive scaffold manufacture (foams and nanofibre hierarchical structures) which provide morphological cell guidance cues, with advanced photo-click technology to tether growth factors/adhesion molecules and electrically conductive elements in defined patterns and gradients, with target resolutions that are relevant to cells (approaching 100 nm). The combination will allow a new generation of stimulatory hierarchical NGCs to be produced with tethered growth factors patterned in the interior of the 3D conduit, as a tool to optimise axonal growth.

Main questions to be answered

With the overarching aim of directing axonal growth and Schwann cell adherence in 3D macroporous/fibrillar scaffolds as improved nerve guidance conduits:

1) What resolutions can be photo-clicked onto fibres (of diameter ~1 micron), aligned fibre mats and macroporous foams of functionalised silk* using 2-photon lithography and what are the speed limitations?

2) Can photo-clicked patterns be obtained in 3D structures using holographic projection lithography, and what resolutions are achievable?

3) Can recombinant reflectin* (one of the highest proton conductors in nature) be clicked to generate electrically stimulatory patterns on silk?

4) Which tethered growth factors (e.g. laminin, laminin-1, NGF, CNF, RGDS) and reflectin, concentrations, shapes distributions, or combinations have positive effect on axonal growth and cell motility?

*Recombinant silk containing lysine groups, and recombinant reflectin will be supplied by collaborators in the Manchester Institute of Biotechnology

ABM CDT Smart nanoparticles as doubly responsive sensors for foreign DNA

Supervisory Team: Dr Lee A Fielding, Dr Samuel Jones

Abstract

Dr Fielding has ongoing work into the preparation of polymeric hydrogels [1] and colloidal diagnostics for healthcare applications (unpublished work). Dr Jones has developed a colorimetric platform to detect DNA and has expertise in working with viruses and anti-viral materials [2]. The combination of complimentary expertise from the Fielding and Jones provides an ideal platform to develop a nanoparticle-based diagnostic which will act as doubly responsive sensors for foreign DNA. The unmet clinical needs which we seek to address to provide a simple test which can rapidly and easily identify whether a sample (e.g. from a patient) or a device (e.g. an implant or ocular lens) is contaminated with foreign DNA species (e.g. Virus, Bacteria or Fungus) [3].

Main question to be answered

This project aims to develop a nanoparticle-based diagnostic which will both gel and display a colour change in the presence of amplified DNA. It is well known that cationic nanoparticles can gel in the presence of anionic natural polymers [4] and a colour change can be triggered when DNA binds to dyes prepared in the Jones' lab [2]. We aim to use both these strategies, in conjunction with isothermal DNA amplification to develop an assay for foreign DNA detection.

This will involve the following stages:

- Preparing designer nanoparticles containing DNA binding motifs which display a colour change when complexed with DNA.
- Demonstrating that these nanoparticles form a gel in the presence of amplified DNA.
- Characterising the gels prepared in relation to relative DNA/particle concentrations and external stimuli.
- Optimising dye/nanoparticle composition in conjunction with DNA amplification.
- Initiating trials in clinic with interested clinicians.

ABM CDT Light-driven biofabrication of 3D stem-cell chondrogenic tissue analogues

Supervisory Team: Dr Marco Domingos, Prof. Sue Kimber, Prof. Rob Lucas

Abstract

Articular cartilage (AC) defects are one of the major causes of immobility and poor quality of life for millions of individuals worldwide. Current medical therapies have proven to be insufficient for the long-term regeneration of AC defects. Alternatively, the application of bioprinting in tissue engineering (TE) allows for the fabrication of complex 3D constructs via the precise spatial deposition of multiple cells and biomaterials. When integrated with human pluripotent stem cells (hPSCs), bioprinting opens up the possibility to generate human chondroprogenitor-containing tissue substitutes that can then be transplanted into AC defects and produce cartilage to repair the defect. cartilage. Despite significant progress, the current differentiation protocols for hPSCs towards chondrocytes still present several challenges, including the high cost, inducing factor batch to batch variation and precise timing of administered growth factors and cellular receptor expression together with poor precision of pathway activation. Based on previous work [1], we hypothesise that through using optogenetics, which combines optical and genetic approaches to control cell signalling and hence phenotype, cell-signalling receptors that are critical for regulation of chondrogenesis can be replaced with engineered light sensitive receptors, allowing activation of signalling by specific light wavelengths. This will enable precision fine-tuning of differentiation in 3D bioprinted cell-laden hydrogels by light. If successful, this innovative approach would enable, for the first time, the dynamic manipulation of cell signalling pathways with high spatio-temporal precision.

Main question to be answered

- Can we translate established 2D optogenetic protocols for the generation of chondroprogenitors organised into 3D tissue forming arrays?
- How is the light transmission required for optogenetic-receptor activation affected by the physicochemical properties of polymeric hydrogels?
- What are the optimal light parameters (e.g. intensity, spatial accuracy, exposure time, etc) capable of driving hPSC differentiation into chondrogenic lineages in 3D hydrogels?

ABM CDT Reverse engineering a human blood-brain barrier platform for studying neurovascular diseases

Supervisory Team: Professor Nigel Hooper, Dr Marco Domingos

Abstract

Dysregulation of the blood-brain barrier (BBB) is an early and critical event in the pathogenesis of neurovascular diseases, such as Alzheimer's disease, vascular dementia and stroke. However, there is a lack of knowledge of the molecular and cellular mechanisms underlying the breakdown of the BBB in these diseases due to the difficulty in studying the BBB *in vivo*. The multicellular neurovascular unit (NVU) is central to the regulation of BBB function in health and its dysfunction in neurovascular diseases. The NVU comprises endothelial cells, pericytes, astrocytes and neurons. Complex and dynamic interactions between these cells and the surrounding extracellular matrix (ECM) regulate BBB (dys)function.

This project will focus on incorporating hydrogels that mimic the physical and chemical properties of the brain ECM, 3D bioprinting and microfluidic technology to recapitulate the capillary blood flow, along with the co-culture of human induced pluripotent stem cell (iPSC)-derived endothelial cells, pericytes, neurons and astrocytes, to reverse engineer a 3D BBB model. This reverse engineered BBB model will be used to investigate the pathological neurovascular dysfunction underlying Alzheimer's and vascular dementia and for studying the transport efficacy of drugs that target the brain.

