The University of Sydney
Faculties of Engineering and Medicine
&
The Bosch Institute

Inaugural Tissue Engineering Symposium (SuTEN)

Darlington Centre
The University of Sydney

Thursday 23rd November 2006

CONTENTS

1 Foreword: Dean of the Faculty of Engineering

Dear Colleagues,

On behalf of the Faculty of Engineering, University of Sydney it is my great pleasure to welcome you to the inaugural Sydney University Tissue Engineering symposium. Biomedical Engineering in general, and Tissue Engineering in particular, are key areas of teaching and research in the Faculty. It is therefore a great pleasure to have this Symposium at the University at this time.

Distinguished national and international scientists will present updates and reviews on a range of scientific and clinical research into Tissue Engineering. The scientific program is composed of invited presentations. We are grateful to all those who have supported the meeting and made it possible. We hope that you will enjoy your scientific day at the University of Sydney. We are also grateful to all those who have supported the Faculty by sponsoring this event (Faculty of Engineering, Bosch Institute, Faculty of Medicine, MICROSEARCH FOUNDATION of AUSTRALIA, ASDM, Pathtech, Chemicon, Corbett, Eppendorf, SYNTHES and Invitrogen)

We welcome you all and trust that you will enjoy a rewarding and stimulating program, both learning and making new colleagues in the area.

Gregory Hancock, AM, FTSE
Dean of Engineering

2 Foreword: Dean of the Faculty of Medicine

Dear Colleagues,

The Faculty of Medicine is happy to sponsor the Sydney University Tissue Engineering Network symposium, which is a joint initiative of the Faculties of Engineering and Medicine and the Bosch Institute.

The Faculty of Medicine is mindful of the enormous strides that have been taken in recent years in the area of tissue engineering at both the basic and applied research levels. Development of strengths in tissue engineering is a focus of the Bio^3 Project, in which the Faculty is a partner. More recently, the newly-established Bosch Institute has made Organ and Tissue Replacement one of its five central Research Themes. The Bosch Institute, which is associated with the Faculty of Medicine, intends to develop the general area of organ and tissue repair, replacement and renewal as a key focus for intellectual development over the next few years.

The program of the symposium is an exciting one, with a number of outstanding speakers from Sydney, interstate and overseas. This symposium will be a superb opportunity to promote interactions between high quality researchers interested in this area.

Bruce Robinson
Dean, Faculty of Medicine (Acting)

3 Program

8.00am		*Registration, Coffee, tea, juice and cocktail muffins*
8.45am		**Welcome and Opening Remarks:** *Professor Nicholas Hunt, Executive Director, The Bosch Institute* *Dr Hala Zreiqat, Symposium Convenor, Biomaterials & Tissue Engineering Research Unit, University of Sydney* *Professor Greg Hancock, Dean, Faculty of Engineering*
8.50am	**SESSION 1:**	**Stem Cells in Tissue Engineering** *Chair: Professor Richmond Jeremy*
8.55am		**Plenary** Professor Peter McDonald Joint Head, Transplant Program, Faculty of Medicine, UNSW *"The use of stem cells to treat heart disease"*
9.40am		Dr Andrew Zannettino, Head, Myeloma and Mesenchymal Research Laboratory, Division of Haematology, Institute of Medical & Veterinary Science, Adelaide *"The biology and therapeutic application of prospectively isolated mesenchymal precursor cells"*
10.05am		Professor John Rasko, Head, Gene & Stem Cell Therapy Program, Centenary Institute of Cancer, Medicine & Cell Biology *"Australia's only non-human primate model of MSCs and the future of cGMP facilities for clinical cell therapy trials in NSW"*
10.30am—10.55am		**Morning Tea**
11.00am	**SESSION 2:**	**Future Directions for the SuTEN** (Discussion Session) *Moderators: Professor Hans Coster, Dr Alex Sharland, Professor Nick Hunt*

11.55am	**SESSION 3:**	**Biomaterials for Different Applications** *Chairs: Dr Andrew Ruys and Professor David McKenzie*
12.00 noon		Professor Tony Weiss Molecular and Microbial Biosciences, Faculty of Medicine, University of Sydney *"Synthetic Elastin"*
12.25pm		Professor Véronique Migonney Laboratoire de biomatériaux et polymères de spécialité, Université Paris13 *"Bioactive PET: a Way to Develop a Smart Ligament"*
12.50pm—1.40pm		**Lunch & Industry Exhibition** Attendees are encouraged to have their passports stamped at each exhibit to go in the draw for several prizes
1.45pm	**SESSION 4:**	**Nanotechnology** *Chair: Professor Elspeth McLachlan*
1.50pm		**Plenary** Professor Rutledge Ellis-Behnke Brain and Cognitive Science, MIT,Boston *"Nano Neuro Knitting: peptides, Nanofiber scaffolds for brain and axon regeneration with functional return of vision"*
2.30pm	**SESSION 5:**	**Skeletal Tissue Engineering** *Chair: Associate Professor Rebecca Mason*
2.35pm		Associate Professor Chris Little, Director, Raymond Purves Bone and Joint Research Laboratories, University of Sydney, Royal North Shore Hospital *"Cartilage Tissue Engineering – the need to target degradative pathways"*
3.00pm		Dr Hong Zhou Senior Research Fellow, ANZAC Research Institute *"Osteoblasts and Tissue Engineering"*
3.25pm—3.55pm		**Afternoon Tea**

3.55pm	**SESSION 6:**	**Orthopaedics and Medical Devices** *Chair: Dr Meg Evans*
4.00pm		Associate Professor Greg Roger CEO, Australian Surgical Design and Manufacture *"Dynamic Tissue Engineering: Orthopaedics and beyond"*
4.25pm		Professor David Little Group Leader, Orthopaedic Research and Biotechnology Unit, Westmead Hospital *"Bone Tissue Engineering by Manipulation of the Anabolic and Catabolic Responses"*
4.50pm	**SESSION 7:**	**Future Directions for the SuTEN:** (Report Back on Discussion) *Moderators: Professor Hans Coster, Dr Alex Sharland, Professor Nick Hunt*
5.35—5.45pm		**Prize Presentations and Closing Remarks**

Symposium Organisers gratefully acknowledge the financial support of the University of Sydney's School of Aerospace, Mechanical and Mechatronic Engineering, Faculty of Medicine, the Bosch Institute, Microsearch Foundation of Australia and our industry sponsors:

4 Abstracts

4.1 The Use of Stem Cells to Treat Heart Disease

Peter S Macdonald FRACP, PhD, MD, Conjoint Professor of Medicine, UNSW
Senior Staff Cardiologist, Cardiopulmonary Transplant Unit, St Vincent's Hospital, Sydney

The prevalence of end-stage heart disease in the Australian community is predicted to increase by 70% over the next decade (from 300,000 to 500,000). This fact, coupled with the limited availability of heart transplantation and mechanical heart asistance, provides a compelling incentive to develop alternative therapies for patients with end-stage heart disease. The use of stem cells to replace lost or dysfunctional cardiac myocytes is an exciting approach that has already progressed to Phase II clinical trials. A range of cell types has been injected or transplanted into infarcted or myopathic myocardium in a variety of experimental models. For example, in one rat infarct model, intravenous injection of human-derived bone marrow stem cells within 48 hours of infarction was shown to induce new blood vessel formation and to stimulate proliferation of pre-existing vasculature in and around the myocardial infarct. The neoangiogenesis resulted in decreased apoptosis of hypertrophied myocytes at the edge of myocardial infarcts, long-term salvage of viable myocardium, reduced collagen deposition and sustained improvement in cardiac function. Several Phase I & II human trials based on this concept have been completed. These trials have established that intracoronary administration of autologous bone marrow-derived stem cells in the days following AMI is safe, however mixed results have been obtained in terms of efficacy. Stem cells can be mobilised from the bone marrow by the administration of GCSF. In a phase I trial, we have administered GCSF to 20 patients with intractable angina who were not amenable to conventional coronary revascularisation. Preliminary results suggest that this is safe and that it provides dramatic relief of angina. In another approach, mesenchymal stem cells transplanted as allografts into infarcted segments of myocardium in rats and rabbits with heart failure, have been shown to become incorporated within and to restore contractile function to the infarct scar. A Phase 1 clinical trial of mesenchymal stem cell therapy for acute MI is underway. A third approach to myocardial cell replacement which has progressed from the laboratory to the clinic has been the use of autologous skeletal muscle myoblasts. The experimental and clinical studies conducted so far have provided proof of principle for the use of stem cells to treat a range of cardiac diseases. Many questions remain regarding stem cell therapy. Is one type of stem cell better than another? How many stem cells are needed? How should they be delivered? Do the stem cells act directly or via paracrine release of growth factors and cytokines? The answers to these and many other questions will only be answered by ongoing experimental and clinical studies.

