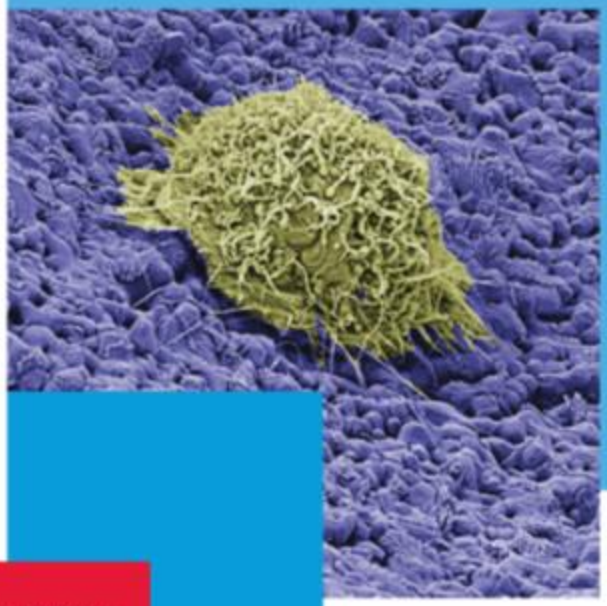
ALLIANCE FOR DESIGN AND APPLICATION IN TISSUE ENGINEERING

THE 5TH TISSUE ENGINEERING SYMPOSIUM

"TISSUE ENGINEERING FOR TISSUE REGENERATION"

THE UNIVERSITY OF SYDNEY – DARLINGTON CENTRE
MONDAY 18TH – WEDNESDAY 20TH AUGUST 2014



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SYMPOSIUM CONVENERS

PROFESSOR HALA ZREIQAT

CHAIR AND FOUNDER OF ADATE
HEAD OF THE TISSUE ENGINEERING AND BIOMATERIALS UNIT
UNIVERSITY OF SYDNEY

PROFESSOR VICKI ROSEN

CONVENER OF ADATE
DEPARTMENTAL HEAD AND PROFESSOR OF DEVELOPMENTAL BIOLOGY
HARVARD SCHOOL OF DENTAL MEDICINE

ORGANISING COMMITTEE

PETER NEWMAN
KHANH HUYNH
KARA SPILLER
MATÉ BIRO
REBECCA MASON
GERALDINE O'NEILL

CONTENTS

FOREWORD: HALA ZREIQAT – CHAIR/FOUNDER OF ADATE

Dear Colleagues

On behalf of ADATE and the University of Sydney, it is my great pleasure to welcome you to the fifth Alliance for Design and Application in Tissue Engineering Symposium.

I would like to extend a most warm welcome to colleagues and friends who have joined us from overseas – from Drexel University, Harvard University, the University of Pennsylvania, from Stanford University, Tufts University, the Center for iPS Cell Research and Application and locally from the University of Adelaide, the University of South Australia, the University of Queensland and the CSIRO. Colleagues from the ANZAC, Garvan and Kolling Institutes, as well as the University of New South Wales all Sydney based, are also participating. Despite the demands of this Symposium, I hope that there will also be time to enjoy something of the unique beauties of our hospitable city and beyond.

There can be no doubt that the challenge of this century for medicine and biomedical engineering will be the regeneration of diseased and damaged tissues and organs. Tissue engineering is a continuing field of research directed towards this achievement. It focuses on the construction of biological substitute matrices containing viable and functioning cells for the restoration, maintenance or improvement of tissue function.

This burgeoning field is inspiring increasing numbers of new investigators and vital support from granting agencies. The coordinated efforts of biologists, physicists, chemists, pharmacists, engineers, computer engineers, material scientists, surgeons and physicians are directed towards life-enhancing outcome. In response to a combination of these opportunities and increasing demand on campus, the Alliance for Design and Application in Tissue Engineering (ADATE) was established in 2006.

This network will continue to enhance opportunities for scientists to expand their intellectual horizons and to build interdisciplinary partnerships. This symposium will draw together scientists and students of tissue engineering from the Faculties of Engineering, Medicine, Science, Pharmacy, Veterinary Medicine and Dentistry.

Distinguished national and international scientists will present exciting progress and debate on a range of scientific and clinical research into Tissue Engineering. Students and postdoctoral fellows will have the opportunity to highlight their discoveries and to build valuable collaboration that will enrich their research. We applaud the Faculties involved and the generous sponsors who have supported the meeting. We trust that you will enjoy this significant scientific symposium at the University of Sydney, organised by the Tissue Engineering and Biomaterials Research Unit.

We welcome you all and trust that you will enjoy a rewarding and stimulating program and also have time to enjoy some of the beauty and cultural riches of this city.

Hala Zreiqat
University of Sydney

PROGRAMME DAY 1 - 18TH AUGUST

LOCATION: Room 124 SIT Building J12, The University of Sydney, NSW, 2006

EARLY CAREER RESEARCH MENTORING SESSION

Inspiration, Motivation and Pursuing Your Vision

ESTABLISHING YOURSELF AS AN INDEPENDENT RESEARCHER

Personal Experience, Career Making Decisions and Things to Remember

CHAIRS: *Kara Spiller, Rebecca Mason, Maté Biro, Peter Newman, Khanh Huynh*

2:00 - 2:05 PM	Hala Zreiqat	*Welcoming speech*
2:05 - 2:10 PM	Kara Spiller	*Introduction to the ECR Workshop*
2:10 - 2:20 PM	Gail Naughton	
2:20 - 2:30 PM	Carol Armour	
2:30 - 2:40 PM	Pamela Yelick	
2:40 - 2:50 PM	Justin Gooding	
2:50 - 3:00 PM	Hala Zreiqat	
3:00 - 3:10 PM	Teruo Okano	
3:20 - 3:30 PM	Sarah Heilshorn	

AFTERNOON TEA

Refreshments Served – Free Discussion

4:10 PM **FREE QUESTION AND ANSWERS**

- *How do we establish a research theme early to help us become independent? How did you develop yours?*
- *How can we identify a supportive environment or mentor to aid in the development of our careers?*
- *What are some tips to develop a unique technology/research path?*
- *How do we ensure successful execution to publications/inventions?*
- *How do we develop collaborations with other researchers?*
- *What are some tips for obtaining grants?*
- *What are some tips for getting promotions?*
- *What are some tips for work/life balance?*

5:00 PM SESSION ENDS

PROGRAMME DAY 2 - 19TH AUGUST

LOCATION: Darlington Centre, 174 City Rd, Darlington NSW 2006

8:00 AM	*Arrival Desk Open – Coffee and Light Breakfast*	
9:00 AM	**Welcome and Opening Remarks: Hala Zreiqat – ADATE co-convenor**	
9:05 AM	**Official Opening by the Hon. Jillian Skinner MP, Minister for Health and Minister for Medical Research**	
SESSION 1 *Stem Cells in Regenerative Medicine*		
CHAIR:	***JASON BURDICK***	
9:10 AM	*Gail Naughton*	Achievements and Challenges in Regenerative Medicine Using Stem Cells
9:40 AM	*Teruo Okano*	Reconstruction of 3D Tissue and Organ by Cell Sheets
10:00 AM	*James Chong*	Novel Cell Therapy to Repair and Regenerate Injured Heart
10:20 AM		**MORNING TEA**
CHAIR:	***CHRIS LITTLE***	
10:50 AM	*Noriyuki Tsumaki*	Cartilage Regeneration with iPS Cell Technologies
11:20 PM	*Aaron Schindeler*	Cell Lineage Tracking in Tissue Engineered Bone
EARLY CAREER RESEARCHER PRESENTATIONS SECTION		
11:40 PM	*Yasuhito Yahara*	Lineage-Tracing of Articular Chondrocytes During Osteoarthritis Development
11:50 PM	*Zufu Lu*	Synergy of Nanomaterials and BMP-2 Signaling in Osteogenic Differentiation
12:00 PM	*Steven Eamegdool*	Superparamagnetic Iron Oxide Nanoparticle Prelabelling of Neural Precursor Cells
12:10 PM	*Kritika Katiyar*	Tissue Engineered Living Scaffolds for Targeted Axonal Outgrowth
12:20 PM	*Gayle Petersen*	Generation of Induced Neuronal and Glial Cells from Adipose-Derived Stem Cells
12:30 PM		**LUNCH AND INDUSTRY SESSIONS**
SESSION 2 *Biomaterials and Tissue Engineering*		
CHAIR:	***HEIKE WALLES***	
1:30 PM	*Sarah Heilshorn*	Injectable Protein-Ceramic Hybrid Gels for Bone Tissue Engineering
2:00 PM	*Jason A. Burdick*	Engineering Fibrous Hydrogels for Cartilage Repair
2:20 PM	*Nico Voelcker*	High Throughput Screening Technologies To Optimise Cell-Biomaterial Interactions
CHAIR:	***TERUO OKANO***	
2:40 PM	*Justin Gooding*	Using Nano-Biomaterials to Influence Biological Processes
3:00 PM	*Jerome Werkmesiter*	New Customized Bioengineered Collagens for Tissue Engineering Applications
3:20 PM		**AFTERNOON TEA**
EARLY CAREER RESEARCHER PRESENTATIONS SECTION		
JUDGES:	***PAMELA YELICK, CHRIS JACKSON, MATÉ BIRO, QING LI***	
3:50 PM	*Takeshi Kimura*	Immunogenicity of Chondrocytes Derived From Human Induced Pluripotent Cells
4:00 PM	*Yu Suk Choi*	Tissue Stiffness Mimicked Biomaterial Design and Stem Cell Mechanotransduction
4:10 PM	*Gabriella Lindberg*	Strategy for Fabricating Cell-Laden ECM Hydrogels in Cartilage Tissue Engineering
4:20 PM	*Paul Bradbury*	A Sting in the Tail: Focal Adhesion Targeting and Mechanotransduction
4:30 PM	*Richard Wang*	*in vivo* Validation of a Novel Skin Substitute for Chronic Wounds
4:40 PM	*Giselle Yeo*	ECM-Functionalized Ti Alloy Substrates for Improved Osseointegration
4:50 PM	*Ali Negahi Shirazi*	Fabrication of Micropatterned Hydrogels with Favorable Physical Stability
7:00 PM		**GALA DINNER** *Wolfies Waterfront, 27 Circular Quay West, The Rocks, NSW 2000* http://docksidegroup.com.au/restaurants/waterfront/location

PROGRAMME DAY 3 - 20TH AUGUST

LOCATION: Darlington Centre, 174 City Rd, Darlington NSW 2006

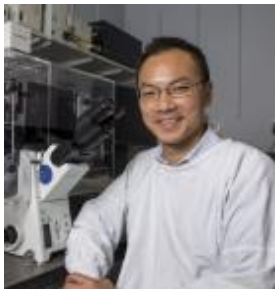
8:00 AM	*Arrival Desk Open – Coffee and Light Breakfast*	
SESSION 3 *Reconstruction of Tissue Architectures*		
CHAIR:	***GAIL NAUGHTON***	
9:00 AM	*Teruo Okano*	Strategies for Improving Vascularization of Engineered Tissues
9:30 AM	*Gail Naughton*	Embryonic-like ECM Stimulates Endogenous Stem Cells and Stem Cell Niche
9:50 AM	*Geraldine O'Neil*	Biophysical Cues Regulating Cancer Cell Migration in 3D
10:10 AM		**MORNING TEA**
CHAIR:	***SARAH HEILSHORN***	
10:30 AM	*Jason A. Burdick*	Engineering Injectable Hydrogels to Influence Cardiac Repair
11:00 AM	*Luiz Bertassoni*	Bioprinting Three-dimensional Vascularized Tissues
11:20 AM	*Toby Coates*	Cell Therapy for Islet Transplantation
EARLY CAREER RESEARCHER PRESENTATIONS SECTION		
JUDGES:	***JASON BURDICK, NICO VOELCKER, MARGARET SMITH***	
11:40 AM	*Aghaei-Ghareh-Bolagh*	Design of Three-Dimensional Tissue Constructs Using Graphene
11:50 AM	*Justine Roberts*	Cytocompatible Biosynthetic Heparin-Poly(Vinyl Alcohol) Hydrogels
12:00 PM	*Tegan Cheng*	A Sugar-Based Injectable Carrier for Bone Formation and Healing
12:10 PM	*Jiao Jiao Li*	Novel Biphasic Scaffold for Repair and Regeneration of Osteochondral Defects
12:20 PM	*Pamela Graney*	Ceramic Scaffolds Induce M1-to-M2 Transition *in vitro*
12:30 PM	*Jelena Rnjak-Kovacina*	Strategy Towards Biomaterial Vascularization
12:40 PM	*Alex Baume*	Infected wound Model for Analyzing Scaffolds
12:50 PM	*Lim Khoon*	Cell Survival in Biosynthetic PVA-Tyramine Hydrogels: Incorporation of Antioxidants
1:00 PM	*William Lu*	Biofunctionalization of PEEK Using Plasma Immersion Ion Implantation Treatment
1:10 PM		**LUNCH AND INDUSTRY SESSIONS**
SESSION 4 *Enhancing Biomaterials and Cell Signalling for Regenerative Medicine*		
CHAIR:	***NORIYUKI TSUMAKI***	
2:10 PM	*Pamela Yelick*	Progress and Challenges in Tooth Tissue Engineering
2:40 PM	*Kara Spiller*	Harnessing the Inflammatory Response for Tissue Regeneration
3:00 PM	*Fariba Dehghani*	Biomaterials and Bone Tissue Engineering
3:20 PM		**AFTERNOON TEA**
CHAIR:	***CHRIS JACKSON***	
3:50 PM	*Justin Cooper-White*	Deterministic Biomaterials for Enhanced Differentiation and Tissue Genesis
4:10 PM	*John McAvoy*	Understanding Lens Development to Regenerate After Cataract Surgery
4:30 PM		**PRIZE PRESENTATIONS AND CLOSING REMARKS**

INVITED SPEAKERS

PROFESSOR GAIL NAUGHTON

Dr. Gail Naughton founded Histogen, Inc. in 2007, and currently serves as CEO and Chairman of the Board for the Company. She has spent more than 25 years extensively researching the tissue engineering process, holds more than 95 U.S. and foreign patents, and has been extensively published in the field.

DR JAMES CHONG



Dr James Chong MBBS, FRACP, PhD is a Consultant Cardiologist at Westmead hospital and leads a research group at the University of Sydney School of Medicine/Westmead Millennium Institute. His research aims to translate findings from the field of Cardiac Regeneration into viable clinical therapies for patients with heart failure.
Dr Chong trained in cardiology at Westmead Hospital before completing a PhD at the Victor Chang Cardiac Research Institute under the mentorship of Prof Richard Harvey. This doctoral training in cardiac development and stem cell biology focused on a previously unidentified population of cardiac stem cells. Post-doctoral training was then performed at the University of Washington, Seattle, USA with Prof Charles (Chuck) Murry. During this period he extended his interests in translational cardiac regeneration to include the use of pluripotent stem cells in small and large animal models of myocardial infarction.

PROFESSOR TERUO OKANO

Teruo Okano is currently the Professor at Tokyo Women's medical University (TWMU) in Tokyo Japan. He received his Ph.D. from Waseda University in 1979. After several years as an Assistant Professor at TWMU, he joined the University of Utah (1984-1988) and later is an Adjunct Professor in the Department of Pharmaceutics since 1994. He returned to TWMU in 1988 as an Associate Professor and became a Full Professor in 1994. He then became Director of the Institute of Biomedical Engineering in 1999 and initiated the present institute, Advanced Biomedical Engineering and Science (ABMES), in 2001. He was the Vice President of TWMU and the Director of ABMES up to March 2014.
He developed temperature-responsive polymeric surfaces for harvesting cultured two-dimensional cell layers. Based on this technology, he has proposed the new concept of "cell sheet engineering" which introduces an alternative path for tissue and organ regeneration.
He received numerous awards including the Clemson Award for Basic Research (1997) given by the Society for Biomaterials (U.S.A.) and Emperor's Medal with Purple Ribbon (National Achievement Award) (2009) from His Majesty of the Emperor of Japan.

