22nd Annual White Rose Work in Progress Meeting

Thursday 17th December 2020

Virtual Online Event

Hosted by
The University of Leeds

Biomaterials & Tissue Engineering Group
22nd Annual White Rose Work in Progress Meeting

Thursday 17th December 2020
Virtual Online Event
Hosted by the University of Leeds

PROGRAMME

TIME	SPEAKER	TITLE
9:15-9.50	**JOINING OF DELEGATES TO ONLINE CONFERENCE**	
9.50-10.00	Welcome by Dr Anthony Herbert, University of Leeds	
	Session 1: Chair – Dr Richard Foster	
10:00-10:15	Joe Woodley	Understanding fibroblast behaviour in 3D fibrous biomaterials
10:15-10:30	David Ramos	The inclusion of microtopographical cues in electrospun scaffolds to mimic the rete ridges stem cell microenvironment
10:30-10:45	David Alexander Gregory	3D printing of Polyhydroxyalkanoates for biomedical applications
10:45-11:00	Caroline Taylor	Aligned Polydroxyalkanoate Blend Fibres for Peripheral Nerve Repair
11:00 - 11:30	**TEA/COFFEE AND POSTER PITCHES 1-5**	
	Session 2: Chair – Dr Jen Edwards	
11:30-11:45	Zhan Yuin Ong	Bioinspired Design of Monodisperse Nanomedicines for Stimuli-Responsive Drug Delivery Applications
11:45-12:00	Amy Harding	Determination of chemical sensitising potential using a defined gene signature set using tissue-engineered human skin equivalents
12:00-12:15	Megan Sharrock	Haemarthrosis in Haemophilia: A Proposed Pathway for the Multifactorial Pathogenesis of Blood-Induced Arthritis
12:15-12:30	Inmaculada Barragan Vazquez	Development of an in vitro immunocompetent human tissue-engineered model of atopic dermatitis
12:30–14:00	**LUNCH AND POSTER PITCHES 6-10**	
14:00-14:30	**Plenary Talk: Redefining Identity of Disease, Tissues and Cells – a Biomaterials Paradigm** **Professor Abhay Pandit** **Director, CÚRAM - SFI Research Centre for Medical Devices; National University of Ireland; Galway, Ireland**	
	Session 3: Chair – Dr Rosti Readioff	
14:30-14:45	Daniel White	A PhD from the Kitchen: Design and Fabrication of a Novel Gel Electrophoresis System for Gene-Activated Matrix Production
14:45-15:00	Samuel Higginbotham	Can Myofibroblast Differentiation be Reversed with Secreted Factors from Adipose Tissue?
15:00–15:30	**TEA/COFFEE AND POSTER PITCHES 11-15**	

	Session 4: Chair – Dr Halina Norbertczak	
15:30-15:45	Kern Cowell	Development and initial testing of a novel automated decellularisation system
15.45-16:00	Debora Morgante	Onlay grafts of acellular matrix to augment the peri-urethral tissue bed for hypospadias repair
16:00-16:15	Patrick Statham	Permeabilising decellularised osteochondral scaffolds for improved chondrocyte penetration
16:15-16:30	Carmen Piras	Self-assembled multicomponent microgels for biological applications
16:30-16:45	**Prizes and presentations**	
16:45	**CLOSE**	

POSTER PITCHES

POSTER	PRESENTER	TITLE
1	William Sanderson	Lack of Standardisation in Mechanical Testing of the Patella Tendon: A Review and Guide
2	Lakshmi Tripathi	Optimisation and Characterisation of Bacterial Cellulose produced by Gluconacetobacter xylinus
3	Emmanuel Asare	A next generation bioinspired device for effective peripheral nerve regeneration
4	Annabelle Fricker	Cardiovascular Tissue Engineering using Natural Polymers
5	Ana Sandoval-Castellanos	Delivery of immobilised NGF and BDNF via a bioactive surface to enhance neurite outgrowth
6	Alexander Boyadjiev	Development of Technologies to Support the Robust and Reproducible Growth of Complex Bioengineered Human Skin Equivalents
7	Asma El Howati	Development of a multi-cellular tissue engineered model of oral lichen planus
8	Syed Mohammad Daniel Syed Mohamed	Kidney Tissue Engineering using Polyhydroxyalkanoates
9	Jonathan Hinchliffe	Semi-artificial pancreas for the treatment of Type 1 diabetes: Perspectives, challenges and solutions
10	Laetitia Raynal	Increasing the potency of biomaterials for tissue growth
11	Nicholas Rose	A Dynamic Biomaterial-ligand Tethering Strategy for Tissue Engineering
12	Fahad Alhamoudi	The HA Particle size and quantity effect on the chemical and Biological Behaviour of Polyurethane and hydroxyapatite scaffolds
13	Jacqueline Solis	Modification of Decellularisation Methods to Assess the Effects of Swelling on the Mechanical Properties of Porcine Tendon
14	Kern Cowell	The development of a 20-year economic model for the cost-effectiveness analysis of using decellularised bone versus fresh-frozen allograft as an acetabular impaction bone graft during a revision hip arthroplasty
15	Sara Memarpour Hobbi	Native nerve cell-derived extracellular matrix for peripheral nerve regeneration

Online posters for each of the above are available at:
https://www.imbe.leeds.ac.uk/biteg-2020/biteg-posters-2020/

Plenary Talk

Abhay Pandit is the Established Professor in Biomaterials and Scientific Director of a Science Foundation Ireland funded Centre for Research in Medical Devices (CÚRAM) at the National University of Ireland, Galway. Prof Pandit has over thirty years of experience in the field of biomaterials. After a seven-year stint in the industry, he has worked in academia for the last 18 years. Prof. Pandit's research is funded by Science Foundation Ireland, the 7th EU Framework programme, Enterprise Ireland, Health Research Board, the AO Foundation and industry sources, which in excess of €100 million. Prof. Pandit was elected to the American Institute of Medical and Biological Engineering (AIMBE) College of Fellows in recognition of his outstanding contributions to the creation of a national centre to develop innovative device-based solutions for the treatment of global chronic diseases. He is the first Irish academic to earn this distinction. He is the author of 27 patents and has licensed three technologies to medical device companies. Prof Pandit has published >290 papers in peer-reviewed high impact journals, >700 conference abstracts with an h-index of 61 and ~14,250 citations. Prof. Pandit has successfully supervised 35 PhD students, 24 postdoctoral researchers with a current cohort of 15 Postdoctoral researchers, 20 PhD students and two research associates.

Redefining Identity of Disease, Tissues and Cells – a Biomaterials Paradigm

Abhay Pandit
Director, CÚRAM- SFI Research Centre for Medical Devices;
National University of Ireland; Galway, Ireland

Abstract

Biomaterials are no longer considered innate structures and using functionalization and biofabrication strategies to modulate a desired response whether it is a host or implant is currently an important focus in current research paradigms. Fundamentally, a thorough understanding of the host response will enable us to design appropriate strategies. The input from the host response needs to be weighed in depending on the host disease condition. Our current inputs have been through a thorough understanding of glyco-proteomics based tools which we are developing in our laboratory. In addition, biomaterials themselves provide immense therapeutic benefits which needs to be accounted in the design paradigm. Using functionalization strategies such as enzymatic and hyperbranched linking systems, we have been able to link biomolecules to different structural moieties. The programmed assembly of biomolecules into higher-order self-organized systems is central to innumerable biological processes and development of the next generation of biofabricated scaffolds. Recent design efforts have utilized a glycobiology and developmental biology approach toward both understanding and engineering supramolecular protein and sugar assemblies.

Podium Presentation Abstracts

Understanding fibroblast behaviour in 3D fibrous biomaterials

Joe Woodley[1], Daniel Lambert[1], Ilida Ortega Asencio[1]
[1]School of Clinical Dentistry, University of Sheffield, Sheffield, UK

Abstract

Introduction
Fibroblasts were once considered simple cells with limited roles in extracellular matrix (ECM) deposition and cellular support. We now know that these cells have key roles in health and disease. Three-dimensional (3D) culture systems have allowed us to investigate fibroblast activity, specifically the relationship between fibroblasts and their natural environment, the ECM. Fibroblasts were first cultured in 3D using collagen hydrogels and studies focussed on proliferation, migration and collagen deposition. In the years that followed, decellularized matrices, synthetic hydrogels and a range of modern biomaterials were used to culture fibroblasts. The interplay of biological features such as integrin binding sites in natural hydrogels, mechanical properties like stiffness and tension in synthetic gels and microarchitectural features like pore and fibre size in novel biomaterials have made understanding the role of the ECM in fibroblast behaviour a challenging task.

Aims
Previous research conducted in our group reported the use of electrospun membranes as potential tools to keep a fibroblast population in a non-active and non-proliferative state. The aim of this project is to manufacture and characterise Polycaprolactone (PCL) fibrous electrospun scaffolds for the characterisation of fibroblast behaviour, beginning with proliferation and viability assays.

Methods
Cell viability was measured and the amount of dsDNA quantified using PrestoBlue™ and PicoGreen™ assays, respectively. To assess whether changes in viability and proliferation were due to increased cell death on electrospun scaffolds, Calcein-AM was used to stain live cells and Ethidium homodimer-1 to stain dead cells.

Results and Discussion
Data from PrestoBlue and PicoGreen assays suggest that 3T3 fibroblasts are less proliferative when grown on our scaffolds. Live/Dead staining shows a modest increase in cell death on the scaffolds, though it is unlikely to be solely responsible for the large fall in viability and levels of dsDNA present on the scaffolds. Further work is required to establish whether the changes in proliferation rates are due to reduced substrate suitability or represent the formation of a quiescent *in-vivo* like population of fibroblasts. The next step will include cell cycle screening studies which will allow us to understand how fibroblasts become less proliferative in our membranes.

