

## Abstract 1

### The role of SQSTM1/p62 in RANKL-activated signaling pathways and osteoclast formation

Kirk HM Yip, Ming H. Zheng, and Jiake Xu

*Molecular Orthopaedic Laboratory, School of Surgery and Pathology, University of Western Australia, Nedlands, WA 6009, Australia*

Paget's disease of bone (PDB) is a chronic lytic bone disorder that exhibits an increased number of nuclei and enhanced activities per cell compared with normal osteoclasts, causing bone pain, deformities, and fractures in patients. Recently, PDB has been reported to be associated with several C-terminal mutations of sequestosome 1 (SQSTM1), also known as p62. However, the role of p62 in osteoclast formation and in RANKL signaling pathway has not been well characterized. In this study, we showed that overexpression of p62 C-terminal deletion with the ubiquitin associated domain (UBA) in preosteoclastic RAW<sub>264.7</sub> cells resulted in higher expression of TRAF6 protein, an adaptor molecule of the RANKL/RANK-activated signaling pathway, and upon RANKL stimulation, exhibited enhanced multinucleation and osteoclast forming *in-vitro*. Interestingly, over expression of the p62 mutant protein resulted in retarded RANKL-induced NF- $\kappa$ B activation, but enhanced NF-AT protein abundance and activity, as well as prolonged phosphorylation of ERK. Using confocal microscopy, we observed that EYFP-p62 was localized to aggregate structures in the cytoplasm and partially co-localized with late-lysosome. In contrast, EYFP-p62 UBA domain deletion mutant was diffusely expressed thorough out the cytoplasm and did not co-localized with late-lysosome. Taken together, our findings indicate that deletion of UBA domain of p62 results in enhanced osteoclastogenesis via the modulation of RANKL-activated signaling pathways in osteoclastic precursor cells.

## **Abstract 2**

### **Identification and Functional Characterization of an Osteoclast-Derived Osteoblastic Factor (ODOF)**

**Ton**

### **Abstract 3**

#### **Protein Kinase C delta (PKC- $\delta$ ) mediates Receptor Activator of NF- $\kappa$ B Ligand (RANKL)-induced signaling pathways required for osteoclastogenesis**

**Cathy Wang, Ming-Hao Zheng and Jiake Xu**

*<sup>1</sup>Molecular Orthopaedic Laboratory, School of Surgery and Pathology, University of Western Australia, Nedlands, WA 6009, Australia.*

Increased formation and activation of osteoclasts underlie many bone lytic disorders such as osteoporosis and Paget's disease. The Protein Kinase C pathway has been suggested to be an important regulator of osteoclastic bone formation, however, the role and subtypes of PKC in RANKL-induced osteoclastogenesis is unknown. In this study, we have screened several PKC inhibitors and found that PKC delta mediates RANKL-induced signaling and osteoclast formation. PKC delta inhibitor, Rottlerin dose dependently inhibited RANKL-induced osteoclast formation, as well as the expression of osteoclast specific genes, calcitonin receptor and cathepsin K. Using nuclear translocation and reporter gene assays, we showed that Rottlerin, inhibited RANKL-induced NF- $\kappa$ B activations in a dose dependent manner. Interestingly, PKC delta activator, Bryostatin 1, enhanced RANKL-induced osteoclastogenesis in a dose dependent manner and partially compensated the inhibitory effect of Rottlerin on osteoclastogenesis. This was achieved by increasing RANKL-induced activation of NF- $\kappa$ B. Furthermore, we showed that overexpression of PKC delta wild type enhanced the NF- $\kappa$ B activation mediated by RANK. In contrast, overexpression of PKC delta dominant negative form reduced the NF- $\kappa$ B activation. In short, our data indicate that PKC delta plays a role in RANKL-induced osteoclast formation. Selective modulation of RANKL signaling pathways by PKC delta may have important therapeutic implications for the treatment of bone diseases associated with enhanced bone resorption.

## Abstract 4

### RECENT DEVELOPMENTS IN LASER SCANNING CONFOCAL ARTHROSCOPY

C W Jones<sup>1</sup>, D Smolinski<sup>1</sup>, D Joy<sup>1</sup>, W McLean<sup>3</sup>, T B Kirk<sup>1</sup>, D Wood<sup>2</sup> and M H Zheng<sup>2</sup>

<sup>1</sup>*School of Mechanical Engineering, University of Western Australia, Perth, Australia*

<sup>2</sup>*Department of Orthopaedic Surgery, University of Western Australia, Perth, Australia*

<sup>3</sup>*Optiscan, Pty. Ltd. Melbourne, Victoria, Australia*

The School of Mechanical Engineering, UWA has developed a novel laser scanning confocal arthroscope (LSCA) that utilises unique fibre optic technology and has numerous potential applications in the fields of orthopaedic research and orthopaedic surgery. The need for mechanical biopsy is obviated by the LSCA's ability to generate 2D and 3D views of tissue microstructure. We have recently reported on new developments and experimental applications of the LSCA. Two major studies have recently been undertaken, the first, an *in vitro* Ovine study, utilised the LSCA for the characterisation of healthy muscle, tendon, menisci and articular cartilage. More recently the LSCA has been used in an *in vivo* longitudinal study of the progression of osteoarthritis (OA) in Ovine stifle joints.

In this first study we report on the *in situ* characterization of chondral and connective tissues in their unaltered physiological environment. The results of our studies demonstrate the common histological features of these tissues and demonstrate the efficacy of LSCA as a rapid method for non-destructive assessment. More recently our work has focused on using the LSCA for the detection of early osteoarthritic changes within ovine stifle joints. The model used was a bilateral lateral meniscectomy a procedure that is known to induce osteoarthritic model in the femoro-tibial joint. Preliminary results show noticeable changes from healthy cartilage to 3-week post-operative cartilage. Chondrocyte cluster formations as expected are easily visible along with fibrillations of varying degrees.

This study is unique in that it is the first time that non-destructive confocal arthroscopy has been employed *in vivo* for longitudinal study in an large animal model. The continuing development of laser scanning confocal arthroscope (LSCA) demonstrates its potential as a tool for assessing orthopaedic tissues *in vivo* thereby obviating the need for destructive tissue biopsy. The instrument has definite application in detecting early osteoarthritic changes and possibly for assessment of the efficacy of cartilage repair techniques such as Matrix Induced Autologous Implantation (MACI).

## **Abstract 5**

### **OSTEOPOROTIC SHEEP AND BALLOON KYPHOPLASTY**

**Beard H<sup>1</sup>, Schultz CG<sup>2</sup>, Moore RJ<sup>1,3</sup>**

*The Adelaide Centre for Spinal Research, Institute of Medical and Veterinary Science<sup>1</sup>;  
Department of Nuclear Medicine and Bone Densitometry, Royal Adelaide Hospital<sup>2</sup>;  
Department of Pathology, University of Adelaide<sup>3</sup>, Adelaide, South Australia.*

Balloon kyphoplasty is a novel procedure that aims to restore vertebral body height by injecting cement into a formed cavity. The aim of this study was to assess the potential of an osteoporotic sheep model for kyphoplasty.

Ten lactating ewes (mean age 8 years) were ovariectomised, injected weekly with dexamethasone (Dexafort, Intervet, Australia) and fed low calcium diet for up to six months and compared with non-ovariectomised and non-treated controls. Dual-energy X-ray absorptiometry (DXA) was used to assess bone mineral density (BMD) in the lumbar spine. Sheep were euthanased at intervals and the entire lumbar spine processed for histology, quantitative histomorphometry and micro-CT (computed tomography). Inflation of a balloon tamp (Kyphon Inc. USA) was attempted ex vivo in a vertebral body.

After 6 months BMD decreased significantly ( $p < 0.05$ ) by  $29.5 \pm 6.5\%$ , trabecular bone volume (L2- L4) decreased from  $29.12 \pm 1.56\%$  to  $20.18 \pm 0.51\%$  and trabecular thickness decreased from  $0.15 \pm 0.01\text{mm}$  to  $0.09 \pm 0.00\text{mm}$ . A balloon tamp was inflated successfully in a vertebral body.

Using DXA and cancellous bone histomorphometry this study has demonstrated significant bone loss in the sheep lumbar spine up to 6 months after ovariectomy and continuous steroid treatment. The successful inflation of a balloon tamp augers well for kyphoplasty studies in this model.

## **Abstract 6**

### **STRUT ANALYSIS OF THE TRABECULAE IN THE TALUS**

**Butler, AM and Walsh, WR**

*Orthopaedic Research Laboratory, Prince of Wales Hospital, University of New South Wales, Sydney, Australia*

Purpose: Studying trabecular bone and its adaptation to alterations in its physiologic and mechanical environment remains an important area of research. Quantifying the architecture of trabecular bone is important for diseases such as osteoporosis. The talus is unique in the human body as it has no muscular attachments and experiences all ground reaction forces. This study uses strut analysis to compare talar trabecular architecture in 3-D. Methods: Three human talii were sectioned (3mm thicknesses) in the anatomical planes. High resolution x-rays revealed the trabecular structure. The x-rays were digitized and the trabecular architecture analyzed using strut analysis software. Results: In the coronal plane the superior regions contained the strongest architecture. There was a significant difference between the superior and inferior regions revealing that the architecture gradually gets weaker. In the sagittal plane, the talar dome, neck and central region of the head possessed the strongest architecture. There was a significant difference between the superior and inferior sagittal zones. In the transverse plane the head contained the strongest architecture while the lateral region was also strong. Conclusions: The strut analysis showed that the architecture gets weaker the further away from the superior articulation. Although bone strength at the ankle joint has been reported previously, as far as the authors are aware, the 3-D approach taken in this study has not been described for the talus. Talus trabecular architecture is complex but the strut analysis shows it to be optimally structured for its load-bearing function as well as highlighting areas of strength.

