

**The effect of acquisition slice thickness on volume measurement accuracy using three-dimensional reconstructed MRI:
evaluation in a novel acrylic meniscal cartilage phantom**

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The fundamental influence of base-image acquisition slice thickness (AST) remains a poorly defined contributor to the determination of three-dimensional (3-D) volume measurement accuracy. Using a novel, paired meniscal cartilage phantom, fashioned from a high-density acrylic polymer, this study aimed to quantitate this relationship in the setting of non-invasive evaluation of the adult human knee.

The study-designed meniscal phantoms were submersed in an aqueous medium and subjected to sectional MR analysis, at various ASTs representative of the routine clinical scanning range. Post-imaging, a 3-D reconstructed image of each menisci, at each AST, was created using commercially-available reconstructive software. A volumetric measurement was obtained for each image and directly compared to the known physical parameters of the phantoms, allowing determination of a relative percentage measurement error (PME).

PMEs achieved ranged from 19.42% (4.0 mm) to 1.05% (1.0 mm) and appeared to follow a near-linear relationship with AST. In reflecting on the available literature, it can be suggested that the PME's achieved using the finer slices may have exceeded clinical relevance (i.e. error range unlikely to negatively influence clinical decision making), and hence be considered a highly reliable measure for surgical management.

In summary, the findings of this investigation demonstrate the achievement of highly precise volume measurement accuracy, attainable using conventional MR slice thickness parameters, thus making these results applicable to the majority of routine imaging sites. With the capacity to delve into sub-millimetre ASTs, there is the theoretic potential to further improve volume measurement accuracy, if a clinically-justifiable need can be identified.

Treatment methods for focal chondral defects of the knee-clinical and histologic evaluation

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Various treatment techniques have been developed in recent decades to stimulate the repair of injured articular cartilage. Carbon fibre implantation (CFI), microfracture (MFX), and autologous chondrocyte implantation (ACI) are some techniques for the treatment of chondral defects in the knee. Despite the abundance of studies has been conducted into these individual therapies, there has been no studies that compared these various treatments. We have performed a retrospective single-surgeon study to analyse the histological and functional results of current articular cartilage repair techniques. Twenty-six patients who underwent different cartilage repair procedures of the knee (16 ACI, 5 MFX and 9 CFI) were biopsied at least one year postoperatively. Standard staining (H&E) was used to examine the sections for cellular morphology of repair tissue type. Additionally, immunohistochemistry was used to determine type-I, type-II, type-X collagen, and S-100 expression, and *in situ* hybridization evaluated Sox-9 and aggrecan mRNA expression. Qualitative improvement on the histology and gene expression occurs with ACI (P=0.001) and MFX (P=0.007) over carbon fibre implantation, however there was no significant difference between ACI and MFX (P>0.05). Additionally, the histology and gene expression outcomes of the three different techniques positively correlated to functional outcomes. This study suggests that ACI and MFX have similar clinical efficacies, whereas CFI may be less indicated for chondral repair of the knee.

Evaluation of natural marine sponges as potential bioscaffolds for the attachment, proliferation and differentiation of osteoblastic cells

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The use of bioscaffolds and cell constructs to support cell growth and tissue regeneration is becoming an ever increasing practice in orthopaedic surgery. However, donor availability and the number of scaffolds suitable for the treatment of osseous injury remains limited. In search of potential bioscaffolds for cell-based bone tissue regeneration we have evaluated the use of natural marine sponges to support the growth and differentiation of osteoblastic cells *in vitro*. For this purpose, 5 unidentified sponge species of genus *Hippospongia* (n=1), *Callyspongia* (n=3), and family Chalinidae (n=1) were selected as candidate scaffolds based on i) hydration potential, ii) fiber matrix architecture, and iii) collagenous composition of spongin fibers. Primary osteoblastic cells seeded onto devitalised sponge matrices were assessed for their ability to attach to, invade, and proliferate in each sponge type using a combination of light, confocal and scanning electron microscopy. In short term cultures (7-days), cellular attachment was observed on all 5 species with cells often aligning along the longitudinal axis of sponge fibers. At 14-days, increased cellular invasion and proliferation was apparent, with osteoblastic cells displaying signs of early-phase matrix deposition. By 21-days culture, osteoblastic cells were found to completely bridge interconnecting spongin-fiber pores, with total encapsulation of sponge skeletons observed in some species. Importantly, the osteoblastic phenotype of these cells was confirmed by staining for alkaline phosphatase. Together with preliminary biocompatibility studies our data indicate that natural marine sponge skeletons may offer a potential new source of bioscaffold for the repair of bone injury.

Characterization of bone marrow stem cell (BMSCs) clones and their potential application for bone tissue engineering

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The heterogenous nature of BMSCs and their ability to differentiate into multiple mesenchymal lineages generate considerable research interest in the area of stem cell isolation and differentiation after in vitro expansion of BMSCs. Most studies on the molecular characterization of stem cell/ progenitor are based upon the cells derived from colony forming unit fibroblast, which is an assumption that the unit is derived from a single cell. The present study is based on clone culture of stem and progenitor cells obtained from single BMSC cell.

Single cell clone cultures were established in 96 well plates. A total of fourteen fast growing robust clones were selected from three bone marrow samples. It was noted that time taken for 30 population doublings varied for each clone ranging from as less as 35 days to 107 days. Evaluation of their differentiation potential showed 5 clones to be tripotential, 8 appeared to be bipotential and the remaining 1 clone differentiated into only one lineage which could be a committed progenitor. These findings have been further confirmed by the expression of differentiation genes. Flowcytometric analysis showed that the clonal populations have a marker expression that is similar to that of BMSC. Most of them readily expressed CD29, CD44, CD90 and CD105 and are negative for hematopoietic markers like CD34, CD45. This study has identified the most preferred cells that could be used for tissue engineering purposes.

TOWARDS BETTER CUTTING TOOLS FOR CANCELLOUS BONE AUTOGRAFT

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The harvesting of autogenous cancellous bone graft often leads to trauma at the donor site, resulting in chronic pain. Heat generation and excessive bone fragmentation during cutting will also increase cell mortality within the graft. These complications can be mitigated by improving cutter design. Cancellous bone graft is commonly harvested using rotational cutting tools. However it is not easy to visualize the cutting process during rotational cutting.

To obtain a better understanding of the mechanics of cancellous bone cutting we have performed orthogonal cutting experiments, using an instrumented laboratory milling machine, on bone samples taken from fresh, frozen bovine femur and patella. Measurements were performed with tool rake angles of 23°, 45° and 60°, and depths of cut in the range 0.1-3mm. Horizontal and vertical cutting forces were measured, and the chip formation process visually recorded.

Continuous and discontinuous chip formation was observed for cutting depths larger than 0.5mm. Chip formation at depths above 1mm was accompanied by bone marrow extruding out of the bone free surface and away from the rake face. This might be beneficial as the bone cells contained in the marrow get carried away from the hot region of cutting. The specific cutting energy was generally low for larger rake angles, and larger depths of cut.

Our results suggest a good choice of rake angle to be about 45 degrees with a depth of cut of not less than 1mm, to ensure continuous chip formation (avoiding excessive fragmentation), bone marrow extrusion and low specific cutting energies.

Accuracy of a computer-assisted navigation system for total knee replacement

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The purpose of our study was to assess the accuracy of an image-free TKR navigation system: 1) in a leg with a normal mechanical axis and, 2) in a leg with an abnormal mechanical axis.

We evaluated the Ci-CAS TKR navigation system of BrainLab and DePuy International. An artificial leg (phantom) was constructed to simulate hip and knee joints. The juxta-articular knee surfaces were composed of a synthetic material (Sawbones – Pacific Laboratories). A highly accurate digital calliper unit (FaroARM Technologies, USA) was used to measure co-ordinates of pre-machined points on the phantom. A software program was developed to calculate actual leg alignment angles. This technique was verified using repeated measurement with variable co-ordinates, giving accuracy to within 0.05 of a degree.

Simulated procedures were then performed with both a normal and abnormal leg mechanical axis. At specific points in the procedure, information was compared between the FaroARM digital measurements and the Ci-CAS system. Repeated serial measurements were undertaken. In the setting of normal alignment, accuracy to within 0.5 degrees was demonstrated. In the setting of abnormal x,y and z plane alignment in both femur and tibia, accuracy to within 0.5 degrees was demonstrated.