PROFESSOR VICKI ROSEN

Dr. Vicki Rosen arrived at HSDM by way of industry, having spent the majority of her research career as a scientist at Genetics Institute, a biotechnology company, where she was part of a research team that identified the bone morphogenetic protein (BMP) genes in 1988. She became a professor in the Faculty of Medicine in 2001, and chair of the Department of Developmental Biology at HSDM in 2005.

Dr. Rosen's lab studies the physiological roles that bone morphogenetic proteins (BMPs) play in the development, maintenance, and repair of musculoskeletal tissues (bone, cartilage, tendon, ligament, meniscus, muscle). The researchers use molecular, cellular, and genetic approaches in a variety of model systems (Xenopus, chick, and mouse) to investigate BMP activities. The investigators believe that enhancing current understanding of BMP biology will lead to the development of novel strategies for repair and regeneration of individual components of the musculoskeletal system, as well as provide new models for examining complex tissue interactions that are required for its function.

PROFESSOR NORIYUKI TSUMAKI

Head of the Noriyuki Tsumaki Lab at the Kyoto Center for iPS Cell Research and Applications.
The Tsumaki laboratory aims to elucidate mechanisms of cell differentiation, to develop methods for the treatment of cartilage disease.

PROFESSOR JASON BURDICK

Jason A. Burdick, PhD is a Professor of Bioengineering at the University of Pennsylvania. Dr. Burdick's research involves the development of hydrogels for various biological applications and his laboratory is specifically interested in understanding and controlling polymers on a molecular level to control overall macroscopic properties. These hydrogels include photocrosslinkable systems based on natural polymers that exhibit spatially and temporally distinct properties and can be processed into fibrous structures, as well as self-assembled materials designed from non-covalent chemical interactions that are useful as injectable hydrogels.
The applications of his research range from controlling stem cell differentiation through material cues to fabricating scaffolding for regenerative medicine and tissue repair. Jason currently has over 140 peer-reviewed publications and has been awarded a K22 Scholar Development and Career Transition Award through the National Institutes of Health, an Early Career Award through the Coulter Foundation, a National Science Foundation CAREER award, a Packard Fellowship in Science and Engineering, and an American Heart Association Established Investigator Award. He is on the editorial boards of *Tissue Engineering*, *Biomedical Materials*, *Biomacromolecules*, *Journal of Biomedical Materials Research A*, and *ACS Applied Materials and Interfaces*.

ASSOC PROFESSOR SARAH HEILSHORN

Sarah Heilshorn is Associate Professor with Tenure in the Department of Materials Science and Engineering and, by courtesy, the Departments of Bioengineering and Chemical Engineering at Stanford University. Prior to joining Stanford in 2006, Prof. Heilshorn was a postdoctoral scholar in the Department of Molecular and Cell Biology at the University of California, Berkeley. She completed her Ph.D. and M.S. studies in Chemical Engineering at Caltech in 2004 and 2000, respectively. She earned a B.S. in Chemical Engineering at Georgia Tech in 1998.
She combines these diverse fields to design new materials that mimic those found in our bodies for applications in tissue engineering and regenerative

medicine. Recent recognitions include the NSF Career Award and the NIH New Innovator Award.

DR AARON SCHINDELER

Dr Schindeler completed his PhD at the Victor Chang Cardiac Research Institute in 1998. His research examined the role of a novel muscle protein in heart and skeletal muscle. As part of this project, he pioneered several new techniques including a method for quantifying actin turnover in cultured cells. His PhD research has been published in the Journal of Cell Biology and Experimental Cell Research. In 2001 he was awarded the St Vincent's Hospital Junior Researcher Award for Excellence. In 2004 his research took a shift in focus from muscle to bone as he took on a postdoctoral position at the Orthopaedic Research & Biotechnology Unit.

Dr Schindeler has been a lead figure in developing a new research project focused on the cellular determinants of bone repair. This began with examining the role of muscle stem cells in bone repair using both cell culture and tissue-specific transgenic mice. Data from this project has been presented at local and national meetings and resulted in a successful NHMRC Project Grant for 2007-8. Dr Schindeler has been successful at attracting grant funding for his research. In the five years since submitting his PhD, he has helped attract over a million dollars of competitive grant funding to his unit.

Dr Schindeler currently holds dual roles as a Research Scientist at the Kids Research Institute and a Lecturer at the University of Sydney. He supervises several postgraduate students and has completed Supervisor Training via the Institute for Teaching and Learning (ITL) at the University of Sydney.

PROFESSOR NICO VOELCKER

Deputy Director of the Mawson Institute. Professor Voelcker drives research on the development of new nanostructured materials with superior properties for applications in biosensors, biochips, biomaterials, tissue engineering and drug delivery. His laboratory has successfully accomplished groundbreaking collaborative research with neuroscientists at the Flinders Medical Centre (FMC) investigating the aggregation of proteins responsible for Parkinson's disease.

PROFESSOR HALA ZREIQAT

Professor Hala Zreiqat is a National Health and Medical Research Fellow, Head of the Biomaterials and Tissue Engineering Research Unit in the Faculty of Engineering, University of Sydney. Her group consists of multidisciplinary team of researchers including engineers, cell and molecular biologists and clinicians. She specializes in developing engineered biomaterials and scaffolds for skeletal tissue applications, and investigating their effect on in vitro and in vivo osteogenesis.

Her team conducts research to gain greater understanding of bone/cartilage and endothelial cells biology when in contact with engineered biomaterials. She has over 80 peer-reviewed publications; 4 review papers; 12 book chapters; and over 120 abstracts in national and international meetings. She is regularly invited to give keynote and plenary presentations at major international and national conferences.

She has organized / chaired a number of major international conferences/ symposia / workshops. She is the immediate past president of the Australian and New Zealand Orthopaedic research Society (2010-2012). Founder & Chair, Alliance for Design and Application in Tissue Engineering (formerly known as Sydney University Tissue Engineering Network - SuTEN, 2006 to present). Amongst her awards are: Leopold Dintenfass Memorial Award, for Excellence in Research (2012); University of Sydney Engineering Deans Research Award (2009).

ASSOC PROFESSOR GERALDINE O'NEILL

Geraldine O'Neill is Group Leader of the Focal Adhesion Biology (FAB) group at the Kids Research Institute and Conjoint Associate Professor with the University of Sydney. The central theme of her research is to understand how cancer cell interaction with the surrounding matrix leads to the progression to metastatic disease, one of the major causes of cancer patient mortality. Her team investigates the cell biology of cancer cell invasion, with a particular focus on brain tumours and neuroblastoma.

PROFESSOR PAMELA YELIC

Pamela C. Yelick, Ph.D. is a Tenured Full Professor in the Department of Oral and Maxillofacial Pathology, Tufts University School of Dental Medicine, where she is the Director of the Division of Craniofacial and Molecular Genetics. Dr. Yelick also holds adjunct appointments in the Cell, Molecular and Developmental Biology, Genetics, and Pharmacology Programs in the Sackler School of Graduate Biomedical Sciences, Tufts Medical School, Boston, MA, and in the Department of Biomedical Engineering, School of Arts and Sciences, Tufts University, Medford, MA.

Dr. Yelick's research focuses on elucidating and understanding molecular signaling cascades regulating the processes of mineralized tissue development, homeostasis, disease and regeneration. Dr. Yelick holds extensive published expertise in basic research pertaining to craniofacial development and regeneration using the zebrafish and mouse models, and in clinically relevant dental tissue engineering research models, using three dimensional dental cell seeded scaffolds implanted into rat and pig models, for eventual applications in dental tissue and whole tooth regeneration. Dr. Yelick has received NIH funding since 1990 and recently received an AFIRM II R01 award.

Dr. Yelick is an internationally recognized leader in dental tissue engineering and craniofacial development, with over 70 peer-reviewed basic research publications, more than a dozen reviews, and over 100 abstracts since the year

2000. She has received national and international acclaim for her research on dental tissue and whole tooth tissue engineering, and has participated in more than 150 Invited Speaker Lectureships.

ASSISTANT PROFESSOR KARA SPILLER

Kara Spiller is currently an Assistant Professor in Drexel University's School of Biomedical Engineering, Science, and Health Systems. A member of the first class of Drexel's accelerated BS/PhD program, Dr. Spiller received bachelor's and master's degrees in biomedical engineering from Drexel University in 2007. As an NSF Graduate Research Fellow, she conducted her doctoral research in the design of semi-degradable hydrogels for the repair of articular cartilage in the Biomaterials and Drug Delivery Laboratory at Drexel (PI: A. Lowman) and in the Shanghai Key Tissue Engineering Laboratory of Shanghai Jiao Tong University (PI: W. Liu), with support from the NSF Doctoral Dissertation Enhancement Program (DDEP).
After completing her PhD in 2010, she conducted research in the design of scaffolds for bone tissue engineering on a Fulbright fellowship in the Biomaterials, Biodegradables, and Biomimetics (the 3Bs) Research Group at the University of Minho in Guimaraes, Portugal (PI: R.L Reis). She then conducted postdoctoral studies towards the development of immunomodulatory biomaterials for bone regeneration in the Laboratory for Stem Cells and Tissue Engineering at Columbia University (PI: G. Vunjak-Novakovic), before returning to Drexel in 2013. Her current research interests include cell-biomaterial interactions, the design of immunomodulatory biomaterials, and international engineering education.

ASSOCIATE PROFESSOR TOBY COATES

The Centre for Clinical and Experimental Transplantation is part of the School of Medicine in the Central Northern Adelaide Renal and Transplantation Service on the 9th Floor of the Royal Adelaide Hospital (East Wing). The group is run by Assoc Prof Toby Coates and our labs are situated in the Hanson Centre Laboratories on Frome Road.
Toby's research focuses on the isolation and transplantation of healthy pancreatic islets as an innovative treatment and potential cure for type 1 diabetes. As part of the Australian Islet Transplantation Consortium, the laboratory prepares and performs assays on purified islets that are ultimately transplanted into patients. To date, the Consortium has transplanted 15 patients across Australia. Factors limiting the success of islet transplantation include suboptimal engraftment, immune reaction and rapid cell death post-transplant. Our laboratory is interested in identifying and combating the causes of islet cell death. We are also interested in basic islet biology, and how function can be protected/quickly restored following transplantation.
The laboratory is based in the Hanson Institute and is part of the Basil Hetzel Institute at the Queen Elizabeth Hospital site and the Robinson Institute. We reside within the Central Northern Adelaide Renal and Transplantation Service, where scientists regularly attend departmental meetings and many of the clinicians are heavily involved with research projects. We recently won a NHMRC project grant beginning in 2009 and we have active collaborations with groups bringing expertise in vascular biology including Dr Claudine Bonder, Centre for Cancer Biology and Cellular Neuroscience Dr Damien Keating, Flinders University.

PROFESSOR JUSTIN COOPER WHITE

Professor Justin Cooper-White is a global leader in using engineering to solve problems in biology. In addition to holding the position of AIBN Group Leader, Professor Cooper-White is Director of the Australian National Fabrication Facility-Queensland Node and the Associate Dean (Research) Faculty of Engineering, Architecture and Information Technology at UQ. He is a past President of both the Australasian Society for Biomaterials and Tissue Engineering and the Australian Society of Rheology.

DR JEROME WERKMESITER

Dr Jerome Werkmeister is a Cell Biologist/Immunologist by training, completing his Ph.D at Monash University in 1978. Prior to joining CSIRO he held various positions at the Kanematsu Institute, Ontario Cancer Foundation, Queens University and Walter and Eliza Hall Institute. He is currently a Chief Research Scientist at CSIRO in the Biomedical Materials and Devices Theme. He is a tissue engineer with expertise in materials and scaffold design, cell and matrix biology, molecular biology and immunology. He has considerable expertise in 3D culture of cells in composite and biological scaffold materials designed and fabricated at CSIRO. He also has expertise in evaluating the tissue response of implanted materials in animal models using specific antibodies to various collagen types developed by CSIRO; understanding structure/function of extracellular matrix, particularly collagen, and has used this knowledge to develop natural biopolymers for biomedical use; novel recombinant uses of collagens as effective scaffolds for cells; platform photo-crosslinking technology for biological self assembling proteins for use as tissue sealants and scaffolds; developing and fabricating new materials, biological, synthetic and textiles, for scaffolds for tissue regeneration. Dr Werkmeister serves on the editorial board of several international biomaterial journals, sits on Standards Australia Technical Committee HE/1/4 on Surgical Implants, and has published 168 scientific papers and a number of patents. He was a co-founder of the Australasian Society for Biomaterials and has been recognised for his scientific contributions to the field of biomaterials science internally by the award of CSIRO Medal twice and externally by the award of Fellow, Biomaterials Science and Engineering.

PROFESSOR JOHN MCAVOY

In the ‘Lens Research Group’ the overarching goal of our research program is to elucidate key developmental mechanisms that govern normal lens development. This information is fundamental for successful recapitulation of the processes required for regeneration of normal lens structure and function after cataract surgery. In particular, the regulation of fibre differentiation has been a major research focus. Our laboratory has over 35 years experience in lens developmental biology research. Whilst both in vitro and in vivo systems have been developed and employed by our lens group to identify key molecular regulators of the fiber differentiation process, of particular note is the development of a lens epithelial explant system. This has proven to be a reliable system for identifying key regulators of lens cell behaviour in the whole animal. Development of this system was initiated during my postdoctoral period in the Nuffield Laboratory of Ophthalmology, University of Oxford (1975-79) where I also received a solid grounding in lens research.
After moving to The University of Sydney in 1979 and establishing the ‘Lens Research Laboratory’, the explant system was instrumental in 1987 in identifying members of the FGF growth factor family as key inducers of fibre

differentiation. This was followed in 1994 by identifying TGFßs as inducers of fibrotic cataracts. Most recently we have shown that during FGF-induced differentiation, Wnt/Frizzled signalling through the planar cell polarity (Wnt-Fz/PCP) pathway has a critical role in promoting the precise alignment/orientation of fibres. Current research aims to exploit these findings with the ultimate aim of being able to promote the coordinated cell behaviour that is required to generate the exquisite three-dimensional organization of fibres (and epithelial cells) and regenerate lens structure and function after cataract surgery.

DR LUIZ BERTASSONI

Dr. Luiz E. Bertassoni graduated from Dental School in Brazil and obtained a PhD in Biomaterials from University of Sydney. He was a postdoctoral fellow at University of California San Francisco, Harvard Medical School and MIT. Currently, Dr. Bertassoni holds a faculty position in Biomaterials and Bioengineering at the Faculty of Dentistry, University of Sydney, where he leads a research group working on various aspects of biomaterials and regenerative medicine. Luiz is a recipient of over 20 national and international research awards and 7 competitive research grants. He is an author of over 20 publications, including research papers and book chapters. Luiz currently serves as ad-hoc reviewer or editorial board member for over 20 scientific journals. His research addresses microscale strategies for tissue engineering, including bioprinting and hydrogel microfabrication; micro- and nanoscale structural and mechanical properties of calcified tissues; and different aspects of microfluidics and ‘organs-on-a-chip’.

