

21st NSW Stem Cell Network Workshop

Bioengineering and Stem Cells

Darlington Centre
City Rd, Sydney
Tuesday, September 23, 2014

SPONSORS & SUPPORTERS

Silver Sponsors



microsurfaces

life
technologies

A Thermo Fisher Scientific Brand

Bronze Sponsors



Workshop Supporters



eppendorf

Network Supporters

The NSW Stem Cell Network gratefully acknowledge the support of:

The NSW Government Office for Health and Medical Research

Diabetes New South Wales

WELCOME

Welcome to the 21st Workshop of the NSW Stem Cell Network.

As with many of the past workshops of the Network, the current one is devoted to a specific theme, namely *Bioengineering and Stem Cells*. The last time a Workshop of this nature was conducted was in April 2005, when we discussed amongst other topics, the growing of human embryonic stem cells on scaffolds made by traditional engineering means. The field has progressed since then and in this Workshop we will hear about sophisticated 3D printers being used for the creation of scaffolds on which a variety of stem can be grown.

Cell tracking has moved forward a great deal in the past decade, with several speakers at the Workshop addressing the topic. This has relevance for future human trials with stem cells, for example, the proposed multicentre Australian cord blood trial for treatment of cerebral palsy.

Bioreactors are needed for scale up of cells needed for transplants into humans, and this is especially so for the use of mesenchymal stem cells. The application of these for the treatment of chronic inflammatory bowel diseases is a topic of the Workshop. New materials are being used to grow stem cells on, for example, endometrial stem cells for treatment of pelvic floor prolapse. We will also hear of other new materials being developed by researchers from the Cooperative Research Centre for Cell Manufacturing.

In this Workshop we also will hear how the relatively new science of microfluidics can be used to culture stem cells. As well, we will hear an up to date account of how to analyse single stem cells, using transcriptomics and other technologies.

Bioengineering techniques as they relate to stem cells are relatively novel in Australia. The Workshop offers an opportunity for proponents of the art to interact with one another, and expand uses of the techniques available. To further this goal, we are encouraging students, especially postgraduate ones, to submit posters, with a prize being offered to the student judged to have the best poster.

We are particularly grateful to Dr Robert Nordon from the Faculty of Engineering from the University of New South Wales for his input on the Program Organising Committee.

We hope you enjoy the Workshop.



Bernie Tuch
Director
NSW Stem Cell Network

PROGRAM

9:00am	Registration opens/ Light refreshment
9:30am	Professor Bernie Tuch—NSW Stem Cell Network <i>Welcoming</i>
9:35am	Prof Bruce Milthorpe—Dean of Science at the University of Technology Sydney <i>Opening Address</i>
Session 1	Single Stem Cell: Imaging, transcriptomics and analysis Chair: Dr Robert Nordon – University of New South Wales
9:45am	A/Prof Jose Polo – Monash University <i>Dissecting the molecular events during reprogramming of somatic cells into induced pluripotent stem cells</i>
10:10am	Dr Robert Nordon – University of New South Wales <i>Cells in focus: biomimicry on a chip</i>
10:30am	Prof Phil Hodgkin – Walter and Eliza Hall Institute of Medical Research <i>Lessons learnt by imaging single lymphocytes</i>
10:50am	Dr Martin Gosnell – Macquarie University <i>Label free interrogation of cell populations by Hyperspectral imaging</i>
11:10am	Morning tea
Session 2	Devices for Stem Cell Expansion & Implantation: Bioreactors, 3D printing & mechanobiology Chair: Dr. Dirk Winnemöller - Miltenyi Biotec Australia
11:30am	Dr Sherry Kothari—CRC for Cell Therapy Manufacturing <i>Smart materials for smart cells - manufacturing the next generation of cell therapies</i>
11:55am	Dr Mike Doran – Qld University of Technology <i>The manufacture of cartilage and bone microtissues from mesenchymal stem/stromal cells, and their assembly into osteochondral tissues</i>
12:15pm	Dr Majid Ebrahimi Warkiani – University of New South Wales <i>High-throughput cell sorting using inertial microfluidics</i>