## Abstract 7

### MECHANICAL PROPERTIES OF THE MIDSHAFT AND FEMORAL NECK FOLLOWING ESTROGEN DEFICIENCY

**Chapman J; Rajaratnam R; Tan S; Payten C; Neil M; Walsh, WR**

*Orthopaedic Research Laboratory, Prince of Wales Hospital, University of New South Wales, Sydney, Australia*

**Purpose:** The increased burden of osteoporotic related fractures to the healthcare system worldwide is without question. Bagi [1] have reported changes in the femoral neck up to 12 weeks following removal of the ovaries in the rodent model. The current study examined the medium and long-term effects of estrogen deficiency in the ovariectomised rodent model. **Methods:** Forty female Sprague Dawley rats were ovariectomised or sham operated at 10 weeks. Equal group were killed at 6 and 12 months following surgery (n=20). Anthropometric measurements, bone mineral density and mechanical properties of the mid shaft femurs and femoral necks were performed. Data was analyzed with a 2-way analysis of variance. **Results:** Significant endosteal expansion at 12 months in the OVX group compared to shams ( $p<0.05$ ) was found. Moment of inertia increased with time but did not differ. Bone mineral density decreased with time and was significantly different compared to Shams ( $p<0.05$ ) but did not differ at 6 and 12 months. Stiffness and ultimate load decreased at 6 and 12 months in the OVX animals compared to 4ad fh(femor di)Tj.037176 Tw 12 0 0 124413.459.7 357.85965 Tal

## Abstract 8

### Identification and Functional Characterization of an Osteoclast-Derived Osteoblastic Factor (ODOF)

Tony C.A. Phan, Renzhi Han, Tamara Davey, Verity J. Smuts, Ming H Zheng, Jiake Xu\*

*Molecular Orthopaedic Laboratory, School of Surgery and Pathology, The University of Western Australia, Nedlands, WA, 6009, Australia*

Bone is a living tissue and is maintained by the coordinate action of osteoblasts and osteoclasts. Intercellular communication between osteoblasts and osteoclasts is the quintessential mechanism in bone remodelling. Here we report the identification and functional characterization of an Osteoclast-Derived Osteoblastic Factor (ODOF), which is expressed by osteoclasts, binds specifically to osteoblasts and elevates cytosolic calcium ( $[Ca^{2+}]_i$ ) resulting in the proliferation of osteoblastic cells. The ODOF gene was identified in RAW<sub>264.7</sub> cell-derived osteoclasts utilising a PCR-selected subtractive hybridisation screening process. Further investigations using reverse transcriptase PCR, revealed that ODOF mRNA was up-regulated during RANKL-induced osteoclastogenesis but was not expressed in osteoblasts or osteoblast-like cells. Recombinant His-tagged ODOF was subsequently produced and labelled with <sup>125</sup>I to ascertain its binding profile. The protein exhibits highly specific binding to primary calvarial osteoblasts with a binding affinity of  $1.7 \pm 0.4$  nM and  $2.7 \times 10^4 \pm 306$  receptors per cell but not with osteoclasts and their precursor cells. Functional studies demonstrated that ODOF stimulates an increase in the growth and proliferation of osteoblastic cells. In contrast, the protein did not promote osteoclastogenesis, osteoclast survival or bone resorption. Mechanistic analysis revealed that ODOF, alone, elevates intracellular  $[Ca^{2+}]_i$  through the PLC-IP3 induced depletion of calcium stores. Moreover, western blot analysis reveals that ODOF induces the activation of the PI3K-Akt and ERK pathways in osteoblasts. Taken together, our results provide evidence for a novel cross-talk mechanism between the osteoclast and osteoblast and indicate that osteoclasts play a role in regulating osteoblastic growth and proliferation.

## Abstract 9

### PERI-ACETABULAR MICROMOTION IN AN *IN-VIVO* ASEPTIC LOOSENING MODEL

RH Edmonds-Wilson, TC Hearn, T MacKenzie, GI Anderson.

*Faculty of Science & Engineering and Department of Surgery, Flinders University, Adelaide.*

We aimed to investigate the peri-acetabular micromotion in an *in-vivo* low morbidity model of focal osteolysis. Seven large dogs underwent uncemented total hip arthroplasty with a custom-made titanium grit-blasted femoral stem and cup. At surgery, a controlled defect was created in the acetabular bone bed using a 4.5mm drill and placing 800 $\mu$ Ls of submicron polyethylene particulates in agarose gel into this defect prior to placing the cup. Radiography and gait analysis were performed regularly to assess osseointegration and return to normal weight-bearing function over six months. The harvested acetabular specimens were potted in polymethyl-methacrylate in a jig allowing four linear variable differential transducers (LVDTs) to contact the bone 2mm sub-adjacent to the metal-backing of the cup, in test 1, and the metal of the cup itself in test 2. This allowed the relative motion of the cup and bone to be calculated after applying loads of either 0.6 or 1.5 bodyweights for 50 cycles at 1Hz through the polyethylene cup liner using a servo-hydraulic materials testing machine (Instron 8511). Inter-test reliability was assessed and found to be less than 10%. Initial testing of relative micromotion between the cup and the bone in the three dorsal positions was  $12.5\pm 3.1\mu\text{m}$ , and  $4.1\pm 3.0\mu\text{m}$  ventrally. Further micromotion and histo-pathological analysis to quantify the extent of the lytic defect is ongoing. The small peri-acetabular micromotion in combination with return to normal weight-bearing strongly suggest that the area of focal osteolysis behind the cup did not result in morbidity in these dogs at 6 months.

## Abstarct 10

### ANTERO-POSTERO DIFFERENCES IN CORTICAL THICKNESS AND CORTICAL POROSITY OF THORACO-LUMBAR VERTEBRAL BODIES

Nick Fazzalari <sup>1,2,3</sup>, Ian Parkinson <sup>1,2,3</sup>, Quentin Fogg <sup>4</sup>, Peter Sutton-Smith <sup>1,2,3</sup>

1. *Division of Tissue Pathology, Institute of Medical and Veterinary Science.*

2. *Bone and Joint Research Laboratory, Hanson Institute.*

3. *Department of Pathology, University of Adelaide.*

4. *Department of Anatomy and Histology, Flinders University. South Australia.*

The relationships between the vertebral body cortex and cancellous bone trabecular thickness have not been well characterised. This study will investigate interrelationships between the cortical shell and cancellous bone trabecular thickness, in the thoracolumbar spine

One hundred and sixty vertebral bodies from the thoracolumbar spine were obtained at autopsy. The average age of the cohort was  $59.3 \pm 22.1$  years (range = 20 – 94 years). Cortical thickness, cortical porosity and trabecular thickness from the adjacent cancellous bone were measured.

At the mid-vertebral body level, anterior cortical thickness was significantly greater than posterior cortical thickness ( $524 \mu\text{m} \pm 352 \mu\text{m}$  versus  $370 \mu\text{m} \pm 283 \mu\text{m}$ , respectively,  $p < 0.0001$ ) and anterior cortical porosity was significantly less than posterior cortical porosity ( $24 \% \pm 14 \%$  versus  $32 \% \pm 16 \%$ , respectively,  $p < 0.0001$ ). Cortical thickness of the mid-anterior region was correlated with trabecular thickness from the middle of the vertebral body ( $r=0.25$ ,  $p<0.002$ ). In contrast, cortical thickness of the mid-posterior region was correlated to trabecular thickness of the cancellous bone adjacent to the mid-posterior cortical wall.

This study provides a novel perspective of thoracolumbar vertebral body bone, where measurement of cortical thickness and cortical porosity in a cohort of skeletally normal individuals revealed structural differences between load bearing anterior and posterior cortical walls. The correlation between cortical thickness and trabecular thickness at different regions suggests that modulators of change to vertebral body bone may affect the cortical and trabecular bone differently. The relationships between cortical and cancellous bone suggest that the middle sectors of the vertebral body play a critical role in load bearing.

## Abstract 11

### **in vitro ReConstruction of Epiphyseal Plate and Its application in the repairing of Epiphyseal Plate injury**

**<sup>1,2</sup>Fei Yin, <sup>1</sup>Y Yu, <sup>2</sup>L Guo, <sup>2</sup>JC Wang, <sup>2</sup>ZL Gao, <sup>2</sup>DS Duan and <sup>1</sup>WR Walsh**

*<sup>1</sup>Orthopaedic Research Laboratories, University of New South Wales, Prince of Wales Hospital, Sydney NSW*

*<sup>2</sup>Orthopaedic Department, China-Japan Union Hospital, Jilin University, Changchun, PR China*

Epiphyseal plate injury may result in deformity of limbs or growth stop. Current treatments focus on preventing the formation of bone bridging within the injury areas. The commonly used materials are adipose tissues, bone cement or synthesized materials. Autologous cartilage transplantation has been tried and the limited resources are the main barrier. With the advance cell engineering techniques, we harvested chondrocytes from the ribs of 4-weeks New Zealand Rabbits, reconstructed by high density centrifugation culture and implanted into the left medial tibial epiphyseal plate defect in 6-weeks New Zealand Rabbits. Empty defects in the right tibia served as controls. The endpoints were 1, 2, 4, 8, 12, 16 and 24 weeks (5 per group per time point).

The tibial angles were evaluated by X-ray at 2, 8 and 24 weeks. The histo-morphology was assessed by H&E and toluidine blue staining.

At 8 weeks, the average tibial angles in the control groups (n=20) showed on X-ray was  $38.80\pm 3.50$  and it was significantly greater than the treatment sides ( $9.01\pm 4.2$ ). Histological staining revealed bone in-growth in the control group at 2 weeks, while the treatment groups presented disorganized cartilage. At 4 weeks cartilage layers were noted in the treatment groups and the cutting edges disappeared.

Autologous epiphyseal plate reconstruction is a cell-based tissue therapy which allows expending the limited resources as well as reshaping and resizing thus provide a significant clinical application.

## **Abstract 12**

### **INFLUENCE OF AGE ON BONE HEALING IN NORMAL AND IMMUNODEFICIENT MICE**

**Gan J., Yu Y., Chen J.B., Gillies R.M., Walsh W.R.**

*Orthopaedics Research Laboratories, Prince of Wales Hospital, University of New South Wales, Sydney, NSW, Australia*

Aging is generally associated with the deterioration of bodily function, including bone healing. The influence of age, especially cell-mediated response on bone repair in immunodeficient animals is not well reported. Thirty young (3-month-old) and adult (5-month-old) nude and normal Balb/c mice were used following ethical approval. A round 1mm-diameter defect was drilled through the medio-lateral aspect of the distal femoral condyle. The animals were sacrificed at day 3, 7, 10, 14 and 21. New bone formation was analysed quantitatively from histology. Normal mice showed new bone formation at 7 days and progressed to complete healing by 3 weeks. There was a significant decrease in new bone in adult normal mice in the first 2 weeks ( $p < 0.05$ ) compared to the young. Nude mice showed new bone formation at 7 days and up to 75% healing at 3 weeks. No significant difference in new bone formation was detected between young and adult nude mice. Assessment of both strains revealed a faster healing rate in young normal mice compared to their nude cousins. However, in the adult animals, normal mice showed delayed healing when compared to the nude. The result suggested that aging suppresses the initial phases of bone healing in normal animals. Absence or low levels of functional T-cells in athymic nude mice may have minimal effect during aging when compared to an intact immune system in normal mice. Despite this, both adult and young nude mice can be used as animal model for clinical research.

## **Abstract 13**

### **Transport of large molecules through articular cartilage subjected to dynamic load**

Bruce Gardiner, David Smith, Peter Pivonka and Alan Grodzinsky

*University of Melbourne*

Osteoarthritis is a common and often debilitating disorder resulting from disease and injury of articular cartilage. Cartilage has limited ability to repair itself due to a lack of blood vessels to enable nutrient transport. Insulin-like Growth Factor-I (IGF-I) is a large molecule with an important role in cartilage growth and metabolism. Experiments have shown that the anabolic effects of IGF-I may be enhanced by dynamically loading the cartilage. We have developed a mathematical model for the transport of IGF-I through cartilage undergoing dynamic loading. The model is based on mixture theory and as such treats the cartilage as a three phase porous continuum: a solid phase (representing the various collagen and proteoglycans), a fluid phase and a solute phase. It is found that enhanced transport of IGF-I through cartilage can be obtained if the capture, and later release, of IGF-I by IGF-I binding proteins embedded in the cartilage matrix is included in the model, otherwise the rate of diffusion and the steady state concentration of IGF-I in the cartilage is the same as for static cartilage. Interestingly, this result refutes the conclusions drawn by previous models (Mauck et al., 2003) that dynamic loading alone (*i.e.* neglecting IGF-I sources/sink effects) can enhance IGF-I transport.