Notes

4.2 Molecular and Cellular Characterisation of Highly Purified Stromal Stem Cells Derived From Human Bone Marrow: Potential Applications in Tissue Engineering

Andrew C.W. Zannettino, PhD.
Myeloma and Mesenchymal Research Laboratory, Bone and Cancer Laboratories, Division of Haematology, Institute of Medical and Veterinary Science, The Hanson Institute and the Department of Medicine, University of Adelaide, Adelaide, Australia.

A large body of evidence demonstrates that stromal tissue derived from adult BM of avian and mammalian species contains clonogenic progenitor cells (CFU-F), some of which are considered multi-potent bone marrow stromal stem cells (BMSSCs) with the capacity to differentiate into a range of mesenchymal cell lineages including adipose tissue, bone, cartilage, tendon and ligament. Despite considerable interest in the biological properties and potential therapeutic applications of these cells, there is no well-defined protocol for the prospective isolation of human CFU-F. Current methodologies for the isolation of primitive BMSSCs are based upon those initially described by Friedenstein and colleagues, which rely upon the adhesion of the stromal progenitor populations to tissue culture plastic and on their subsequent rapid proliferation *in vitro*. Such protocols result in a heterogeneous starting population of adherent BM cells, of which only a minor proportion represent multi-potent BMSSCs. Moreover these protocols select for the progeny of CFU-F, and not for the clonogenic progenitors themselves. Using a combination of magnetic and fluorescence activated cell sorting in combination with an antibody to STRO-1 and vascular cell adhesion molecule-1 (VCAM-1/CD106), we have isolated an almost homogeneous population of BMSSCs from adult human bone marrow. BMSSCs are non-cycling and constitutively express telomerase activity *in vivo* and lack phenotypic characteristics of leukocytes and mature stromal elements. This mesenchymal stem cell population demonstrates extensive proliferation and retains the capacity for differentiation into bone, cartilage and adipose tissue *in vitro*. In addition, clonal analysis demonstrated that individual BMSSC colonies exhibit a differential capacity to form new bone *in vivo*. These data are consistent with the existence of a second population of bone marrow stem cells in addition to those described for the haemopoietic system. As will be discussed, our novel selection protocol provides a means to generate purified populations of BMSSCs for use in a range of different tissue engineering and gene therapy strategies.

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4.3 Cell Therapies: translating research into realities

Professor John E.J. Rasko,
Director, Cell & Molecular Therapies, Sydney Cancer Centre, Royal Prince Alfred Hospital
Head, Gene & Stem Cell Therapy Program, Centenary Institute, University of Sydney
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The Gene & Stem Cell Therapy Program comprises about twenty cell and molecular biologists working on aspects of adult stem cell biology and gene transfer using viral vectors.
Mesenchymal and Hemopoietic Stem Cells
We have established Australia's only non-human primate model of adult stem cell biology. Mesenchymal stem cells (MSCs) are multi-lineage potential cells that can be isolated from the bone marrow with the capacity to differentiate in vitro and in vivo into adipogenic, osteogenic, chondrogenic, and myogenic phenotypes. Mesenchymal stem cells (MSC), as well as showing promise for tissue regeneration and gene therapies, have the potential to facilitate bone marrow transplantation. We have characterized bone-marrow derived MSCs from baboons (Papio hamadrayas) and optimising the viral transduction profile using adeno-asociated virus (AAV) and lentivirus vectors (Chng et al., J. Gene Med., in press). We used a cardiotoxin-induced muscle injury and regeneration NODSCID mouse model to test feasibility and showed that transduced baboon MSCs can be delivered into damaged muscle and engraft in vivo to contribute to ongoing muscle repair. In development are methods to treat cancers using gene-directed enzyme prodrug therapy detected with in vivo biophotonic imaging. In addition, we have studied stem cell mobilisation and autologous peripheral blood stem cell transplantation in the baboons to assess the effect of various cytokines.

Notes

5 Future Directions for the SuTEN

5.1 Question 1: Can the application of nanotechnology into the biomedical field expand the frontier of Tissue Engineering?

Moderator: Professor Hans Coster

5.2 Question 2: What are the as yet unmet clinical needs that can be resolved by Tissue Engineering?

Moderator: Dr Alexander Sharland

5.3 Question 3: How can we build a first-class Sydney University Tissue Engineering program?

Moderator: Professor Nicholas Hunt

6 Abstracts cont

6.1 Synthetic elastin: tissue engineering with an elastic matrix

Professor Tony Weiss
Molecular Biotechnology, MMB G08, University of Sydney

The Weiss laboratory specialises in the tissue engineering of elastin utilising three-dimensional matrices comprising synthetic human elastin. Elastin is the mammalian protein array that is responsible for elasticity in diverse biological locations including elastic arteries, lung, skin and elastic ligaments. Remarkably these diverse locations are served through the expression of a single gene that encodes the monomer precursor of elastin, which is tropoelastin. An essential step in elastin formation requires the rapid association of tropoelastin molecules. Tropoelastin associates at 37°C and is classically cross-linked through strategically placed lysine residues to give the irreversible synthetic elastin. The process of proceeding from tropoelastin to elastin in this monomer to multimer transition resembles plastic polymer assembly and similarly conforms to and takes the shape of the mould within which elastogenic polymerisation occurs.

Using recombinant human tropoelastin, we are making synthetic elastin structures such as sheets, tubes and fibres. Synthetic elastin supports the growth of human cells in tissue engineering applications. For example, elastic sheets support the growth and differentiation of human keratinocytes and the attachment and proliferation of human skin fibroblasts. These effects are not limited to elastin specific cell types as evidenced by the support and growth of ligament derived cells in both 2-D and 3-D culture. Synthetic elastin is best viewed as an elastic, resilient, cell interactive, hydrogel matrix that is biocompatible and conducive to sophisticated tissue engineering applications.

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6.2 Bioactive PET: a Way to Develop a Smart Ligament

Véronique Migonney
Laboratoire de Biomatériaux et Polymères de Spécialité (LBPS/ B2OA/UMR 7052)
Institut Galilée/Université Paris Nord, 93430 Villetaneuse, France

In order to find solutions to the observed “failures” of synthetic anterior cruciate ligaments (ACL), original techniques of weaving and knitting fibers of poly(ethylene terephtalate) (PET) fabrics were first proposed by the Lars Society. As a better understanding of the host-response mechanism has been developed, the scientific community interested in synthetic ligaments has focused on the importance of the quality and control of polymer properties for use in a new generation of synthetic ligaments. The recent results of synthetic ligament implantation are positive with regard to good surgical techniques. However, such surgeries are limited to the small percent of patients that really need a rapid ACL repair (e.g., professional athletes). Therefore in addition to these technical and surgical progresses we proposed to improve the synthetic ligament “integration” by grafting PET with bioactive polymers in order to control the protein adsorption and the fibroblast cell response. This can be achieved by masking the synthetic origin of the prosthesis with the aim of developing a smart well integrated ligament.

In this study, the Lars ACL has been grafted by two bioactive polymers - poly(sodium styrene sulfonate) and poly(methacrylic acid) - in order to demonstrate that the improvement of the fibroblast cell response is related to the modification of the surface chemistry of PET fabrics. *In vitro* assays of fibroblast cell adhesion and proliferation were undertaken on grafted and non grafted fabrics. Results showed that the adhesion strength of cells is sensitively improved whatever the medium chosen for the cell adhesion process - plasma, serum, albumin/collagen/fibronectin mixtures - when the PET is grafted by poly(sodium stryrene sulfonate). Moreover, we demonstrated that collagen is the key-protein at the origin these differences in the cell response. Biomolecular biology assays by PCR were carried out to quantify the gene activation of specific integrins of collagen and showed that the activation of collagen integrins is different on grafted and non grafted fabrics.