PROGRAM

12:35pm	Lunch/ Poster session
Session 2 (cont)	Devices for Stem Cell Expansion & Implantation: Bioreactors, 3D printing & mechanobiology Chair: A/Prof Jerome Werkmeister – CSIRO
1:35pm	Dr Tim Dargaville – Queensland University of Technology <i>Melt Electrospinning Writing as a Tool for Scaffolding Cells</i>
1:55pm	A/Prof Jeremy Crook – University of Wollongong <i>Smart surfaces for stem cell expansion and engineering biosynthetic excitable tissues</i>
2:15pm	A/Prof Jerome Werkmeister – CSIRO <i>New biomaterial meshes and endometrial stem cells for tissue repair</i>
2:40pm	Afternoon tea
3:00pm	Dr Nick Glass – University of Queensland <i>Multiplexed microbioreactor arrays for screening small molecules and cell microenvironments</i>
3:20pm	Dr Janice Fogarty – Cell and Tissue Therapies, Royal Perth Hospital <i>Bioreactor technology for clinical cell manufacture</i>
3:40pm	Concluding comments

A/Prof Jose Polo—Monash University



Associate Professor Jose Maria Polo was born in Buenos Aires, Argentina where he graduated from Buenos Aires University as a Biochemist. In 2002, Jose began his graduate studies at Albert Einstein College of Medicine, New York under the supervision of Dr. Ari Melnick where he worked on the transcriptional mechanism of the BCL6 repression complex in lymphomagenesis and B-cell maturation. In 2008 he obtained his PhD and moved to Boston to the laboratory of Dr. Konrad Hochedlinger at the Harvard Stem Cell Institute to work on reprogramming of adult cells into induced pluripotent stem (iPS) cells. In particular, his work focused in the acquisition of immortality and the existence of epigenetic memory during reprogramming. In June 2011 as a Larkins Fellow, Jose established his independent research group at Monash University. In 2012, Jose was awarded a NHMRC Career Development

Fellowship and in 2014 a Charles Viertel Senior Medical Research Fellowship to continue his work in the molecular mechanism governing the reprogramming process and stem cells epigenetics. As of 2013, Jose holds appointments to the departments of Anatomy and Developmental Biology and to the Australian Regenerative Medicine Institute.

Dissecting the molecular events during reprogramming of somatic cells into induced pluripotent stem cells

Forced expression of the transcription factors *Oct4*, *Sox2*, *c-Myc* and *Klf4* is sufficient to reprogram fibroblasts to a pluripotent state, giving rise to induced pluripotent stem (iPS) cells. iPS cells share many features of embryonic stem cells: they can be cultured indefinitely *in vitro*, are capable of differentiating into any cell type of the three embryonic germ layers and can contribute to chimeric mice upon blastocyst injection. The ability to revert somatic cells to an embryonic state provides a unique tool to dissect the transcriptional and epigenetic events that permit the conversion of one cell type to another. It is well established that nuclear reprogramming requires the resetting of the epigenome and transcriptional network of a specialized cell into that of an embryonic cell. However, the sequence and identity of these molecular events that accompany reprogramming are not well understood. In order to shed light into these events we combined surface markers and fluorescent reporter genes and analysed defined intermediate cell populations poised to become iPS cells at the molecular level. By doing this we uncovered key epigenetic and transcriptional events of cells undergoing nuclear reprogramming. Furthermore, using single cell transcriptomics we investigated how homogenous these molecular changes are in the cell undergoing reprogramming. This data offers new mechanistic insights into the nature and sequence of molecular events necessary to convert adult cells into pluripotent cells.

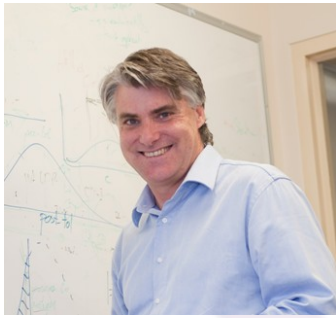
Dr Robert Nordon—University of New South Wales



Dr Robert Nordon is a senior lecturer at the Graduate School of Biomedical Engineering, University of New South Wales. After medical training he obtained a PhD in biomedical engineering, developing a closed, automated bioreactor system for expansion of stem cells for the clinic. The benchtop device known as the Quantum Cell Expansion System™ is marketed by Terumo BCT. His current research lies at the interface between cellular therapies, bioreactors, microfluidics and single cell analysis. He is currently developing microneedle arrays for painless blood collect, microfluidic bioreactors for single cell tracking, transcriptome analysis and large scale cell expansion. Dr Nordon is a partner-investigator with the ARC special research initiative into stem cell science (Stem Cells Australia, SCA), and is the Vice President of the International Society for Cellular Therapy (Australia and New Zealand region).