DAY 2 – 19TH AUGUST

SESSION 1 – STEM CELLS IN REGENERATIVE MEDICINE

PROFESSOR GAIL NAUGHTON
FOUNDER, CHAIRMAN, CEO, HISTOGEN, INC. USA

HUMAN EMBRYONIC-LIKE ECM STIMULATES ENDOGENOUS STEM CELLS AND CREATES A STEM CELL NICHE IN VIVO

We have developed a defined, naturally-secreted soluble (CCM) and insoluble human extracellular matrix (hECM) with embryonic-like characteristics. These materials are secreted by neonatal human fibroblasts grown in microcarrier culture systems under embryonic conditions of hypoxia and suspension, which upregulates a number of substances associated with stem cell niches in the body including various laminins, Collagen 4, CXCL12, NID1, NID2, and NOTCH2. This material has been shown to support proliferation of hESCs and MSCs, and can be manufactured reproducibly under GMP conditions. The CCM contains a number of growth factors including Follistain, Noggin, VEGF, Stem Cell Factors, and Stromal Derived Factor 1alpha which are known to attract MSCs in vivo and stimulate stem cells. Intradermal injections of the CCM have been shown to stimulate follicular stem cells and induce new, prolonged hair growth in three clinical trials with subjects with male pattern baldness. No severe adverse reactions or toxicities have been seen The CCM has also been shown to be osteoinductive, osteoconductive, and angiogenic. The hECM rapidly supports the infiltration of MSCs in vitro and in vivo and upregulate Collagen II and Aggrecan. In full thickness osteochondral defects in rats the hECM induced new bone formation, mature blood vessels, and hyaline cartilage. The quality of the new tissue was excellent, as evidenced by the modified O'Driscoll score ($p<0.001$). These results support the hypothesis that our embryonic-like proteins stimulate endogenous stem cells as well as simulate a stem cell niche in vivo, offering the potential of many therapeutic applications.

PROFESSOR TERUO OKANO
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STRATEGIES FOR IMPROVING VASCULARIZATION OF ENGINEERED TISSUES

We have developed unique tissue culture dishes with the thermo-responsive polymer poly(*N*-isopropylacrylamide) (PIPAAm). Using these cultured cell sheets harvested from the temperature-responsive surfaces, we have established so called "cell sheet engineering" to create functional tissue sheets to treat a wide range of diseases from corneal dysfunction to esophageal cancer and cardiac failure. And also, we have established the new method of 3D tissue and organs by making layered cell sheets.

The “intelligent surface” of these dishes possessed the hydrophobicity similar to regular tissue culture polystyrene dishes at 37°C. However, the surface reversibly became hydrophilic at a lower temperature under 32°C and spontaneously released the cultured cells as a single layer without the need for trypsin or EDTA, thus leaving the cell layer with extracellular matrix (ECM) intact. All the cultured confluent cells were harvested as a single contiguous cell sheet from the thermo-responsive culture dishes and readily applied to other biological and non-biological surfaces[1)]. We here propose this novel system of cells and cell-layers arrangement called “cell sheet engineering.”

We initiated human clinical studies of cell sheet engineering therapy using oral mucosal cell sheet for treatment of cornea epithelium deficient disease and recovery from endoscopic submucosal dissension surgery for esophageal epithelial cancer, periodontal regenerative therapy, cartilage regenerative therapy, and we also succeeded in treating cardio-myopathy using myoblast cell sheet. To make thick 3D tissue, multi-step transplantation at 1 or 2-day intervals gave synchronously beating-thick-myocardial tissues with blood vessels. To imitate *in vivo* environment, the production of media-perfused microvascular beds *in vitro* and the transplantation of layered rat cardiac cell sheets over the artificial bed was investigated. One approach was to use living tissue having a connectable artery and vein system, which was resected from rats[2)]. Other was to create collagen-based microchannels by gelling collagen around paralleled stainless wires and extracting them[3)]. In both cases, culture media was supplied by bioreactors or the tissue, and then triple-layered rat cardiac cell sheets having endothelial cells were put on previously planted cell sheets. Interestingly, capillaries were regenetrated between the cardiac cell sheets and the vascular beds. Blood perfusion analyses clearly demonstrated that red blood cells passed through the capillaries and reached into the cardiac tissue indicating the possibility of *in vitro* functional blood vessel formation and the further development of bioengineered thick myocardial tissues with sufficient vascular network. *In vitro* vascular network formation in 3-D tissues should be a breakthrough technology in regenerative medicine and contribute to future organ engineering.

Cell sheet engineering is a highly promising tool to achieve new therapy and reconstruction of 3D tissue in the fields of tissue engineering and regenerative medicine.

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DR JAMES CHONG
UNIVERSITY OF SYDNEY

NOVEL CELL THERAPY TO REPAIR AND REGENERATE INJURED HEART

Cell therapies specifically targeting heart failure could greatly decrease morbidity, mortality and burgeoning health care costs worldwide. These novel therapies can be broadly grouped into two categories. The first, Adult Stem/Progenitor Cells (ASCs) have a limited ability to form down-stream differentiated cells (termed plasticity). Nevertheless, ASCs have already been used in many clinical trials investigating cardiac repair. Results have shown a favourable safety profile but inconsistent results regarding efficacy. The second category, Pluripotent Stem Cells (PSCs) have

an unquestionable ability to form bonafide, spontaneously contracting, cardiomyocytes. However, as a cardiac regenerative strategy PSCs currently remain in the preclinical arena. This presentation will discuss recent work on a Platelet Derived Growth Factor Receptor-Alpha expressing ASC population[1-2] and on human PSC derived cardiomyocytes (hPSC-CM). Particular focus will be made on recent non-human primate experiments

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PROFESSOR NORIYUKI TSUMAKI
CENTER FOR iPS CELL RESEARCH AND APPLICATION

CARTILAGE REGENERATION WITH IPS CELL TECHNOLOGIES

Articular cartilage covers the ends of bones and provides shock absorption and lubrication to diarthrodial joints. Articular cartilage is an avascular tissue, and has limited capacity for repair. The repair of cartilage damage with healthy hyaline cartilage has been a challenging clinical problem. Since chondrocytes undergo dedifferentiation when expanded in monolayer culture[1], there is a need to develop sources of chondrocytes that can be used for cell transplantation into the cartilage defects in regenerative medicine. We have been trying to generate chondrocytic cells using cell reprogramming technologies. One approach is to convert somatic cells, such as dermal fibroblasts or blood cells, into induced pluripotent stem cells (iPSCs), followed by inducing their differentiation toward chondrocytes. The methods used to induce the differentiation of embryonic stem cells (ESCs) toward chondrocytes can be applied for the differentiation of iPSCs. However, it is still needed to develop methods that can yield hyaline cartilage, rather than fibrocartilage, and that can minimize the risk of tumor formation when the cells are transplanted. We are generating a human iPSC lines that expresses EGFP when the cells have differentiated into chondrocytes, and have been using them to search for the optimal conditions for the chondrogenic differentiation of human iPSCs. Another approach is direct cell type conversion. We found that the transduction of mouse[2] dermal fibroblasts with two reprogramming factors (c-Myc and Klf4) and one chondrogenic factor (Sox9) results in the direct induction of chondrogenic cells without going through the iPS cell state[3]. Transduction of human dermal fibroblasts with the same combination of factors also resulted in the direct induction of chondrogenic cells[4]. This approach may also provide a candidate cell source for chondrocytes that can be used for cartilage regeneration.

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DR AARON SCHINDELER
UNIVERSITY OF SYDNEY

CELL LINEAGE TRACKING IN TISSUE ENGINEERING BONE

Bone tissue engineering strategies use endogenous cells and/or transplanted cells from the host or a donor to regenerate an osseous defect. Many studies using cell transplantation have shown very poor incorporation of transplanted cells into bone defects, despite significant improvements in regeneration. We have been using Cre-Lox approaches to track cells of different lineages in models of de novo bone formation. It has been previously suggested by several groups that cells of the Tie2-lineage can undergo endothelial to mesenchymal transitions to osteoblasts. Evidence from a Tie2-cre Ai9 reporter line show that while these cells are abundant in rhBMP-2 induced ectopic bone, Tie2-lineage cells contribute primarily to endothelial tissues and osteoclasts.

We have also attempted to use inducible Cre-Lox systems to answer other specific questions of relevance to tissue engineering. We generated an inducible Col2-creERT2 Ai9 reporter model to address the transdifferentiation of chondrocytes during endochondral ossification. In addition we have looked at local tissue labelling with tamoxifen using a globally inducible UBC-creERT2 Ai9 reporter model. In both of these systems we can report challenges associated with the inducible technology, in particular non-specific and leaky expression.

These studies highlight the insights that can be gained by cell tracking studies, but also some of the intrinsic challenges with the mouse models.

SESSION 1 – ECR PRESENTATIONS SECTION

LINEAGE-TRACING ANALYSIS OF ARTICULAR CHONDROCYTES DURING OSTEOARTHRITIS DEVELOPMENT

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INTRODUCTION

Chondrocytes which locate above the tidemark in articular cartilage appear neither to proliferate nor undergo hypertrophy under physiological conditions. On the other hand, it has been reported that hypertrophic chondrocytes are detected above the tidemark in articular cartilage in mouse osteoarthritis (OA) models. However, it is not fully understood how the articular chondrocyte differentiate and behave in articular cartilage during development of OA. The purpose of this study is to trace chondrocyte lineage in articular cartilage during development of OA using *Col11a2-CreER; Rosa26-stop^{flox}-EYFP* transgenic mice.

EXPERIMENTAL METHODS

We generated transgenic mice expressing CreER in chondrocytes under the control of type XI collagen α2 chain gene (*Col11a2*) promoter and enhancer sequences (*Col11a2-CreER*). *Col11a2-CreER* mice were mated with *Rosa26-stop^{flox}-EYFP* tester mice. Upon administration of tamoxifen, chondrocytes in the resultant *Col11a2-CreER; Rosa26-stop^{flox}-EYFP* mice are supposed to be marked by YFP expression.

Eight week-old *Col11a2-CreER; Rosa26-stop^{flox}-EYFP* mice were intraperitoneally injected with 0.1 mg/g of body weight tamoxifen for 5 days. Four and eight weeks after injection, mice were sacrificed. Knee articular cartilage was dissected, frozen, sectioned and subjected to analysis of YFP signals by a fluorescence microscope.

In addition, right knees of eight week-old tamoxifen injected-*Col11a2-CreER;Rosa26-stop^{flox}-EYFP* mice were subjected to destabilized medial meniscus (DMM) or sham operations. Four and eight weeks after operation, mice were sacrificed and expression of collagen type X (COL10) and YFP protein were examined by immunohistochemistry.

RESULTS AND DISCUSSION

We detected YFP fluorescence in cells above the tidemark but not in the calcified zone, and COL10 expression in calcified zone but not above the tide mark in articular cartilage of non-operated mice at four and eight weeks after injection of tamoxifen.

DMM operated-*Col11a2-CreER; Rosa26-stop^{flox}-EYFP* mice expressed COL10 both in the zone above tidemark and the calcified zone. Of note, YFP expression was detected in some of the cells which locate in the calcified zone in addition to the cells above the tidemark. On the other hand, YFP expression was not detected in the calcified zone in articular cartilage of sham-operated mice.

These results suggest that osteoarthritic condition created by DMM operation induce hypertrophic differentiation of cells above the tidemark in articular cartilage and may cause migration of cells above tidemark toward calcified zone.

CONCLUSIONS

Cells above the tidemark in articular cartilage do not migrate toward calcified zone for at least 8 weeks in physiological condition.

Osteoarthritic conditions induce hypertrophic differentiation of *COL11A2*-positive chondrocytes above the tidemark.

Osteoarthritic conditions cause migration of chondrocytes above tidemark toward calcified zone.

TISSUE STIFFNESS MIMICKED BIOMATERIAL DESIGN AND STEM CELL MECHANOTRANSDUCTION

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INTRODUCTION

In recent years, ECM stiffness and resulting cell contractility have been identified as potent stem cell differentiation regulators. Successful stem cell-based therapies will require acclimating cells to the abnormally stiff ECM of muscular dystrophy while inducing and/or maintaining myogenesis, fusion, and dystrophin delivery. Here we showed that adipose-derived stem cells (ASCs) more completely undergo extracellular matrix (ECM)-directed myogenesis than their bone marrow-derived counterparts (BMSCs) by expressing myogenic markers 40-fold higher and forming fused muscle *in vitro*; fusion rates, however, still produce too few myotubes to constitute a clinically viable cell source (~2%).

EXPERIMENTAL METHODS

To encourage end-on cell fusion to form aligned skeletal muscle, a mechanically patterned substrate with alternating neuronal- and muscle-like stiffness that mimicks innervated muscle may be a more physically appropriate environment. This mechanically patterned substrate or 'Zebraxis' matrix was fabricated with alternating regions of soft (neurogenic), firm (myogenic), and/or stiff matrix (fibrotic or osteogenic).

RESULTS AND DISCUSSION

ASCs, C2C12 myoblasts, and PC12 neuronal cells all differentially sorted themselves based on their stiffness preference: cardiomyocytes, myoblasts, and ASCs all durotaxed to the myogenic regions of the pattern whereas neurons had opposing behavior. With additional alignment, ASCs fused into multi-nucleated myotubes at a rate almost twice that on static hydrogels. Moreover, a great fraction of ASCs-derived myotubes underwent multiple rounds of fusion due to alignment of their cadherin-rich ends. Most importantly, the multi-nucleated myotubes that form are resistant to transdifferentiation when plated onto a stiffer matrix mimicking a more fibrotic-like stiffness. Singly nucleated ASCs are not, suggesting that additional strategies are necessary to achieve a pure myotube fraction. However differential sorting, enhanced fusion, and multiple fusion events supports using ECM to create spatially-patterned *in vitro* muscle for regenerative uses in fibrotic muscle diseases such as muscular dystrophy.

CONCLUSION

A mechanically patterned hydrogel mimicking tissue specific stiffness in flat surface, e.g. “Zebraxis” can induce lineage specific differentiation. Due to enhanced ASC fusion, our ‘Zebraxis’ hydrogels provide a platform to create tissue engineered, innervated micro-muscle to test cells from disease models, e.g. muscular dystrophy. Our efforts to develop tissue engineered musculoskeletal systems hinge on the understanding gained here about stem cell-microenvironment interactions.

SYNERGISTIC EFFECT OF NANOMATERIALS AND BMP-2 SIGNALLING IN INDUCING OSTEOGENIC DIFFERENTIATION OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS

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INTRODUCTION

Mesenchymal stem cells (MSCs) hold a great promise for the repair and regeneration of large bone defects and non-union bone fractures, which remains a major clinical challenge for orthopaedic surgeons. A deep understanding of the signalling pathways controlling MSCs into osteogenic lineage will significantly advance the MSC-based approaches for bone tissue regeneration. Our strategy to understand these signalling pathways is to mimic the components within bone microenvironment, as they are critical in providing sufficient signals to drive the osteogenic differentiation of MSCs.

In the present study we seek to understand the interactions of three days of bone morphogenetic protein-2 treatment (BMP-2, mimicking transient induction of BMP-2 after bone injury) and bioactive glass nanoparticles (nBG) incorporated polycaprolactone (PCL) coating on hydroxyapatite/β-tricalcium phosphate(HA/TCP) scaffolds (nBG-PCL/HA/TCP scaffolds, mimicking bone nanostructure) in directing osteogenic differentiation of adipose tissue-derived mesenchymal stem cells (ASCs) and the underlying signalling pathways involved .