## Abstract 14

### HEALING BONE WITH THE ADIPOSE DERIVED STROMAL STEM CELLS-THE HISTOMORPHOMETRY OF THE OVINE DEFECT MODEL.

Niechoda, B., Yu, Y., Chen, J.B., Walsh, W.R.

*Orthopaedic Research Laboratories, Prince of Wales Hospital, University of New South Wales, Sydney, Australia*

The objective of this study was to examine the potential of culture expanded, adipose tissue derived stromal stem cell population to heal a critical size confined cancellous bone defect.

It has been demonstrated recently, that the adipose-derived stem cells (ADSCs) are capable of osteogenic differentiation under appropriate conditions *in vitro*. (P.Zuk *et al*, *Tissue Engineering* 2001, 7(2)).

Bone marrow aspirates and subcutaneous adipose tissue were harvested from 42 adult wethers. The population of stromal cells was derived from both tissues. Populations of bone marrow cells and adipose stromal cells were expanded in culture and stimulated with osteogenic medium for 7 days. Cultured cell populations were harvested, mixed with a hydroxyapatite carrier (Pro-Osteon 200R) and deposited into bilateral medial femoral condyle confined cancellous defect.

7 groups was examined: Bone graft+ ADSCs, Bone graft+ BMSCs, Carrier + ADSCs, Carrier+ BMSCs, Bone graft, Carrier, Empty defect.

2 weeks, 4 weeks and 8 weeks time-points were examined.

Histological staining's: H&E and Masson's were performed and quantified statistically. Immunohistochemical staining for BMP4 and BMP7 and their downstream regulators: Smad4 and CBFA1 were evaluated in the defect area and graded in a blind fashion by two trained observers.

There was a progressive and time dependant increase in woven bone formation in the defects treated with ADSCs across all time points. The amount of bone formed in this group was comparable with the amount formed by the use of BMSCs.

The results of this study support the hypothesis that seeding porous hydroxyappatite with ADSCs does enhance bone formation and defect healing.

## Abstract 15

### KINEMATIC JOINT MEASUREMENTS USING SINGLE-PLANE RSA

J.L. Ioppolo<sup>1,2</sup>, N. Börlin<sup>3</sup>, M.D. Baratz<sup>4</sup>, B. Nivbrant<sup>1</sup>, C.R. Bragdon<sup>4</sup>, M.G. Li<sup>1</sup>, R.I. Price<sup>3</sup>, H. Malchau<sup>4</sup>, D.J. Wood<sup>1</sup> and W.H. Harris<sup>4</sup>

<sup>1</sup>*Department of Surgery (Orthopaedics), University of Western Australia*

<sup>2</sup>*Medical Technology and Physics Department, Sir Charles Gairdner Hospital*

<sup>3</sup>*Department of Computing Science, Umeå University, Sweden*

<sup>4</sup>*Orthopaedic Biomechanics & Biomaterials Laboratory, Boston, MA, USA*

Skeletal kinematics are able to yield significant insight into the patient factors, surgical techniques and design of implants used for the management of joint replacement patients. This project involved the basic scientific development of a clinical system for the measurement of skeletal kinematics. Radiostereometric Analysis (RSA) is the established “gold-standard” for measuring the long-term migration of prosthetic hip and knee joint components with respect to underlying bone. However, the use of conventional RSA to perform measure skeletal kinematics is limited. In addition, no clinical methods currently exist for measuring the *in-vivo* wear of polyethylene tibial components.

The precision and accuracy of this novel RSA fluoroscopy system was determined *in-vitro* using two international standard reference phantoms and a single-plane General Electric Flexiview (OEC9800) fluoroscope. A method for determining knee joint wear was evaluated by analysing the instantaneous tibiofemoral centre of rotation over a shortened knee flexion-extension cycle of a woodcut knee phantom implanted with total knee replacement components.

The accuracy of RSA fluoroscopy is proportional to the pixel size of the image intensifier and was approximately 200 microns in the proximal-distal and anterior-posterior directions and approximately 700 microns in the medial-lateral direction. The lower threshold for the *in-vitro* measurement of statistically significant polyethylene tibial component wear was 400 microns.

The technique offers the independent and accurate assessment of the *in-vivo* kinematics of joint implants designed by different manufacturers. Results of this work will assist the surgical decision making and implant design processes by offering direct evidence of the characteristic *in-vivo* skeletal kinematics of a particular implant.

## Abstract 16

### **Expression of growth factors in nude rat bone defects treated with gamma irradiated human bone grafts**

JB Chen, Y. Yu, QJ Li, \*D.A.F. Morgan and W.R. Walsh

*Orthopaedic Research Laboratories, University of New South Wales, Prince of Wales Hospital, Sydney*

*\*Queensland Bone Bank, Holy Spirit Hospital, Brisbane*

Banked allogeneic bone grafts have been used widely in clinic for the repair of bone defects. Massive allografts are thought to function through an osteoconductive pathways, demineralized bone was reported to be osteoinductive. Morselized allograft which has been used to fill bony defects in revision arthroplasty to fulfill a mechanical role, as well as to provide a scaffold for new bone formation has yet to be elucidated the potentiality of osteoinductive function. Gamma irradiation is a widely accepted secondary sterilization procedure. The effect of gamma irradiation on biological properties of morselized allograft bone has not been well reported.

Our previously study found a negative dose-dependent effect of gamma irradiation on bone formation of morselized human bone grafts in nude rat defect model. Evidence of osteoinductivity was found in the defects packed with zero or low dose treated grafts. This study focused on mechanism investigation by testing the expression and distribution of bone morphogenetic protein (BMP) 7, core binding factor  $\alpha$ 1 (CBFA1) and proliferating cell nuclear antigen (PCNA).

CBFA1 and BMP7 stained mainly the osteoblasts and some mesenchymal like cells lining around the intact cortex, the new bones adjacent to the host cortex bridged by the implanted grafts and in the loose connective tissue far from the host bone. A negative correlation of immunostaining of CBFA1 and BMP7 and gamma irradiation was presented by quantitative analysis. The low bone formation rate in the high dose gamma irradiation group (25 kGy) may due to the less expression of BMP7 and/or CBFA1.

## Abstract 17

### **The Expression of TRAIL and TRAIL Receptors in Cultured Synovial Fibroblasts from Rheumatoid and Osteoarthritis**

Anak Agung Sagung Sri Kencana Dharmapatni<sup>1</sup>, David M. Findlay<sup>2</sup>, Andreas Evdokiou<sup>2</sup>, Holding C<sup>1</sup>, Wallwork, M<sup>3</sup>, David R. Haynes<sup>1</sup>

<sup>1</sup> *Dept. of Pathology, University of Adelaide, South Australia, Australia*

<sup>2</sup> *Dept. of Orthopaedics and Trauma, University of Adelaide, and Hanson Institute South Australia, Australia*

<sup>3</sup> *Adelaide Microscopy, University of Adelaide, South Australia, Australia*

In this study, we investigated TRAIL (Tumor necrosis factor related apoptosis inducing ligand) and TRAIL receptor expression on cultured synovial fibroblasts and correlate this with their ability to undergo TRAIL and disease modifying anti rheumatic drugs (DMARDs)- induced apoptosis.

Synovial tissues were obtained from patients undergoing arthroscopy biopsy. Synovial fibroblasts were isolated by digestion with collagenase and dispase for 11/2- 2 hours. After repeated passaging, the cells were used at passage 3 to 6. Immunocytochemistry and immunofluorescence were used to investigate the expression of TRAIL and TRAIL receptors on these cells. Apoptosis was induced with TRAIL, Methotrexate and Actinomycin D or combination of these reagents. DAPI (4'-6-Diamidino-2-phenylindole) staining were used to examine the morphology of cells that undergoing apoptosis. Cell viability was quantitatively examined by crystal violet staining.

We found that both synovial fibroblasts from RA And OA constitutively express TRAIL, TRAIL Receptor 4 and Osteoprotegerin (OPG). Induction of apoptosis with soluble TRAIL (100 to 1000 ng/ml) for 24 hours did not induce cell death in rheumatoid synovial fibroblasts from both rheumatoid (RA) and osteoarthritis (OA). These cells were sensitive to Actinomycin D-induced apoptosis. Combination with soluble TRAIL and pretreatment with TNF  $\alpha$  and IL-1  $\alpha$  enhanced the sensitivity of RASF to Actinomycin D-induced apoptosis.

In conclusion, synovial fibroblasts from RA and OA are relatively resistant to TRAIL induced apoptosis but sensitive to Actinomycin D induced apoptosis. Expression of TRAIL decoy receptors (TRAIL R4 and OPG ) may be involved in the resistance of these cells to TRAIL induced apoptosis.

## Abstract 18

### **EXPLORING ORTHOPAEDIC USE OF 3-D RECONSTRUCTED MRI APPLICATIONS: AN OVERVIEW OF PRELIMINARY VALIDATION IN A CONTROLLED PRECLINICAL MODEL**

Kurmis AP\*, Slavotinek JP, Reynolds KJ, Hearn TC.

*\*Department of Orthopaedics, Division of Surgery, Repatriation General Hospital, Daws Road, Daw Park, South Australia, Australia & School of Informatics and Engineering, Faculty of Science and Engineering, Flinders University, Bedford Park, South Australia, Australia.*

Although three-dimensional (3D) applications of computed tomography are now widely considered commonplace in orthopaedic a

## Abstract 19

### A NEW GFP EXPRESSING OSTEOSARCOMA ANIMAL MODEL

Joanna Lenaghan, Karla Contreras, Anne Nadesapillai, Peter Choong, Joseline Ojaimi

*Department of Orthopaedics, The University of Melbourne, St. Vincent's Hospital, Melbourne*

25–40% of patients suffering from osteosarcoma will succumb to the disease, an overwhelming 98% from pulmonary metastases. Better understanding of micrometastasis holds the key to improved survival outcomes. Our aim has been to develop an animal model of osteosarcoma that will be amenable to more sophisticated imaging, providing 'illumination' of micrometastases and their pathogenesis.

U2OS, a non-transformed, human osteosarcoma cell line was stably transfected with green fluorescent protein (GFP). Cells were selected in G418 for 4 weeks to enrich GFP expression. Transfected and untransfected cell lines were analysed *in vitro* to determine the effects of transfection on the cells proliferative and migratory properties. Balb-c nude mice were inoculated with the U2OS-eGFP cell line using intratibial injection, and were sacrificed at 2, 4 and 6 weeks. Post inoculation the tibia and lungs were examined intact, and as histological sections, under fluorescent microscope.