Cells in focus: biomimicry on a chip

Stem cell science is all about understanding how intrinsic and extrinsic signals direct cell fate. We adopt a naïve ‘reverse engineering’ approach where biological processes are mimicked on a microfluidic chip. For example to understand induction of blood formation from haemogenic endothelium by fluid shear stress we developed a microfluidic device that mimics the embryonic circulation. One can then dissect the response of individual cells by time lapse fluorescence imaging, fate mapping and division pedigree analysis. In the future, the same microfluidic technologies will be scaled for manufacture of specialised cells for clinical therapies.



Professor Phil Hodgkin is an experimental and theoretical immunologist and current head of the Immunology Division at the Walter and Eliza Hall Institute, Melbourne.

His primary research interests centre upon the regulation of T and B lymphocytes. His laboratory develops quantitative methods for exploring lymphocyte behavior including single cell imaging and division-based tracking. His laboratory is developing analytical software tools for simulating the effect of cytokines and genetic changes on lymphocyte growth, survival and differentiation. His goal is to understand the behavior of lymphocytes and

recreate the immune response with computer-based models.

Lessons learnt by imaging single lymphocytes

T and B-lymphocytes process many signals to reach decisions about the strength and type of response to follow after challenge. By combining direct imaging of single cells, genetic manipulation and computer modelling we follow B and T lymphocytes making decisions under different stimulation combinations. Our results reveal surprisingly simple rules for cellular calculation. B cells typically follow an automaton program – cells simply count through a series of divisions, stop, return to quiescence and die. Intrinsic but independent, variation in single B cell times to divide and differentiate automatically vary the number of plasmablasts produced as stimulation is altered. T cell stimulation also leads to automaton proliferation, cessation and death with a large number of signals altering the number of divisions before returning to quiescence. These results reveal how T and B cells exploit stochastic variation of timed processes to manipulate the strength of the population response and the proportion of cells allocated to different tasks. While perhaps impossible to predict the infinite variety of paths taken by single cells and families, knowledge of the underlying stochastic processes, combined with rules of integration acquired by single cell tracking and analysis enables accurate modelling of the population response over time.

Contributing authors: Andrey Kan^{1,2}, Julia M. Marchingo^{1,2}, Jie H.S. Zhou, Susanne Heinzel^{1,2}, John.F. Markham³, Ken R. Duffy, Mark R. Dowling^{1,2}, Philip D. Hodgkin^{1,2}

¹The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia, ²The University of Melbourne, Melbourne, Victoria, Australia, ³Victoria Research Laboratory, National Information and Communications Technology (ICT) Australia, University of Melbourne, Melbourne, ⁴Hamilton Institute, National University of Ireland, Maynooth, Ireland

Dr Martin E Gosnell—Macquarie University



Dr Martin E. Gosnell received his Bachelor of Engineering in Electrical engineering with first class honours from the University of Technology at Sydney in 2001, with major work in control, robotics, vision, learning and pattern recognition. His honours thesis received a score of 100%.

He was awarded the I.E.E. (London) engineering prize in 1998. Martin has worked as an engineer and scientist for CSIRO and by contract to the university of Queensland and Newcastle, and as a design engineer and research scientist for consumer electronics manufacturing companies in the areas of computer brain interface, intelligent recognition systems, specialised laser applications and audio for which he has received eight industry design awards. His interests include

applied mathematics, control, information theory, hyperspectral and image analysis, algorithm and classification system design. His Ph.D. was completed at Macquarie University in 2014 in the area of hyperspectral characterisation of cells. He has successfully developed technology, IP and published work on cellular, tissue and embryonic characterisation and developed translational tools for the medical industry.

He is currently a centre researcher for the ARC centre of excellence in nanoscale biophotonics and provides contractual services through his bioinformatics company Quantitative Pty Ltd.

Label free interrogation of cell populations by hyperspectral imaging

We present a methodology for the identification and detection of endogenous metabolites, enzymes and redox cofactors applied to a variety of cells and tissue with highly sensitive and discriminative results. The method applies hyperspectral imaging techniques to autofluorescent emissions measured at a number of strategic excitation wavelengths. The pixel spectra are analysed using a variety of multivariate analysis using subspace projections to highlight biologically interesting phenomena. The method allows us to produce abundance, redox and colocalisation images. Using revealing subspace projections we can detect mean changes in protein bound fluorophore spectra perhaps influenced by changes in protein conformation or binding sites, and other intracellular environmental conditions such as pH. The analysis can employ multiple modalities, both autofluorescence and labels, and uses dynamic image registration allowing the analysis to focus on autofluorescent emissions from specific organelles. These spectrums are employed to generate a useful and biologically significant feature set from which we can extract two or three highly informative feature variables, application of mixture models and discriminatory analysis form highly sensitive detections and allow discovery of meaningful subpopulations and creation of response and diagnostic scales. We show cellular response to a number of treatments where the addition of galactose is shown to restore mitochondrial function and L-carnitine are shown to reduce mitochondrial ROS. The difference in metabolism between wildtype and genetically modified cells is also shown. Bioanalyses are presented for a range of cells where we discuss changes in levels of NADH relating to complex 1 dysfunction along with up regulation of flavins and ROS, increase in retinoids and detection of increases in levels of early structural protein associated with differentiation and cytochrome C due to stress. We present results for embryos, motor neuron, diabetes, cancer and differentiating stem cells.