METHODS

We first examined the effects of short (three days) BMP-2 stimulation on osteogenic differentiation of ASCs, followed by the combinational effects of short BMP-2 stimulation and nBG-PCL/HA/TCP scaffold by setting up the following groups: (1): ASCs seeded on HA/TCP scaffolds (control group), (2): ASCs seeded on nBG-PCL/HA/TCP scaffolds, (3): ASCs preconditioned with BMP-2 and seeded on HA/TCP scaffolds, (4): ASCs preconditioned with BMP-2 and seeded on nBG-PCL/HA/TCP scaffolds. We further investigated the mechanisms underlying the synergistic effect of BMP-2 stimulation and nBG-PCL/HA/TCP scaffold in inducing the osteogenic differentiation of ASCs.

RESULTS AND DISCUSSION

First, compared to ASCs without BMP-2 pre-conditioning, ASCs treated with BMP-2 (50 ng/mL) for 3 days showed significantly higher levels of Runx-2, collagen type I, osteopontin, and bone sialoprotein gene expression and ALP enzyme activity at 4 and 14 days.

Second, we observed that the combination of BMP-2 pre-conditioning and culturing on nBG-PCL/HA/TCP scaffolds produced the most significant induction of osteogenic gene expression and ALP activity, compared to ASCs grown on HA/TCP scaffolds without BMP-2 pre-conditioning; ASCs grown on HA/TCP scaffolds with BMP-2 pre-conditioning, and to ASCs grown on nBG-PCL/HA/TCP scaffolds without BMP-2 preconditioning. In addition, we investigated the effect of nBG-PCL/HA/TCP scaffold substrate on Wnt-3a expression in ASCs. Compared to ASCs on HA/TCP scaffolds, ASCs cultured on nBG-PCL/HA/TCP scaffolds showed significantly higher Wnt-3a protein expression (3-fold increase) as early as 5 hours after seeding, which increased up to about 5 folds at 24 hours. The role of Wnt-3a signalling in nBG-PCL/HA/TCP scaffold-mediated ASCs osteogenic induction was further investigated by adding Wnt-3a signalling inhibitor (IWR-1) into the culture medium. ASCs pre-conditioning with BMP-2 (3 days) and seeded on nBG-PCL/HA/TCP scaffolds in the presence of IWR-1 significantly decreased the levels of Runx-2, collagen type I, osteopontin and bone sialoprotein gene expression, as well as ALP activity at day 14.

Last, we found that activated β1-integrin protein expression in ASCs cultured on nBG-PCL/HA/TCP scaffolds was 4 and 7-fold higher, respectively, compared to those cultured for 5 and 24 hours on HA/TCP scaffolds, while no change was found for total β1-integrin expression between these two groups; and blocking of β1-integrin signalling in BMP-2 pre-conditioned ASCs cultured on nBG-PCL/HA/TCP scaffolds showed two major effects: (1) decreased Wnt-3a protein expression at 5 and 24 hours, and (2) inhibited Runx-2, collagen type I, osteopontin and bone sialoprotein gene expression, as well as ALP activity at day 14.

CONCLUSIONS

This study revealed that different components in the bone tissue microenvironment constitute a coordinated signalling network which controls stem cell fate into osteogenic differentiation. This highlights the importance of identifying the signals in each component of the bone tissue microenvironment that are spatially and temporally necessary for bone repair and regeneration.

ULTRASMALL SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLE PRELABELLING OF HUMAN NEURAL PRECURSOR CELLS

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INTRODUCTION

Stem cells prelabelled with ultrasmall superparamagnetic iron oxide nanoparticles (USPIOs) can be visualised using magnetic resonance imaging (MRI). This allows for noninvasive long-term monitoring of integration and stem cell fate following transplantation for treatment of neurological damage/diseases[1]. We investigated the biological impact of introducing USPIOs into primary human fetal neural precursor cells (hNPCs) *in vitro*, promising candidates for cellular therapy. In addition, the capability of imaging USPIO-labelled hNPCs with MRI was also investigated.

EXPERIMENTAL METHODS

Viability, cell cycling, proliferation, apoptosis, migration, lineage potential and intracellular calcium concentration was assessed, via Muse Cell Analyzer, Immunohistochemistry, and live-cell imaging. Uptake of USPIOs into hNPCs was assessed by graphite furnace atomic absorption spectrometry, and by TEM and super-resolution laser microscopy. *In vitro* visualisation of USPIO-labelled hNPCs was performed using MRI.

RESULTS AND DISCUSSION

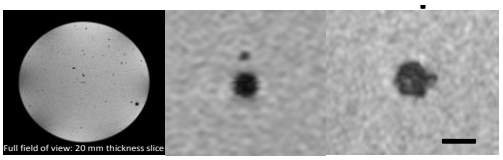

Figure 1: Neurosphere hNPCs were treated with 10 µg/mL USPIOs for 24 h, and imaged with MRI. TE = 3.5 ms. Scale bar = 200 µm.

Our USPIOs displayed superior T_2 relaxivity (368.2 mM^{-1} s^{-1}) compared to currently available USPIOs of similar core size, thus, UPSIO-labelled hNPCs were able to be detected with MRI with excellent contrast properties, at a low USPIO concentration (10 µg/mL). At this concentration, there was significant uptake into hNPCs, but with no adverse effect on the cells' biological function.

CONCLUSION

This study provided a thorough investigation into the relationship between USPIOs labelling conditions and hNPCs function, and found that hNPCs can be efficiently loaded with USPIOs at optimal conditions with favourable biocompatibility, in addition to demonstrating that USPIO-labelled hNPCs can be detected with MRI *in vitro*.

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TISSUE ENGINEERED LIVING SCAFFOLDS FOR TARGETED AXONAL OUTGROWTH

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INTRODUCTION

The nervous system has limited capacity for regeneration, making the effects of neurotrauma or degeneration devastating and often permanent. Successful regeneration often requires an orchestrated reestablishment of neural connections and cellular structure requiring long-distance axonal pathfinding and neuronal/glial migration. Our objective is to create tissue engineered "living scaffolds" comprised of neural cells in an anisotropic 3-D architecture. The leading contemporary living

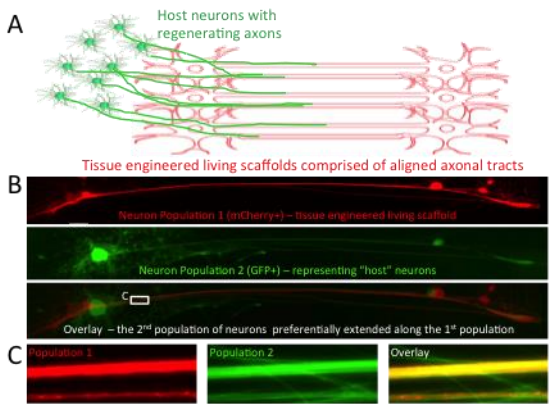

Fig 1. Axon-mediated axon regeneration (AMAR). (A) Schematic depicting host axons growing along preformed axons in a "living scaffold". (B) *In vitro* test bed containing differentially labelled neurons: population 1 (red) and population 2 (green) neurons/axons. (C) Higher

scaffolds for neurorepair utilize aligned axonal tracts[1,2] or glial cells[3] to direct regenerating axons across damaged tissue to appropriate targets. These living scaffolds may mimic key developmental mechanisms, whereby directed axonal pathfinding and neural cell migration occur along pathways formed by other cells. The objective of the current study is to determine the structural and soluble factors responsible for axonal pathfinding along pre-formed tissue engineered "living scaffolds" using a dual *in vitro – in vivo* approach.

EXPERIMENTAL METHODS

Tissue engineered living scaffolds consisting of longitudinally aligned, fluorescently labelled axonal tracts were generated. These living scaffolds were transplanted to bridge 1cm sciatic nerve gaps in rats, and mechanisms of acute axon regeneration were assessed at 2 weeks post-implant. For *in vitro* evaluation, a second population of differentially labelled neurons were added to assess preferential axon growth along preformed axon tracts. We hypothesize that "living scaffolds" drive axonal growth based on juxtracrine signalling involving the combined use of haptotactic, chemotactic, and geometric cues.

RESULTS

We found that regenerating peripheral nerve axons had an affinity to grow directly along living scaffolds comprised of aligned axonal tracts, demonstrating axon-mediated axon regeneration (AMAR). To study this with increased experimental control, we developed an *in vitro* test bed whereby a second population of neurons, representing the "host", preferentially extended axons along the pre-existing axonal tracts (Fig. 1). We are currently systematically modifying the specific structural and soluble factors along axonal tracts to elucidate molecular mediators driving AMAR.

DISCUSSION AND CONCLUSION

We exploit key mechanisms responsible for axonal pathfinding during embryonic development to create tissue engineered living scaffolds for neuroregeneration. These constructs possess specific biological cues to allow for targeted and orchestrated neural tissue regeneration. Therefore this technology offers considerable therapeutic advantages by using living cells in conjunction with biomaterials, which may ultimately facilitate functional recovery in currently intractable neurotrauma and neurodegenerative disease.

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GENERATION OF INDUCED NEURONAL AND GLIAL CELLS FROM ADIPOSE-DERIVED STEM CELLS

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INTRODUCTION

Tissue engineering approaches to treat injury to the central and peripheral nervous systems (C/PNS) are constrained by a lack of suitable transplantable cells, limited self-repair capability and restricted regeneration. Ex-vivo generation of neuronal and glial cells may be possible by direct

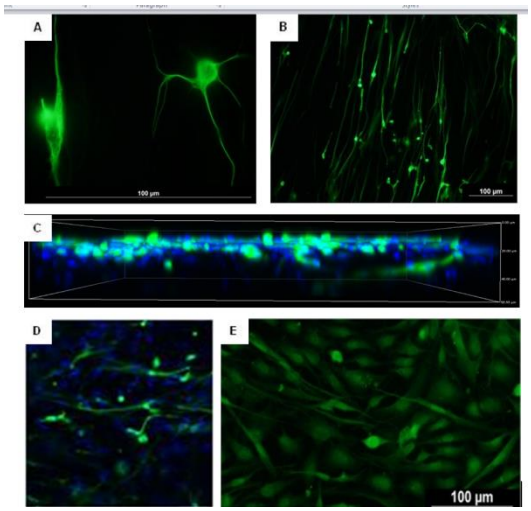
differentiation of adult stem cells through exogenous expression of transcription factors. Transcription factors have been found to be instrumental in the formation of neurons, and their forced expression has been shown to direct neuronal conversion of somatic cells1. Adult stem cells have also been shown to differentiate into Schwann cells by chemical induction alone2. Here we describe the generation of induced neuronal and glial cells from equine adipose-derived stem cells (EADSC), by lentiviral vector expression of novel transcription factors combined with a defined chemical induction media.

EXPERIMENTAL METHODS

EADSC were transduced with lentiviral vectors expressing Brn2, Ascl1, Myt1l and NeuroD1 for neuronal induction, or Krox20, Oct6, Sox10 and Brn2 for Schwann cell induction, under doxycycline control. Transduced cells were seeded in 2D as monolayers and in 3D on a porous polystyrene-based substrate (Alvetex®). Cells were cultured in induction media for up to 3 weeks, prior to immunostaining for neuronal-specific βIII-tubulin, or glial-specific S-100β.

RESULTS AND DISCUSSION

Neuronal-specific βIII-tubulin immunostaining demonstrated unipolar, bipolar and multipolar neurons (A), with extensive neurite outgrowth (B). Neuronal differentiation was also demonstrated in 3D cultures, with evidence of neurite infiltration into the scaffold (C, D). Glial-specific S-100β immunostaining demonstrated elongated, spindle-shaped Schwann cells (E).



CONCLUSION

The forced expression of Ascl1, Brn2, Myt1l and NeuroD1, and Krox20, Oct6, Sox10 and Brn2, induced the direct conversion of EADSC into neuronal and glial cells respectively. These induced cells may contribute to novel tissue engineering approaches for CNS or PNS regeneration, as well as neurological disease modelling and drug screening applications.

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SESSION 2 – BIOMATERIALS AND TISSUE ENGINEERING

A/PROF SARAH HEILSHORN
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INJECTABLE PROTEIN-CERAMIC HYBRID GELS FOR BONE TISSUE ENGINEERING

Stem cell transplantation is a promising therapy for a myriad of debilitating diseases and injuries; however, current delivery protocols are inadequate. Transplantation by direct injection, which is clinically preferred for its minimal invasiveness, commonly results in less than 5% cell viability. As a strategy to protect cells, we have designed a reproducible, bio-resorbable, customizable hydrogel using protein-engineering technology. In our Mixing-Induced Two-Component Hydrogel (MITCH), network assembly is driven by specific and stoichiometric peptide-peptide binding interactions. By integrating protein science methodologies with simple polymer physics models, we manipulate the polypeptide chain interactions and demonstrate the direct ability to tune the network crosslinking density, sol-gel phase behavior, and gel mechanics. This is in contrast to many other physical hydrogels, where predictable tuning of bulk mechanics from the molecular level remains elusive due to the reliance on non-specific and non-stoichiometric chain interactions for network formation. Furthermore, the hydrogel network can be easily modified to deliver a variety of other bioactive payloads such as growth factors, peptide drugs, and hydroxyapatite nanoparticles. We present the use of these materials for the co-delivery of adipose-derived stem cells with hydroxyapatite nanoparticles to promote bone tissue regeneration.

PROFESSOR JASON BURDICK
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ENGINEERING INJECTABLE HYDROGELS TO INFLUENCE CARDIAC REPAIR

Heart disease is a major clinical problem and post myocardial infarction (MI), left ventricular (LV) remodeling ensues and leads to geometric changes that result in dilation and thinning of the myocardial wall. This increases stress in the infarct and healthy tissue and can ultimately result in heart failure. Injectable biomaterials are being investigated to address this clinical problem, including to alter stresses in the infarct region when injected and to deliver biologics, such as stem cells and biomolecules. My laboratory is interested in a class of hydrogels based on the molecule hyaluronic acid (HA) and we have synthesized variations of HA macromers that form hydrogels with a range of mechanical properties and degradation (from a few weeks to stable over many months). This tunability in properties allows us to investigate how material properties (e.g., mechanics and degradation) influence the ability of injectable HA hydrogels to alter stress profiles and LV remodeling and to deliver therapeutic molecules (e.g., TIMP-3, to alter matrix remodeling within infarcts). Most recently, we have designed hydrogels that only degrade in the presence of matrix metalloproteinases (MMPs) to introduce a feedback mechanism for on-demand release of

biological signals. Finally, to permit delivery of hydrogels via catheters, we have developed a class of shear-thinning and self-assembling hydrogels. Ultimately, these iterations on material design are teaching us what important signals are needed in these hydrogels towards the next generation of translatable therapeutics for cardiac repair.

PROFESSOR NICO VOELCKER

MAWSON INSTITUTE, UNIVERSTIY OF SOUTH AUSTRALIA

HIGH THROUGHPUT SCREENING TECHNOLOGIES TO OPTIMISE CELL-BIOMATERIAL INTERACTIONS

The CRC for Cell Therapy Manufacturing, aims to facilitate the cost-effective manufacture and rapid translation of cell therapies into clinical practice.

With a total of $59M in cash and in-kind resources, including a $20M grant from the Australian Government, the CRC aims to provide new treatments and develop new materials-based manufacturing technologies for the treatment of conditions such as diabetes, chronic wounds, cardiovascular disease, and immune-mediated diseases such as graft versus host disease.

The CRC brings together the spectrum of skills and facilities required to turn a promising cell into a viable cell therapy. The national and international partners include research providers, manufacturers, hospitals and charities. Underpinning this partnership is a newly established cGMP manufacturing facility, designed to deliver cell-based therapeutics for the CRC's first-in-man clinical trials.

This talk will first introduce the motivation for forming the CRC, and then highlight the expertise and capabilities within the consortium as well as the target cells and diseases within the CRC. This will be followed by a presentation of the two research programs and the key research projects currently being undertaken in terms of the aims, experimental approaches and partners involved.