This is the first study to assess the accuracy of a clinically validated navigation system. The study demonstrates the high level of in-vitro accuracy of the Ci navigation system in both normal and abnormal mechanical leg alignment settings.

IN VITRO ANALYSIS OF ANTIFUNGAL IMPREGNATED POLYMETHYLMETHACRYLATE BONE CEMENT

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Fungal infection of total joint arthroplasty is a rare but devastating complication. The purpose of this study is to examine the change in mechanical properties with the inclusion of various concentrations of anti-fungal in the cement as well as the release rate from the cement.

Amphotericin powder was mixed in different concentrations with Tobramycin-impregnated Simplex (Stryker). Compressive strength and modulus of standardised specimens was measured. The cumulative dose of amphotericin and tobramycin released measured from various cement/ Amphotericin formulations was measured using UV-V spectrometry.

Incorporation of Amphotericin into the cement showed a statistically increased compressive strength from 107MPa to 123MPa ($p < .001$) with 4 mixes of antifungal per packet. There was no statistically relevant change in modulus or strain to failure. At 72 hours no significant elution of antifungal (<1%) had occurred and the cumulative release of antibiotic had reached a maximum.

Contrary to expectations the mechanical properties of PMMA cement are improved by the addition of Amphotericin B, however the elution of the antifungal is negligible. This is most likely due to the conjugated unsaturation found in amphotericin molecule which will readily react with methyl methacrylate during cement cure. This results in an increase in crosslink density in the bone cement, subsequently improving mechanical strength. The covalent bonds formed between the amphotericin and the cement prevents it from eluting. We suggest that this is not a valid method for treatment of deep fungal infections.

THERMO-MECHANICAL INVESTIGATION OF THE SHORT GLASS FIBRE REINFORCED EPOXY USED AS THE CORTICAL BONE ANALOGUE IN SAWBONES FEMURS

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Tensile and four point bending tests were used to assess the material properties of the short glass fibre reinforced (SGFR) epoxy resin that are used as the cortical analogue in third generation *Sawbones*, at both room (22°C) and body temperatures (37°C).

Standardised specimens were machined from the shaft of *Sawbones* femurs obtaining sections from the anterior, posterior, lateral and medial quadrants in the long axis of the femur. The flexural properties of the specimens were determined using ASTM D6272-02 and the tensile properties were obtained using ASTM D638-02.

The mean(SD) values of the modulus of elasticity in four point bending for room and body temperature specimens were 10.74(0.88)GPa and 3.98(0.9)GPa respectively (P<0.001). The mean(SD) values of the modulus of elasticity in tension for the room and body temperature specimens were 9.4(0.8)GPa and 5.4(1.3)GPa respectively (P=0.02).

This research demonstrates that the modulus of elasticity of SGFR epoxy used in third generation *Sawbones* is highly temperature dependent. A reduction in modulus of elasticity of up to 63 percent was observed when increasing the temperature of the specimens from room to body temperature. When performing *in vitro* total hip modelling ideally the specimens should be at 37°C due to the properties of PMMA cement, however SGFR epoxy *Sawbones* do not accurately represent bone at this temperature. Hence, this material is not an appropriate model for testing the mechanics of implants that use bone cement.

STRAIN IN THE PROXIMAL FEMUR: THE EFFECT OF EXETER STEM IMPLANTATION AND POSITION

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This investigation has been designed to determine the strain patterns in the proximal femur for an intact non-implanted native femur (three specimens) and to compare this to the strain of the proximal implanted femur for both neutral (five specimens) and varus (four specimens) stem geometries.

Sawbones femurs were implanted with Exeter stems. Implant position with respect to the femur was controlled using specially designed positioning devices. Implanted specimens were instrumented with strain rosettes positioned along the lateral and medial aspect of the femur with the third rosette positioned at the medial calcar. A static anatomical loading vector of 650N was applied to the head of the implant for 7½ minutes. Data was gathered during the application of the load and for a further 7½ minutes following the removal of the load.

There was a significant variation in longitudinal strain at the medial calcar with a strain of $-1800\mu\epsilon$ observed on the native femur specimens and $-430\mu\epsilon$ and $-360\mu\epsilon$ measured on the neutral ($P=0.04$) and varus ($P=0.02$) stem position specimens respectively. However, there was no significant difference between the strains of the neutral and varus stem position specimens in the longitudinal direction at the calcar. The magnitudes of the circumferential strains reduce for both the neutral and varus stem positions when compared to the tensile native femur strain. The similarity of the strain at the medial calcar for the neutral and varus specimens may explain why varus malposition of the Exeter stem has not been associated with adverse clinical outcomes.

TENDON TRANSFER FIXATION IN THE FOOT

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Purpose: This study compares a tendon to tendon technique vs. a bioabsorbable interference-fit screw (BIORCI 7X20mm, Smith & Nephew, Memphis, USA) with and without Krakow suture for the fixation of tendon to bone in tibialis posterior reconstructions. The goal is to provide reproducible stable fixation that maintains the tendon in the operatively placed position. We compared the failure load of the various techniques employed for fixation. Investigating current techniques is important to qualify their application. Methods: Eighteen human naviculi were dissected from fresh frozen cadaver feet. Eighteen ovine tendons from same aged sheep were obtained. These were divided into three groups (n=6) representing 1. tendon to tendon group 2. bioabsorbable interference-fit screw 3. bioabsorbable interference-fit screw with Krakow suture. The tendons were reconstructed with the navicular using the various techniques and tested in tension (displacement of 1mm/sec) until failure. Results: The tendon to tendon group had the highest average load to failure (96.9 ± 24.3 N) followed by the interference-fit screw with Krakow suture (63.2 ± 31.5 N). The interference-fit screw-only group had the lowest load to failure (33.4 ± 15.7 N). All bioabsorbable interference-fit screw fixations failed by tendon pull-out regardless of Krakow suture being present. Tendon to tendon fixation, although strongest, is not always desirable due to operative limitations. Using bioabsorbable screws result in decreased operative dissection. Conclusions: Using interference screws showed decreased fixation strength but incorporating the Krakow suture effectively doubled the strength of fixation. This is a simple yet effective method to increase the fixation if this is an issue.

BONE MARKERS IN OSTEOARTHRITIS

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Osteoarthritis (OA) is characterised by progressive degenerative damage to the articular joint cartilage. Evidence is accumulating that bone changes in primary OA may be important in the disease etiology, and that changes in subchondral bone may actually precede cartilage changes. The aim of our study was to measure biochemical markers of bone turnover, and to relate the results to other clinical parameters, in patients with severe OA of the hip.

Blood and urine were collected from patients presenting for hip replacement surgery, both at pre-admission clinic and on the day of surgery, 3-4 weeks later. The markers measured were serum creatinine, osteocalcin, alkaline phosphatase, and urinary pyridinoline and deoxypyridinoline. Serum OPG and RANKL, important molecules in regulating bone turnover, were measured using a commercial sandwich ELISA assay. Patient details recorded included whether their OA was localized or general, co-morbidities, BMI, age and gender.

Analysis of 55 patients found that levels of these biochemical markers were closely similar at the two sampling times, with the exception of osteocalcin, which varied between fasting and non-fasting states. Importantly, 21%, 32% and 40% of the measured values for alkaline phosphatase, urinary pyridinoline and deoxypyridinoline respectively were greater than the normal value range for these markers, suggesting that OA may be a disease of increased bone turnover rate.

This information will be of great assistance in further elucidating the mechanisms that lead to bone changes in OA and other diseases of the skeleton. We are also hopeful that a particular profile of bone markers may be diagnostic of OA and may even be useful as an early indication of the development of this disease.

Effect of screw insertion torque level on cortical bone pullout strength

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During insertion, screw torque can reach levels at which bone failure and stripping occurs. Surgeons reportedly induce torque levels surprisingly close to this limit, tightening to an average of 86% of failure torque. Although high screw forces are desirable for stability, a tradeoff exists between torque and screw force levels, cumulative damage from tightening, and subsequent reduction in holding strength of surrounding bone. The objective of this study was to determine the effect of insertion torque level on bone material properties as assessed by screw pullout strength. Ten pairs of ovine tibiae were tested. One side of each pair was randomly selected for measuring ultimate failure torque (T_{max}) at 3-4 shaft sites along the flat medial aspect. T_{max} values were normalized by bone density (measured by DXA) and used to predict T_{max} at contralateral tibia sites. Screws were inserted at 50%, 70% and 90% of predicted T_{max} ; pullout tests were immediately performed and strength values normalized by cortical thickness. Results showed that 90% T_{max} could be beyond the yield point and reduce pullout strength. Limitations include small sample size and inexact estimation of T_{max} . However, one single test at 90% T_{max} indicating a 50% loss in stiffness (estimated by torque-rotation data) resulted in a pullout strength value 40% less than the average 70% T_{max} pullout strength. Analysis of failure tests indicate tightening to 86% T_{max} leads to an average 50% loss in stiffness. We conclude that screw tightening to clinical levels may lead to damage sufficient to compromise holding strength in surrounding cortical bone.