Dr Sherry Kothari—CRC for Cell Therapy Manufacturing



Dr Sherry Kothari is the Chief Executive and Managing Director of the CRC for Cell Therapy Manufacturing. The CRC for Cell Therapy Manufacturing is focused on the cost-effective manufacture and rapid translation of cell therapies into clinical practice. The CRC will 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.

As well as her appointment at the CRC, Sherry has extensive experience in working within bioscience at the research/industry/clinical interface. Sherry started her career in maxillofacial surgery before undertaking a PhD at the University of Sheffield, focusing on healing at the bone implant interface. After lecturing in Medical Materials and Tissue Engineering at the University of Manchester, she undertook an Executive MBA, to build on her passion for entrepreneurship and translation within the biotechnology sector. Sherry has been a founder and investor in two successful university life sciences spin-out companies and has since played a significant role in introducing an entrepreneurial culture within science and engineering in higher education, including helping mentor and fund early start-up ventures. Sherry has been on the Board of SIFE (Students in Free Enterprise), raising the capacity for invention, innovation, commercialisation, technology acquisition and new business growth. Sherry moved to Australia in 2007. Based at the University of South Australia, she established partnerships across Australia, Asia and Europe. In 2011, she took over as Program Leader for one of the three research programs of the Wound Management Innovation CRC, before taking on her current role at the CRC for Cell Therapy Manufacturing.

Smart materials for smart cells—manufacturing the next generation of cell therapies

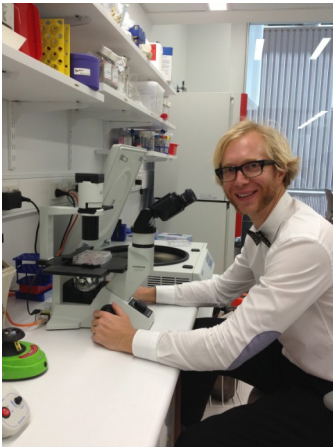
Of an estimated US\$1.25bn invested in regenerative medicine companies in 2012, 85% was in cell therapy companies. The cell therapy industry is conservatively expected to achieve global sales in excess of \$8billion by 2018 and is the fastest growing sector of the human therapeutics industry.

In 2012, seven new cell therapy products were approved by regulatory agencies around the world, compared to the preceding three years, when only five therapies were approved and the period 2002-2008, when there were no new approvals. Today, there are almost 700 cell therapy based preclinical and clinical trials being conducted and an estimated 700 companies worldwide with a focus on some aspect of regenerative medicine.

Despite this significant momentum, relatively few cell therapies actually make it to the clinic. As with any young field and new therapeutic approach, several challenges still remain. The development of a successful cell therapy is a long and arduous process that requires smooth transition to current Good Manufacturing Practice (cGMP) and compliance from what are often non-compliant early stage protocols or starting materials. The current high cost of manufacture of a cell therapy (attributed to high cost of reagents, safety & quality testing and infrastructure) is also a barrier to wide-scale adoption, as is the ability to cost-effectively produce an adequate number of cells for therapeutic purposes. There are also significant regulatory hurdles to overcome.

The CRC for Cell Therapy Manufacturing is addressing these barriers to fast track the development of promising cells into new, potentially curative therapies and make them more affordable and accessible for patients. This presentation will focus on lessons learnt from a case study and how addressing some of the above challenges can facilitate this.

