

HONOURS PROJECTS

2009

DISCIPLINE OF PATHOLOGY

RESEARCH CLUSTERS

- **Neurological Diseases**
- **Bone and Joint – IMVS**
- **Bone and Joint – Dr David Haynes' Lab**
- **Spinal Research - IMVS**
- **Forensic Sciences**
- **Extracellular Matrix Research**

Bone and Joint (IMVS) – Professor Nick Fazzalari, Dr Ian Parkinson, Dr Julia Kuliwaba, Dr Blair Hopwood, Dr Peter Smith

MOLECULAR MECHANISMS OF MICRODAMAGE-STIMULATED BONE REMODELLING

Bone remodelling is achieved by the initial activity of osteoclasts to resorb bone followed by the formation of new bone by osteoblasts. Bone resorption is stimulated in two general ways. First, bone is resorbed in response to a systemic requirement for calcium. Second, and less well understood, is the process whereby osteoclasts are stimulated to remove bone that has sustained microdamage (microscopic cracks in the bone matrix). This project seeks to identify, in human bone samples, the molecules that may link microdamage to the initiation of bone resorption. The project will involve analysis of normal and pathological (Osteoporotic and Osteoarthritic) human bone samples. The identification of regulatory mechanisms that control bone remodelling is essential to design better treatments for musculoskeletal diseases that are characterised by impaired bone quality and/or structure. The project will involve handling human bone tissue samples, fluorochrome labelling of microdamage, bone histology, quantitative bone histomorphometry, and immunohistochemistry.

Supervisors: Dr Julia Kuliwaba, Professor Nick Fazzalari.

THE ROLE OF THE OSTEOCYTE CELL NETWORK IN BONE REMODELLING

Tissue level studies of human bone have not been fully exploited to elucidate the physiology of bone remodelling. In particular, the functional role of the osteocyte cell network in bone is poorly understood and there is increasing evidence that osteocytes play a significant role in directing bone remodelling to a specific location in the bone mineral. The osteocytes may detect bone strain magnitudes to effect bone remodelling. Also, secretion of soluble factors from damaged osteocytes (induced by microcracks) may locally activate the initial osteoclastic resorptive phase of targeted remodelling. This study will explore the inter-relationship between osteocyte cell number and distribution with bone remodelling in human bone tissue. In addition, osteocyte cell expression of proteins postulated to be involved in signalling for osteoclastic bone resorption will be investigated. The project will involve analysis of normal and pathological (Osteoporotic and Osteoarthritic) human bone samples. Specifically, the project will involve handling human bone tissue samples, bone histology, quantitative bone histomorphometry, immunohistochemistry, and confocal microscopy.

Supervisors: Dr Julia Kuliwaba, Professor Nick Fazzalari.

3D IMAGING OF MICROCRACK PROPAGATION IN HUMAN CORTICAL BONE

If the skeleton does not adequately repair bone microcracks, they accumulate, resulting in decreased bone strength, which may lead to an increased risk of fracture (presented clinically as stress fractures and Osteoporotic fragility fractures). The process by which microcracks in bone initiate, propagate and ultimately coalesce leading to failure remains poorly understood. This project will investigate the microscopic tissue changes associated with microcracks during crack propagation in human cortical bone and the influence of bone microstructure (such as the vasculature and osteocyte cell network) on this process. The project will involve handling human bone tissue samples, fluorochrome labelling of microcracks, confocal microscopy, micro-CT imaging, and 3D data computer analysis.

Supervisors: Dr Julia Kuliwaba, Professor Nick Fazzalari.

PREDICTING BONE STRENGTH – CONTRIBUTION OF BONE ARCHITECTURE

The mass-based paradigm for bone strength does not adequately enable the identification of individuals at risk of bone fracture. Other factors must influence the quality of bone. Bone architecture is one such factor, not assessable using current clinical tools. Micro-CT imaging allows assessment of bone architecture *ex vivo* and with further technological advances similar assessment will be possible *in vivo*, in the near future. Understanding the correlation between bone strength and bone architecture will be essential to the clinical application of this architectural information. This project seeks to investigate bone architecture and bone strength. The strength of whole vertebral bodies will be assessed using a mechanical testing device and this will be correlated with data acquired by micro-

CT imaging, before and after testing. The project will involve handling human tissue samples, operation of micro-CT imaging and mechanical testing equipment and histological tissue preparations for analysis.

Supervisors: Dr Ian Parkinson, Professor Nick Fazzalari, Dr Peter Smith.