REDUCING THE FRACTURE MISS RATE: AIDING DIAGNOSIS THROUGH IMAGE PROCESSING

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Accurate detection and evaluation of musculoskeletal trauma is often problematic, regardless of the treating physician's level of experience. While bone fractures are a relatively common occurrence, their presence can often be missed during x-ray diagnosis. For a trauma patient, a delay in diagnosis can lead to ineffective patient management, increased dissatisfaction, and expensive litigation. Fast and accurate detection of long bone fractures is therefore an important orthopaedic and radiologic problem. We have developed a computer aided fracture detection system - initially for the detection of long bone fractures - that in the future will help lower the miss rate.

We have created a number of image processing software algorithms useful for automating the fracture detection process. The first of these is a tool for fully-automatic long bone segmentation that is capable of identifying boundaries - as defined by the AO - between the metaphyseal and diaphyseal segments in an x-ray image. Anatomic structure varies markedly between the segmented regions so different fracture detection criteria are required. Our tools are then able to accurately detect abnormalities, including fractures, in the diaphyseal segment of the longbone. Our experiments on a set of sample images show that these tools can consistently identify the boundaries between bone segments, and then accurately highlight mid-shaft long bone fractures within the marked diaphysis. We believe that in the future this type of scheme will help reduce the number of fractures that are missed during the reading process.

MEASUREMENT OF ACETABULAR MICROMOTION IN A CANINE MODEL OF TOTAL HIP ARTHROPLASTY

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This study assessed peri-acetabular fixation in an *in vivo* low morbidity model of focal osteolysis. Seven dogs received unilateral uncemented total hip arthroplasties with 220µg of polyethylene particles added behind the cup.

The harvested acetabular specimens were potted in polymethylmethacrylate in a jig allowing four motion transducers to contact the bone 2mm sub-adjacent to the metal backing of the cup in test 1, and the metal of the cup in test 2. This allowed the relative micromotion of the cup and bone to be calculated after applying compressive loads of up to 1.5 bodyweights using a servo-hydraulic materials testing machine (Instron 8511).

In five specimens minimal micromotion (<20µm) was observed, well within the level thought to indicate bony fixation. The other two specimens displayed levels between 50 and 100µm.

The contact radiographs indicated good bony support (in the range of 25-30% bone apposition) for three components. For the remainder, there was no bony fixation. Two of the non-contact specimens were known to be grossly loose prior to testing, and corresponded to the components with higher micromotion measurements.

The other two components with no bony fixation, but supposedly excellent micromotion levels, indicate that the method of micromotion assessment may have over-constrained the components. The micromotion data, in combination with the contact radiographs, suggest that the purely compressive loading had a stabilizing effect.

The prognostic value of microvessel density and angiogenic factor expression in human osteosarcoma

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Despite recent advancements in neoadjuvant and adjuvant chemotherapy for osteosarcoma, 25-50% of patients, without evidence of systemic disease at initial diagnosis, subsequently develop metastatic disease, and this remains the major cause of death from this tumour. Several factors that have been identified as independent prognostic markers in osteosarcoma, however the role of angiogenesis still remains a topic of debate. This study aims to evaluate the significance of the degree of angiogenesis and expression of angiogenesis-related genes in osteosarcoma and correlate it with long-term clinical outcome. Archival pre-chemotherapy biopsy tissue of 25 patients with osteosarcoma that were treated at St. Vincent's Hospital, Melbourne was reviewed and the tissue was processed for immunohistochemical identification of microvascular endothelial cells with antibodies directed against CD31 and CD34. Assessment was made of the degree of angiogenesis, as determined by the microvessel density (MVD), and further histological examination was performed looking at the immunohistochemical expression pattern of VEGF (pro-angiogenic factor) and PEDF (anti-angiogenic factor). This was then correlated with patient outcome, in terms of recurrence, metastasis and death. We hypothesise that increased vascularity in osteosarcoma is associated with increased risk of metastasis and poor prognosis and that potential molecular defects in key endogenous anti-angiogenic factors normally expressed in osteoblasts are likely to be responsible for the increased angiogenesis seen in osteosarcoma.

Abbreviations: PEDF – pigment epithelium derived factor; VEGF – vascular endothelium growth factor

Caffeic acid phenethyl ester, a natural component of honeybee propolis induces osteoclast apoptosis and attenuates osteoclastogenesis via the suppression of RANKL-induced NF- κ B and NFAT activity

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NF- κ B is a key regulator of osteoclast differentiation, activation and survival. Caffeic acid phenethyl ester (CAPE), a natural NF- κ B inhibitor from honeybee propolis has been shown to have anti-tumor and anti-inflammatory properties. However, the effect of CAPE on osteoclast formation and RANKL signaling is unknown. In this study, we investigated its effect on the regulation of RANKL-induced osteoclastogenesis and osteoclast survival. Both RAW264.7 cells and primary bone marrow cells were used to examine the effect of CAPE on RANKL-induced osteoclastogenesis. In order to determine the action of CAPE on signaling pathways, we used reporter gene assays for NF- κ B and NFAT activity, and Western blotting for phospho-IK β . To assess rates of apoptosis we measured changes in annexin staining, caspase-3 activity, chromatin and microtubule structure. Our results showed that low concentrations of CAPE (<0.5 μ M) dose dependently inhibited RANKL-induced osteoclastogenesis in RAW 264.7 cells and in bone marrow cell cultures. At higher concentrations, CAPE induced apoptosis of RAW 264.7 cells and RAW 264.7 cell-derived osteoclast like cells (OLCs). Consistently, we found that CAPE increases caspase-3 activity and disrupts the microtubule network in OLCs. Furthermore, CAPE inhibited both basal and RANKL-induced NF- κ B and NFAT activation in a dose dependent manner. This study implies that attenuation of osteoclastogenesis and induction of osteoclast apoptosis through the inhibition of NF- κ B and NFAT activation by this natural compound might be useful for the treatment of osteolysis attended with enhanced osteoclast formation and activation.

Biomarker discovery in osteoarthritis

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Purpose: Osteoarthritis (OA) is the most common joint disorder, producing pain and disability that leads eventually to the destruction of articular cartilage. Although many of the risk factors for OA have been defined (genetic factors, BMI, female sex), the etiology of this disease is poorly understood. Recent data, from examination of bone in OA, from MRI and from circulating bone markers, suggest a role for subchondral bone in initiation and progression of OA. Sensitive disease markers, which will permit diagnosis of OA during the early pre-radiologic stages, and which facilitate monitoring of disease progression, are urgently required. In this study, surface enhanced laser desorption/ionization time-of-flight-mass spectroscopy (SELDI TOF MS) was used to screen for specific serum biomarkers in end-stage OA patients presenting for hip replacement surgery.

Methods: Serum samples from 31 OA patients (median age of 67 years) and 30 age-matched unaffected patients (median age of 64.5 years) were fractionated based on charge (6 fractions) and SELDI spectra were collected from fraction 1 of each patient, in triplicate, and in three molecular weight ranges [1.5-10 kDa(low), 6.5-30 kDa(mid) & 25-150 kDa(high)].

Results: All spectral data were normalized, standardized and subjected to multivariate statistical analysis and data mining procedures, ultimately leading to the detection of a number of peaks that optimally separated the two groups. Multiple peaks were identified from each mass range and combined across all three mass ranges in each patient sample. Twenty-two specific peaks were identified that could discriminate between the two sample sets; 10 peaks (m/z 2-9 kDa), 7 peaks (m/z 7-16 kDa) and 5 peaks (m/z 31-71 kDa) were significantly different ($p < 0.05$) between the two groups. This profile represents a diagnostic fingerprint of the multiple biomarkers that discriminates OA from normal controls. Ongoing analyses involve the correlation of the SELDI profile with patient characteristics.

Conclusions: This study has potentially identified a panel of biomarkers that represents a diagnostic fingerprint of risk factors and/or predictors of OA progression, which we will now seek to validate in patients earlier in disease progression. The identification of validated biomarkers will greatly accelerate therapeutic development for this major public health concern.