Main question to be answered

1) what is the most appropriate natural hydrogel that recapitulates the *in vivo* mechanical (stiffness, elasticity and viscosity) and biochemical (cell adhesion) properties and supports the growth of the multiple NVU cell types?
2) what is the effect of alteration of the ECM (e.g. increased stiffness, reduction in proteoglycan content) on BBB function and amyloid deposition?
3) will alteration of protein activity lead to alterations in BBB integrity and amyloid deposition, and what are the molecular mechanisms underlying this?

ABM CDT Short peptide fibrillation - towards predictive building of synthetic ECM scaffolds

Supervisory Team: Prof. Paul Popelier, Prof. Alberto Saiani

Abstract

One of the key engineering challenges in the life-science and biomedical sectors is the design and manufacturing of bespoke scaffolds for 3D cell culture, tissue engineering and cell/drug delivery, i.e. cell niches. These cell niches underpin a large and growing sector of biotech and biomed industries whether they are used (i) in-vitro for the study of cell behaviour, toxicity testing or tissue engineering, or (ii) in-vivo for the delivery of cells and/or drugs or to promote regeneration of damaged tissues. In the past two decades significant efforts have been made to develop novel biomaterials to build such scaffolds. One such class of material, which has attracted significant interest, is hydrogels as these soft, highly hydrated materials can be engineered to mimic the cell niche. A variety of approaches can be used to design hydrogels, including the self-assembly of short de-novo designed synthetic self-assembling peptides.

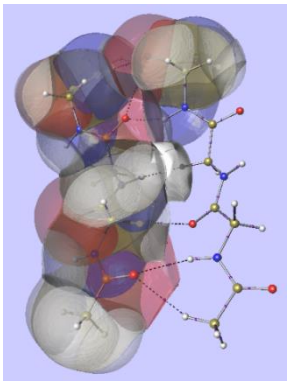
Peptides offer a number of advantages:
(i) peptide synthesis has become a routine procedure making them easily accessible.
(ii) the library of 20 natural amino acids offers the ability to modulate the intrinsic properties of the peptide such as structure, hydrophobicity, charge and functionality, allowing the design of materials with a wide range of properties.
(iii) synthetic peptides are chemically fully defined and easy to purify through standard processes, which is not always the case for natural polymers such as proteins.
(iv) being built from natural amino acids they usually result in low toxicity and low immune response when used in-vivo, and can be degraded and metabolised by the body, which is not always the case for synthetic polymers.

One of the most popular and successful design, as far as hydrogel formation is concerned, was devised by Zhang's group and is based on short peptides (4 to 20 amino acids long) with alternating hydrophilic and hydrophobic residues.

Main question to be answered

The gelation of a short peptide is a two-stage process. First, the peptide needs to be able to self-assemble into fibrillar structures, which in a second step need to entangle and/or associate to form 3D percolated networks that retain water. The early-stage conformational pathway dictates the nature and morphology of the fibrillar structure formed ultimately defining the final properties of the materials.

In this project we computationally investigate, starting from basic principles and using the state-of-the-art approach Quantum Chemical Topology (QCT), the chemical peptide design space (in terms of peptide sequence, property of individual amino acid and conformation space) in the context of β-sheet fibrillation prediction.



The figure shows the so-called topological atoms appearing in two anti-parallel β strands, engaging in hydrogen bonding. The atoms are space-filling (finite volumes) and their specific properties (charge, energy, dipole moment), can be calculated for any configuration. This modern view of atomistic reality offers two activities: interpretation and prediction of structure and dynamics. The former involves: (i) quantification of the local strength of hydrogen bond interaction by quantum topological measures, (ii) Relative Energy Gradient (REG) analysis of rotational energy barriers in the peptide backbone, (iii) electrostatic field analysis of fibril surfaces, and (iv) characterisation of edge effects ("sticky junction") in terms of electron correlation/dispersive effects in pi-pi stacking.

The latter activity (prediction) plucks the first fruit of a completely new force field called FFLUX, which has been designed from scratch over the last decade in order to make a step change in accuracy. It's more reliable architecture, and involvement of machine learning, guarantees long term success in understanding fibril formation, and thus serves as a rational guide to future peptide design.

ABM CDT Designing a new vaginal prophylaxis for the release of biocompatible polymeric virucides

Supervisory Team: Dr Samuel T. Jones, Dr Lee Fielding

Abstract

The prevention of sexually transmitted viral infections such as, human immunodeficiency viruses (HIV), herpes simplex virus–2 (HSV-2) and human papilloma virus (HPV) for example, is important globally. These viruses can have life threatening and/or life altering effects and many are not currently treatable. Prevention rather than treatment is the best approach to dealing with sexually transmitted viral infections. Where vaccines are available, infection rates can be significantly reduced. For those viruses that have no vaccine, prevention *via* other means is necessary. One approach often considered, but not yet implemented successfully for sexually transmitted infections, is the use of vaginal inserts to release antivirals prophylactically. The current state-of-the-art anti-HIV prophylaxis is a vaginal ring insert that releases an anti-retroviral (ARV) drug, which has seen only limited success in Phase-III trials. These antivirals are only effective after infection, are inherently toxic and lead to viruses developing drug resistance, raising concerns that long-term prophylaxis use may lead to drug resistant viral strains.

Through the use of 'kill on contact' antiviral polymers (Polycides) (which have recently been developed in the Jones Lab) it will be possible to tackle the problem of transmission using an entirely new approach. As a prophylaxis our Polycides breakthrough will function before infection occurs meaning there is no opportunity for resistance to develop.

This project will focus on the design of a new hydrogel-based long-term delivery system for these polycides, the study of the release profiles and the *in-vitro* studies to show protection of cells from viral infection.

Main question to answered

Can a hydrogel vaginal insert be synthesised that allows for long-term release of polycides leading to protection from sexually transmitted viral infections?

The project would focus on the development of a cross-linked polymeric hydrogel capable of controlled release of polycides. Typically hydrogels are used in the release of small molecule drugs and their release profiles are typically short. The release of the highly soluble and much larger polycides presents new challenges compared with small molecule drugs. Understanding the release profile is essential for drug delivery *via* a vaginal ring insert in order to be able to advise users of time between replacements.