PROFESSOR JUSTIN GOODING

DIRECTOR AUSTRALIAN CENTER OF NANOMEDICINE, UNIVERSITY OF NEW SOUTH WALES, SYDNEY

MOLECULARLY ENGINEERED SURFACES FOR INFLUENCING CELLS AND MEASURING THEIR RESPONSE TO STIMULI

Exploring cell-surface interactions are vitally important for both understanding cell –adhesion and for designing man made surfaces that interact with cells in a very well defined way. One strategy to perform such studies is to use model surfaces that contain cell adhesive ligands immobilised onto the surface of a self-assembled monolayer (SAM)modified surface1. Such surfaces can mimic the extracellular matrix but with unambiguous presentation of cell adhesive ligands and control over the density of these ligands. Herein will be present a strategy for the preparation of well defined cell adhesive surfaces on silicon, using the cell adhesive peptide arginine-glycine-aspartic acid (RGD) as the cell adhesive ligand. How to make surfaces that switching the accessibility of cell adhesive ligands

and surfaces that can explore the impact of stimuli and the release of matrix metalloprotease (MMP) enzymes from macrophage cells2 will be outlined.

The modification of silicon with a SAM without an intervening oxide layer is achieved via hydrosilylation of alkenes or alkynes. Surfaces are modified with a base monolayer followed by an oligo(ethylene oxide) layer, to make the surfaces resistant to nonspecific adsorption of cells, and then RGD peptides.

The data shows that not only cell phenotype but cell migrant and cell outside-in signalling for bovine aortic endothelial cells is influences by the density of RGD ligands on a silicon surface. The optimal ligand density for signalling was an average spacing of 44 nm which is consistent with the density of RGD ligands in fibronectin. The amount of cell adhesion was sensitive to topography but for a given topography the optimal RGD density remained the same. It is shown that the accessibility of the RGD ligands can be controlled by using electrically switchable surfaces. In the case of macrophage cells, silicon photonic crystal surfaces can monitor the release of MMPs from these cells upon stimulation with LPS.

Cell response is shown to be highly sensitive to surface design.

DR JEROME WERKMEISTER

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION

NEW CUSTOMISED BIOENGINEERED COLLAGENS TAILORED FOR TISSUE ENGINEERING APPLICATIONS

The structure and properties of scaffolds are critical to ensure controlled cell behaviour and tissue regeneration. Protein based materials have high information content and can be designed to meet the end point requirements, particularly for cell based therapies. Mammalian collagen is a good example of a successfully used biomedical protein material but there are concerns regarding product variability and animal sourcing. Recombinant mammalian collagens require complex systems and are difficult to scale up and have very poor protein yields necessary for product fabrication. Recently, a new group of collagens have emerged derived from various bacteria (Fig.1). These proteins require simple systems to produce and are stable without the complex post-translational modifications often required. We have demonstrated how these collagens can be designed to incorporate specific biological domains and scaled up to produce new biomedical collagens at high yield.

Constructs based on the collagen-like protein Scl2 from S. pyogenes included the registration domain (V) and the collagen domain (CL). Site-directed mutagenesis was used to introduce heparin (H) and integrin (I) binding sequences, and to generate multiple CL domains, V-(CL)1-4. DNA clones were subcloned into expression system pColdIII and transformed into E. coli host BL21 for expression. Recombinant bacterial collagens were produced in 2L stirred tank bioreactors. Samples were analysed by SDS-PAGE and for biological function before and after material fabrication. In addition, various chemical strategies could be introduced to tether additional biological motifs and

cross-linking agents to allow further targeting towards specific tissue engineering applications like cartilage repair.

All constructs were readily expressed and protein purified by simple patented scalable procedures based on collagen properties. Heparin and integrin activity were demonstrated by FITC-heparin and L929 cell binding, respectively. Yields of >19 g/L were obtained from high cell density fed-batch production using defined media.

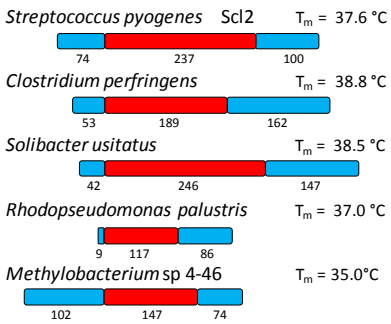

Fig.1: Tm, of bacterial collagens, including V domains (blue) and the central triple helical domain (red)

With increasing length, V-(CL)1-4, there was a reduction in product yields (Fig.2). The products in all cases were stable triple-helices.

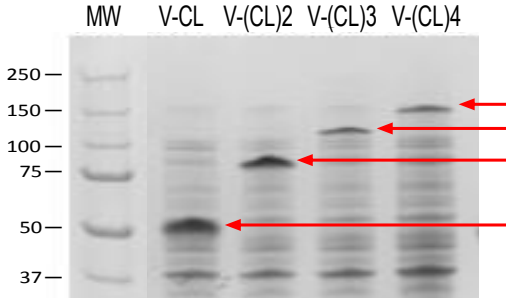

Fig.2: Building larger collagen collagen proteins

The new recombinant bacterial collagens can be tailored to incorporate specific biological domains with various sizes and with high yields suitable for tissue engineering.

SESSION 2 – ECR PRESENTATIONS SECTION

IMMUNOGENICITY OF CHONDROCYTES DERIVED FROM HUMAN INDUCED PLURIPOTENT CELLS

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INTRODUCTION

Articular cartilage damage is caused by trauma or necrosis, and seldom heals due to its limited capacity for repair. There is a need for cell sources for chondrocytes that can be transplanted into the damaged sites in the cartilage. Human induced pluripotent stem cells (hiPSCs) have pluripotency and the potential for self-renewal similar to embryonic stem cells (ESCs), but are not associated with the ethical issues. A hiPSC library is being developed, and will possibly provide allogeneic hiPSC-derived chondrocytes at low cost. However, immunological aspects of the hiPSC-derived chondrocytes are not known. We investigated the immunogenic properties of hiPSC-derived chondrocytes.

EXPERIMENTAL METHODS

We differentiated human hiPSCs toward chondrocytes following the methods previously described for ES cells with modification. We used human primary articular chondrocytes as the control. We performed flow cytometry to analyze expression of immune factor such as major histocompatibility complex (MHC) I/II. To predict and evaluate immune response to the hiPSC-derived chondrocytes when they are transplanted, we employed mixed lymphocyte reaction (MLR) assay. In addition, we assessed immunosuppressive activities of hiPSC-derived chondrocytes by co-culturing them with the activated lymphocytes. To examine the *in vivo* immune response to the cartilage generated by hiPSC-derived chondrocytes, we performed xenotransplantation of the hiPSC-derived cartilaginous tissue into subcutaneous spaces of immunocompetent mice.

RESULTS AND DISCUSSION

The hiPSC-derived chondrocytes did not constitutively express cell surface molecules required for induction of T cell immune responses. Expression of MHC class II was not induced in hiPSC-derived chondrocytes by IFN-gamma stimulation, whereas it was induced in human primary chondrocytes. MLR that hiPSC-derived chondrocytes had limited ability to stimulate proliferation of allogeneic lymphocytes compared with mononuclear cells in peripheral blood. These results collectively suggest that hiPSC-derived chondrocytes have limited immunogenicity. Presence of hiPSC-derived chondrocytes suppressed proliferation of activated lymphocyte in the co-culture system in chondrocyte number-dependent manner, suggesting that hiPSC-derived chondrocytes bear immunosuppressive effects. Xenotransplantation experiments showed that hiPSC-derived cartilaginous tissues were protected from immune cell invasion in subcutaneous spaces of mice for several months.

CONCLUSION

The hiPSC-derived chondrocytes showed limited immunogenicity.

The hiPSC-derived chondrocytes had immunosuppressive potential.

The hiPSC-derived cartilage was protected from invasive immune reaction in the xenotransplantation model.

STRATEGY FOR FABRICATING CELL-LADEN ECM DERIVED HYDROGELS IN CARTILAGE TISSUE ENGINEERING

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INTRODUCTION

Articular cartilage injuries and osteoarthritis (OA) are the most common joint disorders in our day and represent a major cause of pain and disability among both the elderly population as well as young active patients. Although bioengineering stem cells *in vitro* has shown to be a prospective source of advanced therapy to heal OA diseases, significant challenges still remain [1,2]. One key aspect is to provide the cells with instructive elements needed to induce chondrogenesis and extracellular matrix (ECM) formation. In this study, we aim to develop a platform for rapid 3D assembly of photocurable cell-laden ECM derived hydrogels to quantify chondrogenesis, chondrocyte morphology and ECM formation.

EXPERIMENTAL METHODS

Human nasal chondrocytes (1 x 10^6 cells/ml) were loaded in 10 wt% gelatin-methacrylamide (Gel-MA) and crosslinked using photoinitiator (0.05 wt%, Irgacure 2959) in the presence of UV light (365 nm) using a custom made Teflon mould. Live/dead (Calcein-AM/Propidium Iodide) assay was conducted to assess the viability of the encapsulated cells. Proteoglycans and GAG accumulation was visualized using Safranin-O stained histological slices. GAG content was quantified using dimethyl-methylene blue (DMMB) assay. The cell nuclei and F-actin were stained to analyse cytoskeletal architecture using DAPI and Alexafluor488-Phalloidin respectively.

RESULTS AND DISCUSSION

In this study, it was observed that cell-laden constructs with good spatial and temporal control were fabricated from photocurable Gel-MA. The encapsulated cells were highly viable (≈70%) after 21 days of culture, exhibiting a rounded morphology. This lack of microfibrillar F-actin architecture highly suggested that the chondrocyte phenotype was retained. Safranin-O stained sections also showed that the encapsulated chondrocytes were able to secrete new ECM in the Gel-MA based hydrogels. Accordingly, this methodology presents a valuable platform to assess necessary instructive elements needed to improve chondrogenesis as well as organization of matrix components in ECM derived hydrogels.

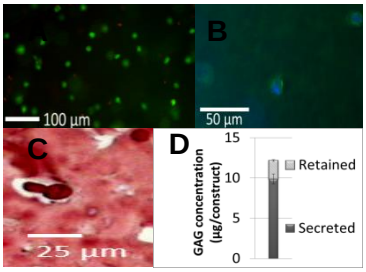

Fig 1. Viability (A), morphology (B), ECM formation (C) and GAG accumulation (D) of chondrocytes cultured for 3 weeks.

CONCLUSION

This study showed that photocurable Gel-MA hydrogels support cell survival and function post encapsulation, and are suitable as constructs for chondrocytes encapsulation. Utilizing this

methodology, future studies will focus on fabrication of hydrogels with bioactive molecules such as heparin and hyaluronic acid to further promote chondrogenesis within the hydrogel.

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A STING IN THE TAIL: FOCAL ADHESION TARGETING AND MECHANOTRANSDUCTION

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INTRODUCTION

Cells probe and sense the mechanical properties of their environment through integrin-mediated focal adhesions, in a process known as mechanotransduction. Recently, we have shown that the adhesion docking protein NEDD9 mediates adhesive force to the extra-cellular matrix and we propose that NEDD9 mediates inside-out mechanotransduction. NEDD9 is targeted to focal adhesions via a conserved c-terminal focal adhesion targeting domain (FAT). The FAT domain is found in other key focal adhesion components including p130Cas/BCAR1 (part of the Cas protein family with NEDD9) and Focal Adhesion Kinase (FAK). To date, little is known about whether the discrete FAT domains differentially regulate function. The aim of the present study was to analyse the functional significance of the structurally homologous C-terminal FAT domains in mediating focal adhesion targeting and adhesion to the extra-cellular matrix.

EXPERIMENTAL METHODS

Domain exchange constructs were engineered, with the FAT domains of p130Cas and FAK substituted for the NEDD9 FAT domain. The function of the NEDD9-FAT fusion proteins was then assessed by transfection into NEDD9 knockout mouse embryo fibroblasts and glioblastoma cells with tetracycline inducible NEDD9 knock-down. Rates of molecular exchange at focal adhesions were determined by Fluorescence Recovery After Photobleaching of fluorescently tagged fusion proteins. Adhesion force to matrix-coated beads was measured using a magnetic tweezer device.

RESULTS AND DISCUSSION

We first confirmed that each fusion construct retained focal adhesion targeting ability. Since the residency time of proteins at focal adhesions has been shown to determine focal adhesion stability and strength, we compared dynamic exchange of the NEDD9-FAT fusion proteins at focal adhesions FRAP. Importantly the FAK FAT significantly reduced NEDD9 molecular exchange at focal adhesions. The p130Cas FAT also slowed dynamics, however to a lesser extent than the FAK FAT. Measurement of adhesion strength via a magnetic tweezer device and the application of linearly increasing force to fibronectin beads pre-incubated with transfected cells revealed that neither the FAK nor p130Cas FAT domains rescued adhesion changes due to NEDD9 depletion.

CONCLUSION

Despite extensive homology of the FAT domains - in particular the high level of sequence homology between the related proteins p130Cas and NEDD9 (57% identity) - the NEDD9 FAT domain appears to serve a unique function in regulating the rate of exchange at focal adhesions and adhesive force to the extra-cellular matrix.

IN VIVO VALIDATION OF A NOVEL SKIN SUBSTITUTE FOR CHRONIC WOUNDS

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INTRODUCTION

Current tissue engineered solutions for soft tissue repair have proven to greatly assist in skin restoration. Although there has been evidence of greatly improved outcomes for patients with acute wounds, such as burns, they are less successful with chronic wounds. The exact reason is uncertain but factors that contribute to wound chronicity, including bacterial load and poor vascularisation, are likely to play a role.

The ideal scaffold for chronic wound applications would support extracellular matrix (ECM) formation, vascular infiltration and have some bactericidal activity. Additional features, such as the ability to deliver growth factors and other functional components, would also be advantageous. In this study we examined the ability of a scaffold, with potential utility for soft tissue regeneration, to address some of the facets of this multi-factorial problem.

EXPERIMENTAL METHODS

This study examined the *in vivo* utility of Variotis, a novel skin tissue scaffold constructed of elastomeric polycaprolactone (PCL), to support granulation tissue formation. Results were compared between the base scaffold (PCL only) and the same scaffold with Bioglass 45S5 as a polymeric additive (PCL/Bioglass, in w/v: 45% SiO_2, 25.5% Na_2O, 24.4% CaO, 6% P_2O_5).

For these studies, sterilised scaffolds (5 x 5 x 10 mm) were implanted subcutaneously in 6 week old male Sprague Dawley rats. Animals (n=4/time point) were terminated at 2, 4, 7, 14, 21, 28 and 42 days post implantation. The scaffolds were then removed and used for analysis of cellular infiltration, ECM formation and inflammatory response at both the protein and gene level. Immunohistochemistry was used to assess collagen I and III accumulation, vessel formation (CD31), and macrophage infiltration (CD68). The gene expression of markers for ECM formation collagen I & III, fibronectin, vessel formation (CD68 and PECAM1), and inflammation (TLR-2 and 4, TNF-α, MCP-1, CD68) were determined by qRT-PCR.

RESULTS AND DISCUSSION

Overall the ECM content and vascularity of both scaffolds increased with time. Histological and immunohistochemical comparisons showed that, relative to the base scaffold, the PCL/Bioglass constructs had increased collagen deposition and density, more blood vessel ingression,

granulation tissue formation deep and throughout the scaffold in the first week, and no apparent fibrous encapsulation.

Real time qPCR results were complementary to the histological and immunohistochemical observations. Additionally the PCL/Bioglass scaffold showed inflammatory upregulation which may accelerate granulation tissue formation. Interestingly there was a time dependent decrease in the expression of TLR-2 and TLR-4 in the PCL/Bioglass constructs; opposite to that of the base scaffold. This suggested a decrease in inflammatory response, potentially related to the antibacterial properties of Bioglass 45S5.