Relationship between progression of osteolysis adjacent to hip prostheses and POLYETHYLENE wear

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Purpose: Knowledge of the extent and progression of peri-prosthetic osteolysis (PPO) is important in monitoring and surgical management of total joint replacement patients, and to determine effects of medical treatments. The aim of this study was to use quantitative computed tomography (CT), with wear and migration analyses, to determine the rate of progression of PPO and its relationship to wear of the polyethylene (PE) cup.

Methods: PPO adjacent to 19 well-fixed Harris-Galante acetabular components was measured at 12-month intervals. Migration analyses were used to exclude cases with migrated components. Volumetric PE wear was determined from digitised X-rays using the Polyware software program. Patient-related factors including age, gender, BMI, activity levels, comorbidities, and joint pain and function were recorded from our Joint Replacement Database.

Results: Lesions in many of these patients were relatively quiescent, while others progressed markedly over a one year period. There was a significant association between progression of osteolysis and total PE wear, PE wear rate and volumetric PE wear in the 12 month period between CT scans ($p=0.026$, $p=0.025$ and $p=0.035$, respectively). None of the other covariates examined was significantly associated with the progression of osteolysis.

Conclusions: CT measurement of PPO progression and accurate estimates of cup wear and stability provide important information to guide clinical management of total hip replacement patients. The data support the involvement of PE particles in bone destruction in these individuals.

MECHANICAL AND ELECTRICAL ENVIRONMENTS TO STIMULATE BONE CELL DEVELOPMENT

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The aim of this project was to evaluate the effects of mechanical strain and indirect electrical stimulation upon the development of bone forming osteoblast cells and any possible synergistic effects of the two stimulants. This aim was achieved by using a novel device, designed and developed with the capability of creating a cell substrate surface strain along with an exogenous electrical stimulant individually or at the same time. Proliferation and differentiation was determined as a measure of cellular development. The indirect electrical stimulation was achieved through the use of pulsed electromagnetic field (PEMF) stimulation while the mechanical strain was produced from the dynamic stretching of a deformable cell substrate. The PEMF signal mimicked a clinically available bone growth stimulator signal. Results showed reduced proliferation and increased differentiation (alkaline phosphatase activity) with SaOS-2 osteoblast-like cell cultures, which were exposed to indirect electrical stimulation. MG-63 osteoblast-like cell cultures also showed reduced proliferation, however they did not show an increase in their differentiation with PEMF exposure. Mechanical stimulation alone did not have a significant effect over either proliferation or differentiation, while a dual mechanical and electrical stimulation resulted in cellular differentiation significantly increasing. It is possible a synergistic interaction between the two stimulants is occurring on a biological level.

Computer assisted tightening of cancellous bone screws

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Internal fixation of fractures often requires the tightening of bone screws to stabilise fragments. Inadequate torque can leave the fracture unstable, while over-tightening results in thread stripping and loss of fixation. The optimal amount of screw torque is specific to each application and is difficult to attain in practice due to the wide variability in bone properties. The aim of this project was to develop an automated system for sensing the properties of a material through its interaction with a bone screw, and to use this data to determine an appropriate level of tightening. A custom test rig was designed and built for bone screw experiments. We established that differences in synthetic bone material density of 0.1 gm/cc in range corresponding to osteoporotic bone could be automatically detected through effects on the rotational characteristics of a cancellous bone screw. Based on this detection, an electric motor controller was demonstrated to change driver speed and stop implant tightening at a variable level corresponding to material density. Ovine cancellous bone specimens were then tested to evaluate the method given the continuously variable density and interface characteristics of bone. Results indicated that plateau current measured during screw insertion is directly related to bone density and is a strong predictor of peak current. Based on these relationships, a control system was programmed and subsequent shutoff tests results were encouraging. We have demonstrated that bone density can be automatically detected through screw rotational characteristics, establishing the basis for adaptive surgical control of screw tightening.

Orthopaedic utilization of 3-DMR imaging:

An investigation in the South Australian clinical setting.

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Despite widespread availability of the basic technology required to convert sectional magnetic resonance (MR) imaging data to three-dimensional (3-D) computer displays, and the increasing frequency of reported use internationally, 3-DMR remains a grossly under-utilized resource in the Australian orthopaedic setting. The purpose of this investigation, performed as a survey-style questionnaire analysis, was to gauge the current level of clinical application of 3-DMR technology in the local environment and to explore barriers to more widespread adoption.

A list of clinical facilities performing onsite orthopaedic surgery in South Australia (SA) was obtained from the Australian Orthopaedic Association. The respective Department Head (public facilities) or Medical Director (private facilities) was identified in each instance, and a study-specific survey questionnaire was forwarded to these individuals. A 92.9% response rate was achieved.

Review of the responses demonstrated that most orthopaedic surgical sites already have the capacity to generate 3-D images from sectionally-acquired data, although such advancements are rarely extended to MR imaging. All respondents suggested a willingness to consider opportunities to incorporate 3-DMR into routine practice at their respective clinical sites, in-part bolstered by the previously-demonstrated advantages of using parallel CT-based applications, if the technology was made readily available and supported locally.

In summary, these findings suggest that there remains great opportunity to explore the further integration and more widespread application of 3-DMR in orthopaedic practice. To best facilitate this, orthopaedic centers require the demonstration of robust scientific validation, cost-effective integration into existing patient management pathways, time-efficacy, and readily available local technical support.

Measuring Patellar Kinematics using Single-Plane Radiostereometric Analysis (RSA) Fluoroscopy

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The aim of this study is to seek an answer to the following question: Can the *in-vivo* kinematics of the patellar be measured following total knee replacement (TKR) surgery using single-plane Radiostereometric analysis (RSA) fluoroscopy? Many complications following TKR are associated with abnormal patellar tracking and measuring its motion would enable the collection of evidence-based data to allow the quantitative comparison between different patellar treatments.

A human subject was implanted with a Profix TKR (Smith & Nephew, Warsaw, IN) and tantalum RSA markers were implanted in the anterior patellar, in the femur slightly proximal to the femoral component, in the tibia slightly distal to the tibial component and in the tibial polyethylene. A standard RSA examination, conventionally used for migration and wear measurements, was performed with the UmRSA software (RSA Biomedical AG, Umeå, Sweden) to initially determine the relative positions of the implanted RSA markers. The human subject was then asked to ascend a single-step during low-dose fluoroscopy screening under full weight-bearing conditions. Fluoroscopy images were acquired at 7.5 frames per second and allowed a fully dynamic reconstruction of patellofemoral motion during the step ascent activity.

The patellar was found to translate 10 mm laterally relative to the femur between 0-30 degrees knee flexion, prior to translating 15 mm medially between 30-80 degrees of knee flexion. During 0-80 degrees of knee flexion, the tibia was found to rotate internally 16 degrees. We postulate that the patellar translated medially after engagement in the femoral groove at 30 degrees of knee flexion due to the internal rotation of the tibia exerting forces on the patellar ligament that connects the anterior tibial tubercle with the patellar apex (the distal region of patellar).

The patellar was also found to rotate 3 degrees laterally between 0-30 degrees knee flexion before rotating 5 degrees medially between 30-80 degrees knee flexion. This type of movement is in contrast to the normal patellar, of which *in-vitro* studies predict¹ that little or no patellar rotation occurs during knee flexion.

This work demonstrates that it is possible to measure *in-vivo* patellar kinematics using single-plane RSA fluoroscopy. The *in-vivo* measurements performed in this work are in accordance with the results of previous *in-vitro* measurements that have been reported in the literature¹. This work is part of a greater study that is analysing the patellofemoral kinematics of groups of patients following implantation with different types of TKR.

1. Chew J.T.H, Stewart N.J., Hanssen A.D., Luo Z.-P., Rand A., An K.-N., Differences in patellar tracking and knee kinematics among three different designs. *Clin. Orthop.* 2003;345:87-98

Baseline Polyethylene Tibial Component Wear Measurements using Single-Plane Radiostereometric Analysis (RSA) Fluoroscopy

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The aim of this study is to measure the *in-vivo* distal penetration of the femoral component into the polyethylene tibial component following total knee replacement (TKR) surgery. Wear debris from tibial polyethylene inserts is thought to be a major factor in aseptic loosening of TKR and fractures and delamination of the tibial polyethylene also represent a major problem. The *in-vivo* measurement of such wear and polyethylene damage is essential for improving the articulation and longevity in TKR.