In vivo experiments.
Subcutaneous implantations of grafted and non grafted fabrics were performed on rats and the inflammatory response after 2 and 8 weeks was analyzed. Results of systematic assays and histology analyses showed that no inflammatory response induced by the grafted bioactive polymer. These encouraging results led us to go on *in vivo* experiments on sheep. 14 grafted and non grafted ACL were implanted on sheep and the responses at 3 months are analyzed.

In conclusion, we develop a new generation of artificial ligaments for ACL reconstruction by grafting anionic polymers, such as poly(sodium styrene sulfonate) (pNaSS) or poly(methacrylic acid) (pMA), onto PET fabrics surfaces activated by ozonation. These “bioactive” polymers are known to exhibit interesting biological properties such as modulation of protein conformation, cell proliferation, bacteria adhesion and no inflammatory response which provide hope for an improved host response when grafted on PET fabrics. This is the “smart ligament”.

Financial support: Bernard Brulez Lars Society , Arc sur Tille, France

Notes

6.3 Nano Neuro Knitting: Using Nanotechnology to Repair the Brain

Rutledge Ellis-Behnke
Massachusetts Institute of Technology, School of Engineering, Boston, USA

Nanotechnology is often associated with materials fabrication, microelectronics, and microfluidics. Until now, the use of nanotechnology and molecular self assembly in biomedicine to repair injured brain structures has not been explored. In order to achieve axonal regeneration after injury in the central nervous system several formidable barriers must be overcome, such as scar tissue formation after tissue injury; gaps in nervous tissue formed during phagocytosis of dying cells after injury and the failure of many adult neurons to initiate axonal extension. Using the mammalian visual system as a model, we report that a designed self-assembling peptide nanofiber scaffold creates a permissive environment not only for axons to regenerate through the site of an acute injury, but also to knit the brain tissue together. In experiments using a severed optic tract in the hamster, we show that regenerated axons reconnect to target tissues with sufficient density to promote functional return of vision, as evidenced by visually elicited orienting behavior. The peptide nanofiber scaffold not only represents a new nanobiomedical technology for tissue repair and restoration, but also raises the possibility of effective treatment of central nervous system and other tissue or organ trauma.

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6.4 Cartilage Tissue Engineering - the need to target degradative mechanisms

Chris Little, Raymond Purves Bone & Joint Research Labs, Institute of Bone & Joint Research, Kolling Institute, University of Sydney at Royal North Shore Hospital, Level 5 University Clinic – B26 St. Leonards, NSW, Australia, 2065

Progressive degradation and erosion of articular cartilage is a central pathological event in arthritis, with loss of this bearing surface being a major determinant in the need for joint replacement. The degeneration of cartilage in osteoarthritis (OA) occurs due to an imbalance between anabolism and catabolism by the resident cells. It is widely acknowledged that cartilage has a limited ability to repair or regenerate particularly with advancing age, and a significant research effort has therefore been directed towards improving the reparative potential of chondrocytes. To this end the effect of numerous growth factors (e.g. IGF-1, TGF-beta, PDGF, FGF, OP-1) on cartilage metabolism have been extensively studied. The potential of these agents to (re)induce a chondrogenic phenotype in chondrocytes or mesenchymal stem cells for potential repopulation of isolated cartilage defects or OA is the subject of ongoing study. Similarly, the chondrogenic potential of different natural and synthetic scaffolds, ± seeding with different cells with or without mechanical loading has been investigated. Surprisingly however, very little attention has been paid in the “cartilage repair literature” to directly controlling the catabolic pathways that are activated in diseased joints, in order to facilitate cartilage repair.

Recent advances have been made in defining degradative mechanisms in cartilage, in particular the enzymes responsible for breakdown of the two principle structural matrix proteins, aggrecan and type II collagen. Late stage cartilage degeneration is characterized by extensive proteolysis of the type II collagen network. It appears from studies using antibodies to specific proteolytic neoepitopes and selective inhibitors, that much of the collagenolysis in OA cartilage is due to the action of MMPs, particularly MMP-13 and their inhibition has been successfully employed to significantly abrogate cartilage degradation in animal models of arthritis. Catabolism of aggrecan is an important early event in pathological cartilage breakdown that likely precedes and may be prerequisite for collagenolysis. Cleavage within the interglobular domain (IGD) by the aggrecanases at ..EGE-ARG... appears to be primarily responsible for aggrecan loss from articular cartilage in vitro and in vivo particularly in diseased states. Mice with inactivation of ADAMTS-5 (but not those with inactivation of ADAMTS-1 or -4) were found to be resistant to IL-1 stimulated aggrecanolysis in vitro and cartilage degradation following induction of both inflammatory arthritis and OA. Similarly, mice in which the IGD sequence was mutated to render it resistant to ADAMTS-cleavage (but not when made resistant to MMP-cleavage) also have significant inhibition of cartilage breakdown in vitro and in induced models of arthritis. We and others have shown that significant upregulation of MMPs and ADAMTS expression occurs in focal diseased cartilage in OA. Importantly in the context of cartilage repair strategies however, it is evident that expression of many of these enzymes is significantly increased in cartilage throughout the joint, adjacent to and well removed from the focal erosive lesions. Subsequent activation of the latent MMPs and ADAMTS sequestered in cartilage may significantly impact on cartilage surrounding a focal repair as well as the repair tissue itself. In a diseased joint with increased inflammatory cytokines and/or abnormal biomechanical loading, cells implanted to effect repair of a defect will have induction of catabolic pathways, and imunostaining of tissue generated following ACI in humans shows increased MMP-cleavage of collagen and proteolysis of aggrecan by ADAMTS.

While growth factors such as IGF-1 and TGF-beta partially suppress cytokine stimulated aggrecan and collagen degradation in vitro, these results have not translated to abrogation of cartilage breakdown in animal models of arthritis. Indeed in some cases (e.g. TGF-beta) augmented degeneration has been noted. There is little/no information on the effect of more direct anti-catabolic therapies (e.g. proteinase inhibitors) on cartilage repair in vivo. Following induction of an acute inflammatory arthropathy in which almost complete and equivalent loss of aggrecan occurs by day 7 in wild type mice and those with aggrecan IGD resistant to ADAMTS

or MMP cleavage, subsequent cartilage degeneration or repair was monitored. We found significant restoration of cartilage proteoglycan at day 28 only in mice with aggrecan resistant to ADAMTS cleavage. Furthermore, this correlated with significantly less cartilage fibrillation and structural damage in these mice compared with the two other strains. These results suggest that just targeting a catabolic process, in this case aggrecan proteolysis, may enhance “repair” of damaged cartilage. With the development of new agents to directly inhibit proteolytic events in pathological cartilage, future studies should be directed towards not only evaluating their effect on inhibiting degradation in arthritis, but also in potentially enhancing cartilage repair strategies.

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6.5 Osteoblasts and Tissue Engineering

Hong Zhou
Bone Research Program, ANZAC Research Institute

Mesenchymal stem cells have potential to be used in cell-based therapies for bone regeneration or bone tissue engineering. However, regulation of lineage commitment of mesenchymal stem cells is still poorly understood and development of biomaterial scaffolds and/or gene therapies to direct mesenchymal stem cells to commit to osteoblasts and to form bone tissue is still very preliminary.

Using a transgenic mice model, in which the glucocorticoids signalling is abrogated in mature osteoblast, we demonstrate that mature osteoblasts is essential for mesenchymal progenitor cells to differentiate away from a default adipogenic into an osteoblastic lineage. Dominant adipogenesis and reduced osteoblastogenesis were observed in calvarial cell cultures from transgenic mice. This phenotypic shift in mesenchymal progenitor cells commitment was associated with reciprocal regulation of early adipogenic/ osteoblastogenic transcription factors, and with a reduction in Wnt7b and Wnt10b mRNA and β-catenin protein levels in transgenic vs. non-transgenic cultures. Transwell co-culture of transgenic mesenchymal progenitor cells with wild-type osteoblasts restored commitment to the osteoblast lineage, as did treatment of transgenic cultures with exogenous Wnt3a. Our findings suggest a novel cellular mechanism in bone cell biology, in which osteoblasts exert direct control over the lineage commitment of their mesenchymal progenitors through Wnt signaling. This glucocorticoid-dependent forward-control function places the osteoblast into the centre of regulating early osteoblastogenesis.