The model has been successful in establishment of GFP expressing tumour, identifiable under fluorescent light. This important animal model is unique in the study of osteosarcoma. It will enable more accurate and easier analysis of tumour evolution *in vivo*, particularly in the detection and study of micrometastasis.

## **Abstract 20 (*Withdrawn*)**

## **Abstract 21**

### **ZOLEDRONIC ACID IMPROVES FEMORAL HEAD SPHERICITY IN A RAT MODEL OF PERTHES DISEASE**

McDonald MM, Sharpe IT, Peat RA, Williams PR, McEvoy A, Little DG

*The Children's Hospital at Westmead, Sydney, NSW, Australia*

As the optimal treatment for Perthes disease remains controversial, a pharmaceutical therapy would be of great benefit. We hypothesized that the bisphosphonate, zoledronic acid (ZA) could maintain femoral head sphericity by changing the balance between bone resorption and new bone formation. In this investigation we test the effect of ZA in an established model of Perthes disease - the spontaneously hypertensive rat (SHR).

120 4-week old rats were divided into three groups of 40: 3 doses of saline monthly, 3 doses of 0.05 mg/kg ZA monthly, or 10 doses of 0.015 mg/kg ZA weekly. At harvest, 15 weeks of age, radiographs were taken and a modified epiphyseal quotient (EQ) measured by a blinded observer. Specimens were DXA scanned and processed for histology.

Radiographs revealed increased mineralization and improved sphericity of femoral heads in ZA groups. DXA measurements documented ZA femoral head BMD was increased by 18% to 21% over controls ( $p < 0.01$ ). Overall EQ was improved in ZA-treatment groups ( $p < 0.01$ ). The proportion of "flat" heads ( $EQ < 0.40$ ) was significantly reduced from 45% to 16% with ZA treatment ( $p < 0.01$ ). On histology, affected femoral heads showed osteonecrosis, ossification delay, or both. There was a similar prevalence of osteonecrosis in control and ZA groups however the prevalence of ossification delay was significantly reduced by ZA ( $p < 0.01$ ). Analysis of EQ revealed the largest improvement in sphericity was in femoral heads with both osteonecrosis and ossification delay ( $p < 0.01$ ).

Zoledronic acid favourably altered femoral head shape in this spontaneous model of osteonecrosis in growing rats.

## Abstract 22

### **ZOLEDRONIC ACID TREATMENT ENHANCES NET VASCULARIZED HARD CALLUS FORMATION IN A CLOSED RAT FRACTURE MODEL**

McDonald MM, Dulai S, Godfrey C, Hamilton B, Little DG

*The Children's Hospital at Westmead, Sydney, NSW, Australia*

It has been assumed that osteoclast activity is a prerequisite for the normal process of endochondral ossification. However recent studies have suggested that endochondral ossification proceeds normally at the growth plate, even in the absence of osteoclasts. We therefore hypothesized that endochondral ossification (soft callus removal) during fracture repair would not be delayed by the potent osteoclast inhibitor zoledronic acid (ZA).

ZA dosing commenced in rats 1 week post closed fracture, with harvests at 2, 4 and 6 weeks. Dosing regimes included: saline, low dose 0.025 mg/kg ZA as bolus (LDB) or divided weekly doses (LDW), and high dose 0.1 mg/kg ZA as bolus (HDB) or divided weekly doses (HDW).

Histomorphometry revealed no significant difference in the percent of avascular cartilaginous (soft) callus, regardless of treatment. All groups showed complete vascular ossification (hard callus) by 6 weeks.

QCT at 6 weeks revealed increases in callus BMC ( $p < 0.01$ ) and callus volume ( $p < 0.05$ ) in all ZA groups over saline. Between 4 and 6 weeks, HDB-ZA callus volume decreased by 8%, indicating remodelling was occurring. In contrast, HDW-ZA caused delayed remodelling, callus volume increasing by 24%.

In conclusion, ZA did not delay fracture callus endochondral ossification, indicating that osteoclast function is not essential to soft callus removal. Single dose bolus treatment provided a better outcome over continuous dosing, allowing for hard callus remodelling while still increasing callus BMC and volume. This study reveals that ZA treatment during fracture repair is clinically safe: it provides a larger hard callus without delaying endochondral ossification.

## Abstract 23

### **AN IN VITRO EVALUATION OF 2- AND 3-FLUTED DRILL BITS: AN INVESTIGATION OF THERMAL DAMAGE, HOLE PLACEMENT ACCURACY AND MECHANICAL PROPERTIES.**

Milne, H R M; Gillies, R M; Bertollo, N; Ellis L P; Stephens, P C; Butler A M; Walsh, WR

*Orthopaedic Research Laboratories, University of New South Wales, Sydney, Australia*

This study compared the drilling characteristics of a 2-fluted Smith and Nephew (S&N) drill bit, a Synthes 3-fluted drill bit and a new 3-fluted 'SurgiBit' drill bit from Orthopedic Innovation (OI).

The in-vitro characteristics studied were;

- Stiffness
- Accuracy
- Feed-rate
- Torque
- Heat generation

The bending stiffness, load and energy to failure were tested. A comparison of the characteristic feed-rate of each drill was made at a range of forces. A thermal camera measured temperature elevations in the bone during drilling. Drill accuracy was examined by measuring skiving distances at drill bit entry angles between 0° and 60°.

The 3- fluted designs showed higher stiffness and maximum force to failure ( $P<0.05$ ). Feed-rates measured during testing were greater in the 3- fluted designs ( $P<0.05$ ). The 3- fluted designs were the most accurate throughout all angle tested. The accuracy of the OI drill bit was consistent at all the angles tested ( $p=0.114$ ). The 2-fluted design became increasingly less accurate at higher angles ( $p<0.0005$ ). Mean torques measured were higher and more variable in the 2- fluted drill bit ( $p<0.05$ ). The 2-fluted drill bits produced a greater thermal increase during drilling.

Higher stiffness may reduce breakage during procedure. The higher feed-rates experienced by the 3- fluted drills reduces drilling time. This may reduce thermal damage. The 2-fluted torque profile suggests a more jarring cutting action which may create micro-damage. This could lead to a structurally compromised drill hole. The results of this study suggest there may be clinical advantages derived from adopting 3-fluted drill bits.

## **Abstract 24**

### **THERMAL RESPONSE OF MENISCUS UNDERGOING IN VITRO RADIOFREQUENCY TREATMENT**

Napper, R; Stass, V; Walsh, W R

*Orthopaedic Research Laboratories, University of New South Wales, Prince of Wales Hospital, Sydney, NSW, Australia.*

Radiofrequency (RF) energy is used clinically to alter the geometry, mechanical and biological properties of collagen based tissue. Although the effects of RF and other thermal treatments have been evaluated with microscopy little is known about the actual thermal distribution produced in the tissue.

Adult porcine menisci were treated for varying powers and times using a monopolar RF probe inserted into an artificial tear on the anterior aspect of the meniscus. The in vitro experiment used thermocouples inserted into the meniscus and infrared thermography to measure the core and surface temperatures during RF treatment and for a ten minute cool down period. Experiments were carried out in a shallow bath of saline solution allowing a complete circuit between the probe electrode and return electrode that was placed beside the meniscus. The depth of saline solution was adjusted to leave the anterior surface of the meniscus open to air allowing accurate infrared measurements.

Results indicate that surface temperatures approximate core temperatures to within 2°C. At the lowest energy treatment of 15 Watts for 30 seconds and highest energy treatment of 30 Watts 60 seconds the maximum temperature reached by the RF probe increased from 34°C to 91°C. The radius of tissue surrounding the treatment probe that was heated to above 30°C from room temperature increased from 3.5mm to 10mm for the extremes of treatment energy. The saline solution was found to act as a heat sink drawing energy from the treated tissue.

## Abstract 25

### OSTEOSCLEROSIS IN MICE LACKING RAB3D IS RELATED TO DISRUPTIONS IN POST-TGN VESICLE TRAFFICKING IN OSTEOCLASTS

Nathan J. Pavlos<sup>1</sup>, Jiake Xu<sup>1</sup>, Dietmar Riedel<sup>3</sup>, Steven L. Teitelbaum<sup>2</sup>, Reinhard Jahn<sup>3</sup>, F. Patrick Ross<sup>2</sup>, Ming H. Zheng<sup>1</sup>

1. *Unit of Orthopaedics, School of Surgery and Pathology, University of Western Australia, Perth, Western Australia*
2. *Department of Pathology, Washington University School of Medicine, St. Louis, USA*
3. *Department of Neurobiology, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.*

Intracellular membrane trafficking is essential to osteoclast function however little is known about the nature and regulation of the transport pathways that govern its structural and functional polarisation. We have recently reported the existence of the small exocytic-related Rab3D GTPase in osteoclasts. Here, to shed light on the possible involvement of Rab3D in osteoclast physiology we examined Rab3D-deficient mice for skeletal anomalies. Strikingly, we identify an osteosclerosis phenotype in these mice; with bones from these animals exhibiting increased total volume, increased trabecular number and reduced trabecular separation. Basal osteoclast numbers were normal, however, the total eroded surface was significantly reduced, suggesting that the resorptive defect was due to attenuated osteoclast activity rather than a disruption in osteoclast formation. Consistently, ultrastructural analyses revealed that Rab3D<sup>-/-</sup> osteoclasts exhibit irregular regulated borders. To further delineate the molecular mechanism(s) underlying this resorption deficiency, we expressed a series of enhanced yellow fluorescent (EYFP)-tagged wild-type and mutant Rab3D fusion chimeras in osteoclasts and examined their subcellular localisations and affects on bone resorption. Rab3D was found to associate with the *trans*-Golgi network (TGN) and an as yet undefined subset of post-TGN vesicles of non-endosomal/lysosomal origin. Moreover, while overexpression of Rab3D wild-type and its mutants had no affect osteoclastogenesis or cellular attachment, expression of the GTP-binding deficient Rab3DN135I mutant, which was largely restricted to the TGN, profoundly impaired osteoclastic bone resorption. These data document the existence of a novel Rab3D-mediated post-TGN trafficking pathway that is required for the maintenance of the ruffled border membrane during physiological bone metabolism.