Four human subjects were implanted with a Profix (Smith & Nephew Inc., Memphis TN) TKR, with tantalum RSA markers implanted in the femur slightly proximally to the femoral component and in the tibial polyethylene. A standard RSA examination was performed with the UmRSA software (RSA Biomedical AG, Umeå, Sweden) to determine the relative positions of the implanted RSA markers. The human subjects were asked to perform a clinical procedure that consisted of flexing and extending their leg approximately 20 degrees under full weight-bearing conditions during fluoroscopy screening. Fluoroscopy images were acquired at 7.5 frames per second and allowed fully dynamic reconstruction of tibiofemoral motion during the shortened knee flexion activity. Displacement curves are produced by examining the distance between the femoral and tibial components as a function of knee flexion. Current results are limited to initial baseline displacement curves.

The *in-vivo* measurement of wear has to be measured as a decrease in distance between the femoral and tibial components. In a knee replacement this is not as easy as in a hip with a circular ball joint. This for several reasons: Firstly, it is almost impossible to obtain exactly the same position of the components at two different examinations. Any difference in anteroposterior position and flexion of femur on tibia will affect the measurements. Secondly, the polyethylene will not wear in a circular spot but over the sliding distance of the femoral condyles. Articulating the knee under load whilst performing dynamic measurements and plotting displacement against the flexion angle therefore serves many purposes: To align the components reproducibly, measure wear over the entire sliding distance, compensate for various flexion angles and finally, multiple readings will give a more correct mean value for the curve.

This is an ongoing, long-term clinical study. We expect that the femoral penetration, measured using displacement curves at subsequent clinical examinations, will deepen with time to represent wear of the polyethylene tibial component.

Gene expression of passaged human articular chondrocytes

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Abstract:

Autologous chondrocyte implantation (ACI) is considered to be one of the best cellular engineering approaches for the treatment of articular cartilage injury. ACI is able to repair cartilage defects with hyaline-like histology and improved function over conventional surgical treatments. ACI employs in vitro cultured autologous chondrocytes to repair articular cartilage injury, but the biosynthetic profile of cultured chondrocytes has been shown to be altered during in vitro monolayer cultivation. Thus, this study investigated the expression profile of several chondrocyte associated gene clusters in serially passaged human articular chondrocytes by quantitative real-time PCR. The gene clusters include extracellular matrix proteins (aggrecan, type I collagen, type II collagen, type X collagen, fibromodulin, fibronectin, and link protein), matrix proteinases (MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4 and ADAMTS-5), proteinase inhibitors (TIMP-1, TIMP-2 and TIMP-3), cytokines (IL-1 β , TGF β , TNF α , and IGF-1), transcription factors (Sox-9, c-fos and c-jun), and intercellular signaling (COX-2, MAPk1, and NOS2). Results obtained by clustering analysis (Euclidean distance) showed that with increasing passage number, the gene expression of the matrix proteins aggrecan, type II collagen, and fibromodulin decreased, while fibronectin and link protein increased. Matrix proteinases, MMP3, 9, 13 and ADAMTS-4, 5, decreased expression especially to passage 6, whilst the proteinase inhibitors, TIMP1, 2, 3, remained constant. Cytokine IL-1 also showed increased expression with serial chondrocyte culture. No significant alternation in TNF- α , TGF- β , IGF-1, or transcriptional factors, Sox-9, c-fos, or c-jun expression were observed. These results suggest that the chondrocytic gene expression profile is altered at various degrees with increasing passage number, though the gene expression levels of transcriptional factors which contribute to hyaline cartilage regeneration remain unchanged. This data may prove important for the future development of a more specific and efficacious cultivation technique for human articular chondrocyte-based therapies.

EVIDENCE OF CIRCULATING DONOR MATERIALS IN RECIPIENTS OF BONE ALLOGRAFT

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Our aim was to investigate the possible transfer of cellular material with the use of bone allograft, by detecting the presence of donor DNA in recipient plasma. Fifty one female patients who received bone allograft from male donors were included in the study. Forty patients who received allograft from the Perth Bone and Tissue Bank were identified, with a blood sample analysed between 6 weeks and 18 months of surgery. In addition, 11 patients were recruited from the Prince of Wales Hospital, Hong Kong SAR. These patients had blood samples analysed both preoperatively and at 1 day, 1, 2, and 3 months postoperatively. The blood samples were analysed via Polymerase Chain Reaction, (PCR), for the presence of the SRY gene on the Y Chromosome. Of the total 51 female patients receiving bone allograft from a male donor, 6 tested positive for the SRY sequence. Of the 6 positive patients, 5 were positive at day 1 postoperatively and negative thereafter, with the remaining patient positive at 3 months postoperatively. All patients received cancellous bone allograft. No preoperative specimens were positive. Our results document, for the first time, the presence of donor DNA in recipient circulation after bone allograft use. This occurs despite thorough preparation of the bone allograft designed to render the graft free of antigenic material. The significance of this finding is unclear. The positive findings at day 1 may be a consequence of operative handling of the graft, while the positive result at 3 months may reflect bone allograft physiology, possibly incorporation.

The role of urothelium in the ectopic formation of bone in skeletal muscle.

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This research aims to study the bone forming reaction that occurs when the lining of the urinary bladder is transplanted into skeletal muscle. By obtaining a greater understanding of this reaction, it may be possible to define and isolate further factors that may be applied as osteoinductive agents in orthopaedic surgery (such as bone morphogenetic proteins).

Using species that have demonstrated strong osteogenic potential, three different series will see three types of tissue from guinea pigs (urothelium, cultured urothelial cells, and cultured urothelial cells labelled with green fluorescent protein), transplanted into congenitally immunodeficient mice (SCID-mice). Mice will then be sacrificed after six weeks for implant site tissue studies.

It is anticipated that transplanted urothelial tissue will induce ectopic ossification in skeletal muscle. It is also anticipated that cultured urothelial cells will induce ectopic ossification, demonstrating that only urothelial cells (and not underlying stroma) need to be present to cause the reaction.

Further, when any new bone formed after the implantation of GFP-labelled urothelial cells is studied, it is hoped that osteoblasts within the newly formed bone will also express GFP. This would strongly suggest the osteoblasts are derived from the urothelial cells themselves, and not from muscle-bound cells (such as mesenchymal stem cells) that have been influenced by the juxtaposition of urothelial cells. This would strongly imply that urothelial cells had “de-differentiated” from their epithelial phenotype by way of an epithelial-mesenchymal transition to a stem-cell phenotype, and then re-differentiated along osteogenic lines.

REGIONAL DIFFERENCES IN BONE MINERAL DENSITY LOSS IN RAT LUMBAR VERTEBRAE SIX-MONTHS POST-OVARIECTOMY

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Osteoporosis is a bone disease that affects the spine and hip in postmenopausal women. Technological improvements make it possible to gather detailed information to better understand this disease. This study aims to use DEXA and micro-ct to quantify regional variations in BMD throughout the individual lumbar vertebrae in ovariectomised rats.

Twenty-four female Sprague-Dawley rats were subjected to either bilateral OVX or sham surgery (n=12) at 10 weeks of age. Twenty-six weeks later the animals were sacrificed, lumbar spines were dissected out and cleared of soft tissue. All specimens were DEXA scanned, micro-ct and H&E stained. For DEXA, the ROI were limited to the cancellous vertebral body, and micro-ct was used to calculate BV/TV, Tb.Th and Tb.Sp.

The mean BMDs of each sham vertebral level were significantly higher ($p < 0.001$) than the corresponding vertebral level in the OVX group. Furthermore, there were significant differences between different vertebral levels within each treatment group. Both the sham and OVX lumbar spines exhibit progressive increase in BMD from superior (L1) to inferior (L6) levels, with the sham BMD increasing more down the lumbar spine than the OVX BMD. This was confirmed in the micro-ct data in which there was a significant difference ($p < 0.01$) in BV/TV, Tb.Sp and Tb.Th in the OVX lumbar spine between superior, mid and inferior regions.

DEXA and micro-ct enables in-depth analysis of the effects of osteoporosis on the lumbar spine in OVX rats. Both measurements confirmed the universal loss of BMD throughout the lumbar spine when compared to the sham rats.