BONE ARCHITECTURE – CORRELATION BETWEEN MINERALISATION AND STRENGTH

The mass-based paradigm for bone strength does not adequately enable the identification of individuals at risk of bone fracture. Other factors such as bone mineralisation must influence the quality of bone. We use a back-scattered electron imaging (BEI) technique to quantify the degree of mineralisation in *ex vivo* bone samples. Extending this technique to micro-CT imaging may allow similar analysis to be carried out *in vivo* in the near future. Understanding the correlation between bone strength and bone mineralisation will be essential to the clinical application of fracture risk. This project seeks to investigate bone mineralisation and bone strength by mechanically testing bone samples and correlating BEI and micro-CT data. The project will involve handling human tissue samples, operation of scanning EM, micro-CT imaging and mechanical testing equipment.

Supervisors: Dr Peter Smith, Dr Ian Parkinson, Professor Nick Fazzalari.

Bone and Joint - Dr David Haynes 8303 3180

Several projects can be offered in the Medical School laboratories of Dr David Haynes in the Discipline of Pathology. These projects involve the study of human tissues using immunohistology and other similar techniques. The present focus is on bone and joint pathologies and developing new treatments to prevent bone loss. In addition, cell culture and animal studies are used to demonstrate functional aspects of the investigation. Techniques used include, complex cell culture, micro CT, live animal CT, intracellular signalling, fluorescent microscopy, electron microscopy, and a range of new molecular biological techniques.

PATHOGENIC BONE LOSS is seen in many diseases that are becoming more common in our aging population. Understanding how bone loss occurs in periodontal disease, rheumatoid arthritis and peri-implant loosening are the focus of our research.

APOPTOSIS is an important process regulating many diseases. Studies investigate how apoptosis may regulate pathogenic bone loss and inflammatory diseases.

BONE CELL METABOLISM is regulated by the interaction of osteoclasts and osteoblasts. Projects study how these cells are regulated in health and disease.

ANTI-BONE LOSS DRUGS are being developed, based on molecular mimics, by our colleagues at the Drug Design and Development Centre Queensland. Projects assess these novel drugs in inflammation and bone diseases.

BIOMATERIALS are a fast growing field of medical research. Projects assess how bone cells interact with novel orthopaedic biomaterials.

The Adelaide Centre for Spinal Research – Assoc. Prof. Rob Moore 82223645

Back pain – almost everybody’s problem

About 80% of persons have at least one episode of significant back pain during their lifetime. No race, country, occupation, gender, age-group or lifestyle offers protection from back pain. Adolescents are not spared and by the age of 15 years have a lifetime prevalence approaching that of adults. This means that patients with back pain are frequently encountered in medical practice and when the cost of investigations, surgery and other forms of treatment is added to the cost of lost productivity due to absence from work it is readily seen that the economic consequences of back pain are enormous.

Why is back pain so common? There are many possible causes but pinpointing the specific cause of pain in a patient is usually difficult and frequently impossible. The spine is structurally, biochemically and pathologically extremely complex and research in the spinal field is a continuous challenge that involves most of the medical specialties, allied health sciences, physics, biochemistry, engineering and, increasingly, molecular and stem cell biology.

The Adelaide Centre for Spinal Research (ACSR) is a specialized research centre within the Hanson Institute and is closely interfaced with the Spinal Service in the RAH. The ACSR enjoys an international reputation for the contribution it has made to the understanding of low back pain, especially in its innovative development of models in the sheep which have been successfully applied to answering specific questions. It continues to produce a regular flow of high-quality publications and has received numerous prizes and awards for the quality of its research. There is strong competition for the Clinical Research Fellowships which have allowed an astonishing number of surgeons from USA, UK, Japan, and many other countries to spend up to two years, and sometimes longer, in the ACSR to gain additional surgical skills and to participate in clinical and laboratory research.

The ACSR offers a diverse range of topics suitable for students wishing to undertake Honours degrees and the choice will be widened by the end of 2007 when the Professor appointed to fill a newly created Chair of Spinal Surgery and an additional Senior Lecturer in the same field will arrive to take up their appointments and initiate their research. Before the end of 2007 the ACSR will take delivery of a purpose built biomechanics testing machine specifically designed to investigate the lumbar spine and will appoint a bioengineer to its team of specialist research supervisors.

Set out below are brief outlines of some of the projects that would be suitable for Honours degree projects. Students considering doing Honours in the ACSR should meet with [Associate Professor Robert Moore](#) before finalizing their choice of topic.

Degenerative Spondylolisthesis

This frequently painful condition, commonly occurring in women but largely unrecognized, is characterized by the forward slippage of the body of L4 vertebra relative to L5. Research is directed at finding out why this occurs, the part played by the orientation of the facet joints, what causes the disc and spinal ligaments to fail to prevent it, and devising surgical or other strategies to prevent, retard or rectify the condition.