## Abstract 26

### INTERACTION OF GTP-BINDING RAB3D WITH TCTEX-1, A LIGHT CHAIN OF THE CYTOPLASMIC DYNEIN MICROTUBULE MOTOR COMPLEX

Nathan J. Pavlos<sup>1</sup>, Jiake Xu<sup>1</sup>, Amerigo Carrello<sup>1</sup>, Karen Kroeger<sup>2</sup>, Karin Ednet<sup>2</sup>, Ming H. Zheng<sup>1</sup>

1. *Unit of Orthopaedics, School of Surgery and Pathology, University of Western Australia, Nedlands, Western Australia*
2. *Western Australia Institute for Medical Research, QEII Medical Centre, Nedlands, Western Australia*

Rab3D belongs to the family of small GTPases involved in the regulation of exocytosis. Previously we have shown that Rab3D is required for the maintenance of the osteoclastic resorptive organelle, namely the ruffled border membrane. Here, to further delineate the mechanism(s) underlying this phenomenon, we have employed a yeast two-hybrid system to identify potential Rab3D interacting proteins. Screening a mouse embryonic cDNA library, 5 clones (M-18, -30, -37, -39 and -40) were positively identified which specifically interacted with the N-terminus of Rab3D. Database searches identified these clones as mouse Tctex-1, a 14 kDa light chain of the multimeric cytoplasmic dynein motor complex. A specific interaction between Rab3D and Tctex-1 was confirmed by GST-pull down and co-localisation studies. Truncation analyses mapped the Tctex-1 binding site to the switch II/GTP-binding motif of Rab3D (amino acids 74-95). Consistently, bioluminescence resonance energy transfer (BRET) analysis demonstrated that Tctex-1 preferentially associated with the GTP-bound conformation form of Rab3D in live cells. When overexpressed, Flag-Tctex-1, GFP-dynamitin, or antisense-Tctex-1 disrupted the spatial distribution of Rab3D *in vivo*. Additionally, Rab3D-secretory granules localise to microtubules and are redistributed by nocodazole treatment into  $\beta$ -COPI-positive Golgi mini-stacks in transfected COS-1 cells. These data lend support to the notion that Rab GTPases and molecular motor proteins act in concert to regulate directional membrane transport and furthermore suggest that Rab3D may functions to recruit and regulate the activity of cytoplasmic dynein Tctex-1, controlling the sorting and microtubule-dependent targeting of post-Golgi secretory granules to the ruffled border membrane during osteoclastic bone resorption.

## Abstract 27

### **HISTOLOGICAL CLASSIFICATION OF 58 PATIENTS WITH PLASTIC - EMBEDDED FULL THICKNESS CORE BIOPSIES 2.6-53.1 MONTHS FOLLOWING AUTOLOGOUS CHONDROCYTE IMPLANTATION (ACI) .**

Barry W. Oakes and Sue Connell

*Department of Anatomy and Cell Biology, Monash University, Melbourne and Ian Henderson, St. Vincents and Mercy Private Hospitals Melbourne, Australia.*

58 patients with a mean age of 38 years at ACI implantation (range 14 - 62 years) had full thickness (including subchondral bone) central (C) and/or marginal (M) core biopsies using a Giebel needle (approx. 2.00mm internal diameter) of their repairing lesions at a mean of 12.2 months post-implantation (range 2.6-53.1 months) due to clinical symptoms requiring a second-look arthroscopy. All patients had the classic Brittberg and Peterson ACI surgery using a periosteal patch. The biopsy tissues were classified as hyaline articular cartilage (HAC), 'hyaline-like' articular cartilage (HLC), fibrocartilage (FC), periosteum and mixtures of the above eg. HLC/FC.

Overall in the knee 64% were either HAC or HLC. In most biopsies examined at >12 months post- ACI the periosteal layer was intact with a gradation from periosteal tissue to HLC to HAC in the deep layer adjacent to subchondral bone. After 12 months the periosteal lay 0.02251 Tw

## Abstract 28

### INFLUENCE OF TESTING CONDITIONS ON SURFACE SHEAR STRAIN IN LONG BONE TORSIONAL TESTS

Pelletier, M H; Okamoto, K; Walsh, W R

*Orthopaedic Research Laboratories, University of New South Wales, Prince of Wales Hospital, Sydney, NSW, Australia*

This study was designed to examine the cortical shear strain distributions during torsional testing (2.5Nm) under load or displacement control and internal or external rotation in rabbit femurs. A 1.7mm thick photoelastic coating was bonded to the surface of seven adult rabbit femora. Specimens were mounted in a servo-hydraulic testing machine such that the anatomical axis of the femur was collinear with the testing machine's rotational axis. A zero load and displacement state was achieved at the beginning of each test. Four loading protocols were applied utilizing axial displacement or axial force control feedback mode in internal or external rotation. Photoelastic measurements were taken at three points along the anterior and posterior diaphyses, the measurements were normalized by the maximum shear strain in each bone and grouped (Anterior, Posterior). The results were then analyzed by ANOVA. Displacement controlled tests produced tensile loads of 51.7N for internal and 34.8N for external rotation. Shear strain was statistically higher during external versus internal rotation posteriorly and statistically lower anteriorly. The anterior surface of the femur also showed an increase in the surface strains using displacement control versus load control. Although not statistically significant all displacement control strains were higher than their load control counterparts. The differences noted are likely due to the geometry of the anterior and posterior femur, these differences suggest that direction of rotation should be matched by side, i.e. internal or external rather than simply positive or negative torque, for all torsional testing to control for potential confounding variables.

## Abstract 29

### **COMPARISON OF STRAINS IN UNDERLYING GLENOID WITH METAL BACKED AND ALL POLYETHYLENE IMPLANTS**

Pelletier, M H; Langdown, A; Gillies, M; Sonnabend, D H; Walsh, W R

*Orthopaedic Research Laboratories, University of New South Wales, Prince of Wales Hospital, Sydney, NSW, Australia*

The aim of this study is to examine the strain differences in intact glenoids and following replacement with a metal backed keeled component and a cemented all polyethylene pegged component with the same articular geometry. Three fresh frozen adult upper extremities were cleaned of all soft tissue. A 2mm thick photoelastic coating was bonded to all but the articular surface of the glenoid. Eight strain gauges were attached around the periphery in a plane 12mm proximal to the glenoid surface. Loads were applied to the glenoids using the humerus in four angles of abduction (0°,30°,60°,90°) corresponding to four loading conditions (222N, 538N, 924N, 1204N). Preoperative measurements were taken from each intact glenoid to represent the native state. Each specimen was reconstructed using a metal backed, then PE component. Specimens were retested following each surgical procedure. Perpendicular strain gauges values and the photoelastic measurements were used to calculate the principal strains at each point. Results were normalized by angle/load and differences were identified with ANOVA. The metal backed and all PE implants showed similar trends, shifting strains superiorly. Differences were seen between the intact and implanted states in all four of the superior gauges and the posteroinferior gauge. Differences between the two implanted states were only seen in the anterior gauges. Glenoid arthroplasty indeed alters the strain state compared to the intact case, this is just one factor that can influence outcome. This study is limited in that the complex kinematics of the shoulder cannot be replicated in vitro.

## Abstract 30

### **PINEALECTOMY AND SCOLIOSIS IN THE CHICKEN: MORPHOLOGICAL ASSESSMENT OF VERTEBRAL BODY DEFORMITY**

NL Fazzalari <sup>1,2</sup>, R Davies <sup>1</sup>, IH Parkinson <sup>1,2</sup>, A Fagan <sup>3</sup>

<sup>1</sup>*Bone & Joint Research Laboratory, Div of Tissue Path, IMVS and Hanson Institute, Adelaide,*  
<sup>2</sup>*Dept of Path,* <sup>3</sup>*Dept of Ortho & Trauma, Uni of Adelaide, Adelaide, SA, Australia.*

#### Purpose of the study

Apical vertebral growth rate and final vertebral shape analyses were performed in a study of scoliosis in pinealectomized chickens.

#### Methods

Pinealectomy or a sham procedure was performed on two day old chickens. Survival was for two and four weeks postoperatively. Radiological and undecalcified histological examination of the apical vertebra of the resultant scoliotic deformity was performed. Growth rate was measured by means of fluorochrome labelling.

#### Results

A significantly smaller vertebral body cross sectional area was observed in birds with scoliosis when compared with those without a curve ( $5.9 \pm 0.5 < 7.8 \pm 2$  [mm<sup>2</sup>],  $p < 0.002$ ).

The height of the apical vertebra in birds with scoliosis was greater than the equivalent vertebra in birds without scoliosis ( $6.7 \pm 1.3 > 5.6 \pm 1.1$  [mm],  $p < 0.05$ ).

These differences were visible by four weeks but were not apparent at 2 weeks of age.

Vertebral body wedging is reflected in the ratio of maximum to minimum vertebral body height in the coronal plane. This was apparent at four weeks ( $1.18 \pm 0.07 > 1.04 \pm 0.03$ ,  $p < 0.001$ ) but not at two weeks.

A growth rate asymmetry was suggested in the coronal and axial plane accounting for the resultant vertebral asymmetry.

#### Conclusions

We have shown that the apical vertebrae in this experimental model of scoliosis are longer and narrower than control vertebrae. The wedging of the apical vertebra may be accounted for by an asymmetrical growth rate in both the coronal and axial plane.

## Abstract 31

### Localization of Pigment Epithelium-Derived Factor (PEDF) in Growing Mouse Bone

Gerald M. Y. Quan, Peter F. M. Choong, Joseline Ojaimi

*Department of Orthopaedics, University of Melbourne, St. Vincent's Hospital Melbourne*

Pigment epithelium-derived factor (PEDF) is a potent anti-angiogenic factor found in a wide range of fetal and adult tissues, where it is thought to play a role in the regulation of angiogenesis during development. The temporal expression of PEDF in bone and cartilage during endochondral bone formation has not previously been reported. In this study, we analysed the expression pattern of PEDF in growing mouse hindlimbs from newborn day one through to maturation at week 9, using immunohistochemistry and *in situ* hybridization. PEDF expression was demonstrated in chondrocytes within the resting, proliferative and upper hypertrophic zones of the epiphyseal growth plate. The pattern of expression was consistent throughout the developmental stages of the mouse. In addition, PEDF was expressed by osteoblasts lining the bone spicules in the ossification zone of metaphyseal bone, as well as by osteoblasts lining cortical periosteum. These novel results demonstrate that PEDF is developmentally expressed in both cartilage and bone cells during endochondral bone formation, and strongly suggest that it may play a regulatory role in the processes of chondrocyte and osteoblast differentiation, angiogenesis and bone remodelling during growth and development of long bones.

## Abstract 32

### THE EFFECTS OF OVARECTOMY AND POLYETHYLENE WEAR DEBRIS ON FRACTURE HEALING IN A RODENT MODEL

Rajaratnam R., Yee G., Yu Y., Matthews J B., Ingham E., Walsh W R

*Orthopaedic Research Laboratories, Prince of Wales Hospital, University of New South Wales, Sydney, NSW, Australia*

Several complications arise associated with total hip arthroplasties including fractures and loosening with a high incidence occurring in osteoporotic patients. The healing of these fractures can be further complicated by the presence of wear debris. This study examines the effect of PE wear debris placed directly into a closed fracture site in an oestrogen deficient rodent model.

At 22 weeks of age, a standard closed right femur fracture was produced in fifty ovx and fifty sham Sprague-Dawley rats following fixation with a k-wire.

1cm<sup>3</sup> Ceridust combined with Hyaluronic acid and sterile saline in a 1:2 ratio was injected directly into the fracture site percutaneously. Control animals received comparable injections excluding the Ceridust. Time-points were 1, 3 and 6 weeks.

DEXA revealed a significant reduction in BMC and BMD ( $p < 0.05$ ) in the left, non-fractured femurs in the sham and ovx animals at 3 and 6 weeks respectively. No statistical differences ( $p > 0.05$ ) in the ultimate load or stiffness were observed between the control and PE treated fractures at either time-point.