Our new observations may identify the relevant mechanism for the reciprocal changes in adipocyte and osteoblast numbers seen in aged bone. As numbers of mature osteoblasts decrease with aging, the strength of their ‘paracrine’ signal would decline, so further decreasing osteoblast differentiation and permitting enhanced adipogenesis. These mechanisms would suggest that osteoporosis and bone fracture repair could be limited by insufficient osteoblast-mediated signalling, in addition to inadequate recruitment of mesenchymal precursors to sites of bone formation and fracture. Treatment of non-union of bone fractures using stem cell treatments may be more effective if coupled with gene therapies, or treatments with paracrine factors, to produce commitment to the osteoblast lineage.

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6.6 Dynamic Tissue Engineering: Orthopaedics and Beyond

Associate Professor Greg Roger
Advanced Surgical Design & Manufacture Limited

This paper will explore the evolution of strategies for Orthopaedic repair and reconstruction over the years. In particular the response of researchers to Orthopaedic needs and the response of Orthopaedics Specialists and companies to new advances in research will be compared. Possible future trends will then be discussed.

Notes

6.7 Bone Tissue Engineering by Manipulation of the Anabolic and Catabolic Responses

David G Little MBBS FRACS(Orth) PhD

The production of bone to fill large defects created by trauma or after the removal of bone tumours remains a lofty goal in orthopaedics. Although research advances continue, the reliable formation and maintenance of tissue engineered bone is not yet at hand.
Our group has considerable experience in maximizing bone repair by modulating the anabolic (bone-forming) and catabolic (bone resorbing) responses. Anabolism can be enhanced by ensuring that both osteocompetent progenitor cells and a pro-osteogenic signal are present at the site of repair. We have used bone morphogenic protein (BMP) as a pro-osteogenic signal in several models. More recently, we have examined a possible role for muscle derived cells in osteogenesis. By utilising an anti-osteoclastic agent (i.e. a bisphosphonate), we can control the catabolic response to maximise the yield of bone for repair.
Our future work will take advantage of the use of biomaterials to optimise the delivery of pro-anabolic and anti-catabolic agents in an implant that also provides an optimal scaffold for cellular invasion. Our goal is to produce implants that reliably form and maintain structurally sound bone tissue from a muscle envelope. The cellular contribution to the engineered bone will come from the patient and be produced *in vivo*, not in a laboratory.
We can envisage three main types of practical application for this technology. First will be the production of bone *in situ* in defects that have adequate muscle coverage. Another will be to produce bone in a muscle envelope for later transfer. The final approach will be to produce bone to replace that lost in donor sites used for vascularized bone transfers. This last approach is attractive as it allows for the reliability of vascularised fibular grafting but the correction of donor site morbidity often experienced due to the loss of the fibula.

Notes

7 List of Attendees

Richard	Allen	Bosch Institute
Bob	Bao	Bosch Institute
Alex	Bishop	Bosch Institute
Judith	Black	Faculty of Medicine
Tailoi	Chan-Ling	Bosch Institute
Arthur	Connigrave	Faculty of Medicine
Hans	Coster	Faculty of Engineering
Merylin	Crossley	DVC Research University of Sydney
Fariba	Dehghani	Faculty of Engineering
Ashish	Diwan	St George Hospital
Qihan	Dong	Bosch Institute
Colin	Dunstan	ANZAC Research Institute
Rutledge	Ellis-Behnke	Massachusetts Institute of Technology, USA
Peter	Evans	ANSTO
Meg	Evans	CSIRO
George	Grau	Bosch Institute
Gregory	Hancock	Faculty of Engineering
Mark	Hoffman	UNSW, Materials Science
Nick	Hunt	Bosch Institute
Richmond	Jeremy	Faculty of Medicine & Bosch Institute
Len	Kritharides	Concord Hospital
Qing	Lee	Faculty of Engineering
David	Little	The Children's Hospital at Westmead
Chris	Little	Royal North Shore Hospital
Harry	Lowe	Concord Hospital
Guy	Lyons	Faculty of Medicine
Peter	Macdonald	Cardiopulmonary Transplant Unit, St Vincent's Hospital
Rebecca	Mason	Bosch Institute
Assaad	Masri	Faculty of Engineering
Geoff	McCaughan	Royal Prince Alfred Hospital
David	McKenzie	School of Physics
Elspeth	McLauchlan	Prince of Wales Medical Research Institute
Veronique	Migonney	Institut Galilée/Université Paris Nord
Philip	O'Connell	Faculty of Medicine
John	Rasko	Centenary Institute
Gerg	Roger	Advanced Surgical Design & Manufacture Ltd
Andrew	Ruys	Faculty of Engineering
Alexandra	Sharland	Bosch Institute
Markus	Siebel	ANZAC Research Institute
David	Sonnabend	Royal North Shore Hospital
Jacquie	Stratford	Bosch Institute
Anne	Swan	Bosch Institute
Stephen	Twigg	Bosch Institute
Phill	Waite	University of New South Wales
Michael	Weible	Faculty of Medicine
Tony	Weiss	Faculty of Medicine
John	Whitelock	University of NSW
Anne	Wilson	Bosch Institute
Peter	Youssef	Faculty of Medicine
Andrew	Zannettino	Institute of Medical and Veterinary Science, Adelaide
Hong	Zhou	ANZAC Research Institute
Hala	Zreiqat	Faculty of Engineering

Lab/Group Members

Negin	Amanat	(David Little Group)
Nasim	Annabi	(Hans/Fariba Group)
Ashutosh	Barai	(Chris Little Group)
Trudie	Biner	(Hala Zreiqat Group)
Dan	Burkhardt	(Chris Little Group)
Terry	Chilcott	(Hans/Fariba Group)
Wei	Dr Wei Li	(Qing Lee Group)
Emily	Fuller	(Chris Little Group)
Gary	Gary Lim	(Hans/Fariba Group)
Ben	Gooden	(Chris Little Group)
Jari	Hyvarinen	(Ger Roger Group)
Miriam	Jackson	(Chris Little Group)
Barbara	James	(Hala Zreiqat Group)
Tomas	Kalincik	
John	Kavanagh	(Hans/Fariba Group)
Annette	Kowiak	
Jennifer	Li	(Hala Zreiqat Group)
Zhe	Li	
Renjing	Liu	(David Little Group)
Jane	Liu	(Andrew Ruys Group)
Shaira	Magdon Ismail	(Andrew Ruys Group)
Peter	Maitz	
Michelle	McDonald	(David Little Group)
Susan	McLennan	(Steven Twigg Group)
James	Melrose	(Chris Little Group)
Suzanne	Mithieux	(Tony Weis Group)
Jean	Nightingale	(Andrew Ruys Group)
Tim	O'Mara	(David Little Group)
Yogi	Ramaswamy	(Hala Zreiqat Group)
Mark	Rybchyn	(Rebecca Mason Group)
Batool	Sajad	(Hans/Fariba Group)
Robert	Salomon	(Andrew Ruys Group)
Aaron	Schindeler	(David Little Group)
Alyson	Seymour	(David Little Group)
Brett	Slater	(Ger Roger Group)
Margaret	Smith	(Chris Little Group)
Susan	Smith	(Chris Little Group)
Edwin	Soh	(Andrew Ruys Group)
Soula	Thliveris	(Hala Zreiqat Group)
Sally	Thomson	(Steven Twigg Group)
Crissy	Tomarelli	(Andrew Ruys Group)
Soma	Vignar	(Andrew Ruys Group)
Max	Voigt	(Ger Roger Group)
Annette	Walkowiak	
Chong	Wang	(Qing Lee Group)
Steven	Wise	(Tony Weis Group)
Robert	Wu	(Hala Zreiqat Group)
Ann	Wu	
Mu	Yao	(Andrew Ruys Group)
Nicole	Yu	(David Little Group)
Shiwei	Zhou	(Qing Lee Group)

8 Professor Rutledge Ellis-Behnke

Professor Rutledge Ellis-Behnke is a principal investigator in the Brain and Cognitive Sciences Department at Massachusetts Institute of Technology and the Department of Anatomy at the University of Hong Kong Faculty of Medicine. He is also Visiting Professor at Sichuan University in Chengdu, China and the New York Eye & Ear Infirmary. His primary interest is using nanobiotechnology to reconnect disconnected parts of the central nervous system (CNS).