CONCLUSION

This study has shown that the Variotis scaffold has promising potential for clinical application through its support of cell growth, ECM deposition and blood vessel formation *in vivo*. Together these results suggest that this PCL based scaffold shows promise as a multiplex platform for supporting soft tissue regeneration.

ECM-BIOFUNCTIONALIZED TITANIUM ALLOY SUBSTRATES FOR IMPROVED OSSEOINTEGRATION

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CHARLES PERKINS CENTRE, SCHOOL OF MOLECULAR BIOSCIENCE AND BOSCH INSTITUTE, THE UNIVERSITY OF SYDNEY

INTRODUCTION

Titanium (Ti) and Ti alloys are progressively used in orthopedic devices due to their mechanical properties and biocompatibility[1]. However, Ti-based implants often fail due to limited adhesion with bone cells at the host interface. Tropoelastin, an extracellular matrix (ECM) protein that can modulate the activity of various cell types[2], presents as an ideal candidate for the biofunctionalization of these materials.

Here, we used a plasma activated coating (PAC) to covalently immobilize tropoelastin[3] to Ti-based surfaces. We show that the surface modification tolerates sterilization and retains biological responses to the functionalized substrates.

EXPERIMENTAL METHODS

Surface modification: Ti-V-Al alloy sheets were PIII treated at 20 kV for 800 s and coated with 20 μg/mL tropoelastin at 4°C overnight.

Sterilization: Tropoelastin-coated surfaces were either steam autoclaved at 121°C, 100 kPa for 20 min; or gamma irradiated at up to 40 kGy.

ELISA: Surface-bound tropoelastin was detected with 1:2000 BA4 anti-elastin antibody and 1:5000 horseradish peroxidase-conjugated anti-mouse antibody, and visualized with the ABTS substrate.

Cell attachment: MG63 cells were incubated on functionalized surfaces in serum-free media for 1 hr at 37°C, then washed to remove unbound cells. Bound cells were fixed with 3% (v/v) formaldehyde, stained with crystal violet and quantified.

Cell proliferation: MG63 cells were cultured on functionalized surfaces for 1, 3, 5 and 7 days. Cells were fixed and stained as described above.

RESULTS AND DISCUSSION

PAC treated Ti binds tropoelastin covalently. Tropoelastin coated on untreated Ti is removed by SDS washing, while majority of molecules are retained on PAC treated Ti. These results are expected where the tropoelastin simply physisorbs onto untreated surfaces. However tropoelastin covalently attaches to PAC treated surfaces (Fig 1).

Bone cells attach to sterilized, functionalized Ti. Tropoelastin improves MG63 cell adhesion to both untreated and PAC treated Ti surfaces. Its cell binding activity is fully retained on PAC treated Ti even after autoclaving (Fig 2A) or gamma sterilization up to 40 kGy (Fig 2B).

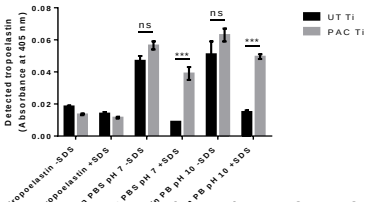

Fig 1. ELISA detection of surface-bound tropoelastin before and after washing in 5% (w/v) SDS at 80°C for 10 min.

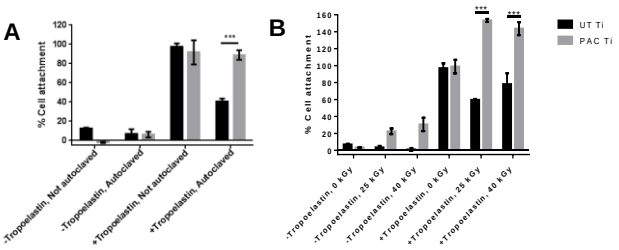

Fig 2. MG63 cell attachment to Ti surfaces, before and after sterilization by (A) steam autoclaving and (B) gamma irradiation.

Bone cells proliferate on sterilized, functionalized Ti. MG63 cells proliferate better on tropoelastin-coated Ti. This benefit persists even until 7 days post-seeding on sterilized surfaces (Fig 3).

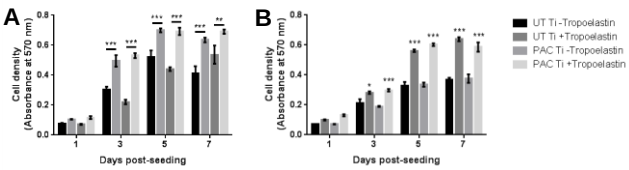

Fig 3. MG63 cell proliferation on (A) steam autoclaved and (B) gamma irradiated Ti surfaces.

CONCLUSION

Ti alloy materials modified with PAC and covalently-bound tropoelastin show improved bone cell adhesion and proliferation. These functionalized surfaces retain full activity even after sterilization.

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FABRICATION OF MICROPATTERNED HYDROGELS WITH FAVOURABLE PHYSICAL STABILITY

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INTRODUCTION

Low mechanical strength and rapid degradation of photo-crosslinkable polymers are their major drawbacks. Robust hydrogels for tissue engineering applications can be fabricated, using interpenetrating polymer network (IPN)[1]. For instance, IPN hydrogel formation using simultaneous chemical crosslinking of poly(lactide-co-ethylene oxide fumarate) (PLEOF) and physical crosslinking of gelatin significantly promoted the adhesion and proliferation of primary osteoblast cells[1]. The slow gelation time and use of redox crosslinking agents in these IPN hydrogels are the main limiting factors in their clinical applications. The aim of this study was to develop a biomimetic hydrogel with high physical stability and mechanical strength, superior biological activity and rapid gelation time.

EXPERIMENTAL METHODS

The IPN hydrogel was fabricated by using photocrosslinking of PLEOF and methacrylated gelatin (GelMA) in the presence of polyethylene glycol-diacrylate (PEGDA) as crosslinker[2]. The concentrations of these compounds were optimised based on compressive modulus and mass retaining of hybrid hydrogel.

RESULTS AND DISCUSSION

Hybrid hydrogels of PLEOF and GelMA were fabricated by simultaneous crosslinking of these two polymers under UV light. The addition of GelMA in the hybrid hydrogel promotes the compression strength of the PLEOF hydrogels by nearly two-fold. In addition, the presence of PLEOF network hydrogel enhances the stability of the protein within the structure of hybrid hydrogel. Gelatin was retained within the structure of hybrid hydrogel for more than 21 days.

The fabricated hybrid hydrogel exhibits a highly porous microstructure as shown in Figure 1-a. This microenvironment supports the proliferation of the osteoblast cells. The number of cells was increased by three times from day 1 to day 7 post-culture. In addition, to promote the cell alignment, we generate a microchannel on the surface of these hybrid hydrogels, by using photolithography technique. Nucleus Hoechst staining of the seeded cells in Figure 1-b show the alignment of the seeded cells 12 hours post-culture.

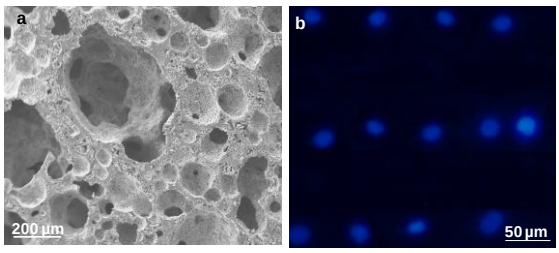

Figure 1 Porous structure (a) and Nucleus Hoechst staining of hybrid hydrogels

CONCLUSION

A porous micro-engineered hydrogel was fabricated by simultaneous photocrosslinking of PLEOF and GelMA. The GelMA retained in the hybrid structure for more than 21 days and improved the cell alignment of osteoblast cells in the surface of microstructure. The results showed the high potential of these hybrids constructs for load bearing tissue engineering and cell-cell and cell-material interaction for a long period of time.

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GALA DINNER

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DAY 3 – 20TH AUGUST

SESSION 3 – RECONSTRUCTION OF TISSUE ARCHITECHTURES

A/PROF GERALDINE O'NEILL

FOCAL ADHESION BIOLOGY (FAB) GROUP - PAEDIATRICS & CHILD HEALTH, CHILDREN'S HOSPITAL, WESTMEAD

BIOPHYSICAL CUES REGULATING CANCER CELL MIGRATION

Mechanical forces contained within brain tissue are predicted to play an important role in regulating the highly invasive behaviour of deadly high grade glioma (HGG) brain tumours. Based on reports of rigidity-dependent migration and invasion in HGG we hypothesized that the glioma cells sense the stiffness of the surrounding tissue and in response generate adhesive force that facilitates movement through the brain parenchyma. We have focused on the pro-invasive focal adhesion molecule NEDD9. Expression of NEDD9 correlates with poor prognosis in glioma patients, however, it is not known whether NEDD9 plays a role in sensing external forces to regulate HGG invasion.

We have used both 2-dimensional (2D) and 3D surfaces to compare NEDD9 effects on cell migration. Adhesive force was measured using a magnetic tweezer device. Finally, we have employed poly-acrylamide hydrogels of defined mechanical properties to analyse the role of NEDD9 in rigidity-dependent HGG phenotypes.

Using 3D collagen gels we show that NEDD9 depletion from mouse embryo fibroblasts (MEFs) and glioma cell lines reduces 3D migration speed. Conversely, NEDD9-/- MEFs show increased migration speed in 2D migration assays. We show that NEDD9 depletion decreases adhesive force and correlated with reduced □1 integrin activation. We therefore suggest that the differences in 2D versus 3D migration speeds may reflect insufficient adhesive force following NEDD9 depletion to overcome the steric hindrance of the 3D matrix. Using poly-acrylamide hydrogels of defined stiffness we show that NEDD9 depletion inhibits rigidity-dependent phenotypes in the HGG cell lines.

We propose that NEDD9 regulates glioma invasion by modulating adhesion force in response to external tissue stiffness. This is a novel mechanism for NEDD9 and opens the door to treating invasive glioma by targeting NEDD9-dependent signalling pathways.

DR LUIZ BERTASSONI
HARVARD-MIT HST / UNIVERSITY OF SYDNEY

BIOPRINTING THREE-DIMENSIONAL VASCULARIZED TISSUES

Fabrication of three-dimensional (3D) organoids with controlled microarchitectures has been shown to enhance tissue functionality. Bioprinting can be used to precisely position cells and cell-laden materials to generate controlled tissue architecture. Therefore, it represents an exciting alternative for organ fabrication. Our group has been interested in developing innovative bioprinting-based microscale technologies, both to improve our understanding of human tissues and to increase our ability to regenerate them with improved efficiency. In this seminar, we will present novel bioprinting techniques to fabricate 3D tissues, such as liver and bone. We will also discuss a new technique developed to bioprint biomimetic microvascular networks embedded within macroscale cell-laden tissue constructs. These techniques have been used to engineer hepatocyte-laden hydrogels on-a-chip and to fabricate osteon- like structures mimicking vascularized bone. The use of these technologies in various regenerative applications will also be discussed.

PROFESSOR TOBY COATES
UNIVERSITY OF SOUTH AUSTRALIA

ENGINEERING THE PANCREATIC ISLET FOR TRANSPLANTATION IN TYPE-1 DIABETES

Pancreatic islet transplantation is an emerging treatment for type 1 diabetes. Currently islets are isolated from deceased organ donors before being transplanted into the liver of highly selected patients with type-1 diabetes. Isolated islets experience a wide variety of environmental stresses that culminate in cell death. Engineering the islet to resist these stresses by suppression of apoptosis, improvement of vascular supply and prevention of rejection by inhibiting auto and alloimmunity is the focus of the work in our laboratory.

Human and rodent islets are isolated from organ donor pancreata using Liberase based enzymatic digestion. A major problem with human islet isolation relates to the death of human islets after isolation and before transplantation. Using smart surfaces and biological mediators we hope to improve islet survival for islet transplantation. A variety of plastics combined with a variety of islet survival factors will be studied including components of islet basement membrane (Laminins, collagens) and heparin sulphates. Islet pro-survival factors (Insulin Like Growth Factor II) and anti T cell strategies (Program Death Ligand 2) have all been used in human islets to provide protection (see Figure1).

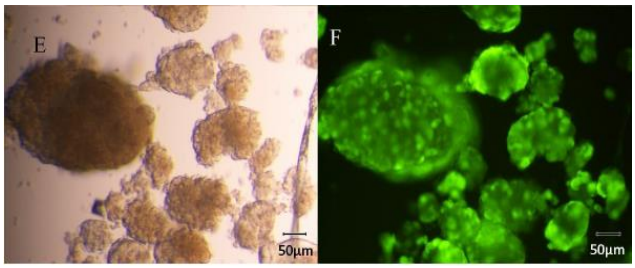

Figure 1: Human pancreatic islets transduced with Program Death Ligand-2 (green Adenoviral GFP PDL-2)

Human pancreatic islets were transfected with adenoviral vectors containing the genes for IGF-II. Transduction of islets with IGFII did not impair islet viability or function. Transduced islets were resistant to cytokine induced apoptotic death in vitro. A minimal mass islet transplant was performed using

rodent islets into immunodeficient diabetic mice, which confirmed the ability of this vector to improve islet survival.

Pancreatic islets can be successfully engineered to provide protection against apoptosis and protection against endogenous cell death pathways and exogenous T cell damage. Developing scaffolds and smart surfaces to prolong or improve islet viability is an important alternative way to protect islets both prior to transplantation and potentially after transplantation.

FURTHER READING

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SESSION 3 – ECR PRESENTATIONS SECTION

DESIGN OF THREE-DIMENSIONAL TISSUE CONSTRUCTS USING GRAPHENE

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INTRODUCTION

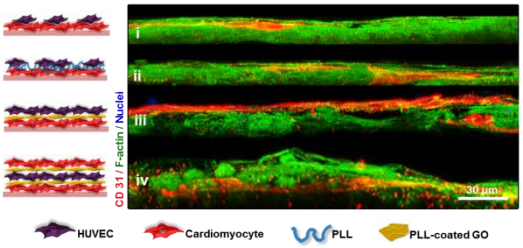
The development of highly organized and functional three-dimensional (3D) complex constructs *in vitro* is of great importance in tissue engineering, since native tissues and organs exhibit highly organized 3D complex architectures composed of extracellular matrix (ECM), different cell types, and chemical and physical signaling cues[1]. In this work we have presented a novel approach to engineer 3D multi-layered constructs using layer-by-layer (LbL) assembly of cells separated with self-assembled graphene oxide (GO)-based thin films.

EXPERIMENTAL METHODS

The first step in the fabrication of multi-layer tissue constructs is to generate a stable and homogeneous cell layer as the bottom most layer. The second step is to deposit PLL-GO particles on the surface of the first cell layer, which provides a cell-adhesive surface for the second layer of cells. Repetition of these two steps, i.e. LbL assembly, easily leads to tissue constructs with 3 or more cell layers[2].

RESULTS AND DISCUSSION

PLL-GO were made by coating GO particles with PLL through strong electrostatic attractions and the partial covalent bonding between the polypeptide chains of the PLL and the sidewalls of the GO. The architecture of the multi-layer cell construct was successfully controlled by the concentration of the PLL-GO, the number of stacked layers and the cell type- and location. The results indicate that our



Schematic of 2L cardiomyocytes and endothelial cells without any ECM layers (i), with pristine PLL (ii), with PLL-coated GOs (iii), and 3L cardiomyocytes and ECs with PLL-coated GOs (iv) at the interface layers between cells.

constructs promote cell adhesion, organization and maturation, and improved cell-cell electrical coupling.