Control ovx fractures showed more cartilage than the sham group at 3 weeks and delayed remodelling at 6 weeks. While the ovx group injected with PE debris showed more fibrous tissue at the fracture gap and inside the diaphysis tunnel showing further delayed healing compared to the sham group with PE.

Our data suggests that delayed healing was evident in the oestrogen deficient group with further delays due to the presence of the PE wear debris.

## Abstract 33

### DEVELOPING SUPERIOR *IN VITRO* MODELS OF BISPHOSPHONATE THERAPY

Schindeler A and Little DG.

*The Children's Hospital at Westmead, Sydney Australia*

Zoledronic acid (ZA) is a potent nitrogen-containing bisphosphonate (N-BP) used in the treatment of osteoporosis and diseases of high bone turnover. *In vivo*, ZA binds rapidly and strongly to the skeleton to suppress osteoclast-mediated bone resorption. Resorbing osteoclasts are able to release and internalise bone N-BP at the bone surface, however other non-resorbing cells may be incapable of achieving this. In fact, it was recently shown that J774 macrophages are unaffected by bound N-BP. Numerous *in vitro* studies have analysed the effects of ZA and other N-BPs on osteoblasts by treating the cell culture media with continuous drug doses. Nevertheless, these drug treatments may not be the most appropriate methods for reproducing the *in vivo* situation.

We have used several approaches to better model the effects of clinical dosing with ZA. Osteoblasts cultured on calcium phosphate-coated discs were not adversely affected by ZA treatment, indicating that drug uptake is indeed limited when ZA is bound. Thus the major exposure of osteoblasts to ZA will occur in the hours immediately following dosing. To model this, we exposed differentiating MC3T3-E1 osteoblasts briefly to ZA and then cultured them normally for 1-3 weeks. Osteoblasts proved highly resistant to all short ZA treatment regimes, even when utilising doses of ZA that prevented mineralisation and/or induced cell death when administered for prolonged periods (i.e. 10-50 $\mu$ M). These methods may lead to more physiologically relevant models of N-BP treatment being adopted in osteoblast culture systems.

## Abstract 34

### HOW MUCH COMPUTED TOMOGRAPHY INFORMATION IS REQUIRED IN ORDER TO AUTO-GENERATE PATIENT-SPECIFIC FINITE ELEMENT MODELS OF THE PELVIS?

+\*Shim, V B; \*\*Pitto, R P; \*\*\* Streicher, R; \*Anderson, I A;

*\*The Bioengineering Institute, University of Auckland, New Zealand*

*\*\* Department of Orthopaedic Surgery, University of Auckland, South Auckland Clinical School, Middlemore Hospital, Auckland, New Zealand*

*\*\*\* Stryker SA, Thalwil, Switzerland*

We are developing techniques for auto-generating patient-specific models of the pelvis. Pelvis models will be used for finite element mechanical simulation as well as computer-assisted-surgery for hip replacement navigation. Our goal is to minimize radiation dosage to the patient using a limited number of computed tomography (CT) slices. Patient CT slices are mainly located in the area of interest, the acetabulum. Extra slices are obtained at specific locations above and below the acetabulum. Regions not covered by patient CT slices are described using Visible Human (NLM, Bethesda, Maryland) CT slices, transformed to match the size and orientation of the patient CT sets. Bony landmark points used in the transformation are selected automatically from the boundaries of the CT images. The finite element models utilize high order (cubic Hermite) basis functions to generate accurate and smooth meshes.

We have applied the algorithm to three patient CT sets (2 males and 1 female) which covered the whole pelvis with 8mm gaps between slices, a total of 27 slices per set. Patient specific meshes constructed using as few as 8 patient slices were constructed. Accuracy of the mesh was tested by calculating root mean squared (RMS) distance between the data points collected from the 27 CT slices (~ 10,000 points) and the fitted surfaces. We have generated smooth FE meshes of the pelvis using only 11 CT slices, achieving a RMS error of less than 3mm. This method will provide a very efficient way for generating models without sacrificing accuracy.

## Abstract 35

### RELATIONSHIP BETWEEN WEAR AND PROGRESSION OF OSTEOLYSIS BASED ON CT, WEAR AND MIGRATION ANALYSIS

\*Stamenkov R, Howie D, Taylor J, Findlay D, Kourlis G, McGee M, Neale S, Pannach S

*\*Dept. Orthopaedics & Trauma, Royal Adelaide Hospital, The University of Adelaide*

Periprosthetic osteolysis remains a significant complication of total hip replacement (THR). Accurate data on the extent and progressive nature of osteolytic lesions is important for the planning of surgical intervention and for monitoring potential medical treatments. The aim of this study was to use quantitative CT, with wear and migration analysis, to determine the progression of osteolysis and to examine the relationship between wear and osteolysis.

EBRA was first used to exclude cases with migrated components to avoid the potential confounding effects of migration on osteolysis measurement. Quantitative CT was used to measure the volume of periacetabular osteolytic lesions and the progression of osteolysis over 12 months. To date, 12 patients (median implant duration 15 years, range 13-16) have been monitored prospectively. Polyethylene wear was determined using Polyware.

Importantly, some osteolytic lesions were quiescent while others progressed markedly. The rate of progression of osteolytic lesions ranged from 0.01 to 10.1 cm<sup>3</sup>/12 months. The increase in osteolysis volume over 12 months correlated strongly with volumetric polyethylene wear rate ( $r=0.71$ ,  $p=0.014$ ). In hips with volumetric wear of less than 50mm<sup>3</sup> /year, the progression of osteolysis was small.

This is the first reported evidence, using quantitative CT and radiographic analysis techniques, which shows differences in the progression of osteolysis with time. This provides important data for patient management. Using this technique, the relationship between the progression of osteolysis and polyethylene wear is more accurately defined. (228/250).

## Abstract 36

### **ULNA DEFECT HEALING WITH A BONE GRAFT SUBSTITUTE AND LOW INTENSITY PULSED ULTRASOUND (LIPUS)**

Stephen PC, Neichoda B, Auld J, Langdown A, Bruce W, Chen J, Yu Y, Pounder N, Walsh, WR

*Orthopaedic Research Laboratories, University of New South Wales, Prince of Wales Hospital, Sydney, Australia*

The potential synergistic effect of LIPUS and bone graft substitutes to improve bone defect healing was explored in this study.

A critical defect model in the adult rabbit ulna was used to compare healing with a tricalcium phosphate bone graft substitute (JAX TCP, Smith & Nephew) and LIPUS. A 1.5 cm defect was made in the right ulna of 18 NZ White Rabbits, and filled with JAX TCP. Half were treated with LIPUS for 20 minutes/day (n = 9). Animals were sacrificed at 4 weeks (n = 3) and 12 weeks (n = 6).

Ulnas were fixitroneed, and graded for evidence of implant resorption and new bone formation. BMD was calculated, and analysed with a 2-way ANOVA. Routine histology (H&E and Trichrome) and immunohistochemistry (Type I collagen, VEGF, PCNA and CBFA-1) was performed.

Defects filled with JAX TCP and treated with LIPUS demonstrated significant amounts of new bone formation compared to controls at 4 and 12 weeks on radiographic analysis. DEXA data revealed a significant increase in BMD with LIPUS treatment at 4 weeks ( $p < 0.05$ ); no difference was observed at 12 weeks. Histological analysis at 4 and 12 weeks confirmed the radiographic findings. Immunohistochemistry confirmed a type I collagenous matrix between the JAX TCP particles in all animals. VEGF, CBFA-1 and PCNA levels were elevated in the LIPUS treated animals compared to controls.

The use of low intensity pulsed ultrasound in combination with bone graft substitutes may offer a viable treatment option in the repair of bony defects.

## Abstract 37

### The Contribution of Cancellous Bone Structure and Turnover to Human Femoral Fragility Fractures

H Tsangari<sup>1,4</sup>, DM Findlay<sup>2,4</sup>, JS Kuliwaba<sup>1,3,4</sup> and NL Fazzalari<sup>1,3,4</sup>

<sup>1</sup>*Div of Tissue Path, IMVS, Adelaide*, <sup>2</sup>*Dept of Ortho & Trauma*, <sup>3</sup>*Dept of Path, Uni of Adelaide* and <sup>4</sup>*Hanson Institute, Adelaide*

Fragility fractures result from reductions in the amount, quality and architecture of bone. However, morphometric investigations have been limited to skeletal sites, which do not fracture, eg. iliac crest bone. Therefore, we have examined bone structure and indices of bone turnover in femoral bone from fractured neck of femur (#NOF) and control individuals. Intertrochanteric bone cores were obtained from 11 female autopsy cases (mean age 76.0±6.3 [SD] yrs) with no evidence of skeletal pathology, and from 15 female patients (mean age 80.7±6.9 yrs) undergoing surgery for a sub-capital femoral fracture. Bone specimens were processed undecalcified into resin and histomorphometry was performed. Interestingly, group comparisons revealed no difference between the female controls and #NOFs at the bone structural level. The static index of bone formation, osteoid surface, was positively correlated with age in the female #NOF group, whereas eroded surface was not age-dependent. In both cohorts, the mean and variance of the bone turnover parameters were greatly increased compared with younger controls. Although eroded and osteoid surfaces were positively correlated in both the female #NOF and control groups ( $r=0.52$ ;  $p<0.05$  and  $r=0.88$ ;  $p<0.001$ , respectively), regression slopes were significantly different ( $p<0.001$ ), suggesting that remodelling may be different in the #NOF group. Although we have previously found differences between #NOF patients and control individuals in the expression of RANK, RANKL and IL-6 mRNA in bone from this skeletal site, these differences did not relate to histomorphometric measures. Ongoing studies will seek to further investigate properties of bone that might contribute to fragility fractures.

## Abstract 38

### INFLUENCE OF IMPLANT FIXATION ON PREDICTED BONE DENSITY CHANGES AROUND A COMPOSITE FEMORAL THA COMPONENT

Turner, A.W.L., Walsh, W.R.

*Orthopaedic Research Laboratories, University of New South Wales, Prince of Wales Hospital, Sydney, Australia.*

In this study, bone remodelling was simulated for a femur implanted with a composite, low bending modulus, fully porous coated stem. The influence of implant–bone ingrowth area on bony remodelling changes was investigated.

Finite element models of a femur and the implant were created from CT scans of a cadaveric human femur and a CAD file obtained from the manufacturer, respectively. Initial bone properties were assigned according to the CT density. A complete joint and muscle force set was used, representing 45% of the gait cycle.

The influence of bone ingrowth area on simulated bone density changes was investigated. The following idealised conditions were examined:

- Fully-bonded contact;
- Proximally-bonded, sliding contact distally; and
- Distally-bonded, sliding contact proximally.

The remodelling simulations were run until remodelling equilibrium. Simulated DEXA images were output and analysed at the seven Gruen zones.