Dr Mike Doran—Queensland University of Technology



Dr Mike Doran has a BSc (Genetics) and a BEng (Chemical) from the University of Alberta Canada. As an engineering graduate I worked as Project Manager for Exxon developing heavy oil fields. In 2002, I relocated to UNSW in Sydney Australia as a PhD Candidate. In 2006 I was awarded a PhD in Biomedical Engineering. Under the excellent tutelage of Dr Robert Nordon, I contributed to one patent and the development of a bioreactor system now marketed by TurumoBCT for stem cell expansion. I am currently a Research Group Leader at the Translational Research Institute (TRI) in Brisbane Australia. I am employed through the Queensland University of Technology (QUT) where I have an ongoing academic appointment through QUT's School of Biomedical Sciences. Since 2009, I have published 30 articles. In 2013/14 I published 9 articles and one video (Trends in Biochemical Sciences). I have three patents

awarded based on concepts and data from my research. I am fortunate to be currently funded to develop stem cell and tissue-engineering solutions for cartilage and bone repair, prostate cancer bone metastasis model systems, umbilical cord blood stem cell expansion and transplant technologies, and synthetic skin for diabetic foot ulcer repair.

The manufacture of cartilage and bone microtissues from mesenchymal stem/stromal cells, and their assembly into osteochondral tissues

Articular cartilage has a limited capacity for self-repair. The effective repair of full-thickness defects may require the implantation of an engineered biphasic osteochondral tissue. Herein we describe the manufacture of both chondrogenic and osteogenic micropellets from bone marrow-derived mesenchymal stem/stromal cells (MSC), and their subsequent assembly into a scaffold-free biphasic osteochondral-like tissue. Using microwell platforms, we have optimised the manufacture of MSC-derived bone and cartilage microtissues. Microtissue formation mimics the natural processes of MSC condensation occurring in chondrogenesis and endochondral ossification. Mimicking these processes significantly enhances both chondrogenesis and osteogenesis. Using a combination of bone and cartilage microtissues as building blocks we have generated zonal macroscopic osteochondral tissues suitable for cartilage defect repair.



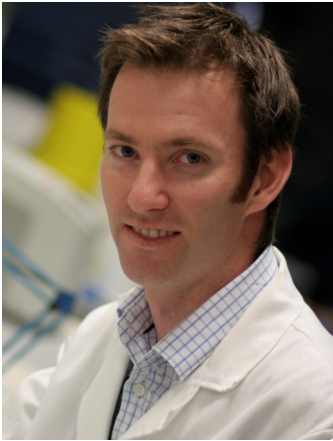
Dr Majid Ebrahimi Warkiani is a new lecturer in the School of Mechanical and Manufacturing Engineering, University of New South Wales (UNSW). He received his PhD in Mechanical Engineering from Nanyang Technological University (NTU), and undertook postdoctoral training at Massachusetts Institute of Technology (MIT). Dr Warkiani PhD research was focused on development of novel isoporous membranes for isolation and recovery of waterborne pathogens using MEMS techniques. At MIT, he expanded his research interest towards biomedical engineering and lab-on-a-chip platforms. Dr. Warkiani is currently working on development of novel microfluidic devices for isolation of circulating tumor cells (CTCs) from blood and subsequent genetic analysis. In addition, he is also collaborating with multi-disciplinary groups at Harvard, MIT

and NUS for development of functional micro/nano-scale tools for study of human diseases, stem cell therapy and point-of-care diagnostics.

High-throughput cell sorting using inertial microfluidics

Cell sorting is critical for many applications ranging from stem cell research to cancer therapy. Isolation and fractionation of cells using microfluidic platforms have been flourishing areas of development in recent years. The need for efficient and high-throughput cell enrichment, which is an essential preparatory step in many chemical and biological assays, has led to the recent development of numerous microscale separation techniques. Size-based passive particle filtration using inertial microfluidics have recently received great attention as a promising approach for particle focusing, filtration and fractionation due to its robustness and high rates of operation. The main advantage of inertial-based microfluidics approaches is that continues-flow separation without clogging can be realized using relatively large microchannels with relatively high resolution. In this seminar, I will describe our recent efforts in development of ultra-high throughput microfluidics systems for separation and fractionation of stem cells. Further, I will show that that high-throughput inertial microfluidics enables efficient sorting of Mesenchymal stem cells (MSCs) as a function of cell diameter, and show that this enables selection and sorting of osteoprogenitor cells from marrow for applications such as bone regeneration. Finally, I will present some of our efforts for large-scale manufacturing and enrichment of MSCs inside perfusion bioreactors.

Dr Tim Dargaville—Queensland University of Technology



Dr Tim Dargaville completed his PhD in polymer chemistry in 2003 at the University of Queensland under the supervision of David Hill. From 2003–2005 he was a research scientist at Sandia National Laboratories in Albuquerque, New Mexico, and in 2006 returned to Australia to take up a position at QUT. He is now a senior lecturer with research interests in the synthesis of novel stealth-like polymers and 3D printed structures for biomaterial and sensor applications.