CONCLUSION

In conclusion, multi layer tissues were successfully fabricated using an LbL assembly technique where PLL-GO thin films were deposited as an adhesive layer in between each cell layer and facilitated the assembly through electrostatic attraction and strong cell adhesion. Compared to previously reported tissue constructs, our approach led to multi layer tissues with enhanced biological activity, mechanical integrity, handling, and retrievability of engineered tissue.

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CYTOCOMPATIBLE BIOSYNTHETIC HEPARIN-POLY(VINYL ALCOHOL) HYDROGELS

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INTRODUCTION

Combining biological and synthetic polymers to make biosynthetic gels allows for the design of hydrogels that provide biological recognition with mechanically robust and controllable material properties. Poly (vinyl alcohol) (PVA), a synthetic polymer, and heparin (hep), a biological polymer found within the extracellular matrix, can be functionalised with a variety of crosslinkable groups, such as aldehydes (AL) and hydrazides (HY). AL and HY groups can undergo mild hydrazone crosslinking at physiological pH, where the only reaction byproduct is water. Thus, this system has significant potential for cell encapsulation.

EXPERIMENTAL METHODS

PVA-HY and PVA-AL [1] and Hep-AL [2] were synthesised according to previous methods. Cylindrical (50µL, Ø5mm) hydrogels were formed by mixing separately dissolved HY and AL (1HY:1AL) polymers at 10wt% (0, 1, 2wt% Hep-AL) in phosphate buffered saline, pH 7.4 for swelling characterisation. Cell growth inhibition assays were performed by placing L929 fibroblasts with 1 mg polymer/mL media for 2d. Viability was assessed with a Vi-Cell counter.

RESULTS AND DISCUSSION

Hydrazone crosslinking led to the gelation of PVA-hep hydrogels within the clinically relevant timescale of <10 minutes at physiological pH (7.4) without any initiator. The gels imbibe increasing volumes of water with increasing hep

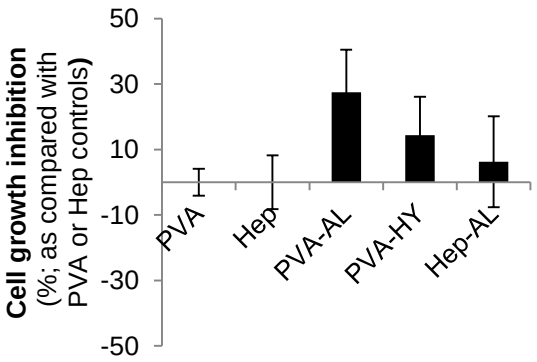

Figure 1. Cell growth inhibition of fibroblasts incubated with PVA-AL, PVA-HY, or Hep as compared to controls (PVA, Hep). Mean ± st dev, n=3.

concentration ($p<0.05$). The mass swelling ratio ($m_{swollen}/m_{dry}$) at 24 hours was 11±1, 17±2, and 25±4, for gels containing 0, 1, 2wt% Hep-AL, respectively. Hep is known to bind and present growth factors and also has the highest negative charge density of any known biological molecule, which likely results in uptake of water and ions. The ability to retain these properties in a biosynthetic gel make PVA-hep gels ideal candidates for soft tissue engineering. Preliminary studies evaluated cell growth inhibition and demonstrated that the inhibition due to PVA-AL (27±13%), PVA-HY (14±12%), and Hep-AL (6±14%) was similar or marginally more inhibitory than the PVA and hep controls. This demonstrates that the functionalised polymers were not cytotoxic, based on an inhibition less than 30% (ISO procedure 10993-5).

CONCLUSION

The rapid gelation, high swelling of the PVA-hep gels, and the cytocompatibility of these functionalised polymers demonstrates their potential for future cell encapsulation studies.

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THE USE OF A SUGAR-BASED INJECTABLE CARRIER IN FUNCTIONAL MODELS OF BONE FORMATION AND HEALING

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INTRODUCTION

We have adopted Sucrose Acetate Isobutyrate (SAIB) as a phase transitioning carrier for the delivery of recombinant human bone morphogenetic protein-2 (rhBMP-2). The SAIB carrier is highly versatile, and we have hypothesized a variety of bone tissue engineering applications. These range from strengthening brittle bones, such as in the genetic disorder *Osteogenesis Imperfecta* (OI), to accelerating open fracture healing.

EXPERIMENTAL METHODS

In a transgenic mouse model of OI, 20µL of SAIB containing 5µg rhBMP-2 was injected intraosseously into the reamed tibia of mice. The effects of rhBMP-2 on increasing the periosteal perimeter of the tibia was compared with no and sham surgery in both OI and wild type littermates.

In a rat model of open fracture healing, a femoral osteotomy with periosteal stripping was performed. 100µL SAIB containing 10µg rhBMP-2, with and without ceramic nanoparticles and bisphosphonate, was delivered directly to the fracture site. The ability of these interventions to and accelerate union was assessed.

RESULTS AND DISCUSSION

The SAIB/rhBMP-2 intervention increased the mean periosteal bone perimeter in the OI mouse model compared to the no treatment and sham surgery groups.

In the rats open fracture model, groups treated with SAIB/rhBMP-2 showed greater local bone formation around the callus and more rapid union than the vehicle treated fractures.

CONCLUSION

SAIB/rhBMP-2 was able to improve bone healing in two models. This resulted in the strengthening of brittle OI bones, and an acceleration of open fractures union. SAIB is thus able to deliver rhBMP-2 in situations where solid carriers would be incapable or impractical. These data support the continued development of SAIB an rhBMP-2 carrier for orthopaedic indications.

A NOVEL BIPHASIC SCAFFOLD FOR THE REPAIR AND REGENERATION OF OSTEOCHONDRAL DEFECTS

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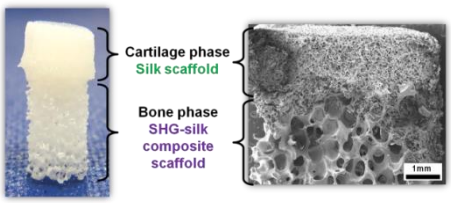
INTRODUCTION

The management and reconstruction of damaged or diseased osteochondral tissue at skeletal joints, which involves both articular cartilage and subchondral bone, have remained a significant clinical challenge. Existing clinical treatments are aimed at alleviating pain and morbidity in the short term, while long term outcomes are rarely ideal and the progression of osteoarthritis often results. Biomaterials-based osteochondral tissue engineering is emerging as a novel treatment strategy that can address the growing unmet clinical need to develop more effective therapies. The present study describes the development of a novel biphasic scaffold for the regeneration of both cartilage and bone segments of the osteochondral unit, with two different phases that imitate the properties of their target tissues. The cartilage phase is a flexible and resilient silk scaffold, anchored to the bone phase which is a mechanically strong and bioactive composite scaffold (silk-coated ceramic scaffold). The properties of this biphasic scaffold were shown to be favourable for promoting osteochondral regeneration.

EXPERIMENTAL METHODS

Sr-HT-Gahnite (SHG) ceramic scaffolds were prepared as previously described[1]. The SHG scaffold was coated with 8wt% silk fibroin aqueous solution to give the SHG-silk composite scaffold (bone phase). The silk scaffold (cartilage phase) was formed by freezing a mixture of silk fibroin aqueous solution and diluted methanol. The SHG-silk scaffold was mounted inside this mixture during silk scaffold formation to integrate the two phases of the biphasic scaffold. Physical, mechanical and biological properties of the biphasic scaffold were evaluated.



RESULTS AND DISCUSSION

The biphasic scaffold had two different phases respectively targeted at cartilage and bone regeneration (**Fig. 1**). Scanning electron microscopy (SEM) examination showed that the two phases of the biphasic scaffold imitated the structural characteristics of native cartilage and bone. Mechanical and degradation testing showed that the biphasic scaffold was mechanically competent and suitable for implantation in load-bearing osteochondral defects. *In vitro* testing showed that the biphasic scaffold supported the growth of human mesenchymal stem cells (hMSCs) and could

induce tissue-specific differentiation responses in the two phases, indicating its ability to encourage the region-specific regeneration of cartilage and bone for effective osteochondral reconstruction.

CONCLUSION

By satisfying the region-specific needs of osteochondral tissue and targeting the regeneration of cartilage and subchondral bone, the developed biphasic scaffold has potential for future clinical application in the reconstruction of osteochondral defects that will block disease progression to osteoarthritis.

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CERAMIC SCAFFOLDS INDUCE M1-TO-M2 TRANSITION *IN VITRO*

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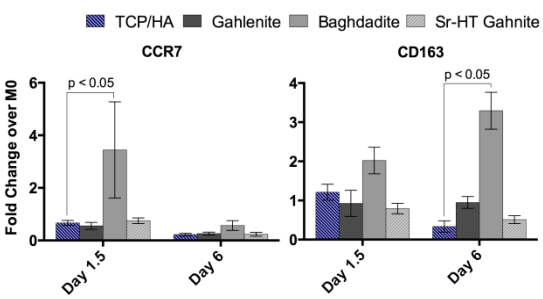
INTRODUCTION

Recently, novel ceramic-based scaffolds have been developed for bone repair that support extensive bone regeneration *in vivo*[1]; however, the mechanism of success is incompletely understood. We have shown that M1 (classically activated) macrophages are required at early stages of healing to initiate angiogenesis, while M2 (alternatively activated) macrophages are needed at later stages to facilitate anastomosis and tissue maturation[2]. The objective of this research is to elucidate the interactions between these scaffolds and cells of the inflammatory response to aid in designing biomaterials that can facilitate healing and tissue regeneration. In this work, the time-dependent effects of macrophages exposed to Gahlenite, Baghdadite and a multi-component ceramic comprised of strontium-doped Hardystonite, Gahnite and a glass phase (Sr-HT-Gh) are investigated and compared to a tricalcium phosphate and hydroxyapatite (TCP/HA) control. Based on their success *in vivo*, we hypothesize that these scaffolds promote the proper M1-to-M2 sequence of macrophage activation needed for adequate bone repair.

EXPERIMENTAL METHODS

Primary human monocytes were isolated from blood and differentiated into macrophages as described previously[2]. On day 5, test scaffolds were steam-sterilized and equilibrated in culture media for 15 min. Macrophages were seeded directly onto the scaffolds at a density of 1×10^6 cells/mL in 15 μL and allowed to attach at 37°C and 5% CO_2. After 1 h, the culture media was adjusted to 1 mL and the samples were incubated an additional 1.5 – 6 days, with a media change on day 3. Unactivated macrophages (M0) exposed to culture media alone served as a control. On days 1.5 and 6, the scaffolds were transferred into TRIzol for RNA extraction and stored at -80°C. Gene expression for 10 markers of macrophage phenotype was conducted as described previously[2]. Data shown represent the mean fold change over M0 ± SEM (n=4). Statistical analysis was performed using a two-way ANOVA and Dunnett's multiple comparisons test with TCP/HA



scaffolds as the control; $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Gene analysis revealed upregulation by Baghdadite scaffolds of M1 (*TNF-α*, *CCR7*) and M2a (*TIMP3*) markers on day 1.5, and M2c marker *CD163* on day 6. Sr-HT Gahnite scaffolds induced upregulation of M2a markers (*MDC*, *TIMP3*) on day 1.5, and suppression of M1 marker *IL-1β* by day 6. No differences were observed between Gahlenite scaffolds and the control.

These findings suggest that Baghdadite and Sr-HT Gahnite scaffolds induce a mixed M1/M2 phenotype. Intriguingly, Baghdadite scaffolds promote an M1-to-M2 transition, consistent with the natural sequence of macrophage activation during normal healing.

CONCLUSION

Overall, these results suggest that Baghdadite and Gahnite scaffolds have potential to maximize bone repair by recapitulating the proper inflammatory response for healing.

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STRATEGIES TOWARD BIOMATERIAL VASCULARISATION

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INTRODUCTION

Biomaterials play a central role in modern regenerative medicine and tissue engineering strategies. They serve as tuneable biophysical and biochemical milieus that direct cellular behaviour and function and can thus be used to replace and regenerate missing or injured tissue, deliver cells, drugs and biological molecules to the site of injury and study biological processes *in vitro* [1]. However, one of the biggest obstacles in translating the advances in biomaterials research to clinical, diagnostic and research applications has been the lack of sufficient vascular tissue regeneration in current synthetic and natural biomaterials [2].

EXPERIMENTAL METHODS

We utilise lyophilised silk sponges with tuneable bulk porosity, pore size, mechanical and degradation properties as a platform to develop a range of vascularisation strategies, including: 1) vascular-like hollow channels, 2) *in vitro* pre-vascularisation (pre-seeding with endothelial cells), 3) *in vivo* vascularisation (arteriovenous loop), 4) VEGF delivery from a slow release depot and 5) ECM-based biomimetic approaches.

RESULTS AND DISCUSSION

We present a range of strategies to vascularise critically-sized silk scaffolds. We demonstrate that the hollow channels engineered in porous silk scaffolds play a pivotal role in enhancing cell infiltration, delivering oxygen and nutrients to the scaffold bulk, and promoting *in vivo* host tissue integration and vascularisation. Further, we demonstrate that a combination of hollow channels and *in vitro* pre-vascularisation or VEGF delivery further enhances host integration and vascularisation.

We also present our preliminary work on utilising the essential role of a vascular niche proteoglycan perlecan in developing novel, biomimetic biomaterials for enhanced biomaterial vasularisation.

CONCLUSION

We have developed a number of novel strategies that will contribute to translating silk and other biomaterial platforms to advanced clinical and research applications.

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INFECTED WOUND MODEL FOR ANALYSING SCAFFOLDS

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INTRODUCTION

Tissue engineered scaffolds that can deliver antimicrobial agents have great potential for healing infected wounds. To rapidly establish the efficacy of such biomaterials, *in vitro* models are required which examine these devices and their interactions with cells and bacteria simultaneously. Current *in vitro* wound models are 2 dimensional[1] and are performed with cells and bacteria cultured in chamber wells with the bacteria isolated from the cultured cells. The present study describes a novel wound model where human dermal fibroblasts (HFb) and *Staphylococcus aureus* (*S. aureus*) are co-cultured on a 3D porous scaffold. The relative growth these cells were monitored using reverse transcription PCR (RT-qPCR).

EXPERIMENTAL METHODS

Scaffolds (Variotis, Biometic) were placed into the wells of a 24 well tissue culture plate. *S. aureus* and HFb were added to each scaffold in 500µl of DMEM. Scaffolds inoculated with either *S. aureus* alone or HFb alone served as controls. After 1 and 5 days the scaffolds were examined for HFb and *S. aureus* cell growth by SEM and RT-qPCR as described below. Each series of studies were performed in triplicate.

To detach HFb, scaffolds were first washed in PBS to remove non-adhered cells and then incubated in of trypsin/EDTA. The bacterial biofilm was removed from the scaffold by sonication. RNA was extracted from the mixed cell population using Trizol (Sigma) and the FastPrep system (MP Biomedicals) in 2ml tubes containing a mixture of glass beads to break up the bacterial cell walls. The RNA was reverse transcribed to complementary DNA (cDNA) and qPCR was then used to quantify cell growth. Specifically 18s and 16s rRNA were amplified as markers for mammalian and bacterial cells respectively. The 18s and 16s primer sets were tested on each sample, including the HFb-only and *S. aureus* only control sets.

RESULTS AND DISCUSSION

When grown in mono-culture the absolute copies/µl of HFb and *S. aureus* increased with time No 18s was detected in the bacterial culture and conversely no 16s was detected in the mammalian culture illustrating the specificity of the RT-qPCR analysis.