Bone remodelling was simulated out to 6 postoperative years. Most density changes occurred within the first 2 to 3 years for all cases. There was very little difference in bone density changes between the fully- and proximally-bonded implants. For the distally-bonded case, more significant bone density changes, relative to the fully-bonded case, were evident at the medial and lateral zones. Medial bone loss was enhanced for the distally-bonded implant while apposition was increased laterally.

This study shows that distal fixation is unfavourable in terms of proximal–medial bone loss for a primary, uncemented, low modulus stem. For this implant, proximal or full fixation is preferable.

## Abstract 39

### VARIATIONS IN MECHANICAL PROPERTIES ALONG THE LENGTH OF THE EXTRAMUSCULAR TIBIALIS ANTERIOR TENDON

Vizesi, F; Gandevia, S; Walsh, W R

*Orthopaedic Research Laboratories, University of New South Wales, Prince of Wales Hospital, Sydney, NSW, Australia*

This study investigates variations in the biomechanical properties of the extramuscular tibialis anterior (TA) tendon from proximal to distal by mechanical testing of specimens from sheep. Particular emphasis is placed on the toe-region of the stress-strain curve as this is where the tendon is believed to operate under physiological conditions and for which there is limited published work.

TA tendons were harvested from the hind legs of mature wethers and cut down into three regions of interest: A (distal), B (central) and C (proximal). Each sample was tested with a constant force of 1N and allowed to creep for 600s to accommodate the majority of tissue viscoelasticity. A sample size of n=9 was employed for each region. The samples were tested at room temperature with regular spraying of phosphate buffered saline to maintain moisture levels and cell viability. The biomechanical properties measured were stiffness and Young's Modulus.

The central region of the TA tendon was significantly stiffer than the proximal and distal regions by more than a factor two. The cross sectional area of regions A, B and C were not significantly different, so the pattern of Young's Modulus was similar to the stiffness. In all cases, statistical significance was established between regions using ANOVA with  $P < 0.02$ .

Under physiological conditions, the tendon is normally loaded in the low strain region and therefore it is important to consider the mechanical properties under these conditions. The results shown here may be considered for modelling of muscles and joints and for development of tendon prostheses that should have comparable mechanical properties to the native tissue.

## Abstract 40

### **PORCINE SMALL INTESTINE SUBMUCOSA IS NOT AN ACELLULAR COLLAGENOUS MATRIX AND CONTAINS PORCINE DNA: POSSIBLE IMPLICATIONS IN HUMAN IMPLANTATION.**

Jimin Chen<sup>1</sup>, Craig Willers<sup>1</sup>, Ming-Hao Zheng<sup>1</sup>

<sup>1</sup> *School of Surgery and Pathology (Orthopaedics), University of Western Australia*

Porcine small intestinal submucosa (SIS) has been recommended as a cell-free, biocompatible biomaterial for the repair of rotator cuff tendon tear. However, we have observed non-infectious oedema and severe pain in patients who have undergone SIS implantation for tendon repair. The aim of this study was to conduct an independent assessment of the safety and efficacy of Restore™ SIS membrane. The Restore™ orthobiologic implant was examined by histology and the Nested PCR technique using porcine immunoreceptor DAP122 gene to examine if SIS membrane contained porcine cells or DNA respectively. The material was also implanted into mice and rabbits for the evaluation of biological reaction and inflammatory response. Restore™ SIS was found to contain multiple layers of porcine cells. Chloroacetate esterase staining showed that some of these cells were mast cells. Nested PCR of the DAP122 gene demonstrated that Restore™ SIS contained porcine DNA material. Subcutaneous implantation of Restore™ SIS membrane in mice, and in rabbits for rotator cuff tendon repair, showed that the membrane caused an inflammatory reaction characterized by massive lymphocyte infiltration. In conclusion, Restore™ SIS is *not* an acellular collagenous matrix, and contains porcine DNA. Our results contradict the current view that Restore™ SIS is a cell-free biomaterial, and that no inflammatory response is elicited by its implantation. We suggest that further studies should be conducted to evaluate the clinical safety and efficacy of SIS implant biomaterials.

## Abstract 41

### **MATRIX-INDUCED AUTOLOGOUS CHONDROCYTE IMPLANTATION: INFLUENCE OF SEEDING DENSITY.**

Craig Willers<sup>1</sup>, Ming-Hao Zheng<sup>1</sup>

<sup>1</sup> *School of Surgery and Pathology (Orthopaedics), University of Western Australia*

Osteochondral injury accounts for approximately 20% of arthroscopic diagnoses of the knee, mainly located on the medial femoral condyles. Conventional treatment is generally symptomatic and/or characterized by inferior fibrocartilage repair. Matrix-induced Autologous Chondrocyte Implantation (MACI) was implemented to encourage regeneration in osteochondral defects in this study. Twenty rabbits were randomly divided into five equal groups to assess their regenerative outcome following untreated injury, membrane-only MACI treatment, MACI at  $10^4$  cells/ml, MACI at  $10^5$  cells/ml, and MACI at  $10^6$  cells/ml after 8 weeks. During primary surgery, 3x3mm defects were established in the medial femoral condyles and autologous chondrocytes for cultivation and re-implantation. After integration of chondrocytes into the type I/III collagen ACI-Maix membrane, treatment was administered by packing fibrin glue into the osseous compartment and introducing seeded ACI-Maix into the articular cartilage void. Upon sacrifice and processing, regenerative tissues were investigated by H&E, polarised light LM, Alcian Blue (proteoglycan), and type II collagen immunohistochemistry, then scored using a modified ICRS scoring system. Untreated defects could only manage an inferior fibrocartilaginous repair with no restoration of osteochondral architecture at all time points. Membrane-only MACI regeneration was characterised by cartilage of reduced thickness and matrix synthesis. MACI treatment produced regenerative tissue nearly identical across the three seeding densities and at all three time points, with reliable hyaline-like morphology and healthy matrix production. This project suggests that seeding densities below the commercially accepted density of  $10^6$  cells/ml are capable of regenerating tissue of the same calibre. This may be clinically significant in reducing perioperative cultivation times.

## Abstract 42

### **Paradigm of cartilage regeneration by matrix-induced autologous chondrocyte implantation (MACI): a histological assessment.**

Ming-Hao Zheng<sup>1</sup>, Craig Willers<sup>1</sup>, Lyn Kirilak<sup>1</sup>

<sup>1</sup> *School of Surgery and Pathology (Orthopaedics)  
University of Western Australia*

Conventional treatment regimes for articular cartilage injury only manage biomechanically inferior tissue comprised mainly of fibrocartilage. The development of autologous chondrocyte implantation has seen improvements in patients outcomes over conventional therapy, but complications associated with periosteum has led to the search for alternative scaffolds for the seeding of autologous chondrocytes. We have conducted an objective assessment of matrix-induced autologous chondrocyte implantation (MACI) patients by histological examination. Seven biopsies were analysed at 48 hours, 21 days, 6, 8, and 12 months postoperatively. Scanning electron microscopy and RT-PCR confirmed ACI-Maix type I/III collagen membrane efficiently integrates chondrocytes into its matrix and maintains the chondrolineage phenotype (aggrecan and collagen II expression). Results of sequential histology and collagen II staining at the five time points showed that MACI induces the regeneration of cartilage-like tissue as early as 21 days, with hyaline-like cartilage formed at 6 months. In summary, we have shown that MACI is a reliable paradigm for the regeneration of articular cartilage.

## Abstract 43

### **Human Chondrocytes Express Protease-Activated Receptors: Possible Involvement of Thrombin-induced Cell Proliferation and Migration.**

Renzhi Han<sup>1</sup>, Lyn Kirilak<sup>1</sup>, Craig Willers<sup>1</sup>, Ming-Hao Zheng<sup>1</sup>

<sup>1</sup> *School of Surgery and Pathology (Orthopaedics)  
University of Western Australia*

Fibrin sealant, with thrombin as its active component, has recently been recommended for use as tissue glue in autologous chondrocyte implantation (ACI). However, the effect of thrombin on chondrocyte proliferation, and the expression of associated protease-activated receptors (PARs) in human chondrocytes remains unclear. In this study, we sought to investigate the expression of PARs 1-4 within cultured autologous chondrocytes, the effect of thrombin on intracellular calcium and cellular proliferation, and the migration of human chondrocytes in co-culture with fibrin sealant. Immunocytochemistry and RT-PCR was used to assess PARs expression in chondrocytes, thrombin and PAR agonist calcium response was assessed by fluorescence, the effect of thrombin was measured by the alamarblue assay, and chondrocyte migration was evaluated in co-culture with collagen membrane and fibrin sealant. We demonstrated that PARs 1-4 are indeed expressed by cultured human chondrocytes. Thrombin and PAR-1 agonist peptide both induced a fast intracellular Ca<sup>2+</sup> elevation in human chondrocytes. In addition, thrombin, in concentrations ranging from 0.1 U/ml to 1 U/ml, was shown to significantly promote proliferation of cultured human chondrocytes after 48 hours, with maximum efficacy at 1.0 U/ml thrombin. Migration of human chondrocytes into fibrin sealant after co-culture with collagen membrane and fibrin sealant was observed after 48 hours. Taken together, these data suggest thrombin promotes proliferation and migration of human chondrocytes, at least in part through PAR-1 mediated cell signalling. Collectively, this data advocates the use of fibrin sealant as a component of the ACI technique.

## Abstract 44

### **ACTIVATION OF MAP KINASE p38 AND UP-REGULATION OF CYTOKINES TNF- $\alpha$ AND IL-1 $\beta$ AT INJURED GROWTH PLATE**

FH-H Zhou, BK Foster, XF Zhou\*, and CJ Xian

*Department of Orthopaedic Surgery, University of Adelaide Department of Paediatrics, Women's and Children's Hospital, North Adelaide 5006; and \*Flinders University Department of Human Physiology, GPO Box 2100, Adelaide 5001.*

The growth plate cartilage is a fragile area of the growing long bone and has a limited ability to regenerate after fracture. Previously, we have characterised the injury responses and cellular mechanisms for the bony repair of the injured growth plate however, the underlying molecular mechanisms remain unknown. Activated p38 mitogen-activated protein kinase (MAPK) interacts with proinflammatory cytokines to transduce injury induced stress signals and regulate cell functions. In this study, we examined activation of p38 and expression of TNF- $\alpha$  and IL-1 $\beta$  at the injured proximal tibial growth plate of young rats by Western blot analysis, real-time RT-PCR and immunohistochemistry. Results showed 4-6 folds increase in phosphorylation of p38 from 8 to 24 hours post injury and levels declined by day 3. Immunolocalisation of activated p38 showed nuclear and cytoplasmic staining in neutrophils and macrophages at the injury site, and in chondrocytes at the adjacent growth plate. The activation of p38 during this inflammatory phase coincided with up-regulated expression of TNF- $\alpha$  and IL-1 $\beta$  mRNA 24 and 8 hours post injury, respectively. In addition, both TNF- $\alpha$  and IL-1 $\beta$  were immunolocalised in the inflammatory cells at the injury site. Our results suggest that activation of p38 may be associated with up-regulation of IL-1 $\beta$  and TNF- $\alpha$  during the inflammatory response at the injured growth plate. Due to their known functions in affecting cell migration and bone cell differentiation, their up-regulation may play a role in regulating subsequent responses for the bony repair of injured growth plate.