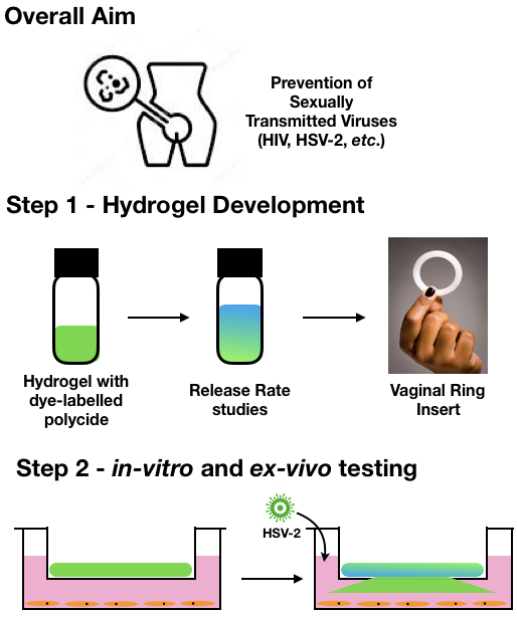


Figure 1 – Overview of proposed route to

The student will be required to synthesise and test, in a lab setting, several hydrogel compositions with dye labelled polycides. Regular discussion with the external advisor will allow for rapid identification of the most suitable hydrogel systems. Lab-based studies will allow for the ideal release rate profiles to be identified before moving to *in-vitro* testing, which will involve both toxicity testing and prevention of infection studies over a period of time. If successful protection of cells is achieved, tests with *ex-vivo* vaginal tissues will be performed. By the end of this project a vaginal ring insert will have been generated, ready for *in-vivo* testing.

ABM CDT Development of a bioelectronic wound dressing to aid chronic ulcer wound repair

Supervisory Team: Prof. Sarah Cartmell, Dr Lee Margetts, Dr Kimberly Mace, Dr Jonny Blaker

Abstract

The incidence of chronic wounds are increasing due to a growing and ageing population. Patients who are more susceptible to chronic ulcers include the elderly and diabetics. Over £2billion was spent on wound care in 2014 alone. Advanced wound care products are needed on chronic wounds as without intervention approximately 50% of these wounds do not heal.

The role of electrical regimes in skin has been documented, with local ionic currents playing a part in directing epidermal cell migration when closing full thickness wounds.

It has been recently documented that externally applied electrical stimulus can influence cell activity, both increasing cell proliferation and increasing differentiation and extracellular matrix production. This project will investigate the development of a fibrous conductive polymer wound dressing with a capacitive applied electrical field, that has the potential to aid the healing of chronic ulcers.

Main question to answered

1) What is the current state of the art regarding application of electrical regime to wounds?
2) Can the use of conductive polymers improve the wound repair by either altering the status of macrophages resident in the chronic ulcer or by increasing the proliferation / extracellular matrix production by dermal fibroblasts?
3) Can the use of an applied electrical regime via the conductive polymers improve the wound repair?
4) What is the optimal design of a conductive polymer wound dressing to ensure homogeneity of electrical field to be applied throughout the wound?
5) What is the mechanism of action of any proof of concept data obtained in this project?

ABM CDT Developing CXCR4-targeted drug delivery biomaterials for treatment of chronic lymphocytic leukaemia (CLL)

Supervisory Team: Dr Simon Webb, Dr John Burthem, Prof. Sabine Flitsch

Abstract

Present therapies for CLL can induce major disease regression but almost all cases will have a significant residual population of neoplastic cells [6]. Consequently, treatment duration is prolonged, incurring high financial cost, while the variability of residual CLL lymphocytes predisposes patients to the emergence of treatment-resistant clones. Targeted delivery of therapeutic agents within liposomal materials offers an innovative solution to this problem: selective delivery of therapeutic agents to CLL cell populations while protecting the contents from degradation, significantly enhancing drug effectiveness [7].

The proposal will exploit a new molecular motif (BAT1) that has recently been developed by Burthem and Webb [1,3]. BAT1 presents a bis(cyclam) that binds to the CXCR4 receptor with high affinity, and it has a hydrophilic tether that permits its linkage to surfaces through a primary amine terminus. The circulating cells of CLL have high expression of CXCR4, and the interaction between CXCR4 and its ligand CXCL12 controls cell migration and enhances the survival of CLL lymphocytes [8]. BAT1 therefore has several modes of action: (a) it blocks direct survival signals caused by CXCL12 [9] and impairs CLL cell emigration from the bloodstream into tissues; (b) its targeting function allows BAT1-liposomes to deliver their therapeutic contents to the CLL cells. We have recently shown that attachment of BAT1 to liposomes provides a multivalent display that supports binding to CXCR4-expressing cells from patients, leading to targeted delivery of the exemplar drug doxorubicin with high (relative) specificity and blocked migration of these CXCR4-expressing cells [1].

Main questions to be answered

1) In addition to our selective delivery of doxorubicin [1], we wish to deliver new therapeutic agents known to be effective against CLL (e.g. ibrutinib, fludarabine, mitoxantrone); these will be incorporated in the liposomes. The materials properties of the liposomes will be modified to optimise their carriage of different therapeutic cargos, and to promote endosomal escape.
2) Additional modifications of the liposomal surface to improve circulation and targeting will be explored. Glycolipids and cleavable PEG stealth lipids will be used in addition to BAT1 modification [2,4,5]. Lipid-attached glycan molecules, synthesised through a high-throughput chemoenzymatic approach [4], will be included in the liposome formation; these are selected to increase CLL-targeting specificity, adding functional capability or directing uptake-pathway.
3) To expand the concepts developed in 2) and further exploit this technology, the modification of other biomaterials with BAT1 and glycan motifs will be assessed. For example, carboxymethylcellulose (CMC) is a key component of wound-healing bandages and is currently studied by joint Webb/Flitsch and Webb/ConvaTec PhD students. The surface modification of CMC with S-Lex glycan could promote local accumulation and activation of cells of the adaptive immune system, which could significantly benefit both immune responses to tumours and wound treatment.