Rim Lesions

The ACSR has successfully developed a sheep model of the degenerative changes which follow the creation of a small tear in the outer (rim) part of the annulus of the intervertebral disc. There is lack of agreement as to the potential for healing of these tears. The project will examine pathological evidence of healing occurring spontaneously using archive material. The effect of rim lesions on hoop stress in the disc will be explored biomechanically and attempts will be made to promote healing by various forms of stabilization.

Organization of the Annulus of the Intervertebral Disc

The disc is a complex structure that is critically important in maintaining spinal strength and mobility. Its failure always results in consequential changes in other parts of the spinal motion segment. The disc is made up of an outer annulus which comprises onion-like layers of helicoidal collagen fibres alternately running at an angle of 60° to each other. The mechanism whereby the layers can slide across each other during spinal movements while maintaining a strong bond between the layers is not well understood. The project will review the structure of the annulus, look at the bonding arrangements, and will explore the biomechanical behaviour of the normal annulus and the annulus in which separation of the lamellae is artificially induced.

Are intervertebral discs capable of spontaneous regeneration?

Intervertebral disc degeneration is being targeted as a suitable condition for trials of regenerative medicine, but the introduction of a new population of cells or molecules would not be warranted if discs showed no innate potential to recover from a degenerated state. It would be useful therefore to have an understanding of the natural capacity of the disc to regenerate. Lumbar discs of sheep, aged from 16 weeks to three years, will be injected with a proteolytic enzyme to induce moderate disc degeneration. The progression of degeneration and possible spontaneous regeneration of these discs, as well as non-injected control discs will be monitored by X-ray and MRI prospectively after injection. It is hypothesized that the discs of the younger animals will have a greater regenerative capacity than those of older animals. These tissues will be subject to histological analysis *post-mortem*. This study will provide important data on what age group or which discs would best be targeted for therapeutic interventions. Additional studies in which treated discs are analyzed further with biochemistry and functional biomechanics are planned before therapeutic interventions are investigated.

Osteogenic properties of cells from osteoporotic bone

Osteoporosis is a generalised condition of decreased bone mass and quality that is characterised by an increased likelihood of fractures, especially in the spine. To address this growing health problem in an aging population, the ACSR has developed a sheep model to explore the underlying mechanisms of this disease. There is however little information about ovine bone physiology and the molecular pathways associated with bone loss. The aim of this study is to characterize osteoblasts from these osteoporotic sheep. Trabecular bone samples from osteoporotic and normal sheep will be cultured *in vitro* to promote outgrowth of osteoblast-like cells. The osteogenic potential of first passage cells will be assessed using established assays for cell proliferation, maturation, mineralization, matrix quality and expression of genes coding for specific bone proteins. This study will improve our understanding of the pathological mechanisms of osteoporosis and will assist the development of new treatments for this condition.

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RISK FACTORS FOR PULMONARY THROMBOEMBOLISM IDENTIFIED AT FORENSIC AUTOPSY AND SIGNIFICANCE OF BODY MASS INDEX

A wide range of lethal diseases is responsible for unexpected death. Of the approximately 1400 coronial autopsies performed in South Australia every year, cases of unexpected death due to pulmonary thromboembolism are relatively common. Although there have been well-established predisposing risk factors identified in clinical studies, their significance in contemporary post-mortem populations has not been analysed in detail. It is known that age, trauma, surgery, immobility, sepsis, pregnancy, malignancy and disorders of coagulation all increase the risk of deep venous thrombosis (DVT) with subsequent pulmonary thromboembolism (PTE). Obesity has also been proven to be an independent risk factor in both males and females. This project would entail retrospective analysis of cases of PTE for risk factors

comparing possible changes over time. In addition, the role of obesity will be assessed using BMI measurements for cases with PTE and without (age and sex-matched controls). This will also provide data on the possible effects of the significant increase in BMI in the local coronial autopsy population over recent years. This work will necessitate some time being spent at Forensic Science SA.

ANALYSIS OF THE NATURE AND EXTENT OF INJURY PATTERNS IN SOUTH AUSTRALIAN HOMICIDE VICTIMS FROM 1980 TO 2005.

Homicides in South Australia most often involve blunt trauma, strangulation, stabbing and shooting. On occasion there may be more than one lethal mechanism and considerable numbers of non-lethal injuries. All homicide cases in South Australia undergo formal coronial autopsies at Forensic Science SA. Detailed external and internal evaluations are performed with ancillary testing, which includes toxicological testing of blood and tissues and sampling for DNA analyses. All injuries are photographed, sketched and described in considerable detail. Although epidemiological monitoring of homicides is undertaken on a state-by-state basis to assist in the compilation of national data, less information is available on injury patterns. Retrospective analysis of injury patterns to include the type of fatal lesions present will be conducted over a 25-year period to assess whether changes in causes of death, and in the degree of violence, have occurred over time in the local South Australian population. Analyses will include an evaluation of the number and severity of injuries, the use of multiple weapons and the relationship to drug usage. This work will necessitate some time being spent at Forensic Science SA.