HAND PARESTHESIA IN SUBACROMIAL IMPINGEMENT SYNDROME

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We have noticed that some patients presenting with impingement syndrome complain of tingling and numbness in their affected arm & hand. The association of paresthesias with impingement syndrome of the shoulder has not been studied before. 100 consecutive patients (mean age 54.7yrs, mean duration of symptoms 2.5 yrs) undergoing surgery were enlisted for the study. 54% had paresthesias during the course of the shoulder problem. Of these, 62.3% patients had radial sided symptoms, 30.2% ulnar-sided symptoms, while 7.5% involving all fingers. A highly significant association was observed between the occurrence of paresthesias and previ

AN ANALYSIS OF BONE GRAFT SUBSTITUTES IN A CRITICAL SIZE DEFECT MODEL

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The in vivo response of a new class of micro and macro porous hydroxyapatite materials was examined in a standard rabbit tibial defect.

Bilateral anteromedial critical size defects (5mm by 15mm) were created in sixty, 12 week old, NZ white rabbits using a standard model. The defects were filled with hydroxyapatite(HA) based materials of varying porosity (Apapore 60% and 80% porous), a biphasic HA –tricalcium phosphate material (Osatura 80% porous) or a silicate substituted HA material (Pore Si). Radiographic, torsional testing, histomophometric analyses and light microscopy were performed at 2, 4 and 12 weeks to assess healing, mechanical properties and percentages of bone, implant and void. Data was compared to ProOsteon 200R examined in the same model using the same endpoints.

No differences were noted between groups at each time with respect to radiographic appearance or mechanical data. The biphasic HA material demonstrated implant resorption by 12 weeks unlike the HA materials. The incorporation of silicate to the HA material increased bone formation within the defect. Comparison of the HA based materials to ProOsteon 200R (calcium carbonate and HA) revealed increased bone formation in this class of bone graft substitutes at 12 weeks.

Porosity and material composition play an important role in the in vivo performance of bone graft substitutes. Pure HA materials resorb at a slower rate compared to biphasic HA materials. Increasing porosity had a positive effect on new bone formation.

IS IT BETTER TO BE HOT?

MECHANICAL PROPERTIES OF BONE CEMENTS AT VARYING CURING TEMPERATURES

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It has been suggested that warming femoral prosthesis prior to implantation may reduce cement porosity and hence reduce cement microfractures and potentially improve long-term results. This study investigates the effects of temperature during curing of different bone cements.

Four cements (CMW1, Endurance, Palacos R-40, Simplex) were tested following ASTM F451-99 standards. The cements were placed into four preheated moulds at 18°C, 37°C, 40°C and 50°C respectively following mixing, and allowed to cure at these temperatures for 1 hour and the final 23 hours at 18°C. Faxitron radiographics, apparent density, ultrasonic modulus and compression testing were performed for each cement at each temperature (n=25 per group). Additional samples were used to assess micro and macroporosity with micro Computer Tomography (μ CT).

Whilst some of the cements were indeed influenced by the temperature during curing this was not universal across all cements and temperatures. In general, increasing the temperature during the initial one hour of curing increased the mechanical properties due to a reduction in porosity and increase in density. CMW1 and Endurance were more susceptible to temperature changes with respect to changes in physical and mechanical properties.

Heating femoral stems prior to cementing to influence polymerisation and interface bonding opens a number of questions regarding the effect of temperature, implant material, surface roughness and cement type. This study revealed temperature is an important factor but may not be transferable across different cements as their compositions vary.

RELATIONSHIP BETWEEN PROGRESSION OF OSTEOLYSIS AND POLYETHYLENE WEAR AND PATIENT-RELATED FACTORS

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Knowledge of the extent and progression of osteolysis is important in deciding the timing of surgical intervention, the type of surgery and also for monitoring the potential effects of medical treatments. The aim of this study was to use quantitative computed tomography (CT), with wear and migration analyses, to determine the rate of progression of osteolysis and to examine the relationship between osteolysis and polyethylene wear and patient-related variables.

The annual progression of osteolysis adjacent to 19 well-fixed Harris-Galante acetabular components was determined. Migration analyses were used to exclude cases with migrated components. Volumetric polyethylene wear was determined from digitised X-rays using the Polyware software program. Patient-related factors including age, gender, BMI, activity levels, co morbidities, and joint pain and function were recorded from our Joint Replacement Database.

Lesions in many of these patients were relatively quiescent, while others progressed markedly over a one year period. There was a statistically significant association between progression of osteolysis and total polyethylene wear, total polyethylene wear rate and volumetric polyethylene wear rate and volumetric polyethylene wear in the 12 month period between CT scans ($p=0.026$, $p=0.025$ and $p=0.035$ respectively). None of the other covariates examined was significantly associated with the progression of osteolysis.

CT measurement of osteolysis progression and accurate estimates of cup wear and stability provides important information to guide clinical management of patients with periprosthetic osteolysis.

Ac45, a V-ATPase Accessory Subunit Interacts with Vo Domain Subunits and is necessary for Osteoclastic bone resorption

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Solubilization of bone mineral by osteoclasts is dependent on the acidification of the extracellular resorption lacuna by means of a multimeric vacuolar type proton pump (V-ATPases). Besides such specialized function the V-ATPases is also essential for acidification of diverse intracellular compartments that includes the Golgi apparatus, endosomes, lysosomes and secretory granules. The core structure of V-ATPases comprises of two functionally and structurally distinct domains, V₁ and V₀. The peripheral cytoplasmically oriented V₁ domain is responsible for ATP hydrolysis which provides the energy for the translocation of protons across the integral membrane bound V₀ domain. Here, we have identified an accessory subunit, Ac45 that interacts with the V₀ domain and is involved in V-ATPase-mediated function in osteoclasts. Ac45 was localized to the ruffled border region of polarized resorbing osteoclasts and partially colocalized with pH-dependence of V-ATPase

Truncation mutants of RANKL within the TNF-like core domain inhibit RANKL-induced osteoclast differentiation and activation

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Receptor activator of NF- κ B ligand (RANKL) is a crucial factor necessary for osteoclast differentiation and activation. In this study we have examined the role of the TNF-like core domain of RANKL in osteoclast differentiation and activation. To this end, a series of truncation mutants of the TNF-like core domain of RANKL were expressed as GST-fusion proteins, and their biological activities assessed using a number of pro-osteoclastogenic systems. Osteoclastogenesis assays revealed that while GST-rRANKL (aa160-318) containing the full TNF-like core region strongly induced osteoclast formation, RANKL truncation mutants GST-rRANKL (aa239-318), (aa160-268), (aa160-291), (aa246-318) display significantly decreased osteoclastogenic activity. Consistently, the decrease in osteoclast number correlates with decreased TRACP activity and reduced calcitonin receptor and cathapsin K gene expression. Furthermore, competition studies revealed that all RANKL truncation mutants were capable of inhibiting RANKL (aa160-318)-induced osteoclast formation but with different efficacy, RANKL mutant (aa246-318) being the most potent. RANKL mutant (aa246-318) was also found to competitively decrease RANKL (aa160-318)-induced osteoclastic bone resorption *in vitro*. Interestingly, GST pull down studies reveal that all RANKL mutants have reduced binding affinity to RANK. In addition, all RANKL mutants display significant reduction in the activation of crucial osteoclastic signalling pathways including NF- κ B, ERK, JNKs and have decreased I κ B α degradation as compared to the full-length protein. Together, our data indicate that RANKL mutants within the TNF-like core domain may act as competitive inhibitors of RANKL-induced osteoclast differentiation and activation and thus may offer potential therapeutic approaches to combat bone lytic disorders.

Autologous Tenocyte Implantation for Massive Rotator Cuff Defect: Histological Assessment in the Rabbit

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Introduction: Large or recurrent rotator cuff tendon tears are difficult to treat effectively. Collagen bioscaffolds have recently become available to reinforce tendon repair. This study compares the suitability of two collagen bioscaffolds for autologous tenocyte implantation, and evaluates the reparative response induced by these biomaterial constructs in a rabbit model.

Methods: Tenocytes were isolated from rabbit patellar tendon, cultured, and seeded onto either Restore SIS or ACI-Maix collagen membrane. Scanning electron microscopy was used to examine tenocyte integration and growth within the bioscaffold over time. Massive rotator cuff defect was created in fifty NZW rabbits and treated by either: (A) suture of autologous cuff tendon (control), (B) ACI-Maix implantation, (C) Autologous tenocyte-seeded ACI-Maix implantation, (D) SIS implantation, (E) Autologous tenocyte-seeded SIS implantation. Gross and histological evaluation was performed at four and eight weeks postoperatively.