## Abstract 45

### OSTEOGENIC SIGNAL TRANSDUCTION PATHWAYS IS MODULATED BY SURFACE CHEMISTRY MODIFICATION OF TITANIUM IMPLANTS

Zreiqat, H<sup>1</sup>, Valenzuela, S<sup>2</sup>, Evans, P<sup>3</sup>,

*<sup>1</sup>University of New South Wales, School of Medical Sciences, Sydney, 2052, N.S.W, Sydney, Australia*

This study was undertaken to assess possible mechanism(s) by which surface chemistry modification of commonly used orthopaedic material (titanium alloy (Ti-6Al-4V)) with magnesium affect the adhesion, proliferation and differentiation of human bone derived cells (HBDC).

Primary HBDC were obtained from outgrowths of normal trabecular bone removed at primary hip replacement. Bone chips were cultured in  $\alpha$ -minimal essential media supplemented with 10% fetal calf serum and 0.1 M L-ascorbic acid phosphate. Highly polished disks (Ti-6Al-4V, 15mm diameter x 1mm thick) modified with either Mg were used. HBDC were plated on implant disks in serum-free media for 2, 4 and 6 hrs. Scanning electron microscopy (SEM) was performed to determine cell morphology on the different materials. At the predetermined time points, cells were lysed on the specific surfaces, total proteins were extracted and equal protein amounts were separated by SDS/PAGE. Subsequently, Western blotting with anti-type I collagen, -alkaline phosphatase, -actin, -phospho-Erk1/2 and c-fos, and -fra-2 antibodies were conducted.

HBDC cultured on Mg-modified Ti-6Al-4V had a different activation pattern of protein and cytoskeletal elements as well as in the signalling pathway studied. These signalling pathways regulate gene expression for establishing an osteogenic phenotype. Incorporation of Mg into commonly used orthopaedic implants leads to changes in signalling transduction in osteoblasts which may contribute to their increased ability to synthesize matrix proteins and form bone on implant materials. Therefore, surface chemistry modification of metallic implants with divalent cations may alter the molecular component of implant surface and promote optimal osteogenesis

## Abstract 46

### **Osteoprotegerin (OPG) is localised to the Weibel-Palade Bodies of Human Vascular Endothelial Cells and is reduced at sites of inflammatory bone loss.**

Zannettino ACW<sup>1</sup>, Holding CA<sup>2</sup>, Diamond P<sup>1</sup>, Atkins GJ<sup>3</sup>, Findlay DM<sup>3</sup>, Kostakis P<sup>1</sup>, Farrugia A<sup>1</sup>, Gamble J<sup>4</sup> and Haynes DR<sup>2</sup>

<sup>1</sup>*Myeloma and Mesenchymal Research Laboratory, Division of Haematology Hanson Institute, Institute of Medical and Veterinary Science, Frome Road, Adelaide, Australia.*

<sup>2</sup>*Department of Pathology, Adelaide University, Adelaide, South Australia, 5005, Australia.*

<sup>3</sup>*Department of Orthopaedics and Trauma, Adelaide University, North Terrace, Adelaide, South Australia, 5000, Australia.*

<sup>4</sup>*Vascular Biology Laboratory, Division of Human Immunology, Hanson Institute, Institute of Medical and Veterinary Science, Frome Road, Adelaide, Australia.*

Recent studies demonstrate roles for osteoprotegerin (OPG) in both skeletal and extra-skeletal tissues. Although its role in preventing osteoclast formation and activity is well documented, emerging evidence suggests a role of OPG in endothelial cell survival, and the prevention of arterial calcification. This study shows that vascular endothelial cells *in situ*, and human umbilical vein endothelial cells (HUVEC) *in vitro*, express abundant OPG. OPG expression by endothelial cells is markedly reduced in tissue near sites of bone loss in rheumatoid arthritis and periodontal disease. In HUVEC *in vitro*, OPG co-localises with P-selectin, within the Weibel-Palade bodies, where it is detectable using an antibody that recognises the dimeric form of human OPG. Treatment of HUVEC with the pro-inflammatory cytokines, TNF- $\alpha$  and IL-1 $\alpha$  resulted in mobilization and secretion of OPG protein into the culture supernatant. Furthermore, using competitive RT-PCR and OPG-specific ELISA, TNF- $\alpha$  treatment of HUVEC resulted in a sustained increase in OPG mRNA and protein over the 24 hour treatment period. Importantly, reciprocal immunoprecipitation experiments revealed that OPG is physically associated with vWF both within the WBP and following secretion from endothelial cells. Interestingly, this association was also identified in human peripheral blood plasma. In addition to its interaction with vWF, we show that OPG also binds with high avidity to the vWF reductase, thrombospondin (tsp-1), raising the intriguing possibility that OPG may provide an important "link" between tsp-1 and vWF in regulating vWF polymer size. In summary, the intracellular localization of OPG in association with vWF, in HUVEC, together with its rapid and sustained secretory response to inflammatory stimuli, strongly support a modulatory role in vascular injury and inflammation and haemostasis.

## Abstract 47

**Receptor activator of nuclear factor kappaB (RANK) and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) expression in tissues obtained from sites of peri-implant osteolysis characterised by CT.**

**Holdings C.A.(1), Shrestha K.R.(2), Dharmapatni K.(1), Stamenkov R.(2), Neale S.D.(2), Findlay D.M.(2), Atkins G.J. (2) Howie D.W (2) and Haynes D.R.(1)**

*(1) Department of Pathology, Adelaide University, South Australia, 5005, Australia.*

*(2) Department of Orthopaedics and Trauma, Adelaide University, South Australia, 5000, Australia.*

RANK and TNF- $\alpha$  are key factors regulating bone resorption in disease. RANK is a crucial factor that induces osteoclastogenesis. TNF- $\alpha$  stimulates both the inflammatory response and osteoclastogenesis either in synergy with RANK activation or alone. The aim of this study was to investigate the expression of RANK and TNF- $\alpha$  in tissues from sites of osteolysis identified from CT scans of patients undergoing total hip replacement. High-resolution spiral multislice CT, with a metal artefact suppression protocol was used to select tissue and to measure the volume of osteolytic lesions around titanium cementless acetabular and femoral components of total hip arthroplasties. Immunohistochemical analysis of formalin fixed sections from 8 patients undergoing revision of total hip prosthesis was performed using monoclonal antibodies directed against RANK and TNF- $\alpha$  (R&D Systems). Control tissue consisted of synovial tissue samples taken from patients with osteoarthritis. Sections were evaluated by light microscopy and polarised light was used to detect polyethylene particles. Both RANK and TNF- $\alpha$  were strongly expressed in revision tissues adjacent to osteolytic lesions and were associated with large multinucleated osteoclast-like cells, and macrophages. Control tissue stained very weakly for both RANK and TNF- $\alpha$ . Under polarised light, large numbers of polyethylene particles were distinctly visible within the RANK expressing multinucleated osteoclast-like cells. This study demonstrates the expression of both RANK and TNF- $\alpha$  is elevated in tissues from sites of peri-implant osteolysis, and that these molecules are associated with large multinucleated cells that had phagocytosed large numbers of small polyethylene particles, implicating polyethylene wear as a cause of peri-prosthetic osteolysis.

## Abstract 48

### **TRAIL and TRAIL Receptor Expression by Chondrocytes from Degenerate and Healthy Cartilage in Osteoarthritis.**

Anak Agung Sagung Sri Kencana Dharmapatni<sup>1</sup>, David M. Findlay<sup>2</sup>, Andreas Evdokiou<sup>2</sup>, Holding C<sup>1</sup>, Wallwork M<sup>3</sup> David R.Haynes<sup>1</sup>

<sup>1</sup> *Dept. of Pathology, The University of Adelaide, South Australia, Australia*

<sup>2</sup> *Dept. of Orthopaedics and Trauma, The University of Adelaide, and Hanson Institute, South Australia, Australia*

<sup>3</sup> *Adelaide Microscopy, The University of Adelaide, South Australia, Australia*

This study investigated the expression of Tumour Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL) and TRAIL receptors on chondrocytes and cartilage from patients with OA. We also examined the effect of TNF  $\alpha$  and IL1 $\alpha$  on the expression of TRAIL and TRAIL receptors on chondrocytes. The sensitivity of chondrocytes to TRAIL induced apoptosis was also investigated.

Tissue was taken from areas of healthy and degenerate cartilage from patients undergoing total knee replacement. Immunohistochemical detection of TRAIL and TRAIL receptors was carried out on frozen sections of cartilage. Immunofluorescence staining was carried out on cultured chondrocytes. Apoptotic cells after treatment with soluble TRAIL was visualised using DAPI (4',6 diamidino-2 phenylindole dilactate) staining. Cell viability was measured by crystal violet staining.

TRAIL,OPG and TRAIL R4 was expressed in both healthy and degenerate cartilage. Immunofluorescence staining confirmed that chondrocytes constitutively express TRAIL,TRAIL R4 and OPG. Treatment with TNF $\alpha$  but not IL1 $\alpha$ , upregulated TRAIL expression. Both IL1 $\alpha$  and TNF $\alpha$  upregulated expression of TRAIL R4 and OPG. Approximately 10-25 % of the chondrocytes demonstrated apoptosis after TRAIL treatment.

TRAIL can be detected in chondrocytes and both degenerate and healthy part of cartilage from patients with OA. However, only small amount of chondrocytes undergo apoptosis after induction with soluble TRAIL. This may be due to the expression of the decoy receptors OPG and TRAIL R4. These findings indicate that chondrocyte apoptosis mediated by TRAIL may have a role in the degeneration of cartilage in OA but the importance this in OA is yet to be determined.

## **Abstract 49**

### **DEVELOPMENT OF RSA FOR THE IN-VIVO ASSESSMENT OF THE MECHANICAL PROPERTIES OF HEALING FRACTURES**

Mellick J Chehade

*University of Adelaide; Department of Orthopaedics and Trauma, Royal Adelaide Hospital, Adelaide, South Australia*

The accurate in-vivo assessment of the mechanical properties of healing fractures remains a major challenge. Radiostereometric Analysis (RSA) is able to very accurately measure changes in positions of bone or implants using plain radiography. There are a few reports of its use in trauma but only with respect to changes in the position of bone or implant position with respect to time. A measure of fracture stiffness can be determined by simultaneously measuring a deforming force and the resultant change in the position of the tantalum markers defining the position of fracture fragments under investigation. The “dynamic test” position is therefore compared with the unloaded static position. This concept has potential clinical application in many fracture or ligamentous trauma situations allowing clinical management decisions to be made on the basis of accurate objectively determined mechanical data. Defining clinical endpoints to fracture healing and load restraints during fracture healing will be possible. It will also allow objective comparisons between different treatment modalities. A research program is currently underway in Adelaide to establish RSA as both a clinical and research tool in Orthopaedic Trauma and our early experience will be presented.