The inclusion of microtopographical cues in electrospun scaffolds to mimic the rete ridges stem cell microenvironment

David H Ramos[1]*, Sheila MacNeil[2], Frederik Claeyssens[2] & Ilida Ortega Asencio[1]
[1]The School of Clinical Dentistry, University of Sheffield, Sheffield, UK
[2] Kroto Research Institute, North Campus, University of Sheffield, Sheffield, UK

Abstract

Human skin requires a highly efficient regeneration mechanism to serve as a protective barrier. Adult stem cells use their regenerative capabilities to proliferate and differentiate to support skin maintenance. In the skin, adult stem cells reside on microenvironments known as rete ridges that enhance nutrient diffusion and improve the mechanical properties of the dermal-epidermal junction (DED) [1]. Our research group has reported the potential of using electrospun membranes to mimic both the ECM structure and the morphology of the native topographical cues found in soft tissues including skin [2][3]. The aim of this project is to incorporate topographical features to electrospun scaffolds for exploring their effect on skin regeneration using skin tissue engineered models.

Polycaprolactone (PCL) was used to fabricate topographically controlled electrospun scaffolds using 3D-printed collectors. The effects of introducing topographical cues in cell metabolic activity were measured using human dermal fibroblasts (HDF) and keratinocytes (HDK). Skin tissue-engineered models were used to evaluate the performance of the scaffolds to mimic the topography of the rete ridges. Histology and Immunocytochemistry were used to analyse the models. The TCES were successfully fabricated and tested *in vitro*. The metabolic activity of HDF and HDK was higher on the TCES in comparison with a plain PCL scaffold. Histological analysis showed the formation of ridge-like structures for groups that were implanted with TCES, as well as an improvement in cell infiltration and tissue integration in comparison with plain PCL scaffolds.

In summary, the inclusion of topographical cues in the scaffold design is a promising approach to recreate to a degree the native morphology of the rete ridges in the skin. Our data show the potential of using our microfabricated scaffolds to promote cell infiltration and the generation of niche-like structures that resemble in morphology and function the skin native microenvironment.

References

[1] K. T. Lawlor and P. Kaur, *Int. J. Mol. Sci.*, 2015.
[2] Í. Ortega, A. J. Ryan, P. Deshpande, S. MacNeil, and F. Claeyssens, *Acta Biomater.*, 2013.
[3] I. Ortega Ascensio, S. Mittar, C. Sherborne, A. Raza, F. Claeyssens, and S. MacNeil, *J. Tissue Eng.*, 2018.

3D printing of Polyhydroxyalkanoates for biomedical applications

David Alexander Gregory1 *, Annabelle Fricker1 , Emmanuel Asare1 , Ipsita Roy1,2
1 Department of Material Science and Engineering, Faculty of Engineering, University of Sheffield, Sheffield, UK
2 National Heart and Lung Institute, Faculty of Medicine, Imperial College London, London, United Kingdom

Abstract

In recent decades 3D printing (additive manufacturing) has started to become an integral part in the development of 3D scaffolds for biomedical applications. This has resulted in advanced material development for use with 3D printing technologies. An important challenge in tissue engineering is the move from classical 2D to 3D scaffolds, which mimic the natural habitat of cells. [1] Some key biomedical applications include wound healing patches, patient specific implants and devices. Successful implementation of these scaffolds depends on the biomaterials, their properties and the manufacturing technologies used. In this context, Polyhydroxyalkanoates (PHAs) have exhibited exceptional value. [2, 3]

PHAs are natural, thermoplastic, easily processable, biopolymers sustainably produced via bacterial fermentation and exhibit excellent biocompatibility towards a large variety of cell types. [4] Moreover, their mechanical properties are tuneable and they degrade non-toxically via surface erosion, making them excellent candidates as scaffold materials. [2]

Here, we demonstrate the successful 3D printing of PHAs via fused deposition modelling of various CAD-designed structures for use in tissue engineering. [1] Further, we demonstrate the ability to print novel multi-material constructs with PHAs and alginate. The printed constructs are investigated for cell viability and tensile properties are tested. Finally, the prospects of PHA-printed scaffolds for next generation in-vitro and in-vivo studies are discussed.

References:

[1] E. Asare, D. A. Gregory, A. Fricker, E. Marcello, A. Paxinou, C. S. Taylor, J. W. Haycock, I. Roy, in The Handbook of Polyhydroxyalkanoates, Vol. 3 (Ed: M. Koller), CRC Press, 2020, 231.
[2] A. V. Bagdadi, M. Safari, P. Dubey, P. Basnett, P. Sofokleous, E. Humphrey, I. Locke, M. Edirisinghe, C. Terracciano, A. R. Boccaccini, J. C. Knowles, S. E. Harding, I. Roy, J Tissue Eng Regen Med 2018, 12, e495.
[3] Q. A. Majid, A. T. R. Fricker, D. A. Gregory, N. Davidenko, O. Hernandez Cruz, R. J. Jabbour, T. J. Owen, P. Basnett, B. Lukasiewicz, M. Stevens, S. Best, R. Cameron, S. Sinha, S. E. Harding, I. Roy, Frontiers in Cardiovascular Medicine 2020, 7.
[4] P. Basnett, E. Marcello, B. Lukasiewicz, R. Nigmatullin, A. Paxinou, M. H. Ahmad, B. Gurumayum, I. Roy, J Funct Biomater 2020, 11.

Aligned Polydroxyalkanoate Blend Fibres for Peripheral Nerve Repair

C. S Taylor[1], M. Behbehani[2], A. Glen[1], R. Nigmatullin[3], P. Basnett[4], F. Claeyssens[1], I. Roy[1] and J.W. Haycock[1]

[1]Department Materials Science & Engineering, University of Sheffield, UK.
[2]The Electrospinning Company, Unit 5, Harwell Campus, Harwell, Didcot, UK.
[3] Advanced Composites Collaboration for Innovation and Science, University of Bristol, Bristol, UK.
[4]Applied Biotechnology Research Group, School of Life Sciences, University of Westminster, London, UK.

Abstract

Nerve guide conduits (NGCs) are a favourable alternative repair strategy, to autografts, as they avoid issues such as donor site morbidity. Hollow NGCs lack natural guidance cues and so the addition of guidance scaffolds, such as electrospun fibres, is a popular research approach shown to increase nerve regeneration distances[1]. Polyhydroxyalkanoates (PHAs) are a favourable synthetic material, for tissue engineered scaffolds, due their biocompatibility and favourable mechanical properties.

P(3HB) and P(3HO) were produced by bacterial fermentation and characterised as previously described[2]. Electrospun aligned PHA fibres, with diameters of 5 and 8µm, were fabricated and quantified by SEM and microCT for fibre alignment and diameter. Rat Dorsal Root Ganglion (DRG) bodies were extracted and explanted whole on to the ends of a 3D in vitro fibre testing method[3]. DRGs were labelled for β III tubulin and S100β for quantification.

Aligned PHA fibres supported neurite outgrowth, and primary Schwann cell migration from DRG explants. The highest average neurite outgrowth length was measured at 3.94 ± 0.38 mm on 5µm P(3HB):P(3HO) 50:50 fibres. Blends of PHAs can be fabricated into aligned fibre scaffolds using electrospinning, with known fibre diameters and are a promising material to aid and improve nerve regeneration.

This work was funded by the European Community's Seventh Framework Programme (FP7–NMP–2013–SME–7) for NEURIMP under grant agreement no 604450.

REFERENCES:

1. Bell J et al. Tissue Eng Part B Rev. 18: 116-128, 2012.
2. Basnett et al. Microbial Biotechnology 10(12), 2017.
3. Behbehani et al. Int J Bioprint, 4(1):123, 2018.

Bioinspired Design of Monodisperse Nanomedicines for StimuliResponsive Drug Delivery Applications

Zhan Yuin Ong [1,2] *

1 School of Physics and Astronomy
2 Leeds Institute of Medical Research at St. James's, School of Medicine, University of Leeds

Abstract

Nanomaterials such as gold and porous silica nanoparticles have emerged as exciting tools in drug delivery due to their biocompatibility, multifunctionality, and unique physical properties. However, a major challenge for the clinical translation of these promising biomaterials lies in the need for simple, cost-effective, and scalable methods to produce highly uniform nanoparticles with built-in functionalities such as cell targeting and stimuli-responsive capabilities. In this regard, we have recently developed facile bioinspired approaches utilising amino acids and their analogues to synthesise monodisperse multibranched gold nanoparticles and porous silica nanoparticles. I will first discuss the design of L-dopa functionalised multibranched gold nanoparticles and their application for the selective photothermal therapy of breast cancer cells [1]. The incorporation of gold nanoparticles into a hydrogel formulation for the light activated release of antimicrobial peptides to enhance their therapeutic potential against pathogens will be further demonstrated [2]. Finally, a onepot amino acid/polymer polyelectrolyte templated method for the synthesis of monodisperse cancer targeting and pH-responsive porous silica nanoparticles in the sub-100 nm range which is favourable for avoiding premature clearance and promoting tumour accumulation via the enhanced permeation effect will be discussed [3]. Taken together, these studies bring forward new insights for the design of an improved class of highly uniform and multifunctional nanomaterials with tuneable and stimuli-responsive drug release capabilities.