Results: Scanning electron microscopy of the ACI-Maix membrane showed the loose organisation of collagen fibres of its rough side, a feature conducive to cell adhesion and integration. Furthermore, one, three, and five day sequential analysis after seeding showed progressive tenocyte integration into the membrane and matrix neosynthesis. Scanning electron microscopy of the SIS membrane exhibited its relatively smooth surface of highly compacted collagen fibres. Sequential analysis of the SIS membrane after seeding showed monolayer cell integration with progressive proliferation and observed matrix neosynthesis. At four weeks, all ACI-Maix and SIS membrane reparative outcomes were characterized by partially absorbed membrane, subsequent lymphocytic inflammatory response, and immature bone trough remodelling. By eight weeks, inflammation had subsided and the regenerating tendon illustrated improved remodelling by histology. Autologous tenocyte implantation with both membranes improved reparative tendon histology and grade at eight weeks in comparison to membrane-only implants, and was histologically equivalent to the control group. All fifty rabbits regained normal gait two weeks postoperatively, and no tendon rupture occurred in any rabbit groups.

Conclusion: Autologous tenocytes seeded onto both ACI-Maix and SIS membrane show cell behaviour suitable for cell-based therapy. Although both implants showed inflammatory response in the rabbit initially, resorption occurs with time. The bioscaffold-induced healing response of rotator cuff tear is improved by the addition of autologous tenocytes in the rabbit. We therefore suggest autologous tenocyte implantation may be a clinically useful alternative treatment for massive rotator cuff tears.

Microarray study of bone marrow stromal cells from osteoporosis

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Ageing of the human skeleton is characterized by decreased bone formation and bone mass. These changes are more pronounced in patients with osteoporosis. We propose decreased bone formation as a consequence of ageing and osteoporosis may be explained by different genetic profiling of bone marrow stem cells, which results in decreasing of osteogenic differentiation.

To test this hypothesis, microarray experiments were designed to identify the genetic profile of bone marrow stromal cells from juvenile, geriatric, osteoporotic, and normal adult rats. Osteoporotic animal model was generated from ovariectomy. Bone marrow stem cells were isolated by flushing the femurs and subcultured by the first passage. Total RNA was extracted from the first passaged cells and further processed for the microarray study. The animal model was verified by examination of the animal's tibia and femur histology. Identified genes were further verified by quantitative PCR.

There were 1025 genes detected with significant differences between normal and osteoporotic rats. There was more than 2-fold up-regulation in 194 genes and a 2-fold down-regulation in 108 genes in osteoporosis compared with normal rat. Lipoprotein lipase (Lpl), cellular retinoic acid binding protein 2, osteoglycin precursor, osteoclast inhibitory lectin, retinoic acid inducible protein 3, Neuropeptide Y (Npy) are the most relevant genes identified in regulating cell differentiation and osteogenesis, which were differently expressed in osteoporosis compared with normal rat. Therefore, difference in genetic profiling of bone marrow stem cells plays a potential role in osteogenesis in osteoporosis.

Biochemical aspects of degenerative cartilage in osteoarthritis

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The exact etiology and pathophysiology of osteoarthritis (OA) remains elusive, although it is characterized by degeneration of articular (hyaline) cartilage. A primary role for subchondral bone in the etiopathogenesis has been proposed with possible communication between the subchondral bone and deep cartilage. The purpose of our study was to analyze molecular changes during the progression in cartilage degeneration and to reveal the molecular alterations to histological changes.

Five patients with radiographic Grade IV osteoarthritis of the medial compartment undergoing total knee replacement were included in this study. Osteochondral samples from the tibiae were obtained from the arthritic and relatively normal compartments of the knee joints for comparison. Samples were decalcified and prepared for immunohistochemistry and chondrocytes were isolated and cultured for the mRNA expression of type I collagen, osteocalcin, osteopontin, alkaline phosphatase, bone sialoprotein and bone morphogenetic protein 2 and 4, et al.

The most significant finding on immunohistochemical analysis was strong expression of molecules for mineralization in the chondrocytes in Grade IV OA in the deep layers of cartilage except type I collagen. Up-regulated expression of bone matrix protein genes were found during the osteo-differentiation of chondrocytes from OA patient. The appearance of the chondrocytes and the staining distribution suggest that during the progress of OA chondrocytes differentially produce matrix proteins like osteocalcin and bone sialoprotein, which induces articular cartilage mineralization and subsequent replacement of articular cartilage by subcondral bone tissue through the change of collagen type.

THE EFFECT OF AUTOLOGOUS GROWTH FACTORS ON THE PROLIFERATION OF OSTEOBLAST-LIKE CELLS

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Autologous growth factors (AGF) derived from platelets may be of great therapeutic benefits on bone formation. More studies are needed for published conflicting results. This study was to investigate the short-term effect of AGF on osteoblast-like cells.

Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) was prepared using Interpore Cross ACCESS™ system from 120ml of whole blood collected from 10 volunteers. AGF was released by mixing PRP or PPP with thrombin (0.5unit/ml). Saos-2, and U2OS cells were maintained in media with 10% FBS. The cells were stimulated with 0% & 10% of FBS, 4%, 0.8%, 0.16% of PRP and PPP for 48hours after starving 12hours. The cell proliferation rate was measured with MTT assay and compared using one-way ANOVA.

The platelet count of PRP was 5.7 times that of whole blood. The effects of AGF on cell proliferation were listed as following (OD, $\bar{x}\pm S$): U2OS cells: 0.185±0.004, 0.073±0.009; 0.185±0.012, 0.181±0.018, 0.086±0.006; 0.137±0.018, 0.098±0.010, 0.060±0.003; Saos-2 cells: 0.054±0.006, 0.037±0.006; 0.073±0.002, 0.056±0.005, 0.043±0.005; 0.062±0.009, 0.049±0.006, 0.037±0.006 for 10% & 0% of FBS; 4%, 0.8%, and 0.16% of PRP and PPP. PRP significantly increased proliferation of U2OS cells compared with PPP, while Saos-2 cells did not.

The distinct effect of AGF on proliferation of U2OS and Saos-2 cell lines was noted. It should be careful to explore the effects of growth factors on different cells.

A VALIDATED SUBJECT-SPECIFIC MODEL OF THE HUMAN KNEE JOINT

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Validated finite element (FE) models are invaluable tools for obtaining mechanical behaviour of structures, where it may otherwise be difficult or impossible to acquire. Their application is vast and their development is ever increasing.

A subject-specific FE model of a human knee was developed using CT and MR images of a cadaveric leg. Images were segmented and transformed into the correct coordinate system using Amira (Indeed Visual Concepts, Berlin).

The knee model comprises the femur and tibia and soft-tissue components of the knee including the articular cartilages, menisci and ligaments (cruciate and collateral). The bony structures were modeled as rigid bodies and the cartilages and menisci were represented by 10-noded tetrahedral elements. Ligaments were modeled as nonlinear spring elements. Passive knee motion was initiated by applying a small load to the femur, with the tibia constrained in all directions. Pre-processing was undertaken in MSC Patran (MSC Software Corp., Santa Ana, CA) and the analysis was computed using ABAQUS (Abaqus, Inc., Pawtucket, RI).

The model was validated using 3D coordinate data obtained using an electromagnetic tracking device from the cadaver under various flexion angles.

Finite element modeling plays an important role in the biomechanics discipline. Development of such computer models allows simulation of real-life mechanical behaviour. This can potentially aid in the clinical field as a predictive tool.

BILATERAL congenital pseudoarthrosis of the clavicles without mutation of the Cbfa1/Runx2 gene

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Purpose of the study: We have identified a 21 year-old male with bilateral pseudoarthrosis of the clavicles, presumed to be congenital. Since the genetic basis of pseudoarthrosis of the clavicle has not been identified, we investigated the possible involvement of mutation of the *Cbfa1/runx2* gene. *Cbfa1/runx2* is a transcription factor that plays an essential role in osteogenesis, and mutant forms have been linked to cleidocranial dysplasia (CCD), a congenital condition characterized by skeletal anomalies that include hypoplastic and/or aplastic clavicles.

Methods: A shoulder to neck CT was obtained and 3D reconstruction of images was performed using the ®True Life Anatomy software. DNA was extracted from peripheral blood white cells and the gene was amplified by PCR, using intron- and exon-specific primers, and these gene segments were sequenced.

Summary of results: All 7 exons of the *Cbfa1/runx2* gene were examined by direct sequencing of PCR products generated from DNA samples from the proband and his parents. No mutations were identified in any coding region or splice-donor/acceptor sites in the *Cbfa1/runx2* gene in any of the samples.