ABM CDT Development of an advanced biofabrication platform for cartilaginous tissue regeneration

Supervisory Team: Dr Stephen Richardson, Dr Marco Domingos, Dr Bilal Barkatali

Abstract

Fibrocartilaginous tissues, such as the meniscus and intervertebral disc (IVD), promote healthy locomotion and protect adjacent bony tissue by distribution of high levels of mechanical load. Damage to these tissues leads to long-term disorders which represent some of the most significant socioeconomic problems facing the UK and wider Western world today. For example, over 1.5 million people across the USA and Europe suffer from injuries to the knee meniscus every year with the majority progressing to early osteoarthritis, while degeneration of the IVD is the leading cause of back pain. Current surgical interventions offer poor long-term outcomes, with patients having a meniscectomy being 10x more likely to develop early osteoarthritis that the general population and back pain surgery offering no better long-term pain relief than no intervention. As a result of these limitations novel regenerative therapies are required.

Potentially the most promising novel approach employs 3D bioprinting to create tissue engineered constructs (scaffolds) with patient-derived cells and growth factors to support and stimulate the regeneration process of the tissue. However, most strategies developed so far have failed to generate constructs capable of mimicking both the structural as well as functional organization of the native fibrocartilaginous tissue, whilst being sufficiently robust to withstand the extreme biomechanics experienced within the knee joint or spine. This project aims to overcome these limitations by developing an advanced biofabrication platform where 3D bioprinting techniques, bioinks and stem cell technology will be combined towards the generation of biomimetic fibrocartilaginous substitutes with enhanced mechanical performance.

Main questions to be answered This project will focus on developing an integrated biofabrication platform for the automated manufacturing of fibrocartilaginous tissues with enhanced biomechanical performance. This will be achieved through the rational design of novel tough, printable, cell-compatible bioinks and subsequent combination with 3D bioprinting technologies capable of emulating the structural and functional features of the native load-bearing tissue microenvironment. The student will choose either meniscus IVD as an exemplar tissue and focus on the following primary questions:

1. What is the optimal bioink formulation capable of meeting, simultaneously, the requirements for high shape fidelity printing and cell viability?
2. Which parameters can be manipulated during the design of the bioinks (e.g. cross-linking degree) and printing process (e.g. scanning speed) to allow for the creation of stiff fibrocartilaginous tissue mimics which are capable of withstanding physiologically relevant loading?
3. Which biochemical moieties (e.g. adhesion proteins) and biofabrication strategies (e.g. multi-material extrusion in suspension baths) can be implemented to support survival and differentiation of seeded cells (mesenchymal stem cells or native fibrocartilaginous cells) and production of appropriate, functional extracellular matrix?

A range of techniques will be available to address these questions, including AFM, rheological/dynamic mechanical testing; bioprinting; ; MSCs and fibrocartilaginous tissue cell culture.

ABM CDT Development of hexagonal boron nitride reinforced light curable dental resin composites

Supervisory Team: Dr Xiaohui Chen (Helen), Prof. Julie Gough, Prof. Nick Silikas

Abstract

Dental caries is one of the most prevalent global diseases that continues to be a major problem for both adults and children. The global restorative dentistry market size has grown from $12.52 billion in 2014 to $15.6 billion in 2019 with an average annual growth rate of 4.5%1.

Resin composites are the most commonly used direct restorative material for restoring both posterior and anterior teeth. Despite modern resin composite restorations offer pleasing aesthetics and satisfactory clinical longevity, an annual failure rate of 1-4%2 was reported mainly due to fracture or secondary caries. Replacement of failed restorations consumes a significant amount of clinical time and imposes high financial costs for both health care systems and patient (e.g. £62.10 under NHS for 2020).

Graphene has been introduced to resin composites and resulted in improved physico-mechanical properties3,4 and increased antibacterial activities. However the potential for clinical application was significantly limited by aesthetics. A pilot study5 (led by the applicants) incorporating hexagonal boron nitride (hBN), a structural analogue of graphene with similar layered structure into UDMA resin composites, showed increased flexural strength without detrimental impact on curability. The study indicated that hBN has the potential as a reinforcing filler for dental resin composites and generated interests both in academia and industry. However, numerous challenges still remains in achieving the optimal resin composite formulation. Although hBN has been incorporated in epoxy resin composite system, there is very limited published literature for dental applications. Cytotoxicity and wear characteristics of hBN resin composites are important for clinical application however remain unknown.

References:

1Global Restorative Dentistry Market Report 2019

2Should my composite restorations last forever? Why are they failing? Brazilian Oral Research, 2017; 31 (suppl): e56.

3Graphene for the development of the next-generation of biocomposites for dental and medical applications. Dental Materials, 3017, 33(7), 765-774.

4Graphene composite with dental and biomedical applicability, Journal of Nanotechnology, 2018, 9, 801-808.

5Hexagonal Boron Nitride UDMA resin composite, IADR 2018.

Main questions to be answered

This fundamental translational research project therefore aims to develop hexagonal boron nitride (hBN) reinforced light curable dental resin composites with improved mechanical properties that are biologically safe for clinical application. A series of model resin composites with hBN and boron barium silicate glasses will be formulated and a range of clinical relevant properties will be characterised following ISO standard ISO 4049-2019 Polymer based restorative materials and methods recommended by Academy of Dental Materials. A collection of commercial resin composites will be used as control.

The questions to be answered include:

1.Reinforcing mechanism of hBN for light curable resin composites
2. Degradation of hBN reinforced resin composites
3. Cytotoxicity of hBN reinforced resin composites
4. The influence of hBN on the wear characteristics of resin

ABM CDT Suspended additive manufacturing of complex wounds for precision therapy testing

Supervisory Team: Mr Jason Wong, Dr Marco Domingos, Dr Adam Reid

Abstract

Complex wounds (leg and pressure ulcers, trauma wounds and burns) are common, high morbidity and costly problems. In the UK >1.6 per 1000 of the population are affected by complex wounds at any one time with an estimated annual cost to the health service of over £5 billion. The overall management of complex wounds in Greater Manchester is projected to be £300 million by 2020/21. The psychological burden of repeated healthcare visits, discomfort of dressing changes, and stigmatisation of illness is significant, making this a major healthcare priority. This is an area with a poor track record in translational medicine, and limited evidenced therapies to meet healthcare needs. The scientific field lacks relevant preclinical models encompassing the complexity of these heterogeneous wounds. 3D Bioprinting offers a potential route to generate 3D tissue models capable of mimicking the native wound tissue structural and functional heterogeneity by the precise spatial deposition of multiple materials, cells and bioactive compounds. However, most of the bioprinting techniques do not allow for direct manufacturing of constructs under physiological conditions. Using a novel suspended manufacturing system, cell-loadable materials can be printed directly inside fluid gels which act as scaffolding systems whilst being supplemented with cell culture media. Using patient complex wound samples to map the cellular heterogeneity allows for precision modelling of preclinical experiments and assay development relevant to advance clinical care.