Melt electrospinning writing as a tool for scaffolding cells

Melt electrospinning writing (MEW) is a relatively new additive manufacturing technique for fabrication of porous scaffolds with applications in tissue engineering and regenerative medicine. MEW is similar to traditional solution electrospinning but instead of using a polymer dissolved in a solvent as the feed material, MEW uses a solvent-free polymer melt. This melt is extruded through a narrow gauge needle attached to a high voltage supply such that the resulting electrified molten jet is attracted to a grounded collection plate as the polymer crystallises. In writing mode, the translational movement of the collector plate is programmed via computer such that layer by layer writing of the fibres is possible. Typical fibre diameters are 5-10 μm and overall porosity often exceeds 85% making structures prepared by MEW suitable for scaffolding of cells either directly attached to the polymer fibres or encapsulated within a gel residing in the pores of the scaffold. In this presentation the state-of-the-art of MEW and types of scaffolds achievable using this technique will be discussed together with a brief series of our results showing encapsulation of fibroblasts and how this may be extended to stem cell encapsulation.

Contributing authors: Dr Tim Dargaville¹, Prof Dietmar Hutmacher¹, Prof Paul Dalton²
¹Queensland University of Technology, QLD, ²University of Würzburg, Germany

A/Prof Jeremy Crook—University of Wollongong



Associate Professor Jeremy M Crook is a Chief Investigator of the new ARC Centre of Excellence for Electromaterials Science (ACES) at the University of Wollongong, an Associate Professor (A/Prof) and Principal Fellow at the Australian Institute for Innovative Materials, Intelligent Polymer Research Institute, and Illawarra Health and Medical Research Institute, and holds an honorary appointment as A/Prof and Principal Fellow in the Department of Surgery - St Vincent's Hospital at The University of Melbourne (UoM). Jeremy was awarded a PhD (Dep't of Pathology, UoM) in 1998, after which he undertook a post-doctoral appointment as a Fogarty International Centre Medical Research Fellow at the US National Institutes of Health (NIH). Working under renowned

researchers Drs Daniel Weinberger and Joel Kleinman in the Clinical Brain Disorders Branch of the National Institute of Mental Health, Jeremy was awarded the NIH "Fellows Award for Research Excellence". In 2002, Jeremy was recruited by the stem cell biotechnology company ES Cell International Pty Ltd (ESI), where he was appointed Project Manager in Melbourne, followed by Program Manager of Platform Technologies in Singapore. During this time Jeremy oversaw the development of the world's first clinically compliant pluripotent stem cell lines (Cell Stem Cell, 2007). Since working at ESI, Jeremy has been a Group Leader in the A*STAR Institute of Medical Biology (IMB) in Singapore, where together with preeminent stem cell scientist Dr Alan Colman he established the Stem Cells Disease Modelling Laboratory. In addition to Group Leader at IMB, Jeremy held the position of Head of the Singapore Stem Cell Bank. In ACES, Jeremy's laboratory is undertaking research relating to human pluripotent and neural stem cells for disease modelling and *ex vivo* tissue engineering, and incorporates novel biomaterials for improved cell culture and differentiation, early phase drug discovery, diagnostics, and regenerative medicine.

Smart surfaces for stem cell expansion and engineering biosynthetic excitable tissues

Biocompatible natural and synthetic materials offer new opportunities for stem cell research and regenerative medicine by providing smart surfaces for controlling the interface between cells and their substratum. The use of smart biomaterial surfaces for delivering chemical and non-chemical cues that direct human stem cell fate will be especially important. At the ARC Centre of Excellence for Electromaterials Science (ACES) we are using biomaterials to create mechano-, electro- and chemo-active surfaces to engineer and re-engineer excitable tissues such as "brain-like" neural tissue. My presentation will canvass the research being undertaken at ACES, including the combination of clinically relevant human stem cells with smart polymers such as conductive polymer films and polymeric gels produced by three-dimensional bioprinting as promising strategies to culture and differentiate cells for *in vitro* modelling, drug screening, toxicity testing and potentially transplantation therapy.

A/Prof Jerome Werkmeister—CSIRO



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 172 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.