As expected, co-culture of HFb and *S. aureus* decreased the growth of the HFb considerably when compared to the HFb in mono-culture. This is due to exotoxins produced by the bacteria. The 16s also decreased over the culture period. This may be explained by competition for nutrients with the fibroblasts resulting in thicker biofilm formation.

CONCLUSION

The novel infected wound model enabled simultaneous monitoring of growth of *S. aureus* and fibroblasts on a 3D scaffold. This model can be used for rapidly assessing the efficacy of antibacterial wound healing scaffolds, dressing and drug delivery devices.

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IMPROVING CELL SURVIVAL IN DEGRADABLE BIOSYNTHETIC PVA-TYRAMINE HYDROGELS: INCORPORATION OF ANTIOXIDANTS

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INTRODUCTION

Covalent incorporation of biological molecules into synthetic hydrogels is an emerging strategy to engineer cell encapsulation constructs for long term use. However, this process often involves chemical modification of the biological molecules which can cause degradation and denaturation[1]. Therefore, we hypothesised that non-chemically modified proteins could be covalently incorporated into synthetic gels for improved biological performance. We have previously shown that unmodified proteins were successfully incorporated into a degradable synthetic hydrogel, made from poly(vinyl alcohol) (PVA) grafted with pendant tyramine groups (PVA-Tyr)[2]. However, the crosslinking process was shown to be detrimental to cells in preliminary 3D cell encapsulation studies. Therefore, the aim of this research was to improve encapsulated cell survival. The hypothesis was that the incorporation of anti-oxidative proteins, sericin and gelatin, protects cells from the potentially harmful radicals formed during the crosslinking.

EXPERIMENTAL METHODS

PVA-Tyr was synthesised according to a previously published protocol[2]. Hydrogels (20 wt% total macromer) were fabricated with 2 mM tris(2,2'-bipyridyl) ruthenium (II) chloride hexahydrate and 20 mM sodium persulphate under visible light (15 mW/cm^2) for 3 minutes. Gels were 20% PVA-Tyr or 19% PVA-Tyr with either 1 wt% sericin (PVA-Tyr/1S), 1 wt% gelatin (PVA-Tyr/1G), or 18% PVA-Tyr with 1wt% of both proteins (PVA-Tyr/1S/1G). Murine dermal fibroblasts were encapsulated at a concentration of 1 million cells/ml. Live-dead assay (Calcein-AM/Propidium Iodide) was used to assess the viability of cells.

RESULTS AND DISCUSSION

The cells did not survive the photo-encapsulation in pure 20% PVA gels, which was hypothesised to be due to the radicals generated (Fig 1). As both gelatin and sericin have anti-oxidatant properties it was theorised that incorporating these proteins

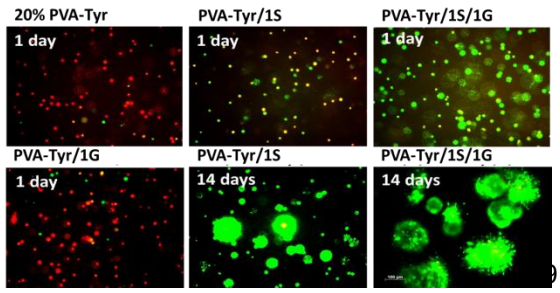

Figure 1: Live-dead images of encapsulated cells. Scale bar = 100um.

could protect the cells by scavenging radicals. Gelatin incorporated at 1 wt% had no effect on initial cell survival. Sericin incorporated at the same wt% successfully promoted cell survival during the encapsulation process (Fig. 1). A higher concentration of gelatin (5 wt%) was required to have similar protective effects (data not shown). Longer term studies showed that sericin alone was not enough to support long term bioactivity, and that the encapsulated cells were only able to spread and form cell-cell contacts when both sericin and gelatin were combined in the gels. Other cell types (Schwann cells and cardiac myocytes) have also been encapsulated using this system and showed promising results.

CONCLUSION

This study showed that sericin was able to promote the survival of encapsulated cells in PVA-Tyr gels during the crosslinking process. However, both proteins were required to facilitate long term cellular activity in the gels, which will be the focus of our future studies.

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BIOFUNCTIONALIZATION OF PEEK VIA PLASMA IMMERSION ION IMPLANTATION (PIII)

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INTRODUCTION

Poly-ether ether ketone (PEEK) has emerged as a leading biomaterial due to its biocompatibility and mechanical properties. However, due to the inertness of PEEK, bioactivity remains suboptimal and interest had been focused on improving this aspect. Plasma Immersion Ion Implantation (PIII) was demonstrated improve the bioactivity of many polymers while providing a one-step method to covalently immobilize proteins onto the treated surfaces. The aim of this project is to apply PIII treatment on PEEK and explore methods of achieving better cell responses.

EXPERIMENTAL METHODS

The modified surfaces were evaluated by means of contact angle, ELISA and ATR-FTIR detection coupled with SDS washing. Cell culture studies including attachment, spreading, proliferation and gene expression were performed to assess the biological responses.

RESULTS AND DISCUSSION

Contact angle studies show that hydrophobic recovery after longer PIII treatments occurs faster but stabilizes after 2 weeks. Protein studies demonstrated that proteins remain detectable on the surfaces after harsh washing conditions confirming that proteins are covalently linked to the modified surfaces. Cell culture results show that the treated surfaces alone increased the bone cell attachment rate by over 80% with significantly improved spreading and proliferation. The covalently linked ECM proteins on the treated surfaces were also shown to remain highly biologically active.

CONCLUSION

The current results had shown that cell attachment to PEEK can be largely improved by using PIII. These findings will help us further understand the effects of the PIII treatment process on PEEK in which the outcomes may help us improve its bioactivity for bone related applications.

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SESSION 4 – ENHANCING BIOMATERIALS AND CELL SIGNALLING FOR REGENERATIVE MEDICINE

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STRATEGIES FOR WHOLE TOOTH TISSUE ENGINEERING

Tooth development is an exceptionally complex process, during which an initial soft tissue tooth organ eventually gives rise to erupted, functional teeth composed of highly mineralized dentin, enamel and cementum tissues. Our published report in 2002 demonstrated, for the first time, the possibility of using Tissue Engineering approaches to bioengineer functional teeth. Since that time, we have been working to devise methods for optimized dental tissue engineering with the goal of created biological based, fully functional, human replacement teeth. Here we review the advantages and disadvantages of a variety of biodegradable scaffold materials and designs for dental tissue and whole tooth tissue engineering applications. We describe novel three dimensional in vitro tooth models, and in vivo rat and porcine implantation models, to facilitate our goal to generate functional biological tooth substitutes in humans.

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HARNESSING THE INFLAMMATORY RESPONSEFOR TISSUE REGENERATION

The natural inflammatory response during normal bone repair or after biomaterial implantation is critical for the proliferation of blood vessels. Our work and that of others show that the inflammatory response and subsequent angiogenesis can be modulated through manipulation of a type of inflammatory cell, the macrophage, using biomaterial properties1. The goal of this work was to develop an immunomodulatory biomaterial based on decellularized bone that encourages vascularization and new bone formation.

We prepared scaffolds that first promoted the M1 phenotype of macrophages followed by the M2 phenotype, which is the natural sequence observed in normal healing. To achieve this sequential profile, we conjugated the M2-promoting cytokine interleukin-4 (IL4) to bone scaffolds via biotin-streptavidin binding, and attached the M1-promoting interferon-gamma (IFNg) to the scaffolds by physical adsorption.

Primary human macrophages were seeded on the scaffolds and phenotyped after 3 and 6 days. Experimental groups included: Negative control, IFNg only, IL4 only, and their combination. To observe the effects on vascularizatioin in vivo, scaffolds were implanted subcutaneously in C57/BL6 mice (n=3 per group) for two weeks. Samples were analysed histologically and immunohistochemically for the presence of endothelial cells via CD31 staining.

Physical adsorption of IFNg caused early M1 polarization and conjugation of IL4 caused late M2 polarization, as evidenced by gene expression analysis for 10 markers of the M1 and M2 phenotype and ELISA analysis of 4 secreted markers. Interestingly, however, the combination of IFNg and IL4 did not cause robust sequential M1 and M2 polarization, suggesting that the conflicting signals impeded macrophage polarization and that future generations of these biomaterial should more distinctly separate the M1 and M2 phases.

After two weeks of in vivo cultivation, scaffolds that released IFNg (either alone or in combination with IL4) were more vascularized than negative controls or IL4 only groups, as evidenced by CD31 staining, although the differences were not significant, likely a result of small sample size. Considering that IFNg has previously been shown to inhibit angiogenesis2, these results suggest that vascularization was mediated by M1 macrophages, which are highly angiogenic.

This study represents proof of concept that macrophage behaviour can be manipulated for beneficial effects on vascularization. Future studies include more distinct temporal separation of the M1 and M2 phases and evaluation in an orthotopic bone defect model.

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THERMORESPONSIVE INJECTABLE HYDROGELS WITH TUNABLE PROPERTIES

Injectable biopolymer hydrogels display great promise for *in vivo* tissue engineering due to their high mass transfer capabilities, biological similarity to natural extracellular matrix, and minimally invasive method of delivery [1,2]. Low mechanical strength, lack of control on the gelation behavior and the use of cytotoxic crosslinking reagents are their main drawbacks. The aim of this study was to fabricate injectable elastin based hydrogels with tunable gelation and favorable biological properties by covalently bonding elastin with a thermoresponsive copolymer.

A thermoresponsive copolymer, poly(N-isopropylacrylamide-*co*-2-hydroxyethyl methacrylate/polylactide-*co*- N-acryloxysuccinimide-*co*-oligo (ethylene glycol) monomethyl (PNPHO) was synthesised with protein-reactive groups by free radical polymerisation technique. The feasibility of covalently bonding PNPHO copolymer with elastin and formation of hydrogel by this conjugation was studied. In addition, the properties of elastin-*co*-hydrogels were altered to match different clinical requirements.

The synthesis of PNPHO copolymer was confirmed with ^{1}HNMR spectra with evidence of proton peaks for all segments [2]. Moreover, FTIR analysis showed that the succinimide ester groups in PNPHO formed covalent bonds with amine groups of elastin. Results in Figure 1-a demonstrate

that by increasing the temperature to 37 °C, the precursor solution formed hydrogel. By changing the composition of PNPHO copolymer, it was feasible to tune the gelling time of the hydrogels in the range of 2 to 12 minutes that is desirable for the clinical applications. In addition, the dermal fibroblast cell encapsulation efficiency of the hydrogel was nearly 85%. The staining of cell nuclei in Figure 1-b showed that the number of encapsulated cells was also significantly increased from day1 to day 3.

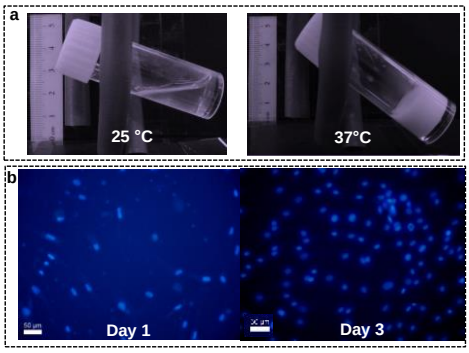

Figure 1. The thermoresponsive behaviour of hydrogels (a) and proliferation of encapsulated cells within the hydrogels from day 1 to day 3 (b).

In this study, a thermoresponsive injectable elastin based hydrogel was fabricated. The gelation behaviour of hydrogels is very versatile because it is tuned to different applications. High viability of encapsulated cells and their proliferation for at least 3 days confirmed favorable biological properties of elastin-*co*-PNPHO hydrogels. All these results justified the high potential of these hydrogels for different *in vivo* tissue regeneration applications.

MORE READING

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AN INJECTABLE HYDROGEL SYSTEM INCORPORATING FREE OR COVALENTLY-BOUND SULPHATED POLYSACCHARIDE FOR INTERVERTEBRAL DISC (IVD) REGENERATION

Hydrogels show significant promise as materials both for cell delivery and to promote tissue regeneration. We detail here the development of an injectable hydrogel system for encapsulation and delivery of mesenchymal progenitor cells (MPCs).

8-armed PEG polymers was functionalised to varying extents with 3-(4-Hydroxyphenyl) propionic acid (HPA). Hyaluronic acid (HA) and a sulfated polysaccharide (SP) were similarly functionalized. A recombinant fibronectin (rFN) was covalently introduced without modification simply through available tyrosine residues. The resulting PEG-HPA/HA/SP/rFN conjugates were cross-linked via horseradish peroxidase (HRP) and H2O2.1 Degradation rates, mechanical properties and architecture of the gels were assessed using rheometry (e.g. Fig. 1A,B) and cryo-SEM (e.g. Fig 1C,D). hMSCs were seeded onto (2D), and into (3D) the gels and cultured in maintenance or inductive media for up to 21 days.

The versatility of this flexible hydrogel system was exemplified through variations in the degree of HPA substitution, polymer concentration, type and concentration of crosslinking reagents, which resulted in a range of mechanical properties and gelation kinetics for these gels. Co-crosslinking of the PEG-HPA conjugates with the rFN encouraged attachment and spreading of hMSCs, in both 2D (Fig. 1E) and 3D (Fig. 1F) formats, with good viability. The presence of the HA and SP was shown to

increase induce chondrogenic gene upregulation and protein and GAG matrix secretion (enhanced by tethering the SP to the matrix), in the absence of inductive media, but to inhibit osteogenesis.

With tunable gelation kinetics, degradation, mechanical properties and functionality, these injectable hydrogels show significant potential for in vitro culture and in vivo delivery of a range of stem cell types and for IVD regeneration.

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UNDERSTANDING MECHANISMS OF LENS CELL SELF-ASSEMBLY: WILL THIS HELP US REGENERATE LENS STRUCTURE AND FUNCTION AFTER CATARACT SURGERY

How the lens generates and maintains its polarised three-dimensional cellular architecture is a poorly understood part of its developmental program; yet, precise regulation of this characteristic feature is critical for function. During morphogenesis cells become organized into a polarized, spheroidal structure with a monolayer of epithelial cells overlying the apical tips of elongated fibre cells. During growth, epithelial cells proliferate and progeny that shift below the lens equator elongate into new fibres that become polarised/oriented towards the epithelium and undergo directed migration to the poles[1]. this study set out to investigate interactions between epithelial cells and fibre cells in order to better understand mechanisms that underlie their assembly into a polarised spheroidal structure.

Epithelial explants from neonatal rats were used to study the polarised behaviour of lens cells. Explants were treated with 200 ng/ml fgf2 to promote fibre differentiation. after 4-6 days, epithelial and fibre cells were identified by immunofluorescent localisation of cell-specific markers. Components of wnt-frizzled and notch/jagged signalling pathways were identified by immunofluorescence and western blotting.

Using confocal microscopy, we showed that polarized/oriented behaviour of elongating fibres in fgf-treated explants is coordinated by islands of epithelial cells and provide evidence that this is mediated by an epithelial-derived wnt. We also show that a reciprocal interaction occurs whereby elongating fibres, by means of a jagged/notch signaling pathway, promote proliferation of the associated epithelial cells and maintenance of the epithelial phenotype[2]. We propose that this interaction in our experimental system mimics interactions between epithelial cells and fibre cells in vivo that are critical for maintaining the two distinct lens cell compartments and their assembly/growth into a spheroidal polarised structure.

This study identifies mechanisms, intrinsic to the lens, that play key roles in regulating self-assembly of epithelial and fibre cells into their distinctive polarized arrangement. This information is fundamental to defining the specific conditions and stimuli needed to recapitulate developmental programs and promote regeneration of lens structure and function after cataract surgery.

MORE READING

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CLOSING REMARKS

THANKS! LOOKING FORWARD TO SEEING YOU NEXT TIME!