He received his PhD in neuroscience from MIT, BS from Rutgers University and graduated from Harvard Business School's International Senior Manager's Program (AMP/ISMP). Prior to returning to school to pursue his PhD, Professor Ellis-Behnke held various management positions including Senior Vice President of a public company for testing and consulting services and Co-founder/CEO in 1995 of one of the first internet companies to do online commerce in computer memory.

Professor Ellis-Behnke is a Fellow and founding board member of both the American and International Academies of Nanomedicine, Associate Editor of Neurology for the journal Nanomedicine, Scientific Advisory Board member for the Glaucoma Foundation, member of the China Spinal Cord Injury Network, Society for Neuroscience, American Chemistry Society, Harvard Business School Health Industry Alumni Association and Sigma Xi.

In addition to his work in neuroscience and nanomedicine he introduced the TabletPC to the classroom at MIT and the University of Hong Kong as part of the migration to the paperless classroom to deliver all course material and texts to the students digitally.

9 Professor Veronique Migonney

Professor University Paris 13th, Galilée Institute, France
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PROFESSIONNAL ACTIVITIES and RESPONSABILITIES

- Director of the Laboratory of Biomaterials and Polymers at Galilée Institute, University Paris 13th
- Full professor, Chemistry and Biomaterials, Department of Chemistry, Galilée Institute,
- Assistant director of Galilée Institute
- Manager of the "Biomaterials" Master Degree, University Paris 13th
- Member of the council and of the scientific committee, University Paris 13th, Galilée Institute
- Member of the council and of the scientific symposia committee at French Society for Biomedical Engineering
- Supervision of Students Post-doctoral Fellows (4),PhD students(20), Undergraduate Students(40)

PUBLICATIONS

Papers : 63
Patents : 15
Communications : 125
Invited lectures : 30

10 Summaries of Research Activities

10.1 Associate Professor Tailoi Chan-Ling

Bosch Institute, Discipline of Anatomy

Expertise: Developmental Neurobiology, glial-vascular biology, angiogenesis, retinal biology, Application of hematopoietic stem cells (HSCs) in would repair

Field of interest: Stemming vision loss with stem cells.

Technology in the lab: Triple-marker immunochemistry, retinal whole-mount technique, In vivo and in vitro assays of cell proliferation utilizing BrdU, apoptotic assays using TUNEL and activated caspase 3, cell specific markers for the astrocytic, oligodendrocytic, neuronal, microglia and vascular lineages, hybridoma culture and storage, sensitive detection methods for tracking of green-fluorescent protein (gfp) labeled cells, confocal and deconvolution microscopy, advanced digital imaging.

Animal models: Model of CNS aging using aged rat retina, Kitten model of Retinopathy of Prematurity (ROP).

Summary:
Age-Related Macular Degeneration (ARMD) is the leading cause of blindness in the elderly. With aging of the population, the incidence of vision loss as a result of ARMD will increase significantly. Vision loss & resultant loss of independence results in deterioration of quality of life for the sufferer & their care providers as well as being a financial burden for the Australian community. Our studies are aimed at maximizing repair functions of HSCs in stemming vision loss.

The significance of our work lies in the observation that HSC can be recruited into an area of injury & generate the unique cell populations that are required for repair of the injured tissue. HSC transdifferentiation & repair may also have a role in hereditary retinal degenerations due to primary retinal pigment epithelium (RPE) dysfunction, such as retinitis pigmentosa. The application of HSC as a form of cell-based therapy has great clinical value since it involves an autologous transplant via the re-introduction of a patiens own HSC thus eliminating any immunological reaction. Currently methods exist to increase the levels of HSC released from an individuals bone marrow. These cells can be removed by apheresis, genetically manipulated ex vivo & re infused into the peripheral circulation. These genetically manipulated HSC would home to the injured RPE, repopulate & repair. An alternative scenario would be that a patient would have their endogenous levels of HSC increased by administration of GM-CSF (already clinically used). Our experimental studies on rodents undertaken in collaboration with Professor Maria Grant, University of Florida represent important milestones to translate these studies to patients with ARMD and other sight threatening retinopathies.

10.2 Professor Hans Coster and A/Prof. Fariba Dehghani

Bioengineering and Biophysics Research
School of Chemical and Biomolecular Engineering
The University of Sydney, Sydney, 2006

Research activities
The research activities of the Bioengineering group in the School of Chemical and Biomolecular engineering covers a wide range of fundamental and applied research areas. The research includes:

Biomaterials and Biomimetics

- Development of advanced solvent free techniques for manufacture of biomaterials used as polymer scaffolds for soft and hard tissue engineering applications.
- Engineering of surface nano features for fabricating composite materials to
    - Promote cellular interaction as well as tissue proliferation.
    - Enhance the mechanical and physical properties of implants as well as drug delivery devices.
    - Tailor biomaterial characteristics to enhance longevity and wearing properties of implants and to promote anchoring to the bone and other organs.
- Sterilization and micro-cleaning of biomaterial and bionic constructs using low temperature and solvent free processes.
- Synthesis and engineering of biopolymers and composite materials with interpenetrating polymer network for drug delivery and scaffold fabrication using neoteric solvents such as carbon dioxide.
- Design of hybrid organic-silicon devices for Bio Field Effect Transistors (BIOFETs).
- Fabrication of implantable bionic devices such as Artificial Pancreas.

Nano/Micronisation

- Development of benign dense gas techniques for fabrication of nano and micro particles with narrow particle size distribution for processing various materials including polymers, metals, ceramics, and pharmaceuticals, utilising;
    - Rapid Expansion of Supercritical Solution (RESS)
    - Precipitation from Gas Saturated Solution (PGSS)
    - Gas Anti-Solvent (GAS)
    - Supercritical Assisted Atomisation (SAA), etc.

Biophysics and Nanostructural Engineering

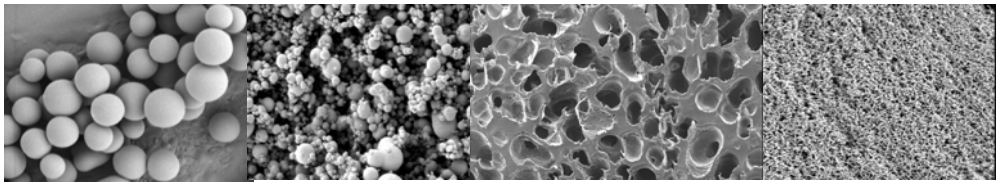
- Fundamental and applied research into molecular films, functionalisation of surfaces, hybrid organic-silicon materials using advanced impedance spectroscopy, neutron and X-ray reflectometry techniques
- Engineering of biosensors based on nanostructural modification of surfaces on inorganic substrate materials such as silicon.
- Development of novel separation technologies to produce electrical cell fusion (hybrid cell types – hybridomas) using dielectrophoretic manipulation of cells and particles.

Drug Delivery

- Processing and formulation of pharmaceutical compounds to enhance drug bioavailability, efficacy and promote patient compliances utilising:
    - solvent free processes with potential intrinsic sterility in conjunction with hydrogels, liposomes
    - stimuli responsive polymer systems for controlled drug delivery and polymer scaffold fabrication.

Biocatalysis and Biosequestering

- Promoting catalysis and biological sequestering using Dielectrophoretic agitation. We will explore the application of dielectrophoresis to obtain improvements in the interactions of catalysts and biological molecules in small liquid volumes. It involves the application of AC electric fields to induce translation and rotation in small particles (dielectrophoresis).
- Synthesis of high value compounds at moderate temperatures using biocatalysis and micro organisms. We aim to fabricate pharmaceutical compounds such as chiral drugs and antibiotics, biomaterials, nano inorganic compounds, polymers such as polysaccharides.



10.3 Dr Qihan Dong

Head, Prostate Cancer Research Program
Department of Endocrinology and Sydney Cancer Centre
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To investigate why metastatic prostate cancer cells can survive and grow in the absence of androgens, we have set up a system to isolate prostate cells in the absence of androgens. We believe that the system can be used eventually for isolation of normal or cancerous stem cells.

Field of interest: The adult stem cell theory has been established for decades but the isolation of stem cells has been achieved only in a few organs. We would like to establish a foundation for (i) facilitating the isolation of prostate cancer stem cells, which will help to develop an effective therapy to eradicate the cancer ultimately; (ii) deciphering the mechanism of androgen-independence that is closed related to the relapse of the cancer after androgen ablation treatment; and (iii) understanding of the role played by prostate epithelial stem cells in the onset of prostate cancer. In addition, this project has wider implications as many of the techniques can eventually become common place when stem cell or indeed cancer stem cell work is undertaken.