EXAMINATION OF THE EFFECTS OF HEAT ON ARTERIAL DIAMETER

Drivers of vehicles and pilots of aircraft may be involved in lethal crashes in which there is subsequent incineration of the body. While there is often loss of limbs and superficial tissues, deeper organ structures such as the heart are often preserved. An important medicolegal question is whether an underlying condition, such as coronary artery atherosclerosis, precipitated the accident. Assessment of microscopic tissue structure is often complicated by coagulative necrosis from heating, however the effect of heat on blood vessel diameter has not been quantified. Evaluation of the possible shrinkage of elastic blood vessels following heat exposure will be undertaken to evaluate whether vessel diameters observed at autopsy are an accurate indicator of vessel diameters prior to death.

Extracellular Matrix Research Group – Dr Mark Gibson 8303 5337, rm N330, Medical School North

Extracellular matrix (ECM) provides strength, support, resilience and protection to tissues and organs within the human body. In addition ECM has a profound influence on tissue development, morphogenesis and structure; and on processes such as tissue remodelling and repair, and cellular differentiation and migration. All cells within the body interact with, and are influenced by, adjacent matrix. The ECM consists of a complex mixture of protein molecules the composition of which is tailored to suit the function of individual tissues. ECM is a major component of tissues such as skin, lungs, blood vessels, bone and cartilage but is also important in organs such as kidney and liver.

Dr Gibson's research has focussed on two important structural ECM elements widespread in the body, fibrillin microfibrils of elastic fibres and collagen VI microfibrils. He has identified, cloned and characterised many of the molecules associated with these structures. The elucidation of the structure and function of these molecular complexes is important for understanding a wide range of human cardiovascular, lung and musculoskeletal diseases such as emphysema, aortic aneurysms, atherosclerosis and muscular dystrophies. In addition, recent research has highlighted the roles of these microfibrils in controlling and implementing the biological impact of TGF β and related growth factors, (important

modulators of matrix synthesis, structure and function) during tissue development, remodelling and homeostasis; and in major diseases. These latent growth factors are stored in the matrix and activated during tissue remodelling and repair. Inappropriate TGF β activation causes major fibrotic diseases such as pulmonary fibrosis, glomerulonephritis, liver cirrhosis, and keloid formation as well as a number of common often fatal genetic disorders such as Marfan syndrome. The mechanisms involved are poorly understood. Recent evidence indicates that a family of proteins called latent TGF β binding proteins (LTBPs) control the targeting and activation of latent growth factors stored on the fibrillin microfibrils. Our laboratory is currently developing a number of transgenic mouse, molecular interaction and cell culture models to study the full function of LTBPs in tissue development and homeostasis, and in growth factor activation.

We have a range of Honours project options tailored to suit each student's background, interests and aspirations. Areas of research which could involve specific honours projects include:-

- a) Immunohistochemical, electron microscopic and morphological analysis of transgenic and knock out mouse models.
- b) Molecular binding analyses involving engineering and purification of recombinant proteins, molecular binding assays and kinetics, binding site identification and molecular modelling.
- c) Cell culture models determining the effect of blocking or amplifying target genes on matrix assembly and growth factor activation and expression. This approach involves transfecting specific cell lines with inducible expression or antisense constructs, immunohistochemical and SDS-PAGE analysis of elastic fibre components, and growth factor activity assays.

Each project will teach the student a range of techniques in molecular and cell biology and provide a strong basis for a continuing research career in biomedical sciences.

<p>Neurological Diseases – Professor Robert Vink, Dr Corinna Van Den Heuvel, Professor Mounir Ghabriel</p>

The Centre for Neurological Diseases is a multidisciplinary research facility made up of staff from various areas in the University of Adelaide, the Royal Adelaide Hospital, the Institute of Medical and Veterinary Science and the Hanson Institute who share a research interest that revolves around traumatic brain and spinal cord injury, stroke, peripheral nerve injury, and degeneration, particularly Parkinson's and Alzheimer's Diseases. The Centre is located in modern, spacious laboratories on the 2nd floor of the Hanson North Building, and is equipped with an extensive range of equipment for tissue processing, histology, immunohistochemistry, digital imaging, western blotting, tissue culture, and in vivo animal experimentation.