Main questions to be answered

1) What are the cellular phenotypes that make up a complex wound and what is the composition of its matrix? This will be answered by sampling patient wounds and performing spatial transcriptomics on the samples to identify the cellular phenotype and spatial make-up of the wound
2) What cellular phenotypes and matrix components are common to most complex wounds. This will be answered by looking at numerous complex wound to generate a unifying “make up” of cells and matrix to be emulated through suspended additive manufacturing techniques.
3) Can we produce a “complex wound” model based on common components of the wounds using suspended additive manufacturing? This will be answered by multiple cell line suspended printing in hydrogels that have a simplified matrix and cellular profile to human complex wounds
4) Can wound therapies influence the complex wounds models? This will be answered by testing known therapies (cell based or antimicrobials) on these complex wound surrogates.

ABM CDT Peripheral nerve reconstruction: NO hope for advanced regeneration
Industry Project Partner

Supervisory Team: Prof. Julie Gough, Dr Adam Reid

Abstract

Traumatic peripheral nerve injury (PNI) is common and mainly affects the upper limbs of young economically active adults, of both genders. PNI presents serious economic consequences for the patient, and society as a whole, and despite modern microsurgical techniques, functional restoration is always incomplete. For the patient, the outcome is impaired hand sensation, reduced motor function alongside pain and cold intolerance; furthermore, the enduring nature of these symptoms frequently results in psychosocial morbidity and a lifelong impairment of well-being.

The greatest reconstructive challenge lies with the approximately 1 in 5 PNI patients with a 'nerve gap' (i.e. a nerve defect caused by the trauma) where direct repair of the two nerve stumps is not possible. Nerve gaps may be reconstructed using autologous nerve grafts, which sacrifice sensory function in parts of the lower limb in an effort to restore critical function in the hand. The outcomes following nerve grafting are poor and worsen with increasing gap distance.

We have developed a polymer nerve repair device '*Polynerve*' that has a simple easily upscalable manufacturing methodology; by incorporating grooves on the inner lumen of the conduit, it attempts to address the biology of the Schwann cell – guiding regeneration following PNI. We have completed Phase 1 clinical trials in Manchester with excellent outcomes on small nerve gap injuries.

Now, we seek to augment the biological response at the site of injury to favour regeneration. Nitric Oxide (NO) is a potent free radical that regulates multiple biological processes with involvement in neuronal and vascular regeneration likely via macrophage interaction. It is known that macrophage-induced new blood vessel formation leads Schwann cell-mediated regeneration of peripheral nerves; thus, NO is a potential intervention to augment PNI regeneration.

Main question to be answered

The main questions to be answered are:

1. Does inclusion of NO affect the mechanical and groove morphology properties of the current conduit?
2. Does NO release from the conduit at physiologically/pharmacologically relevant concentrations and rates?
3. What is the impact of NO release on Schwann cell phenotype/behaviour and new blood vessel formation?
4. Does the inclusion of NO at the optimised *in vitro* level enhance nerve repair in a rat *in vivo* model?
5. Does the combination of NO and the scaffold have an acceptable safety profile (ISO1993)

Projects based at the University of Sheffield

ABM CDT Positioning of multiple cell types to investigate inter-cell communication

Supervisory Team: Prof. Dan Allwood, Prof. John Haycock, Dr Munitta Muthana, Dr Frederik Claeyssens

Abstract

Intercellular communication is a major factor for directing the control, development and response of eukaryotic cells *in vivo*. Examples include: in skin tissue, where models containing fibroblasts results in a more mature stratified keratinocyte epithelium; in neuroscience, neuronal and glial cell communication is responsible for growth and differentiation; and with cancer cells and host cells within the tumour microenvironment (TME), where communication can affect the behaviour of cancer cells and promote tumour progression by conferring cancer cells with the ability to migrate, invade, and metastasise. Furthermore, the system of neuronal and Schwann cells requires intercellular contact for Schwann cell myelination and differentiation.

There are, however, many unanswered questions about the nature of intercellular communication and existing co-culture models place cells randomly, making systematic study impossible. An improved understanding of intercellular communication will help uncover the tools used in cell-cell interactions and is essential for investigating cell evolution and dynamics (e.g. for nerve re-growth or understanding cancer growth). This understanding would also assist in the development of drugs to interrupt communication with diseased cells (e.g. cancer cells) to improve clinical outcomes.

A major obstacle to studying inter-cell communication quantitatively is a lack of suitable tools. The development of an appropriate platform to study cell-cell interactions promises to have widespread clinical impact relevant to a range of contexts, including neuronal function, tissue regeneration, cancer and stem cell differentiation.

Main question to be answered

The two main questions posed by this project are:

1. How can different cell types be positioned with defined proximity to each other?
2. To what extent does the cell positioning ability allow us to study inter-cell communication in clinically-relevant systems?

Answering Question 1 must consider that an ideal *in vitro* system should:

a. Allow different cell types positioning with pre-determined separation or contact
b. Be compatible with all cell types
c. Allow microscopy analysis of interacting cells
d. Support large numbers of cell interactions to create sufficient expressed factors for biochemical analysis
e. Be compatible with microfluidic systems

The project will investigate using patterns of magnetic materials and chemically modified regions of a surface to define trap locations for different types of cell. Previous studies have only ever considered one cell type at once, although these show that cell viability is generally good.

Question 2 will be tackled using example cell systems, e.g. neuronal and Schwann cells, or human breast and prostate cancer cells with tumour-infiltrating primary host cells, including human endothelial cells, fibroblasts and immune cells. These will be co-cultured at different cell-type-separations and investigated either by microscopy (single cell pairs) or biochemically (large cm-scale arrays of traps).

ABM CDT Bone Clip: Developing an angiogenic biomaterial to increase bone healing

Supervisory Team: Prof. Gwen Reilly,Dr. Frederik Claeyssens

Abstract

Medical science has increased life expectancy by ~30% in the last 100 years. This increase in life expectancy is unfortunately not matched by a growth in health expectancy (the number of years in good health) and currently there is, on average, an 8-10 years gap between our life and health expectancy in the UK. During this period, many of us will suffer age-related diseases, which reduce our quality of life and come with a substantial socio-economic cost. For example, osteoporotic fractures cost €37 Billion and result in over a million of years in good health (quality adjusted life years) lost in the EU in 2010.[1] This project presents a conceptually simple approach to tackling delayed bone healing by using a proangiogenic biomaterial to clip around the damaged bone and stimulate angiogenesis to accelerate bone regeneration. In essence, we aim to develop and evaluate a periosteal bone substitute for clinical applications. This PhD project will build unique expertise to evaluate a tailored solution to this healthcare problem.

In this project we will investigate a synthetic, easy to handle biomaterial can be produced at scale, to release small stable molecules to stimulate angiogenesis when clipped around a non-healing bone defect to initiate bone repair.

Main questions to be answered This project will study angiogenesis driven bone formation through harnessing innovative fabrication platforms based on polycaprolactone-based polymerised high internal phase emulsions (polyHIPEs). These porous materials can be additive manufactured to include a prototype vasculature. The periosteal substitute material needs to be highly porous to encourage endogenous cell migration and to be easy to load with proangiogenic agents if required. [2-5] The programme of work is described under the following objectives:

1) To optimise the production polyHIPE-based porous membranes via additive manufacturing supporting ingrowth of new blood vessels.
2) To optimise angiogenic factor loading in polyHIPE membranes demonstrating temporal release and evaluating biocompatibility and proangiogenic activity using cultured endothelial cells.
3) To evaluate loaded and unloaded polyHIPEs with proangiogenic agents for angiogenesis using the CAM assay and the bone defect model.

We will aim to produce a periosteal substrate which can be produced at scale from a well-accepted biodegradable material which can be sterilised and stored as an off-the-shelf sterile material. We hypothesise that by paying attention to the microfabrication of this PCL-based material we can achieve a relatively sophisticated membrane that can be produced at scale and presents a straightforward regulatory route to the clinic.

ABM CDT Microfabricating next-generation corneal membranes via the inclusion of partially-enclosed artificial niche structures

Supervisory Team: Dr Ilida Ortega Asencio,Dr Frederik Claeyssens

Abstract

Corneal disease affects millions of people worldwide with higher prevalence in developing countries. Corneal transplantation and the use of membranes as cell carriers (amniotic membrane, AM) have been relatively successful. Unfortunately, AM is costly and its availability is limited.

Researchers at Sheffield have been working together with LV Prasad Institute (LVPEI, India) with the aim of delivering new alternatives for simplifying corneal treatments and therefore increasing their accessibility. One of our approaches has been the development of a synthetic AM substitute that includes microfeatures to mimic the limbal niches of the cornea. Limbal stem cells are believed to reside in the limbus in well-define microenvironments or niches; these niches provide physical support to the limbal stem cells. Our first prototype niche-containing materials were regarded by our clinical collaborators at LVPEI as potentially very useful to assist them at the time of surgery. These niche structures could be use as guiding points and as points for securing tissue explants to the delivery membranes avoiding the use of fibrin glue (fibrin is expensive and not available in many countries and requires considerable expertise in its use).

Therefore, in this project we aim to design and manufacture a new microfabricated corneal membrane with improved partially enclosed niche designs, able to retain the corneal tissue explants delivered during SLET surgery (Simple Limbal Epithelial Transplantation). The project also will aim to understand the biological contribution of incorporating such niche structures to the membranes using an ex vivo corneal model previously developed and optimised at Sheffield.

Main questions to be answered

The main questions to be answered will be :

1) Main Manufacturing Challenges:

a) Can we use electrospinning and 3D-printing approaches to design a new niche structure which is partially enclosed and able to self-hold a tissue explant (~500 µm size)?

b) Can we incorporate these niche designs within a cell delivery membrane and can the degradability and the mechanical stability of this microfabricated membrane be controlled?

2) Biological Questions:

a) How will epithelial and stromal cells residing in the tissue explants respond to different niche morphologies and sizes?

b) Would these niche structures have an impact in differentiation/migration pathways?

c) How these niche structures will impact on the regeneration of a wounded corneal epithelium?

ABM CDT 3D Printed Bespoke Biodegradable Drug Eluting Coronary Artery Stents Produced using Natural Polymers

Supervisory Team: Prof. Ipsita Roy Dr Vanessa Hearnden, Dr Javaid Iqbal

Abstract

Coronary artery disease (CAD) is caused by narrowing of arteries due to the hardening of cholesterol, fats, and other components of the blood, causing inadequate supply of oxygen rich blood to the heart, leading to myocardial infarction. The most promising treatment of CAD is angioplasty which involves mechanical widening of narrowed blood vessels followed by the deployment of a coronary artery stent. The stent is deployed in its collapsed state and inflated inside the narrowed blood vessel, thus restoring normal blood flow (Figure 1).

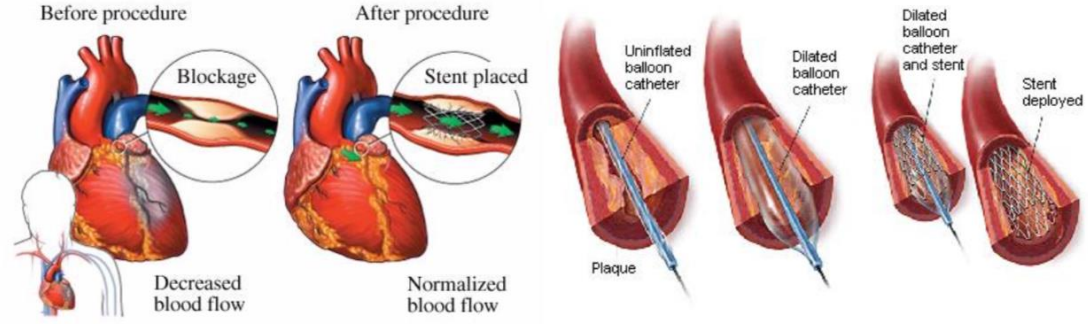


Figure 1: The Stent Placement Technology (a) Arterial blockage (b) restored blood flow post stent deployment (c) ballooning without stent, (d) ballooning with stent (from http://www.bostonscientific.com/lifebeat-online/cardiac-procedures/angioplasty-and-stents.html http://supplements-daily.com/tag/heart-stent/)

There are three types of coronary artery stents, bare metal stents (BMS), drug eluting stents (DES) and the Bioresorbable Vascular Scaffolds (BVS). BMS and DES are metallic stents that result in foreign body reactions including inflammation, in-stent restenosis, thrombosis and stent jailing. BVS are made using bioresorbable polymeric materials which are tailored to resorb once the artery is fully repaired and its function is restored, hence preventing all the above-mentioned problems caused by the BMS and DES. However, the first BVS, ABSORB, produced by Abbott had to be withdrawn due to relatively poor clinical outcomes. The ABSORB stents had limited expansion, suffered from fracture problems, low radial strength, sub optimal strut width and negative recoiling.

The aim of this project will be the development of novel Polyhydroxyalkanoate (PHA)-based 3D printed biodegradable coronary artery stents with tailorable mechanical properties, degradation rates, produced using patient specific CT scans and hence bespoke to specific patient needs, an absolutely unmet clinical need.

Main questions to be answered:

1. Optimal conditions for the production of a range of different PHAs including scl-PHAs, mcl-PHAs and scl-mcl-PHAs (copolymers).
2. The optimal PHA blend/PHA copolymer with the required mechanical properties for a coronary artery stent (high Young's modulus and reasonable elongation at break, enabling the stent to inflate and provide the required stiffness and radial strength).
3. The optimal PHA blend/ copolymer with the required degradation rate. Ideally the stent needs to degrade completely within 2 years.
4. The *in vitro* biocompatibility and haemocompatibility of the developed material
5. The optimal conditions for 3D printing of the stent using the chosen PHA blend/copolymer.

Figure 2: 3D printed PHAs

6. *In vitro* functionality of the 3D printed stents to be tested using a Biomechanical Reactor System (BMRS) provided by BioCompatible Engineering Solutions where the conditions within a coronary artery will be simulated.

This project involves the use of a unique family of biomaterials, Polyhydroxyalkanoates (PHAs). These are a highly biocompatible and biodegradable family of natural polymers, produced by bacterial fermentation and have huge potential as advanced biomedical materials. PHAs exhibit a range of mechanical properties, hence PHA blends or PHA copolymers can be used to achieve the optimal demanding mechanical properties required for a stent. Also, the rate of degradation of the PHAs can be tailored to match the required degradation time of two years. The degradation products of PHAs are natural metabolites, such as 3-hydroxybutyryl-CoA, hence are non-toxic. Finally, and not the least, PHAs degrade via surface degradation, and hence degrade in a controlled manner, a highly desirable property for a stent.

ABM CDT Bioactive nerve guides – anti-inflammatory and anti-scarring devices

Supervisory Team: Prof. John Haycock, Dr Fred Claeyssens, Prof. Fiona Boissonande, Dr Simon Atkins

Abstract

Injury to peripheral nerves through trauma, and sometimes surgery, results in over 300,000 cases each year in the EU. In contrast to the central nervous system, proximal motor and sensory axons have some ability to repair. Individuals who sustain injury with no loss of tissue can be treated by directly suturing proximal and distal ends together, as end-end anastomosis. A fundamental understanding of the molecular and cellular responses to injury is essential when designing approaches for repair, especially for implantable nerve guide conduits (NGCs). We have reviewed NGC performance in detail[1], with conclusions supporting biomaterials improvements in NGCs a realistic approach (e.g. versus cell therapy). NGCs are typically made from inert biomaterials (e.g. polyesters, collagen), and do not stimulate neuronal or Schwann cell adhesion, migration or differentiation for nerve repair. Consequently, existing devices are poor at supporting regeneration. A major challenge is to increase regeneration distance from a few millimetres to critical gap distances of 10-20 mm. For clinically practical improvement, simple innovations in the biomaterial chemistry, in combination with fabrication methods for making porous and flexible materials to reflect the mechanical properties of nerve are proposed and will be investigated in this project. In this project we also will address the problems of inflammation and scarring associated with nerve repair. Devices will therefore delivery key anti-inflammatories (a-MSH, IL-10) and/or an anti-scarring compound (M6P) known to improve functional repair. Devices will be evaluated *in vitro*, and *in vivo* in this PhD project, with a route to following on a clinical study of lingual nerve reconstruction.

Main question to be answered

1. **Synthesise PGS blends with controlled degradation rates and mechanical properties as candidate materials for NGCs. The main questions are: a) suitability of PGS as a nerve implant biomaterial and b) value in exploiting soft mechanical properties of PGS for this purpose.** PGS has been explored for cardiac patches and for retinal transplantation regeneration, but little to date on nerve repair. We recently published on PGS for supporting neuronal and Schwann cell growth *in vitro* and nerve repair *in vivo,* using a 1:1 ratio of glycerol:sebacic acid. This created a flexible polymer with favourable mechanical properties for soft nerve repair (3.2 MPa). PGS will be formulated as a low molecular weight prepolymer, which cross-links to produce a fully cross-linked elastomer (using in house methods[2]). A range of blend ratios with varying modulus to support the growth of neuronal and Schwann cells will then be investigated. Primary neuronal and Schwann cells will be cultured on surfaces for 4 days to facilitate neurite sprouting, using methods developed in-house (e.g.[3]) for selection of optimal blends.

2. **Fabricate PGS blends to form NGCs by microSL. The main question is on identifying an optimal PGS blend suitable for making NGCs by 3D printing.** Optimal blends will be investigated in detail for NGC manufacture. Templates will be built by micro-sterolithography (microSL), which enables accurate and rapid construction of 3D scaffolds to make NGCs. We have published on core methods for PGS NGC manufacture[4], and will extend these to investigation of porosity. Dimensions will be fabricated suitable for a critical mouse sciatic nerve 6 mm injury gap (8 mm length x 0.9 mm internal diameter x 250 µm wall thickness).

3. **Investigate the problems of inflammation and scarring associated with nerve repair. The main question is whether an increase in inflammation will impede regeneration, and whether local delivery key anti-inflammatories (a-MSH, IL-10) and/or an anti-scarring compound (M6P) will improve functional repair.** Acute injury triggers an inflammatory response necessary for early repair, however chronic inflammation is known to be damaging, and can also lead to scar formation. We will therefore combine the scaffold properties of nerve guides, with the therapeutic delivery of a-MSH (a potent anti-inflammatory peptide), IL-10 (an anti-inflammatory cytokine) and/or mannose-6-phosphate (an anti-scarring compound). We have separately published on the roles of a-MSH, IL-10 and M6P as potential therapeutic agents.

4. **Evaluate prototype NGCs using a dorsal root ganglion 3D *in vitro* chick model. The main question is whether the chick dorsal root ganglion model will allow quantitative evaluation of porous constructs and identify optimal devices according to PGS blend and degree of connected porosity.** Analysis will be undertaken for devices using an *in vitro* 3D chick dorsal root ganglion model established in our group (Behbehani et al, 2018). The model uses chick DRGs isolated at embryonic day development 12 (EDD12), and allows axon and Schwann cell migration distance to be determined. Variables in PGS blend and porosity (using a fixed number of optimally aligned fibres) by light sheet microscopy will be undertaken.

5. **Evaluate selected NGCs using thy-1 YFP mouse and rat *in vivo* models. The main question is to identify if an optimal PGS blend and porous density from *in vitro* selection (above) supports axon regeneration *in vivo*.** *In vivo* evaluation will be conducted on NGCs using genetically modified mice with a subpopulation of axons that express yellow fluorescent protein (YFP). Studies in our lab using this model allow quantification of axon regeneration across injury sites.[3,4] A critical 6 mm sciatic nerve gap injury will be created using a subset of optimally performing devices will be evaluated at 12 weeks following implantation.

ABM CDT Biomimetic responsive signalling systems

Supervisory Team: Prof N. H. Williams, Prof G. J. Leggett

Abstract

The transmission and amplification of chemical signals across lipid bilayer membranes is central to many biological processes, from the development of multicellular organisms to information processing in the nervous system. This signal transduction is often associated with an amplified signalling cascade. The ability to reproduce such processes in artificial systems has potential applications in sensing, controlled drug delivery and communication between compartments in tissue-like constructs of membrane compartments. Furthermore, the development of systems that can modulate biomolecule activity, protein immobilisation, and cell adhesion and migration at the liquid–solid interface will be tremendously useful in diverse biological and medical applications. Mimicking the dynamic properties of biological systems requires the creation of responsive artificial systems that can control the presentation of regulatory signals to dynamically regulate biological functions in response to applied stimuli.

For example, the ability to detect local changes in pH is important in the detection of disease; so the development of methods for rapid, non-invasive assessment of changes in pH in complex media is important. As well as detecting changes in the contacting medium or the presence of specific signalling molecules, it is desirable to modulate protein or cellular interactions with synthetic surfaces that can respond to the signal by producing the controlled release of any desired therapeutic – leading to a more effective sensing and responding system.

Main questions to be answered

The main goal of this project will be to create a biomimetic transducer system which can respond to the complex environment of biological media, amplifying any signal and reacting to release novel materials as desired. The system will be generic, so that different targets can be addressed, and any small molecule released in reaction to the surrounding environment. The system will be dynamic, capable of amplifying the chosen signal and of returning to a resting state when the surrounding environment returns to its original condition. In earlier work, we have established a novel and versatile system which functions within vesicles. We aim to integrate this system within suspended membranes near patterned surfaces, so that the solid state can react to “wet” biological conditions.

ABM CDT Development of an in vitro musculoskeletal model of human ageing

Supervisory Team: Dr Nicola Green, Dr Fred Claeyssens, Prof Gwen Reilly, Dr Aileen Crawford

Abstract

Globally, musculoskeletal (MSK) conditions are the leading contributor to disability and are commonly linked with depression and negative impacts on quality of life. MSK conditions have a significant economic impact worldwide and their prevalence is predicted to rise with an increasingly ageing global population.

Currently drug treatments for MSK conditions are screened using monocultures or, at best, co-cultures of cells growing in monolayers in vitro. However, the cell-cell and cell-matrix interactions within the tissues together with the differing mechanical and structural properties of these tissues at the interface between muscle, bone and cartilage mean that monolayer models fail to capture these interactions. MSK ageing also induces extracellular matrix changes but simple 2D monocultures are incapable of recapitulating these changes in any meaningful way. As a consequence, researchers often turn to animal models. However, important differences in size, anatomy and biomechanics limit their relevance and hinder successful outcomes in the MSK drug development.

Attempts to develop 3D models have been described in the literature, but success in this area is limited with many versions incorporating the cells from only one tissue type and therefore omitting the interactions between cell types. There is therefore an unmet need for a reliable, biologically relevant in vitro model of the MSK interface that can reduce and replace animal models, reflect the ageing process and thereby accelerate the development of MSK treatments.

Main questions to be answered

The project will build an in vitro MSK model containing three tissue types: bone, cartilage and muscle. Cells will be grown on 3D microporous polyHIPE (polymerised high internal phase emulsions) scaffolds, with graded mechanical properties to mimic those of the tissues. Electrospun, cell impermeable barriers will be used between regions of polyHIPE to limit cell infiltration while still allowing cell signalling to occur to ensure spatially controlled growth of cells. This will allow us to answer the following:-

1. Can a model be constructed which recapitulates the basic physiology of bone, muscle and cartilage? This will be assessed through (i) cell metabolic activity and proliferation, (ii) expression of relevant chondrogenic, osteogenic and muscle gene expression, appropriate extracellular matrix (ECM) production and mineralisation (for bone model), (iii) histological evaluation, (iv) cell imaging
2. Can ageing by induced through exposure to compounds known to promote the process of ageing? Advanced glycation endproducts (AGEs) and reactive oxygen species (ROS) are produced spontaneously during metabolism. They play important roles in MSK ageing in vivo and in vitro.
3. Can the healthy and aged models be used to asses cell response to compounds used in MSK treatments e.g. ibuprofen, known to reduce bone healing and cartilage synthesis; and vitamin D, which may affect bone and cartilage regeneration?