Expertise: Prostate physiology & pathology, prostate stem cell theory, and prostate androgen target genes

Technology:

(1) Establishing procedures to culture primarily prostate cells - We have established culture of prostate epithelial and stromal cells (to provide the inductors for initiation of acini formation).

(2) Flow cytometry
We have demonstrated that CD133 positive cells and SP cells are present in prostate epithelial cells, which is consistent with previous reports. We have also found cells with high activity of ALDH in epithelial cells.

(3) Establishing *in vitro* bioassay to determine prostate acini-regenerating activity - The epithelial cells were co-cultured with stromal cells in the presence of dihydrotestosterone (DHT). Two weeks after co-culturing, numerous acini-like structure were formed. Immuno-histochemical staining illustrated that there were differentiation toward luminal phenotype because cells positive for cytokeratin 8 and PSA were present in the formed acini-like structure.

(4) Establishing technical procedure for an *in vivo* bioassay - To demonstrate that the isolated cells are able to regenerate prostate tissue *in vivo*, we have set up a procedure to implant cells to renal capsule of immuno-compromised mice (NOD/SCID mice) where the cells are allowed to start tissue regeneration.

10.4 Professor Georges E. R. Grau

Vascular Immunology Unit, Bosch Institute
Discipline of Pathology
The University of Sydney, Medical Foundation Building (K25), 92-94 Parramatta Road, Camperdown, NSW, 2042
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Research Overview
Understanding better the mechanisms of microvessel pathology in cerebral malaria and other inflammatory diseases. On this basis, proposing new therapeutic approaches.

Overview
Fatal malaria is one of the most destructive and potentially correctable disease burdens in the world, affecting mainly children. A major complication is cerebral malaria (CM). Current therapies are limited by our lack of knowledge of the pathophysiological events. Our studies aim at increasing the understanding of pathogenetic mechanisms of CM, and may therefore lead to new approaches to the prevention or prompt treatment of potentially fatal malaria.

Background:
We recently demonstrated that microparticles (MP), which we found to be present in greatly increased concentrations in the peripheral blood of Malawian children with CM (JAMA 2004), are crucial elements of pathogenesis in experimental murine CM (Am J Pathol 2005). Based on these findings, we now aim, in vitro and in vivo, to demonstrate that by modulating the process of vesiculation (i.e. by reducing the production or release of MPs, or by blocking their toxic effects) we can reduce the pathological processes characteristic of CM. The ultimate aim is to reach a novel intervention for preventing the progression of severe malaria towards a fatal outcome and for hastening recovery from severe malarial disease.
In our current projects, we therefore intend to unravel the mechanisms of MP production and action, to delineate pharmacological ways to interfere with these mechanisms, and to define the pathophysiological consequences of excessive MP production.

Research Approach and Equipment
Mouse models of disease, tissue culture, flow cytometry, gene expression analysis, immunohistochemistry, in vivo imaging technologies, Nuclear Magnetic Resonance and biochemical approaches in our research.

Research Projects
The following projects are available for Honours Students in 2007.

Project Title: Assessing microparticles as effectors in the microvascular lesion of cerebral malaria.
Supervisors: Professor Georges Grau (Pathology) and Dr. Valéry Combes (Pathology)
Project description:
Co-cultures of human brain microvascular endothelial cells with parasites and blood cells, in which purified microparticles will be added, to assess the functional consequences. Analysis of immunological and physiological parameters, such as monolayer permeability and trans-endothelial electrical resistance.

Project Title: Investigating mechanisms of microparticle production by microvascular endothelial cells and defining clinically useful inhibitors**.**
Supervisors: Professor Georges Grau (Pathology) and Dr. Valéry Combes (Pathology)
Project description:
Co-cultures of human brain microvascular endothelial cells with parasites and blood cells, in which various inhibitors of intracellular pathways will be added, to assess their ability to reduce the number of MP released upon stimulation by parasites, or to alter their phenotype. Apart from classical inhibitors, we already have new candidate molecules with obvious effects on MP. Analysis of immunological and haemostatic parameters of the MP produced.

Project Title: Defining the implication of hypoxia in the pathology of cerebral malaria
Supervisors: Professors Georges Grau and Nick Hunt (Pathology)
Project description:
Using our well characterised model of cerebral malaria (Trends Immunol 2003), several immunological and pathological pathways have been unravelled but numerous questions remain unsolved. In particular, the extent to which the obstruction of brain microvessels by parasites and host cells lead to hypoxia in the surrounding parenchyma. Dr. V. Combes and S. Parekh in our lab have shown in preliminary experiments that tissue hallmarks of hypoxia can be detected in mice that are genetically susceptible to CM, but not in strains known to be resistant. Injection of probes in mice and follow up of tissue changes, notably in the brain, by immunohistochemistry. Comparison, using quantitative image analysis, of mice infected with the CM-inducing parasite, PbA or with the non-encephalitogenic parasite, K173. Also, comparison of brains from mouse strains exhibiting various genetic susceptibility towards CM.

Recent interesting publications

Hunt NH & Grau GE. Cytokines: Accelerators and brakes in the pathogenesis of cerebral malaria. Trends Immunol 2003 (24): 491-499.

Coltel N, Combes V, Hunt NH, Grau GE. Cerebral malaria -- a neurovascular pathology with many riddles still to be solved. Curr Neurovasc Res. 2004 (2):91-110.

Schofield L, Grau GE. Immunological processes in malaria pathogenesis. Nat Rev Immunol. 2005 (9):722-35.

Wassmer SC, Cianciolo GJ, Combes V, Grau GE. Inhibition of endothelial activation: a new way to treat cerebral malaria? PLoS Med. 2005 (9):e245.

Penet MF, Viola A, Confort-Gouny S, Le Fur Y, Duhamel G, Kober F, Ibarrola D, Izquierdo M, Coltel N, Gharib B, Grau GE, Cozzone PJ. Imaging experimental cerebral malaria in vivo: significant role of ischemic brain edema. J Neurosci. 2005 (32):7352-8.

Combes V, Coltel N, Alibert M, van Eck M, Raymond C, Juhan-Vague I, Grau GE, Chimini G. ABCA1 gene deletion protects against cerebral malaria: potential pathogenic role of microparticles in neuropathology. Am J Pathol. 2005 (166):295-302.

10.5 Dr Colin Dunstan, Dr Hong Zhou and Prof. Markus Seibel

Bone Biology Program, ANZAC Research Institute, Concord Hospital, Concord, NSW 2139

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Currently in our laboratory we have established in vitro and in vivo models for the evaluation of bone metabolism.
In vitro models established are available to study osteoblasts differentiation and bone formation, and osteoclasts and bone resorption. For osteoblast studies we have cell lines including mouse stromal cell line (ST2) and mouse pre-osteoblast cell line (MC3T3-E1) and primary osteoblast culture systems utilising neonatal mouse calvaria or adult mouse bone marrow as the source of osteoblast precursors. Cultures can be evaluated for gene expression and for the ability to form bone nodules, as a measure of osteoblast function. We have culture systems also established to study osteoclast differentiation utilising either a cell line (RAW 264.7 mouse macrophage derived cells) or primary osteoclast cultures using cells derived from mouse spleen or bone marrow. We do not routinely conduct studies of osteoclast function by pit assay but this could readily be established if required.

For in vivo studies, we have animal models established for studying osteoporosis, glucocorticoid induced bone loss, inflammatory joint disease, and cancer metastasis to bone. The ANZAC Research Institute has mouse and rat facilities with capability to generate and maintain genetically modified mice in an pathogen free environment. We are experienced in modifying bone remodelling either by increasing bone turnover with low calcium diet or by decreasing turnover by inhibition of bone resorption using osteoprotegerin or bisphosphonates.