Conclusions: The genetic basis for congenital pseudoarthrosis of the clavicles remains to be determined. It is likely that finding the gene or genes associated with this condition will provide fresh insights into skeletal development and into the unique and interesting clavicular bone, in particular.

MECHANICAL BEHAVIOUR OF LOCKED AND NON-LOCKED BONE PLATES

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This study aims to compare the static mechanical properties of fracture plates designed for use with regular bicortical screws and locking head screws; the straight 10-hole TC100 and PeriLoc bone plates respectively (Smith & Nephew).

Human cadaveric distal tibiae were selected as the model for this study and DEXA scanned to group match for bone quality. A transverse fracture was simulated and the plates were positioned on the lateral aspect of the bones, 65mm from the distal end and secured with 3 screws on either side of the fracture. The reconstructed bones were then tested in static torsion and bending. The pressure footprint underneath the plates was measured with a pressure sensitive device (Tekscan, Boston, USA)

The reconstructed tibiae with the TC100 plates were stiffer compared to the PeriLoc plates in both four point bending and torsion ($p < 0.05$) (Figure 1 and 2). Similarly, the bones reconstructed with the TC100 plates had higher failure torque compared to the PeriLoc ($p < 0.05$). All samples failed via crack propagation through the bone originating from one of the screws. The pressure footprint of the locked plate was much lower than the non-locked plate.

The healing of fractures is a complicated system in which the stiffest system is not necessarily the best and in fact, reduced stiffness may be an advantage for healing and remodelling of the fracture. Considering the reduced pressure footprint on the bone, the locking plate may have a biological advantage over the non-locking plate.

ANTIBIOTIC AND ITS EFFECT ON ACRYLIC BONE CEMENT

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The limited availability of bone cement containing antibiotics leads to surgeons adding varying amounts during mixing. The aim of this study is to determine the effects of antibiotics on commercially available bone cements.

Two PMMA bone cements were evaluated in this study; Simplex (Stryker, Ireland), and VersaBond (Smith & Nephew, USA). Three groups were tested; 1) PMMA (No Antibiotic), 2) PMMA with 1g Flucloxacillin and 1g of Vancomycin (5 wt%), and 3) PMMA with 3g Flucloxacillin and 3g Vancomycin (15 wt%). Cement specimens were prepared according to ASTM F451-99a; *Standard Specification for Acrylic Bone Cement*. The cement dowels were tested in uni-axial compression and scanned using Micro CT to assess compressive strength, porosity, and Euler characteristics.

The addition of antibiotics had an overall negative effect on the compressive properties of both cements. When compared to the unaltered mix; the compressive properties of VersaBond were effected at both antibiotic levels, while Simplex was effected only at the higher dose. When comparing both antibiotic loaded conditions no differences were seen. This is interesting as the high dose was three times the low dose. Porosity was increased with the addition of antibiotic. The Euler characteristics showed isolated solids with VersaBond and isolated pores with Simplex. Micro CT images between cement types were very different.

The increased porosity seen could aid in elution characteristics, and there was little difference in mechanical properties between the loaded and highly loaded states. These two results provide some support for increasing the amount of antibiotic used in arthroplasty.

Apoptosis and Peri-implant Osteolysis: Expression of Trail and its receptors in revision tissue adjacent to osteolytic lesions

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The unregulated bone resorption that occurs in osteolytic regions surrounding failed joint implants is associated with an increased number of active osteoclasts that persist within the invading soft tissue. It has been proposed that these osteoclasts may prevail *in situ* due to an altered expression of a normal apoptotic signalling apparatus. The aim of this study was to investigate the expression of the apoptotic factor TRAIL and its four membrane bound receptors in tissues obtained from sites of peri-implant osteolysis in patients undergoing revision surgery for failed hip prosthesis. Immunohistochemical analysis of formalin fixed sections from 10 patients undergoing revision surgery was performed using monoclonal antibodies directed against TRAIL, and the TRAIL receptors R1, R2, R3 and R4. Control tissue consisted of synovial tissue samples taken from patients with osteoarthritis undergoing primary hip replacement surgery. Sections were evaluated by light microscopy using a scoring system that has been used in several of our publications. Elevated levels ($p < 0.005$) of staining for TRAIL, TRAIL-R1, TRAIL-R3 and TRAIL-R4 was found in tissues from failed prosthesis, with staining associated with both multinucleated osteoclast-like cells and mononuclear inflammatory cells. The increased expression of both decoy receptors TRAIL-R3 and R4 may contribute to the perpetuation of the osteoclasts within the sites of osteolysis, despite the increased presence of TRAIL and death receptors capable of transmitting the apoptotic signal. Targeted intervention of the TRAIL decoy receptors may represent a potential therapeutic target in diseases where there is unwanted bone destruction by osteoclasts.

ABSTRACT - INVITED SPEAKERS

Which ADAMTS enzyme is the major aggrecanase?

Studies with ADAMTS-4 and ADAMTS-5 deficient mice

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Aggrecan is the major proteoglycan in cartilage and it endows this tissue with its unique capacity to bear load and resist compression. In arthritic cartilage, aggrecan is degraded by one or more "aggrecanases" that are members of the ADAMTS (A Disintegrin And Metalloproteinase with ThromboSpondin motifs) family of zinc-dependent proteinases. Although ADAMTS-1, -8 and -9 have weak aggrecan degrading activity *in vitro*, they are not thought to be the primary aggrecanases because aggrecanolysis is not protected in ADAMTS-1 null mice with experimental arthritis, and cleavage by ADAMTS-8 and -9 is significantly less efficient than cleavage by ADAMTS-4 or -5. ADAMTS-9 has not been detected in adult human cartilage. Conversely, there is good evidence to suggest that ADAMTS-4 and -5 are the primary aggrecanases. ADAMTS-5 is expressed constitutively in cartilage, synovium and joint capsule and ADAMTS-4 is induced by catabolic cytokines in cartilage and synovium. Immunoprecipitation of conditioned media from interleukin-1-stimulated bovine cartilage with anti-ADAMTS-4 and anti-ADAMTS-5 antibodies inhibits aggrecanase activity by 75% and 15% respectively. However, the relative importance of these two enzymes in cartilage pathology is not known. In order to resolve this point, we have generated mice deficient in either ADAMTS-4, or ADAMTS-5 catalytic activity, and investigated aggrecan degradation *in vitro* and *in vivo*.

A TANGLED SKEIN - COMPLEX TISSUE INTERPLAY IN OVINE MODELS OF OSTEOARTHRITIS AND MENOPAUSE

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Investigations using the ovine meniscectomy model of osteoarthritis have revealed extensive involvement of joint tissues other than articular cartilage, including subchondral bone (SCB) sclerosis, gross and microscopic synovitis, and altered ligament gene expression. Similarly, ovariectomy of ewes induces structural changes in many connective tissues including articular cartilage and intervertebral disc, in addition to the expected bone remodeling (which can be marked in the immediate subchondral region). When the meniscectomy and ovariectomy insults are combined, as in a model of postmenopausal osteoarthritis, cartilage degeneration is exacerbated without altering effects on SCB structure. However, metabolic and vascular lesions suggest that gender and particularly menopausal status may have an important influence on the SCB and synovial responses in osteoarthritis.

Can we disguise an implant to promote skeletal tissue integration?

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Despite major advances in prosthetic technologies, often implants fail, mainly due to bone erosion at the interface of skeletal tissue and the device. There is a need for fabricating materials to be "biologically" designed to ensure a strong anchorage of the device material in bone tissue, a key process for short- and long-term stability of implants. We propose that improved strength and integration rate of an implant in osseous tissue will be achieved by specifically altering the interfacial chemistry of a biomaterial with bioactive molecules. In our studies we use novel calcium phosphate ceramics divalent cations and/or divalent cations (magnesium or zinc) to modify the surface of titanium implants without altering its bulk properties. We showed that surface chemistry modification of titanium potentiate bone growth in vitro and in vivo. The intracellular signalling cascade triggered as a result of surface chemistry modification of biomaterials remains largely unknown. Key signaling proteins in osteoblasts such as extracellular regulated kinase 1/2 and activated protein-1 pathways were upregulated when bone cells were cultured onto the modified substrata. These data suggest that surface chemistry modification of titanium with bioactive agents may contribute to successful osteoblast function and differentiation at the skeletal tissue/device interface.