References:

[1] Z.Y. Ong*, S. Chen, E. Nabavi, A. Regoutz, D.J. Payne, D.S. Elson, D.T. Dexter, I.E. Dunlop, A.E. Porter, Multibranched gold nanoparticles with intrinsic LAT-1 targeting capabilities for selective photothermal therapy of breast cancer, ACS Appl. Mater. Interfaces 9(45) (2017) 39259.
[2] S.C. Moorcroft, L. Roach, D.G. Jayne, Z.Y. Ong*, S.D. Evans*, NanoparticleLoaded Hydrogel for the Light-Activated Release and Photothermal Enhancement of Antimicrobial Peptides, ACS Appl. Mater. Interfaces 12(22) (2020) 24544.
[3] G. Newham, R.K. Mathew, H. Wurdak, S.D. Evans, Z.Y. Ong*, Polyelectrolyte Complex Templated Synthesis of Monodisperse, Sub-100 nm Porous Silica Nanoparticles for Cancer Targeted and Stimuli-Responsive Drug Delivery, J. Colloid Interface Sci. (2020) https://doi.org/10.1016/j.jcis.2020.10.133.

Determination of chemical sensitising potential using a defined gene signature set using tissue-engineered human skin equivalents

A L. Harding[1*], C Murdoch[1], S Danby[2], M Z Hasan[3], H Nakanishi[3], T Furuno[3], S Hadad[4], R Turner[5], H E. Colley[1].

[1]*School of Clinical Dentistry, University of Sheffield, Sheffield, UK;*
[2]*Sheffield Dermatology Research, Department of Infection, Immunity & Cardiovascular Disease, University of Sheffield, Sheffield, UK;*
[3]*Rohto Pharmaceutical Co., Ltd., Safety Design Centre, Kyoto, Japan;*
[4]*Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK;*
[5] *Research Software Engineering Team, University of Sheffield, UK.*

Background: There are no physical or visual manifestations that define skin sensitivity; a subjective diagnosis is made based on the evaluation of sensory clinical presentations including burning, prickling and itching. Adverse skin sensation in response to topically applied products is common and can limit the use of dermatological or cosmetic products.

Aim: The purpose of this study was to evaluate the use of human skin equivalents (HSE), based on immortalised skin keratinocytes in combination with multivariate analysis to discriminate between chemicals that act as sensitisers, and those that do not.

Methods: Test compounds, pre-defined as known sensitisers or non-sensitisers, were applied topically to full thickness HSE or human *ex vivo* skin for 24 h and their effects on the morphology, viability and permeability barrier determined using histology, lactate dehydrogenase (LDH), and trans-epithelial electrical resistance (TEER), respectively. The gene signature of 22 genes (previously identified as upregulated in response to sensitisers) were determined using RT-qPCR and analysed using principle component analysis (PCA).

Results: Histological staining revealed tissue damage from topical exposure to sensitisers and was confirmed by an increase in LDH and reduction in TEER. RT-qPCR in combined with PCA showed the discriminatory potential of the 22-gene panel to classify compounds into sensitisers and non-sensitisers. Furthermore, linear discrimination analysis refined the number of genes from 22 to 7 (IL-6, PTGS2, ATF3, TRPV3, MAP3K8, HMGB2 and MMP-3), to enable a more high-throughput analysis.

Summary: This data offers promise as an *in vitro* prediction tool for the screening of new chemical compounds, although analysis of a large chemical test set is required to further evaluate the system.

Haemarthrosis in Haemophilia: A Proposed Pathway for the Multifactorial Pathogenesis of Blood-Induced Arthritis

M. Sharrock[1], H. Fermor[1], A. Redmond[2] & C. Brockett[1,2]

[1] *Institute of Medical and Biological Engineering, University of Leeds, Leeds, UK*

[2] *Leeds Institute of Rheumatology & Musculoskeletal Medicine, Chapel Allerton Hospital, Leeds, UK*

Abstract

Haemophilia is a well-known hereditary disorder that continues to gain increasing interest in the research of coagulopathies. Despite this, the major complication of haemarthrosis is massively underrepresented in existing research. Overtime, bleeding into the joint space can cause disabling, polyarticular haemophilic arthropathy which represents the largest cause of morbidity in haemophilia patients. To gain further understanding on this common complication, a rapid review was conducted to uncover the underlying mechanisms at play in haemarthrosis to determine how early changes within the synovial joint progresses to end-stage haemophilic arthropathy. A literature search was conducted with inclusion and exclusion criteria applied to the journal articles retrieved from the search. By collating journal articles within this research area, both indirect inflammatory and direct degenerative forms of damage were pinpointed which resulted in changes to the articular cartilage, synovium, subchondral bone and blood vessels in synovial joints. Both independent and synergistic interactions occur within these tissues that were highlighted in four key pathways: indirect synovitis, macrophage-mediated inflammation, direct cartilage damage and subchondral bone resorption. The pathogenesis of haemarthrosis and its progression from recurrent bleeding to haemophilic arthropathy within the synovial joint is complex and multifactorial consisting of inflammatory and degenerative pathways of a biological and mechanical nature. By providing an in-depth overview of the proposed pathway of blood-induced arthritis, this can guide the direction and necessity of future research needed on the biological and mechanical effects of hemarthrosis in synovial joints.

Development of an *in vitro* immunocompetent human tissue-engineered model of atopic dermatitis

Inmaculada Barragan Vazquez[1*], Simon Danby[2], Helen E. Colley[1], Craig Murdoch[1]

[1]*School of Clinical Dentistry, University of Sheffield, Sheffield, UK*
[2]*Dept of Infection, Immunity & Cardiovascular Disease, University of Sheffield, Sheffield, UK*

Atopic dermatitis (AD) is a common inflammatory skin disorder. The pathogenesis of AD is a combination of defects in skin barrier and an enhanced sensitivity to allergens leading to an over-exaggerated inflammatory response dominated by T-helper type 2 (Th2) cells. The interplay between keratinocytes, Th2 cells and dendritic cells (DCs) is crucial in driving an escalating cycle of inflammation. There is currently no cure for AD and so treatment focuses on management that involves avoidance of triggers, emollient therapy or use of topical corticosteroids. Therefore, new treatments to improve the long-term control of AD are needed. Mouse experimental models have been developed to replicate aspects of AD pathophysiology, however, they are not representative due to their different responses to drug treatments that contribute to a high failure rate for drug development. The aim of this work was to generate tissue-engineered human skin equivalents containing functional immune cells to support drug development.

Purified peripheral blood human monocytes were differentiated into monocyte-derived dendritic cells (Mo-DC) using GM-CSF and IL-4. Purified naïve CD4 T cells were CD3/CD28 activated, stimulated with IL-2 and polarised into Th2 cells using IL-4 and anti-IFNγ. Cell phenotypes were assessed by qPCR, ELISA and flow cytometry for key cell markers. To generate *in vitro* tissue-engineered human skin models, type-1 collagen scaffolds containing primary dermal fibroblasts and T-cells, were seeded with immortalised dermal keratinocytes and Mo-DC, and cultured at an air to liquid interface before analysis.

Characterisation of Mo-DC showed successful differentiation from monocytes by expression of CD207, CD1a and CD11c. Th2 displayed increased CCR3, CCR4, CD119, CD154 and CD4, and secreted increased levels of IL-4, IL-5, IL-6, IL-13 and thymic stromal lymphopoietin (TSLP). Tissue-engineered skin models based on immortalised dermal fibroblasts and keratinocytes displayed a keratinised, stratified squamous epidermis on top of a well-populated fibroblast containing dermis that histologically mimicked human skin. T-cells were successfully incorporated into the dermis and Mo-DC into the epidermis.

In conclusion, multiple immune cells can be differentiated from peripheral blood human monocytes and cultured in a 3D environment together with other skin cells. The next aim is to show immune cell functionality in response to human allergens.

A PhD from the Kitchen: Design and Fabrication of a Novel Gel Electrophoresis System for Gene-Activated Matrix Production

D. White[1,2]*, N. H. Thomson[3], H. L. Femor[4] and G. A. Feichtinger[2]

[1]ESPRC Centre for Doctoral Training in Tissue Engineering and Regenerative Medicine, School of Mechanical Engineering, University of Leeds, LS2 9JT

[2]Division of Oral Biology, School of Dentistry, University of Leeds, LS2 9LU

[4]Molecular and Nanoscale Physics Group, School of Physics and Astronomy, University of Leeds, LS2 9JT

[3]Institute of Mechanical and Biological Engineering, University of Leeds, LS2 9LU

Abstract

Due to the limited regenerative capacity of cartilage, patients that develop severe osteochondral (OC) defects require surgical intervention to restore the natural biomechanics of the joint. However, current graft treatments rely on donor tissue availability and regenerative therapies such as autologous chondrocyte implantation (ACI) and matrix-assisted ACI (MACI) require costly *in-vitro* cell expansions. Gene-activated matrices (GAMs) are a promising, graft-free and acellular alternative to current treatments for OC defects that use intelligently distributed vectors encapsulating gene-encoding therapeutic payloads to instruct stem cell differentiation.

Recently, a novel electrophoretic approach has been developed to produce anisotropic gradient mineralised hydrogels as GAMs for *in-situ* OC defect regeneration at low cost. This approach is designed to spatially control coprecipitation of plasmid-DNA- (pDNA) loaded calcium phosphate nanoparticles (pDNA-CaP-NPs) and pDNA-loaded magnesium phosphate nanoparticles (pDNA-MgP-NPs) as osteogenic and chondrogenic layers respectively. The produced GAM would therefore consist of layered nucleic acid payloads encased in phosphate minerals that spatially control cell transfection and biomineralisation of the gel matrix *in-vivo.*

A novel gel electrophoresis system has been designed to produce these GAMs as cylindrical agarose gels in a simple two-step ‘loading’ process. However, fabrication of the system has been significantly impacted by the national lockdown from March 2020 that, amongst other resources, resulted in the closure of university workshops. Fabrication of the system was therefore relocated to within the home. This presentation therefore follows the efforts of fabricating the system at home and the laboratory-based validation tests that demonstrate the functionality of the system and its ability to produce GAMs suitable for OC defects.

Can Myofibroblast Differentiation be Reversed with Secreted Factors from Adipose Tissue?

S. Higginbotham[1]*, V. L. Workman[1], N. H. Green[1], D. W. Lambert[2], V. Giblin[3], V. Hearnden[1]

[1] Department of Materials Science and Engineering, The University of Sheffield.
[2] School of Clinical Dentistry, The University of Sheffield.
[3] Department of Plastic Surgery, Sheffield Teaching Hospitals NHS Trust, Sheffield.

Abstract

Hypertrophic scarring lowers patient quality of life and costs an estimated $12 billion worldwide annually[1]. This form of scar, often a result of catastrophic or chronic injury, leads to excessive activation of myofibroblasts and collagen deposition[2]. Adipose tissue contains an active cellular component, the Stromal Vascular Fraction (SVF), that may aid wound healing and tissue regeneration. It has been observed that autologous grafting of adipose tissue under scars leads to tissue regeneration, it is hypothesised that the SVF engenders these changes[3].

The aim of this study was to characterise the bioactive factors secreted by different, clinically relevant, formulations of adipose tissue and investigate these factors' ability to reverse myofibroblast differentiation. To achieve this, cell culture medium was conditioned with formulations of adipose tissue and probed via cytokine array to characterize secreted bioactive molecules. Additionally, human dermal fibroblasts activated to myofibroblasts via TGFβ-1 treatment were cultured with the conditioned medium. Western blotting and immunofluorescence were used to quantify myofibroblast reversal.

Anti-fibrotic molecules, such as Hepatocyte Growth Factor and CXCL9, were found, via the cytokine array, to be secreted by adipose tissue. These molecules were found at higher levels in unprocessed fat, implying that the SVF may not be as responsible as thought for tissue regeneration. Conditioned medium from adipose tissue appeared to cause reversal of myofibroblast differentiation. Future work will focus on elucidating the mechanisms behind this effect, starting by inhibiting candidate molecules from this study.

[1] Sen *et al.* (2009) Wound Repair and Regeneration. 17 763-771
[2] Darby *et al.* (2014) Clinical, Cosmetic and Investigational Dermatology. 4 (7) 301-311
[3] Spiekman *et al.* (2014) Plastic and Reconstructive Surgery. 134 699-712

Development and initial testing of a novel automated decellularisation system

K. Cowell[1]*, J. Chandler[2], A. Herbert[1], P. Rooney[3], R. Wilcox[1], H. Fermor[1]
[1]iMBE, University of Leeds
[2]iDRO, University of Leeds
[3]NHS Blood, Transplant, Tissue and Eye Services

Abstract

Decellularisation is the removal of cells and DNA from biological tissue and has been regarded as a solution to the immune reaction and poor regeneration that can occur from the use of xenografts and allografts. To allow the clinical uptake and commercialisation of decellularised tissue there a number of issues that need to be addressed. The current small batch, open processing methods used to produce decellularised scaffolds are labour and time intensive, requiring daily solution changes and taking up to six weeks to complete. The aim of this study was to develop a scalable, automated, closed system to produce decellularised grafts.

To test the recently developed automated decellularisation system, porcine bone plugs were obtained from six pig legs. Thirty were decellularised using the novel automated system, thirty decellularised using a manual solution changing process and eighteen bone plugs were left as native controls. To assess the quality of the decellularised bone plugs, DNA and fat concentrations were quantified to ensure successful decellularisation, H&E and DAPI stains were used to identify any remaining cells or cellular material, sterility broths were used to ensure both processes were completed aseptically and removal of cytotoxic reagents was evaluated using both extract and contact cytotoxicity tests.

The automated, closed system successfully completed all washes aseptically with cytotoxicity testing indicating the successful removal of the wash solutions. Both the automated and manually decellularised bone plugs sufficiently reduced the DNA concentration and there was no histological sign of cells or cellular material. This indicates that the automated decellularisation system can successfully decellularise porcine bone tissue, matching the manual decellularisation process. The next step of this study will be to optimise the decellularisation process by using the automated system to evaluate the effects of shorter wash lengths on the decellularisation of porcine bone before testing different types of tissue such as allograft bone.

ONLAY GRAFTS OF ACELLULAR MATRIX TO AUGMENT THE PERI-URETHRAL TISSUE BED FOR HYPOSPADIAS REPAIR

Debora Morgante[1,2], Anna Radford[2], Syed Khawar Abbas[3], Eileen Ingham[4], Ramnath Subramaniam[2], Jennifer Southgate[1]

[1]Jack Birch Unit, Department of Biology and York Biomedical Research Institute, University of York;

[2]Paediatric Urology, Leeds Teaching Hospitals Trust and Paediatric Surgery, Hull University Teaching Hospitals NHS Trust;

[3]Central Biomedical Services and [4]Institute of Medical and Biological Engineering, University of Leeds.

Introduction

Hypospadias is a common birth defect requiring surgical repair and associated with long-term complications. Cross-linked or non-cross-linked decellularised tissue matrices were hypothesised to provide natural substrates to augment the tissue bed in surgical situations where the native tissue bed is insufficient to support an enduring repair.

Methods

Single patches of acellular, non-cross-linked biomaterial developed from full thickness porcine urinary bladders (Porcine Acellular Bladder Matrix; PABM) or commercial cross-linked acellular matrix derived from porcine dermis (Permacol™) were implanted in a peri-urethral position in male pigs. After three months, macroscopic and microscopic evaluations including a novel objective quantitative analysis of immunohistochemically-labelled tissue sections were performed to assess tissue integration outcomes.

Results

All pigs survived the post-operative period with no complications. Macroscopic evaluation revealed no evidence of residual PABM, but Permacol™ remained apparent. Microscopically, there was no sign of any adverse or inflammatory tissue reaction. Permacol™ grafts showed evidence of partial encapsulation and very sparse cell infiltration. By contrast, PABM implants showed uniform cellularisation, including neovascularisation, throughout the implanted biomaterial. Total cell counts as well as the densities of infiltrating cells resulted significantly higher in PABM than in Permacol implanted grafts.

Discussion

The strong, compliant structural and integrative properties of PABM support its use in surgical situations where there is an insufficient tissue bed to withstand repair. In particular we propose an application in primary complex hypospadias repair, where rapid integration as a supportive layer is predicted to reduce complications and the need for revision surgery.

Ref: Bolland et al. *Development and characterisation of a full-thickness acellular porcine bladder matrix for tissue engineering*. Biomaterials. 2007;28:1061-70. PMID:17092557

Permeabilising decellularised osteochondral scaffolds for improved chondrocyte penetration

P.Statham [1]*, E.Jones [2], L.Jennings [1] & H. Fermor[1]
[1]Institute of Medical and Biological Engineering, University of Leeds
[2] Leeds Institute of Rheumatic and Musculoskeletal Disease, University of Leeds

Abstract

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

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

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

Untreated scaffolds showed cell attachment to the superficial layer but no penetration beneath this. However, both collagenase treated and lyophilised samples showed a greater cell density in the superficial and middle zones of the cartilage, as well as on top of the superficial layer. Viability as measured by alamarBlue, was consistent between untreated, collagenase treated and lyophilised samples.

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

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

Self-assembled multicomponent microgels for biological applications

Carmen C. Piras,[1*] Alasdair G. Kay,[2] Paul G. Genever[2] and David K. Smith[1]
[1]*Chemistry Department, University of York, Heslington, York, YO10 5DD*
[2]*Biology Department, University of York, Heslington, York, YO10 5DD*

Abstract

Hydrogels with spatio-temporally controlled properties are appealing platforms for biological and pharmaceutical applications. We herein report multicomponent hybrid gel beads formed by a low molecular weight gelator (LMWG) based on the dibenzylidene sorbitol (DBS) framework (namely 1,3:2:4-di(4-acylhydrazide)-benzylidene sorbitol (DBS-$CONHNH_2$)) and the biopolymer alginate, in which the spatial arrangement of the two networks could be directed.[1] DBS-$CONHNH_2$ is a thermally triggered LMWG that self-assembles in response to a heat-cool cycle. Alginate forms hydrogels when cross-linked with multivalent cations (*e.g.* Ca^{2+}). Since the two gelators have orthogonal methods of preparation, a specific spatial arrangement of the two networks within the hybrid gel beads could be imposed by temporally controlling the gelation process, forming (1) core-shell structured gels or (2) interpenetrated networks. Interpenetrated gel beads were obtained by an emulsion method that could be adapted to give spherical gel particles of controllable sizes with diameters in the mm or μm range (respectively milli- and micro- beads). The prepared microbeads have reproducible diameters around 800 nm and are stable in water at room temperature for months. We applied these innovative gel microbeads for the release of the bioactive molecule heparin. The hybrid microgel beads could successfully retain and release heparin in cell culture medium in the presence of human mesenchymal stem cells, impacting cell growth. Such formulations may be a sophisticated versatile platform for applications in tissue engineering and regenerative medicine.

References

1. Piras, C. C., Slavik, P., Smith, D. K., *Angew. Chem. Int. Ed.*, **2020**, 59, 853.

Poster Presentation Abstracts

Lack of Standardisation in Mechanical Testing of the Patella Tendon: A Review and Guide

W.J. Sanderson[1]*, R.J. Foster[1], J.E. Edwards[1], R.K. Wilcox[1] & A. Herbert[1]
[1]*Institute of Medical and Biological Engineering, University of Leeds*

Abstract

The patella tendon is commonly used as a graft material for anterior cruciate ligament reconstruction (ACLR). The primary function of the graft is to restore the mechanical behaviour of the knee joint. However, this approach to ACLR has drawbacks, including donor site morbidity, initial necrosis, degeneration or slow incorporation. Tissue engineered patella tendon grafts have the potential to avoid some of these disadvantages, but only limited evidence exists regarding the mechanical properties of such materials in the context of ACLR. Therefore, it is essential that a robust and repeatable methodology be developed for the mechanical testing of the patella tendon, as well as for the tissue engineered grafts derived from this tissue.

A review was undertaken to investigate the different mechanical tests that can be used to investigate the biomechanics of the patella tendon, and the wide variety of approaches to each test that have been described. The purpose of this review was to survey the existing literature, in order to define the state-of-the-art in mechanical testing of the patella tendon, and to highlight the most commonly used testing configurations and protocols.

Ramp-to-failure testing was found to be most commonly performed (included in over 90 % of publications), followed by stress relaxation and cyclic testing (~25 % of publications). Within each type of testing, further variation in test protocol (pre-conditioning regime, strain rate, and maximum strain) was examined. This analysis revealed the wide variability in testing protocols used to characterise the mechanical behaviour of the patella tendon. This has led to a lack of standardisation and the potential for misinterpretation of mechanical data. These variations could have important implications for the comparison of studies conducted using different protocols.

Based on the outcome of our analysis, we propose a standardised protocol for the tensile testing of the patella tendon. We also highlight a lack of published data on potentially relevant mechanical properties and testing configurations. Finally, we outline the validation studies that are required to systematically compare the mechanical measurements obtained using different methodological approaches.

Optimisation and Characterisation of Bacterial Cellulose produced by *Gluconacetobacter xylinus*

Lakshmi Tripathi*, Emmanuel Asare*, Vijayendran Raghavendran*,
David A Gregory, Ipsita Roy**
Department of Material Science and Engineering, Faculty of Engineering, University of Sheffield, Sheffield, UK
* These authors contributed equally to this work
**Corresponding author: l.tripathi@sheffield.ac.uk

Abstract

Bacterial cellulose (BC) is an excellent biomaterial with a high degree of purity, as it does not contain other components such as lignin and hemicellulose that are present in plant-derived cellulose. MC presents a highly porous and crystalline nanofibrillar structure due to its high water retention capacity, hydrophilicity and excellent mechanical properties and is therefore suitable for various applications. However, a high production cost and low-yield has limited the large-scale production of BC and its commercial application. Therefore, optimisation of the bioprocess is important for the scale-up of the BC production. In our present study, we optimised BC production by *Gluconacetobacter xylinus* (NCIMB 5346) under static-conditions using different growth media and culture conditions. The optimised culture conditions resulted in BC yield of 662 g L^{-1} wet weight which was comparatively higher than previously reported yields of BC by *G. xylinus*. The SEM analysis of the dry pellicles showed an ultrafine microfibrillar structure. BC was chemically characterized by XPS and FT-IR analysis. The rheological measurements showed that the material behaved as a hydrogel with a viscoelastic property. Further, due to its purity, biocompatibility and non-cytotoxicity the cellulose produced can be used in tissue engineering and biomedical applications.

A next generation bioinspired device for effective peripheral nerve regeneration

E. Asare[1*], C. Taylor [1], D. A. Gregory[1], L. Tripathi[1], J. Haycock[1], I. Roy[1,2*]

[1]Department of Material Science and Engineering, Faculty of Engineering, University of Sheffield, UK

[2]National Heart and Lung Institute, Faculty of Medicine, Imperial College London, UK

Nerve guidance conduits (NGCs) are attracting interest for their promise to overcome the drawbacks of the traditional peripheral nerve injury (PNI) treatment technologies. However, materials used in current NGCs are deficient in their compatibility with surrounding tissues, thus warranting the search for better NGC materials. This project aims to address this incompatibility by developing a biomimetic NGC prototype with improved support for optimal PNI recovery. To achieve this goal, Polyhydroxyalkanoates (PHAs) and bacterial cellulose (BC) have been produced via microbial fermentation. PHAs are a relatively unexplored family of natural polymers that are rapidly gaining interest among the best-studied biomaterials owing to their wide-ranging desirable properties such as their superior biocompatibility, non-immunogenicity and excellent biodegradation profiles making them ideal candidate materials for NGC[2,3]. Similarly, BC is a versatile hydrogel that is highly biocompatible and structurally dynamic among other desirable properties[1]. Preliminary results from our lab have established the superiority of PHAs in nerve regeneration over some of the commercially available state-of-the-art materials[3]. In our study, we optimized the production of PHAs and BC resulting in at least a 10-fold increase in yield compared to the average values reported in literature. The novel PHAs and their blends have been confirmed to be highly biocompatible *in vitro* with NG108-15 cells. Additionally, in this study, a variety of constructs ranging from multi-channel to internally grooved designs have been successfully designed for future 3D printing using Fused Deposition Modelling. These will in future be printed and tested *in vitro* and *in vivo* for biocompatibility and functionality.

References:

1Raghavendran, V., Asare, E. & Roy, I. Bacterial cellulose: biosynthesis, production, and applications. *Advances in Microbial Physiology (in press)* (2020).

2Lizarraga-Valderrama, L. R. *et al.* Unidirectional neuronal cell growth and differentiation on aligned polyhydroxyalkanoate blend microfibres with varying diameters. *Journal of Tissue Engineering and Regenerative Medicine* **13**, 1581-1594, doi:10.1002/term.2911 (2019).

3Lizarraga-Valderrama, L. R. *et al.* Nerve tissue engineering using blends of poly(3-hydroxyalkanoates) for peripheral nerve regeneration. *Engineering in Life Sciences* **15**, 612-621, doi:10.1002/elsc.201400151 (2015).

Cardiovascular Tissue Engineering using Natural Polymers

Annabelle T.R. Fricker[1]*, David A. Gregory[1], Sian E. Harding[2] & Ipsita Roy[1,2]
[1]*Department of Materials Science and Engineering, Faculty of Engineering, University of Sheffield, Sheffield, UK*
[2]*National Heart and Lung Institute, Faculty of Medicine, Imperial College London, London, UK*

Abstract

Cardiovascular diseases (CVDs) cause the greatest number of deaths worldwide, resulting in a large burden on healthcare systems[1]. Myocardial infarction (MI) is one of the most fatal results of CVDs, as it can cause ultimate heart failure. Current treatments are able to mitigate the MI symptoms but do not repair the damaged tissue. Here we propose the use of a tissue engineered cardiac patch to deliver cells which can repopulate the area of myocardium damaged upon MI.

Polyhydroxyalkanoates (PHAs) are a family of biocompatible and bioresorbable polymers which are produced using bacteria[2,3]. A medium chain length PHA called poly(3-hydroxyoctanoate-*co*-3-hydroxyoctanoate), P(3HO-*co*-3HD), was produced using *Pseudomonas* species and mechanically and thermally characterised. It was found to have desirable properties including a low melting temperature and a high elasticity, making it ideal for cardiac tissue engineering as it closely replicated the native myocardium. P(3HO-*co*-3HD) was also confirmed to be non-cytotoxic via cell assays using C2C12 myoblasts. Multimaterial cardiac patches were produced by 3D printing of P(3HO-co-3HD) with a natural alginate hydrogel encapsulating C2C12 cells. Further work in this project will involve the inclusion of human induced pluripotent stem cell-derived cardiomyocytes and endothelial cells as the cellular components of the cardiac patch, and further *in vitro* and *in vivo* studies will be carried out to determine the potential of this cardiac patch in the application of post-MI treatment.

References

1. Laflamme MA & Murry CE. Heart regeneration. Nature 473, 326-335, 2011
2. Rai R, Yunos DM, Boccaccini AR, Knowles JC, Barker IA, Howdle SM, Tredwell GD, Keshavarz T, Roy I. Poly-3-hydroxyoctanoate P(3HO), a Medium Chain Length Polyhydroxyalkanoate Homopolymer from Pseudomonas mendocina. Biomacromolecules 12, 2126, 2011
3. Bagdadi AV, Safari M, Dubey P, Basnett P, Sofokleous P, Humphrey E, Locke I, Edirisinghe M, Terracciano C, Boccaccini AR, Knowles JC, Harding SE, Roy I. Poly(3-hydroxyoctanoate), a promising new material for cardiac tissue engineering. Journal of Tissue Engineering and Regenerative Medicine 12, 495, 2018

Delivery of immobilised NGF and BDNF via a bioactive surface to enhance neurite outgrowth

Ana Sandoval-Castellanos[1], Frederik Claeyssens[1], John Haycock[1]
[1]*Department of Materials Science and Engineering. The University of Sheffield. Sheffield. United Kingdom*

Abstract

Peripheral nerve injury remains a major cause of disability that compromises the life quality and health of 1 in 1000 patients [1]. A novel strategy to improve the performance of NGCs includes the addition of neurotrophins. However, the short half-life of neurotrophins limits the regeneration of the nerve [2][3]. The development of a sustained delivery platform would allow for a controlled release of neurotrophins and thus, enhance neurite outgrowth. We present the fabrication of a bioactive surface using electrostatic interactions to bind neurotrophins [4] and improve their release. The aim of this study was to 1) develop a bioactive surface enriched with positive amine groups and in turn heparin. This platform was then loaded with NGF, BDNF, or in combination for local release, and thereafter 2) evaluate their effects in neurite outgrowth.

Passive conjugation of heparin into pre-coated amine surface 96 well plates was carried on and characterized by water contact angle and XPS analysis. Concentrations of 1 pg/mL, 1 ng/mL, 10 ng/mL, 100 ng/mL and 1 µL/mL of NGF, BDNF and NGF and BDNF were immobilised passively on bioactive surfaces. The release profiles were measured by ELISA assay. Chick embryo dorsal root ganglia (DRGs) were cultured on bioactive surfaces for 7 days. DRGs were stained for cell nuclei and βIII-tubulin protein to measure neurite outgrowth [5].

Changes in hydrophilicity confirmed the presence of amine groups and later heparin conjugation. XPS analysis showed the presence of sulfur and nitrogen. ELISA assays showed a minimal 24 h release of NGF. Surfaces immobilized with NGF at 1 ng/mL showed no growth factor release after 24 h and 48 h. By day 7, 1% of NGF was detected. DRGs grown on the surface with immobilized 1 ng/mL of NGF developed neurites that were significantly longer in comparison to control surfaces or surfaces with BDNF and NGF / BDNF. Our results showed that bioactive surfaces with immobilized NGF at 1 ng/mL supported the largest neurite. In summary, locally delivered neurotrophin surfaces are a promising approach to stimulate nerve regeneration using bioactive nerve guides [5].

References

[1] R. Hughes, *Pract. Neurol.*, 2008.
[2] S. Madduri, K. Feldman, T. Tervoort, M. Papaloïzos, and B. Gander, *J. Control. Release*, 2010.
[3] S. Tang, J. Zhu, Y. Xu, A. P. Xiang, M. H. Jiang, D. Quan, *Biomaterials*, 2013.
[4] B. Casu, A. Naggi, and G. Torri, *Carbohydrate Research*, 2015.
[5] A.M. Sandoval-Castellaos, F.Claeyssens, J.W. Haycock, *Biotechnol. Bioeng*, 2020.

Development of Technologies to Support the Robust and Reproducible Growth of Complex Bioengineered Human Skin Equivalents

A. Boyadjiev[1], Kirsty Goncalves[1], Victoria Maltman[1], Lucy Smith[1], Henry Hoyle[1] & Stefan Przyborski[1]

[1]Department of Biosciences, Durham University

Abstract

The dermis is the underlying submucosal compartment of the skin and is responsible for supporting the overlying epidermis both structurally and trough trophic signals. It provides biophysical support through the extracellular matrix secreted by the fibroblasts. In reconstructed human skin models the ECM plays a crucial role in preventing keratinocyte infiltration and promoting epidermal development. We have used defined media supplemented with growth factors to promote increased ECM deposition in a porous 3D scaffold. The aim of this is to develop a robust foundation with which a complex epidermis can be constructed, consisting of not only keratinocytes but also supporting cell types such as melanocytes and Langerhans's cells. Neonatal human dermal fibroblasts were cultured in an Alvetex® Scaffold for 2 and 4 weeks in either low serum Medium 106 or Dulbecco's modified eagle medium with 10% serum. Both media were further supplemented with 100 µg/ml ascorbic acid and 10 ng/ml transforming growth factor-β1. Analysis was done by immunohistochemistry. In addition, cell counting and MTT assay were used to assess HDFn growth in 2D culture using both media conditions. Our data suggests an increase in ECM deposition in the high serum DMEM condition in comparison to the low serum M106 condition, which will be confirmed by performing further quantified analysis. An increase in collagen-1/3 deposition within the dermal compartment after both 2 and 4 weeks of dermal formation with high serum DMEM was observed. Quantification of 2D cell growth by counting and MTT assay demonstrated increased HDFn viability and initial rate of proliferation with cells cultured in DMEM. These results show that serum supplemented DMEM has the potential to increase dermal maturation through ECM deposition and promote improved ECM deposition over time, thus increasing dermal robustness. These results will be applied to the generation of a full-thickness reconstructed skin equivalent using a 2, 3 and 4-week-old dermis to determine if increased ECM deposition over time will produce a more robust dermis capable of supporting a complex epidermis. Notably, DMEM has shown promise to act as a universal skin cell culture medium, with the overall goal to optimise this medium for the generation of a pigmented and immune-competent reconstructed human skin model. We hypothesise that this feasible due to trophic factors and ECM components generated by dermal fibroblasts supporting the development of these novel skin models.

Development of a multi-cellular tissue engineered model of oral lichen planus

Asma El Howati, Craig Murdoch, Helen E. Colley
School of Clinical Dentistry, University of Sheffield, UK

Abstract

Introduction: Oral lichen planus (OLP) is a common T-cell immune-mediated mucocutaneous disease of unknown aetiology. Despite advances in OLP research, investigations into this condition are hampered by the lack of effective experimental models. *In vitro* tissue-engineered models have been developed to replicate the oral mucosa and different oral pathological conditions. However, currently there are no tissue-engineered models of OLP.

Aim: To develop a tissue-engineered oral mucosal model containing activated T-cells to replicate OLP for use in the development of novel treatment strategies.

Methods: Naïve CD4+ T-cells were isolated from buffy coat and their purity and viability assessed. CD4+ T-cells were activated in Green's or RPMI medium with PMA/Ionomycin or CD3/CD28 beads and their optimal activation status measured by CD69 using flow cytometry. Naïve CD4+ T-cells were polarised into Th1 and phenotype confirmed with flow cytometry, ELISA, q-PCR and western blot for key Th1 markers. Full-thickness tissue-engineered oral mucosal (TEOM) models were created using immortalised oral keratinocytes (FNB6) and normal oral fibroblasts.

Results: T-cells were isolated with high purity (85-95%) and viability (>90%). Upon activation, CD69 expression was increased with similar levels in RPMI or Green's medium and the cells proliferated in culture over 14 days. CXCR3 surface marker, measured by flow cytometry, was highly expressed in both the activated and the polarised cells, only the polarised cells secreted Th1-related cytokines, IFN-γ and TNF-α. In addition, Th1-related transcriptional factors T-bet and STAT1 were detected in increased levels in the polarised cells. H&E stained sections of TEOM revealed a multi-layered stratified squamous epithelium on top of a fibroblast-populated collagen scaffold. T-cells extracted from the collagen maintained viability >85%, over 14 days in culture.

Conclusion: T-cells were successfully isolated with high purity and viability and polarised to Th1 phenotype. Future work will involve incorporating polarised T-cells into TEOM and validating with OLP markers. We will use the model to develop novel treatment strategies.

Kidney Tissue Engineering using Polyhydroxyalkanoates

Syed Mohammad Daniel Syed Mohamed[1], Gavin Welsh[2], Ipsita Roy[1]

[1] Department of Materials Science and Engineering, Faculty of Engineering, University of Sheffield, Sheffield, UK

[2] Bristol Renal, Translational Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK

Abstract

Kidney failure happens when the kidney loses its normal function, mainly to filter blood and regulate electrolyte balance, i.e., to maintain the normal chemical balance of the body. Besides the conventional haemodialysis, bioartificial kidney development utilising real kidney cells has been initiated, not only to maintain the filtering functions but also to restore the physiological activities a normal kidney would perform. Prevalent kidney conditions include nephropathy, glomerulosclerosis, and minimal change disease lead to the malfunction of the glomerulus, the main filtering component within the kidney.

This research is an attempt to develop a bioartificial kidney with the potential of constructing a 'wearable kidney' in the future. [1] Polyhydroxyalkanoates (PHAs) are natural polymers of bacterial origin produced using bacterial fermentation, selected as a prospective biomaterial to for kidney tissue engineering. PHAs are known to be biocompatible toward a broad array of human cells, and have been widely utilised in biomedical applications. [2] A specific type of PHA will be selected for this application, based on their physical properties such as the rigidity, thermoplastic behaviour, and durability. Initially, this research will explore two types of glomerular cells for the tissue engineering approach, the glomerular endothelial cell line (GEnCs), and the human conditionally immortalised podocytes. [3] In future, other cell types and additive manufacturing will be combined with the ultimate aim of the development of a mature bioartificial kidney.

References

1.Tarantal, A.F. and C.A. Batchelder, *Scaffolds for kidney tissue engineering.* Handbook of Tissue Engineering Scaffolds, Vol 2, 2019: p. 477-492.

2.Lizarraga-Valderrama, L.R., et al., *Tissue Engineering: Polyhydroxyalkanoate-Based Materials and Composites.* Encyclopedia of Polymer Applications, Vols I-Iii, 2019: p. 2652-2675.

3.Kitching, A.R. and H.L. Hutton, *The Players: Cells Involved in Glomerular Disease.* Clinical Journal of the American Society of Nephrology, 2016. **11**(9): p. 1664-1674.

Semi-artificial pancreas for the treatment of Type 1 diabetes: Perspectives, challenges and solutions

Jonathan Hinchliffe[1],Moyin Odugbemi [2],Victor Gault [3] & Ipsita Roy[1]
[1]Department of Material Science and Engineering, Faculty of Engineering, University of Sheffield, Sheffield, UK
[2]School of Life Sciences, University of Westminster, London, UK
[3]Faculty of Life & Health Sciences, Ulster University, Coleraine, UK

Type 1 diabetes is described as an autoimmune condition which, through pathological immune system action, causes destruction of the β-cells of the pancreatic islets of Langerhans, reducing insulin production capacity[1]. Diabetes has proven to be a major challenge to both local and international healthcare systems, with large financial and social impacts[2]. Whilst both insulin injections and pancreas transplants remain the gold standard for treatment, patient compliance, cost of daily use and immune-suppressant drugs reliance (in the case of transplantation) remain challenges to the long-term effective treatment of the disease[3]. A proposed solution to this problem has been immunoisolation, where semi-permeable membranes separate the immune system from implanted (potentially allo or xenogeneic) tissue, allowing for tissue homeostasis through the free movement of nutrients, oxygen and therapeutic agents, whilst blocking immune cells and chemical systems access to the system[4]. This technique has been refined, transitioning from bulky intravascular devices to microencapsulated structures, which allow for greater oxygen perfusion into the system[5]. However, challenges to achieve effective therapeutic activity remain, including hypoxia prior to revascularisation, generating significant tissue degradation[6].

This work has explored the use of Polyhydroxyalkanoates for the creation of a semiartificial pancreas, aiming to provide an effective therapy for Type 1 diabetes. The viability and functionality of BRINBD11 cells (pancreatic cell line) have been ascertained on novel Polyhydroxyalkanoates. 2D and 3D structures have been studied and as expected, the 3D substrates have been found to be preferred by the cells[7]. Similar studies have also been carried out using alginate[5]. In future, multimaterial additive manufacturing will be used for the development of an effective semiartificial pancreas.

References: [1]Emery and Rimoin's Principles and Practice of Medical Genetics, Academic Press, 2013, pp. 1-58, ISBN 9780123838346, https://doi.org/10.1016/B978-0-12-383834-6.00090-2., [2]Stedman, M., Lunt, M., Livingston, M., Fryer, A.A., Moreno, G., Bailey, S., Gadsby, R. and Heald, A., 2019. The costs of drug prescriptions for diabetes in the NHS. *The Lancet, 393*(10168), pp.226-227.[3]Herkert, D., Vijayakumar, P., Luo, J., Schwartz, J.I., Rabin, T.L., DeFilippo, E. and Lipska, K.J., 2019. Cost-related insulin underuse among patients with diabetes. JAMA internal medicine, 179(1), pp.112-114. [4]Scharp, D.W., Mason, N.S. and Sparks, R.E., 1984. Islet immuno-isolation: the use of hybrid artificial organs to prevent islet tissue rejection. World journal of surgery, 8(2), pp.221-229.[5]Lum, Z.P., Krestow, M., Tai, I.T., Vacek, I.V.A.N. and Sun, A.M., 1992. Xenografts of rat islets into diabetic mice. An evaluation of new smaller capsules. Transplantation, 53(6), pp.1180-1183 [6]Nourmohammadzadeh, M., Lo, J.F., Bochenek, M., Mendoza-Elias, J.E., Wang, Q., Li, Z., Zeng, L., Qi, M., Eddington, D.T., Oberholzer, J. and Wang, Y., 2013. Microfluidic array with integrated oxygenation control for real-time live-cell imaging: effect of hypoxia on physiology of microencapsulated pancreatic islets. Analytical chemistry, 85(23), pp.11240-11249. [7]Odugbemi, M., 2018. Biopolymers for bioartificial pancreas (Doctoral dissertation, University of Westminster).

Increasing the potency of biomaterials for tissue growth

L. Raynal[1,2] & C. D. Spicer[1,2]
[1]*Department of Chemistry, University of York, Heslington, York, YO10 5DD*
[2]*York Biomedical Research Institute, University of York, Heslington, York, YO10 5DD*

Abstract

In recent years, it has been shown that synthetic biomaterials are not optimal for tissue repair due to their lack of many intrinsic biological properties. In order to improve the potency of biomaterials and increase the reach of tissue engineering (TE), tethered biomolecules such as proteins have been targeted to enhance the biochemical properties of the materials. However, when attached non-specifically (without control over which amino acids are modified) proteins suffer a drastic loss of activity. Our goal is to be able to modify growth factors proteins in a highly specific manner, to provide biomaterials with the potent biological functionality required for cells to form functional new tissue. To target the growth factors FGF-2 and VEGF, a library of peptides is used to perform ligand-directed chemistry at a single site on a protein (**Figure 1A**).[1,2] The peptide ligands are modified with a catalyst that can attach a wide range of functional groups to the protein surface, including fluorophores, photocrosslinkable groups, or specific chemical groups for attachment to a biomaterial (**Figure 1B**). This chemistry should provide a more precise and unique way to modify each protein and allow their use in TE without a loss of activity.

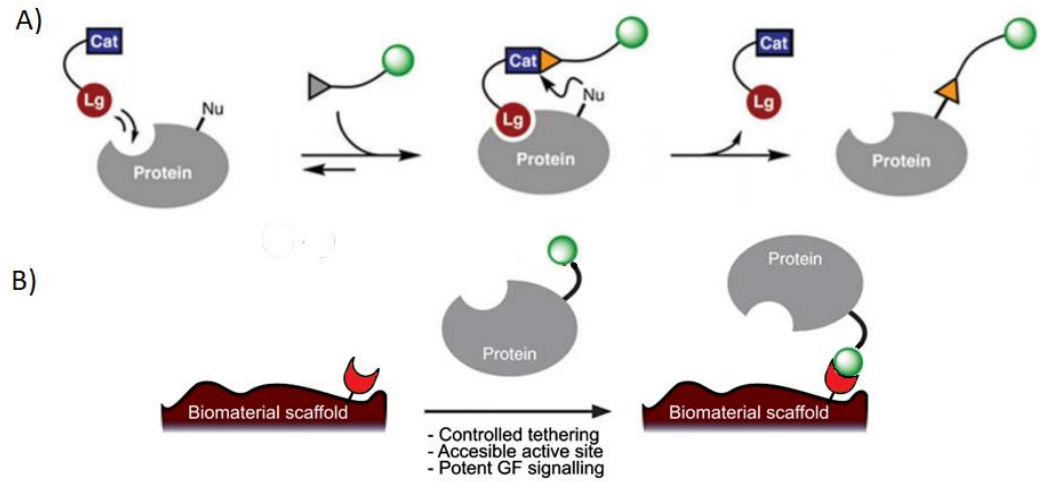

Figure 1: A) Scheme to modify selectively a protein through ligand-directed chemistry, B) Controlled tethering to a biomaterial, with Lg being the binding ligand, Cat the catalyst and Nu a nucleophilic amino acid nearby the active site

A Dynamic Biomaterial-ligand Tethering Strategy for Tissue Engineering

N C. Rose[1,2], C. D. Spicer[1,2]
[1]*Department of Chemistry, University of York, York, YO10 5DD*
[2]*York Biomedical Research Institute, University of York, York, YO10 5DD*

Abstract

Tissue engineering is an emerging and promising regenerative approach that uses materials to repair or replace damaged tissues for the treatment of disease or injury.[1] The effectiveness of a material depends on its ability to signal to cells to direct cell growth and development.[2] Cells must be provided with a number of different biochemical signals in order to form mature tissue, through complex signalling networks that are difficult to recreate in synthetic materials. The ability to attach and detach proteins from a material in an iterative and dynamic manner would therefore represent a powerful way to control tissue growth and recreate natural biochemical signalling pathways for tissue development and maturation.

The overarching aim of this project is to develop chemistries that allow us to reversibly attach bioactive proteins to hydrogels to direct cell behaviour. We are particularly interested in the use of *ortho*-boronobenzaldehydes due to their ability to form stabilised, yet dynamic, boronoimines (Fig 1). A library of boronoimines will be formed and screened using a Förster resonance energy transfer (FRET) assay, using a cyanine dye (Cy3 and Cy5) FRET pair, to measure rate of bonding and stability, which will then be used to conjugate proteins to biomaterial surfaces, and then direct cell behaviour. The project has a long-term goal to develop a modular biomaterial platform that can be used to help combat chronic diseases such as osteoarthritis, heart disease, and chronic wounds, by providing cells with potent biological stimuli for tissue engineering.[3,4]

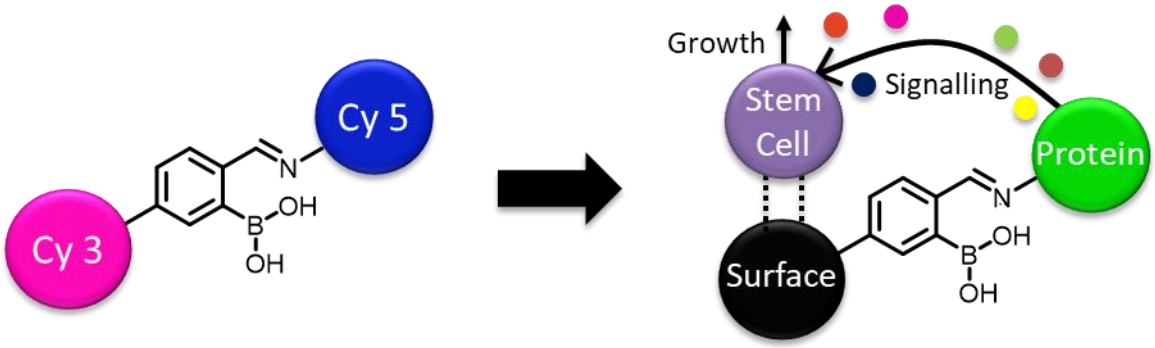

Figure 1: Schematic showing the structure of *ortho*-boronobenzaldehydes and their use to conjugate proteins to a surface to allow for cells signalling needed for tissue development and maturation

[1]R. Langer *et al.*, *Science.*, **1993**, 260, 920–926. [2]**C. D. Spicer** *et al.*, *Chem. Rev.*, **2018**, 118, 7702–7743. [3]S. J. Forbes *et al.*, *Nat. Med.*, **2014**, 20, 857–869. [4]L. A. L. Fliervoet *et al.*, *Adv. Drug Deliv. Rev.*, **2016**, 106, 63–72.

The HA Particle size and quantity effect on the chemical and Biological Behaviour of Polyurethane and hydroxyapatite scaffolds

FH Alhamoudi [1,2], Y Almoshawah[2] , AA Chaudhry[4] , GC Reilly[3] and I U Rehman[2]

1 Dental Technology Department, King Khalid University, KSA,
2 Engineering Department, Lancaster University, UK,
3 INSIGNEO institute for in silico Medicine, University of Sheffield, UK,
4 Interdisciplinary Research Centre in Biomedical Materials (IRCBM), Pakistan

Introduction

Bone bioengineering involves microstructural development and assessments, biomechanical evaluation, multiscale and mechanobiological approaches in skeletal research and has clinical significances in a number of medical applications. Integration of cells and vascularisation plays a pivotal role in bone regeneration. In maxillofacial area, specifically orbital floor, injuries and birth defects can cause bone deformities in the head and face that are difficult to repair or regenerate. Treatment methodologies include use of polymers, metal, ceramics on their own and in combinations mainly for repair purposes, but little attention has been paid to identify suitable materials for orbit floor regeneration.

Methods

In this study, polyurethane (PU) and hydroxyapatite (HA) micro or nano with different percentages (25%, 40% & 60%) were used to synthesise bioactive tissue engineering (TE) scaffolds using solvent casting and particulate leaching method. Physical characterisation of TE scaffolds was investigated by tensile tests and SEM studies. Chemical and structural properties of PU and PU/HA scaffolds were evaluated by FTIR- PAS method and Surface properties of bioactive scaffold were analysed using FTIR-ATR method. Cell viability, collagen form, VEGF protein amount and vascularisation of bioactive TE scaffold were studied.

Results & Discussion

FTIR-PAS characterization confirmed the presence of HA in composite scaffolds, while ATR confirmed the significant amount of HA at the scaffolds top surface in micro-HA. However, nano-HA had a better disparity than micro-HA. The SEM images confirmed the interconnectivity. Increasing the content of HA up to 40% led to improve the mechanical properties, but nano-HA was more promising than that of micro-HA. The 60% HA made the scaffolds so hard and easy to be broken. The cell viability (using MG63) showed no significant difference between PU and PU/HA scaffold either micro or nano. For CAM assay, there were significant different between PU and PU/HA scaffolds, where PU/HA scaffolds showed more vascularisation.

Conclusion

This study describes the PU/HA bioactive scaffold neovascularization potential. The development of new biocompatible materials with tailored properties will be useful in tissue engineering especially in orbital floor regeneration.

Acknowledgments

Financial support was provided by King Khalid University; Ministry of Education; Kingdom of Saudi Arabia. Cell Viability test was conducted in Kroto Research Institute; Sheffield University.

Modification of Decellularisation Methods to Assess the Effects of Swelling on the Mechanical Properties of Porcine Tendon

Jacqueline Solis[1,2], Jennifer H. Edwards2[1,2], Hazel Fermor[1,2], Claire Brockett[1] & Anthony Herbert[1]

[1]*Institute of Medical and Biological Engineering, School of Mechanical Engineering, University of Leeds, UK*

[2]*Institute of Medical and Biological Engineering, School of Biomedical Sciences, University of Leeds, UK*

Abstract

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

In this study, end stage PBS washes in the standard protocol were substituted with Ringer's solution to determine if tissue swelling could be reduced without negatively affecting its mechanical properties, while achieving the same degree of cellular removal. pSFTs decellularised using the modified protocol were compared to pSFTs decellularised using the standard protocol and native tendons.

Geometrical measurements exposed no differences in tissue swelling between decellularised groups. Histological evaluation showed efficient decellularisation in both groups, with no observational differences in histoarchitecture. Stress relaxation testing resulted in reduced relaxation moduli for both decellularised groups. The mechanical properties obtained from strength testing were significantly reduced for both decellularisation groups compared to native pSFTs, but not statistically different between them. However, the toe region modulus of the modified group was not significantly different from native pSFTs. Decellularised pSFTs using the standard or modified process have suitable mechanical properties to act as a viable graft for ACLR.

1. Bollen, S. Epidemiology of knee injuries: diagnosis and triage. British Journal of Sports Medicine 34, 227, 2000; 2. Acosta-Olivo, C et al. Clinical Evaluation of Patients with a Delayed Treatment of Anterior Cruciate Ligament Rupture. The Open Orthopaedics Journal 13, 244, 2020; 3. Safa, BN, Meadows, KD, Szczesny, SE and Elliot, DM. Exposure to buffer solution alters tendon hydration and mechanics. Journal of Biomechanics 61, 18, 2017; 4. Screen, HRC et al. The Influence of Noncollagenous Matrix Components on the Micromechanical Environment of Tendon Fascicles. Annals of Biomedical Engineering 33, 1090, 2005; 5. Screen, HRC et al. The influence of swelling and matrix degradation on the microstructural integrity of tendon. Acta Biomaterialia 2, 505, 2006.

The development of a 20-year economic model for the cost-effectiveness analysis of using decellularised bone versus fresh-frozen allograft as an acetabular impaction bone graft during a revision hip arthroplasty

K. Cowell[1], J. Chandler[2], A. Herbert[1], P. Rooney[3], R. Wilcox[1], H. Fermor[1]

[1]iMBE, University of Leeds

[2]iDRO, University of Leeds

[3]NHS Blood, Transplant, Tissue and Eye Services

Abstract

In over a quarter of the revision hip arthroplasties completed in the UK every year a bone graft is used to replace missing or removed bone. A fresh-frozen bone allograft is the most common graft choice currently used. However, new technology has been developed to produce decellularised bone allografts with potentially better graft incorporation. As these grafts cost more to produce, an economic model has been developed to analyse whether the potential health benefit of these alternative bone grafts outweighs their increased cost.

A 20-year economic model was constructed to estimate the costs and benefits of using different graft types for impaction bone grafting in a revision hip arthroplasty. Using a base case set of parameters an incremental cost-effectiveness ratio (an estimate as to how much any health benefit provided by an alternative treatment will cost) was calculated. Further incremental cost-effectiveness ratios were calculated by changing each of the key parameters to an extreme value to test the limits of the model. Additionally, by estimating a distribution for each key parameter such as surgery success rate and health benefit, 10,000 varying cases could be produced, then averaged to calculate an additional incremental cost-effectiveness ratio.

The model indicated that if a decellularised bone graft was produced for £4502.78 with a re-revision rate of less than 0.0064%, then it would most likely be more cost-effective than a fresh-frozen allograft. However, there are additional cost savings and long-term benefits of using decellularised tissue beyond the scope of this economic model. Decellularised bone tissue could be processed alongside other tissues in a large commercial system further reducing the costs and can also be stored at room temperature long term unlike fresh-frozen allografts that require storage at -80ºC.

Native nerve cell-derived extracellular matrix for peripheral nerve regeneration

S. Memarpour Hobbi[1], S. Atkins[2], F. Claeyssens[2], J.W. Haycock[2], & F.M. Boissonade[1]

[1]School of Clinical Dentistry, University of Sheffield, S10 2TA

[2]Kroto Research Institute, University of Sheffield, S3 7HQ

Abstract

Background and Aims. The repair of peripheral nerve injuries is limited by current clinically available nerve guide conduits (NGCs), which do not provide adequate levels of regeneration compared to the gold-standard of a nerve autograft. Studies suggest that the use of individual extracellular matrix (ECM) components can improve nerve regeneration in NGCs (1), yet fewer studies have investigated the effect of a more native-like extracellular matrix as a scaffold for nerve regeneration, containing all the components present in a peripheral nerve. Therefore, in this project we aimed to produce a native-like ECM to act as a scaffold within nerve guide conduits.

Methods. To produce the native-like ECM, embryonic chick-derived dorsal root ganglia (DRG) were cultured on tissue culture plastic, which resulted in the deposition of an ECM along the DRG neurones (DRG-ECM). The cellular components of the DRG-ECM were removed by a mild decellularisation protocol, resulting in a naturally-obtained decellularised ECM (dECM), maintaining features of the native peripheral nerve tissue.

Results. Decellularisation was evaluated by the removal of DAPI-stained nuclei, while collagen was evaluated by the Sirius Red assay. The dECM was evaluated for nerve regeneration using a neuronal cell line (NG108-15), and showed improved cell attachment, neurite extension and neurite length compared to control tissue culture plastic (Fig. 1), highlighting the potential for a naturally nerve cell-derived ECM in nerve repair.

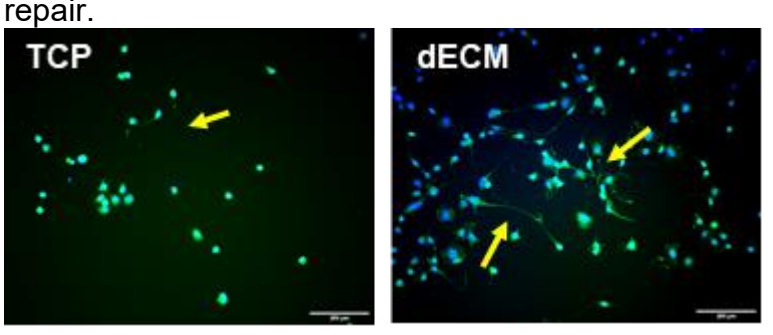

Figure 1. Neuronal cells grown on the decellularised matrix (dECM) show increased neurite extension and cell attachment. Neurites highlighted by yellow arrow. Blue: DAPI stain for nuclei, Green: Phalloidin-FITC stain for F-actin filaments of neurites. Scale bar 200µm.

References. (1). Bell, JH, & Haycock, JW, Tissue Engineering Part B: Reviews, 18(2), 116-128, 2011