10.6 Professor Nicholas H Hunt

Molecular Immunopathology Laboratory, School of Medical Sciences, Faculty of Medicine and Bosch Institute, University of Sydney

Address: Medical Foundation Building (K25), University of Sydney
Email: nhunt@med.usyd.edu.au
www.pathology.usyd.edu.au/Pathology2004/Staff/Pathology_Staff.html

Senior Researchers: Drs Helen Ball, Chris Austin, Angeles Sanchez-Perez
PhD students: Jenny Miu, Silvia Weiser, Leia Hee, James McQuillan

Areas of interest: We are not a Tissue Engineering laboratory. Our primary interest is in immunopathology, particularly in relation to infectious diseases. The cerebral and pulmonary complications of severe malaria are a focus of our investigations.
In general, however, we are interested in areas that are relevant to Tissue Engineering, including: mechanisms of inflammation; endothelial cell biology; tissue protective mechanisms. We have mouse models of malaria and bacterial meningitis.

Our technical expertise includes: small animal handling; immunohistochemistry; laser capture microdissection; gene expression analysis (quantitative RT-PCR, microarrays); tissue culture; flow cytometry.

10.7 Associate Professor Len Kritharides

Department of Cardiology C22 - Repatriation General, Concord
The University of Sydney NSW 2006 Australia

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Research Interests
I have clinical and basic research interests. My clinical research includes non-invasive imaging of myocardial function by echocardiography, CT coronary angiography (particularly to study the behaviour of saphenous vein grafts in vivo), the study of neutrophil activation *in vivo* in response to cardiac surgery, and nuclear imaging. My basic research involves macrophage biology, in particular the secretion of proteins and cholesterol from macrophages.

The relevance of my research to tissue engineering is slightly tangential. I am interested in the biological response of vein grafts- and potentially other materials- when placed in the arterial circulation, and in their imaging. The inflammatory response, defined by imaging or systemic markers is likely to be important in testing the tolerability of new materials in vivo.

A particular interest is the inflammatory response to drug-eluting stents in vivo, particularly in view of recent evidence suggesting late stent thrombosis and infiltration may arise from the polymers used to deliver the drug on the stent.

Technology
Assessment of vein graft wall thickness by CT angiography, nuclear scanning for inflammation with gallium or labelled white cells, flow cytometry analysis of neutrophil activation.

Preferences for workshops
Nanotechnology

10.8 Associate Professor Chris Little & Dr Richard Appleyard

Raymond Purves Bone & Joint Research Laboratories (RPBJRL, Director: Chris Little)
Murray Maxwell Biomechanics Laboratory (MMBL, Director Richard Appleyard)

These labs together form the research arm of the University of Sydney Department of Orthopaedics and Traumatic Surgery at the Royal North Shore Hospital. The research focus of these laboratories is the study of diseases of the musculoskeletal system covering 3 main areas:

1. Joint disease in particular osteoarthritis (OA): mechanisms of cartilage breakdown, changes in synovial fluid and bone remodelling.
2. Spinal disorders in particular intervertebral disc degeneration.
3. Degenerative and traumatic disorders of tendon, ligament and meniscus.

These laboratories are unique in bringing together expertise in cell biology, biochemistry, and biomechanics. A particularly strength has been in the development and use of a range of animal models of musculoskeletal disease and in vitro culture models to investigate cellular changes with onset and progression of pathology. In these systems we are investigating the molecular and biochemical changes that occur and their correlation with the biomechanical properties of the tissues. Outlined below are some ongoing projects at our laboratories:

- Ovine meniscectomy model of osteoarthritis: uni or bilateral lateral or medial meniscectomy induces progressive OA. Evaluation of articular cartilage, subchondral bone and synovial fluid changes in disease using biochemistry, cartilage biomechanics, histology, immunohistology, tissue culture, molecular biology (RT-PCR and real time RT-PCR).
- Ovine annular lesion model of experimental disc disease: controlled partial thickness incision of the outer annulus fibrosus leads to progressive degeneration of the disc. Evaluation using biochemistry, biomechanics, histology, immunohistology, cell culture, RT-PCR.
- Sheep shoulder tendon injury model of rotator cuff disease: partial thickness incision of infraspinatus tendon leads to degenerative change in the remaining tendon. Evaluation using biochemistry, biomechanics, histology, immunohistology, RT-PCR and in situ RT-PCR.
- The role of tensile loading and proteoglycan metabolism on tendon degeneration. Unique cyclic tensile loading culture system using wild type and genetically modified mice. Evaluation using biochemistry, biomechanics, histology, immunohistology, and PCR.
- Proteolysis of small leucine rich repeat proteoglycans in cartilage, disc, meniscus and tendon degeneration in humans. Evaluation using biochemistry, protein purification and sequencing and generation of novel antibodies to proteolytic fragments.
- Arthritis models in mice including collagen induced inflammatory arthritis and a novel meniscal destabilization model of OA. Evaluation using clinical scoring, ground reaction force, histology and immunohistology, micro-CT, and micro-array analysis of genome-wide changes in cartilage in wild type and genetically modified mice.
- In vitro models of progressive cartilage degeneration: full thickness ovine articular cartilage and isolated chondrocytes cultured to induce progressive degradation. Ongoing studies include correlating changes in biochemistry with biomechanical properties of the tissue using a novel hand held dynamic indenter and examining the role of a novel serine proteinase in the activation of MMPs. Evaluation using biochemistry, biomechanics, histology and immunohistology, PCR and siRNA gene silencing.

10.9 Associate Professor David G Little, Dr Aaron Schindeler, Ms Michelle McDonald, Dr Negin Amanat & Dr Craig Godfrey

Orthopaedic Research and Biotechnology
The Children's Hospital at Westmead

Our group has a focus on bone repair. We have both preclinical animal model systems and in-vitro cell and tissue culture systems as the mainstay of our experimental systems. Our interventions are aimed at increasing bone tissue anabolism and or decreasing bone catabolism to optimise the amount and strength of bone repair tissue.

Equipment/Facilities

Animal Facilities
Facilities are those of the Westmead Campus including transgenic mouse facility, small animal facility and large animal facility.
Animal models of fracture healing, critical defect healing, distraction osteogenesis, heterotopic ossification.

Radiological Assessment
Stratec XCT Research SA pQCT Scanner
Piximus Small Animal DXA Scanner
Faxitron High Resolution Digital XR Cabinet

Tissue Assessment
Decalcified paraffin and non-decalcified resin embedding
Standard and immunostaining techniques
Bioquant Image Analysis System (Histomorphometry)

Cell and Tissue Culture Systems
Tissue Culture Laboratory - experience in culturing primary calvarial osteoblasts, bone marrow osteoprogenitors/mesenchymal stem cells, osteoblastic and osteosarcoma cell lines, RANKL-induced primary osteoclasts, primary osteoblast-osteoclast co-cultures, primary muscle cells and muscle cell lines
ELISA assays for in vitro and in vivo bone formation and resorption markers
qPCR and western analysis of osteogenic markers

10.10 Dr Harry Lowe

Interventional Cardiologist, Concord Repatriation General Hospital, Concord, NSW, 2139 and Honorary Research Scientist, Centre for Vascular Research , University of New South Wales Randwick, NSW, 2052

Expertise / Expertise

- Clinical Cardiology
- Interventional Cardiology
- Gene Targeting
- Animal Models of Cardiovascular Disease
- Atheroembolism

Technology provided by lab:

Animal models.

- Rat models of cardiovascular disease.
- Restenosis, myocardial ischemia perfusion, myocardial infarction, stroke,
- 5/6 renal failure,
- diabetes (streptozotocin, zucker)
-

Technologies

- Quantitative Assessment / Small Particle Analysis
- Immunohistopathology

10.11 Dr Guy Lyons

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Room W350 Blackburn Bldg D06
University of Sydney

Interests: skin cancer; head and neck cancer; extracellular matrix; matrix metalloproteinases; transcription; cancer progression; cell differentiation; epithelial-mesenchymal transition; engineered human skin

Expertise: proteinases; recombinant DNA; gene transduction

Facilities: PC2 lab; cell culture; general molecular biology

Animal models: tumour xenografts; UV skin carcinogenesis

10.12 Associate Professor Rebecca Mason

Skin & Bone Laboratory (Endocrine Regulation)

Associate Professor R S Mason and her group study the mechanisms which regulate calcium and phosphate concentrations and bone mass using cellular and molecular techniques. These studies are directed towards better understanding the pathogenesis of osteoporosis and other bone and dental disorders and to improved treatment methods. Mechanisms involved in skin protection from sunlight by vitamin D compounds, which are also important for healthy bones, are studied in skin cell and in vivo systems

Expertise in bone- osteoporosis, bone turnover, vitamin D and in skin - vitamin D, photoprotection and pigmentation.