New biomaterial meshes and endometrial stem cells for tissue repair

Pelvic organ prolapse (POP) is common, and 11-19% of all women will be treated with one or more reconstructive surgical repair operations. It is largely a consequence of childbirth injury and is exacerbated by aging and obesity. Synthetic polypropylene (PP) mesh used to augment vaginal repair surgery has improved anatomical outcomes but the frequency of adverse events is unacceptable. We are examining a novel tissue engineering approach using endometrial mesenchymal stem cells (eMSC) combined with new meshes designed to meet the biological and biochemical properties of the damaged fascial wall. New meshes were designed from a suite of polymers including polyetheretherketone (PEEK), polyamide (PA) and PP and were evaluated for mechanical tissue compliance, particularly selecting those with significantly less stiffness, more flexibility and decreased permanent deformation after cyclic loading. Of these, a polyamide/gelatin mesh (PA+G) was selected for further investigation. The eMSC can be effectively expanded in 2D and 3D culture and on these polyamide (PA) meshes and require selective matrix coatings and defined culture conditions including hypoxia; the eMSC can be maintained in culture as stem cells by various small molecule signaling inhibitors and induced to differentiate to SMC and fibroblasts on addition of appropriate signals including TGF β 1, PDGFB and CTGF to produce new tissue. In a subcutaneous rat model of wound repair implanted with matrix-coated PA meshes, the eMSC promoted early neovascularisation, stimulated a rapid influx of pro-inflammatory M1 macrophages which rapidly switched to a M2 regenerative phenotype with fewer macrophages around the meshes with time. Collagen synthesis increased with implantation time; with the cell-seeded meshes, the neotissue was more organised with minimal fibrosis that resulted in lower stiffness and greater distensibility. We are now developing a pre-clinical large animal model of vaginal repair surgery using an ovine model to evaluate our new meshes with seeded eMSC.

Contributing authors: Jerome Werkmeister¹, Sharon Edwards¹, Kai Su¹, Daniela Ulrich², Jacinta White¹, Ker-Sin Tan², Anna Rosamilia², John Ramshaw¹, Caroline E Gargett²

¹CSIRO Manufacturing Flagship, Clayton, Victoria; ²Ritchie Centre, MIMR-PHI Institute of Medical Research, Clayton, Victoria

Dr Nick Glass—University of Queensland

Dr Nick Glass completed his Bachelors of Science in Mechanical Engineering at the University of Alberta (Canada) and a Master's of Science in Electrical and Computer Engineering with a specialty in MEMS and nano-systems. He then went to Monash University (Australia) for his Doctor of Philosophy in Mechanical and Aerospace Engineering with a focus on microfluidics and lab-on-a-chip systems. Since then has been working in the Cooper-White Group at AIBN for 2 years now on Microbioreactor and microfluidic device development. Primarily, he has been working the use of high throughput microfluidic microarrays for the study of the differentiation of human embryonic stem cells and induced pluripotent stem cells toward renal lineages.

Multiplexed microbioreactor arrays for screening small molecules and cell microenvironments

Effective use of human stem cells in regenerative medicine and drug screening depends on our ability to effectively direct both their undifferentiated expansion and differentiation into desired lineages. Exquisite control over stem cell fate is needed to efficiently produce sufficient, defined cell populations for such applications. We have developed scalable, valveless, continuous-flow microbioreactor arrays that both provide a full-factorial set of exogenous factor compositions, and also allow controlled accumulation of paracrine factors, to cells seeded into these arrays. These arrays have been used to survey up to 8100 individual, perfused cellular microenvironments in parallel, permitting rapid, unbiased screening of combinations of growth factors and available (and new) small molecule agonists and antagonists. Through screens of multi- and pluripotency maintenance, mesendodermal differentiation of human pluripotent stem cells, osteogenic differentiation of mesenchymal stem cells, and human cardiomyocyte proliferation as examples, we demonstrate the unique ability of this platform to separate, visualise, identify and modulate paracrine effects that are not otherwise readily accessible. Importantly, optimization of these culture conditions with the arrays is readily translatable to conventional static culture protocols, exemplifying the immediate practicality of the microbioreactor array platform. This multiplexed microbioreactor platform deciphers factor interplay and signalling hierarchies that control stem cell fate, and is applicable as a universal microenvironmental screening platform for bioprocess optimisation, media formulation design, quality control for cellular therapeutics and cell-based drug toxicity and discovery.

Contributing authors: Titmarsh, D.¹, Frith, J.¹, Glass, N.¹, Padmanabhan, H.¹, Hudson, J.¹, Hidalgo, A.¹, Ovchinnikov, D.¹, Porrello, E.¹, Wolvetang, E.¹, and Cooper-White, J.^{1,2,}*

¹. The University of Queensland, St. Lucia, Brisbane, QLD 4072, Australia, ². Division of Materials Science and Engineering, CSIRO, Clayton, VIC 3169, Australia

Dr Janice Fogarty – Cell and Tissue Therapies, Royal Perth Hospital



Dr Janice Fogarty is the Medical Scientist in Charge of Cell and Tissue Therapies WA (CTTWA) a biotherapeutic manufacturing facility that she helped develop and commission in 2005. Janice has over 20 years experience in haematology and transplant, holds a diploma in tissue banking management and is an adjunct lecturer at the University of Western Australia.

She manages the cleanroom facility operation on a daily basis and oversees annual recertification. CTTWA holds a Therapeutics Goods Administration (TGA) license to manufacture therapeutic goods in the class of human blood and tissue. CTTWA currently manufactures a number of products including heart valves, skin, serum eye drops and therapeutic cells. In addition, CTTWA receives, processes, stores and distributes autologous and allogeneic haemopoietic products for transplantation locally for three hospital transplant programs. CTTWA is also the WA processing and packaging centre for the Australian Bone Marrow Donor Registry (ABMDR), distributing grafts locally, nationally and internationally.

CTTWA has been developing cell therapy and tissue based products that are under clinical trial evaluation including a TGA license for mesenchymal stromal cells.

Bioreactor technology for clinical cell manufacture

Cell therapies are an emerging field of biotherapeutics. The clinical demand for these cultured expanded cell products is increasing at a dramatic rate. Currently, our facility manufactures mesenchymal stromal cells (MSC), fibroblasts and other cell types under GMP using culture flask and factory based methodology. This manufacturing method is heavily labour dependant and is not a completely closed system. In order to meet the increasing demand for cell therapies, implementation of bioreactor technology is essential to improve and increase manufacturing capability. Bioreactor technology offering closed system culture expansion removes some of the risks associated with current traditional methods of manufacturing. The implementation of bioreactor based technology will allow facilities to increase manufacturing capacity to meet escalating clinical demand for cell therapies.

Currently, there are a number of automated and semi automated devices in development and evaluation for the production of expanded cells. For adherent cells, these include the Terumo Quantum hollow fibre perfusion system, stirred bioreactors using microparticles and microcarriers, multi-plate plastic stackers such as the Integrity Xpansion system, the Mabio Clinicell culture cassette and the Rotary Cell Culture System using microcarriers. Our facility has reviewed available devices for suitability for the manufacture of clinical products and is proceeding with the evaluation of the Terumo Quantum hollow fibre perfusion system. It can be operated outside of a cleanroom environment using sterile docking of tubing and is currently the only bioreactor system listed on the Australian Register of Therapeutic Goods (ARTG). Since our centre is licensed by the Australian regulator, the Therapeutic Goods Administration (TGA), to manufacture MSC, the Quantum bioreactor also meets our regulatory requirements. CTTWA has been optimising this system over the past 2 years.

POSTERS

1. Stem Cell Expansion Studies for the Treatment of Leukemia

Authors: Mouna Hamad, Harry Manoli, Mohammad Irhimeh, Ali Khademhosseini, Ali Abbas.

2. Regulating Adipose Stem Cell Differentiation by Electrical Stimulation in Novel Stereolithographic Scaffold Structures

Authors: Miina Björninen, Kerry Gilmore, Jani Pelto, Minna Kellomäki, Susanna Miettinen, Dirk Grijpma, Gordon Wallace, Suvi Haimi.

3. Human Umbilical Cord Perivascular Tissue: The Effect of Cryopreservation on HUCPVC

Author: Michael Sivell.

4. Bone Marrow Stem Cell Transplant in Thalassaemia Patients; A Retrospective Analysis of the Last Four Years

Authors: Sohan Singh and Salem AlShemmari.

5. The Investigation of Cytotoxicity of Highly Luminescent Water Soluble Quantum dots in Colon Cancer Cells

Author: Fatemeh Mir Najafi Zadeh, Deborah M. Ramsey, Fan Wang, Shelli R. McAlpine, Peter Reece and John Arron Stride.

NOTES

Be a Member of the NSW Stem Cell Network

The NSW Stem Cell Network is a professional community with an interest in all forms of stem cells.

The Network strategy is to work with the scientific, health and medical research communities, the higher education sector and business to promote growth and innovation to achieve positive outcomes for the people of NSW. Our work encompasses science, medicine, ethics, law, business and public awareness of stem cells.

Contact Details

www.stemcellnetwork.org.au

stemcellinfo@stemcellnetwork.org.au

(02) 9552 9981

**NSW Stem Cell Network,
26 Arundel St, Glebe, NSW, 2037**