Current studies: calcium and strontium as modulators of bone turnover; vitamin D and analogs in photoprotection. Technology: Human primary osteoblasts from long bones and osteoclasts from peripheral monocytes. Osteoblasts in multilayered mineralising cultures, osteoclast resorption of dentine.

Also cell lines MG63 (human osteosarcoma); RAW - rodent pre-osteoclast line. Human primary skin cells: keratinocytes, melanocytes and fibroblasts. UV irradiation set-up. Vitamin D receptor KO mice.

10.13 Professor John E.J. Rasko

Director, Cell & Molecular Therapies, Sydney Cancer Centre, Royal Prince Alfred Hospital
Head, Gene & Stem Cell Therapy Program, Centenary Institute, University of Sydney
Locked Bag No 6, Newtown NSW 2042, AUSTRALIA
Tel: +61 2 9565 6156 Fax: +61 2 9565 6101

Cell and Molecular Therapy Laboratories, RPA Hospital

Cell therapies derived from patient cells or tissues and manipulated (including genetic modification) outside the body for transplantation builds upon decades of experience in tretaing haematological malignancies. However, cell therapies are an emerging technology controlled by tight government regulation which will require that they be of a Good Manufacturing Practice (GMP) grade for use in clinical trials. The CMTL design comprises four cleanrooms with PC2 containment to support clinical trials involving manipulated human cells. Designed in consultation with the Therapeutic Goods Administration (TGA) the lab environment provides for ex-vivo cell manipulation for human clinical trials involving selection, expansion, gene modification of cells, and/or other cell culture.

10.14 Dr Alexandra Sharland, Dr Alex Bishop, Professor Richard Allen, Associate Professor Steve Chadban

Collaborative Transplantation Research Group

Our group studies many different aspects of transplantation immunobiology. These include the roles of cellular stress pathways, innate and adaptive immunity in ischaemia-reperfusion injury, and both acute and chronic rejection of allografts. The group also has a strong interest in molecular and cellular mechanisms of transplantation tolerance, and in the human anti-pig cellular xenoresponse.

We use both small animal (rat, mouse) and large animal (pig, primate) models of kidney, liver, and heart transplantation, and have access to all the necessary facilities for these and other surgical procedures. We are starting to conduct studies using genetically modified animals in collaboration with Ian Alexander from the Gene Therapy group at the CMRI, Westmead. From early 2007, we will have a PC2-rated microsurgery suite with adjoining laboratory areas, which will allow the gene transfer work to be carried out on the University's main campus. Other techniques which are routinely performed by our group include flow cytometry, immunohisto/immunocytochemistry and immunofluorescence microscopy, RT-PCR, molecular cloning and expression, cell culture, *in vitro* assays of immunity, recombinant protein production and western blotting.

Many of our group are clinicians, or have strong clinical affiliations, making it possible for promising results from preclinical studies to be carried through to investigator-initiated clinical trials.

10.15 Associate Professor Stephen Twigg

University of Sydney, Department of Endocrinology Faculty of Medicine Royal Prince Alfred Hospital

Expertise:
In protease systems, matrix turnover and growth factors

Fields of interest:

- growth factors, MMPs and TIMPs that regulate diabetes complications
- regulation of ECM turnover by growth factors and MMPs and TIMPs.

Technology that your lab provides:

- animal models
- molecular and biochemistry techniques
- tissue culture incl. primary cell cultures

Animal models:

- small and large animal models of diabetes, including wound healing, nephropathy and cardiomyopathy

10.16 Professor Tony Weiss

Molecular Biotechnology, MMB G08, University of Sydney

Synthetic elastin: tissue engineering with an elastic matrix

The Weiss laboratory specialises in the tissue engineering of elastin utilising three-dimensional matrices comprising synthetic human elastin. Elastin is the mammalian protein array that is responsible for elasticity in diverse biological locations including elastic arteries, lung, skin and elastic ligaments. Remarkably these diverse locations are served through the expression of a single gene that encodes the monomer precursor of elastin, which is tropoelastin. An essential step in elastin formation requires the rapid association of tropoelastin molecules. Tropoelastin associates at 37°C and is classically cross-linked through strategically placed lysine residues to give the irreversible synthetic elastin. The process of proceeding from tropoelastin to elastin in this monomer to multimer transition resembles plastic polymer assembly and similarly conforms to and takes the shape of the mould within which elastogenic polymerisation occurs.

Using recombinant human tropoelastin, we are making synthetic elastin structures such as sheets, tubes and fibres. Synthetic elastin supports the growth of human cells in tissue engineering applications. For example, elastic sheets support the growth and differentiation of human keratinocytes and the attachment and proliferation of human skin fibroblasts. These effects are not limited to elastin specific cell types as evidenced by the support and growth of ligament derived cells in both 2-D and 3-D culture. Synthetic elastin is best viewed as an elastic, resilient, cell interactive, hydrogel matrix that is biocompatible and conducive to sophisticated tissue engineering applications.

Expertise: protein biochemistry of connective tissue proteins with emphasis on elastin.

Field of interest: elastin, synthetic elastin and elastic hydrogels for tissue engineering

Technologies in lab: recombinant DNA technology, expression, molecular biology, biochemistry.

10.17 Dr Hala Zreiqat, Dr Andrew Ruys & Dr Qing Lee

a) Biomaterials and Tissue Engineering Research Unit (Head, Hala Zreiqat)
b) Biomaterials Synthesis and Testing Research Group (Group Leader, Andrew Ruys)
c) Computer Aided Biomedical Engineering" (CABE) Unit (Head, Qing Lee)

Units at the Faculty of Engineering, School of AMME, the University of Sydney covering the interfaces between materials science, tissue engineering and cell biology include:

(a) Biomaterials and Tissue Engineering Research Unit:

The principal areas of research interest are in developing biomaterials and examining tissue responses to implanted biomaterials and the protein and cell interactions at interfaces:

1. Evaluation of the skeletal tissue/device interface. The changes in skeletal tissue when in contact with biomaterial (polymer, ceramic and metal). Studies include histological and immunostaining of undecalcified sawn sections of bone with implant. With our expertise we are able to obtain microscopic sections ranging from very large specimens such as sheep femur and human hips to arterial stents in mice.
2. The effect of topographical and chemical modification of biomaterials at the nano- and micro-levels, including divalent cation (magnesium, zinc and strontium), hydroxyapatite and their modulation on bone, cartilage and endothelial cells. Studies involve the use of tissue culture of primary human osteoblasts/osteoclasts, human endothelial cells line, molecular biology (qRT-PCR), *in situ* hybridization, Western blotting, histology and immunohistochemistry (paraffin and plastic embedded sections of hard and soft tissue).
3. The design of scaffolds for skeletal tissue regeneration.

(b) Biomaterials Synthesis and Testing Research Group

Spinal Disk Prosthesis development; Drug Delivery Implants; Hydroxyapatite Bioceramics; Wear Testing of the Prosthetic Knee; Bioactive Coatings (sputtering, electrochemical deposition, electrophoretic deposition, chemical vapour deposition); Biodegradable Load-Bearing Orthopaedic Implants; Ceramic-Metal Functionally Gradient Materials; Laser-Lithography Surface Engineering of Biomaterials for Optimal Cellular Response; Diamond-Like-Carbon Coatings for Wear Resistance and Haemocompatibility; Carbon-Based Biomaterials and Ceramics Matrix Nanocomposites.

Synthesis facilities: include one of the most well-equipped bioceramics synthesis facilities in Australia, numerous furnaces and ceramic processing facilities, and extensive facilities for biopolymer synthesis and thin-film coating deposition.

Biomechanical testing facilities: include 8 Instron tensometers of various capabilities, a large and well equipped Rheology laboratory, and a voice-coil dynamic fatigue tester (the ELF3400), one of only two voice-coil dynamic fatigue testers in Australia, with capabilities up to 5kN and 50nM, sensitive load cells down to 20N, and a simulated body fluid testing chamber attachment.

(c) Computer Aided Biomedical Engineering" (CABE) Unit:

A high-performance in-house cluster system consisting of 24 dual-core CUP is developing, which allows very complex 3D scaffold multiscale modelling and topological optimisation. The construction of computer-aided rapid prototyping system is under reviewing.

Many thanks to our industry sponsors: