

CORS Paper Session 2: Bone •

Moderators Mark Glazebrook, NS, and Cari Whyne, ON

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An In-vivo Evaluation of the Effect of a Hydroxyapatite Coating With and Without the Use of BMP-7 on Extracortical Bone Bridging Using a Canine Segmental Defect Model

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Purpose: Extracortical bone bridging and ingrowth have been shown to reduce stresses on the stem and cement mantle of tumor endoprostheses. The purpose of this study was to assess the effect of bone morphogenetic protein 7 (BMP-7) delivered by Peri-Apatite[®] (PA, Stryker Orthopaedics) hydroxyapatite coating on porous segmental replacement prostheses.

Method: Eighteen mature mongrel canines were implanted with unilateral segmental replacement prostheses made of a cobalt-chromium (Co-Cr) alloy and coated with two layers of sintered Co-Cr alloy beads (diameter 600 to 800µm). The control group consisted of a plain porous coated segmental prosthesis without any PA coating. Group 2 consisted of a PA-coated segmental prosthesis coated with buffer solution. Group three consisted of a PA-coated segmental prosthesis loaded with rhBMP-7 (Stryker Biotech) in a buffer solution carrier. Group 1 had the implant only. Group 2 had the buffer solution evenly applied to the porous coat and group 3 had 2.9 mg of BMP-7 in liquid buffer solution evenly applied. The canines were allowed to fully bear weight without restrictions. The femurs were retrieved at twelve weeks for radiographic and histologic analysis. **Results:** Gross and radiographic data of the retrieved specimens showed that all six PA-coated implants augmented with BMP-7 had complete bone bridging; only one of the PA-coated implants and only two of the plain porous implants were completely bridged. There was a greater percentage of bone apposition for the BMP-7 augmented PA-coated group compared to both the plain ($p=0.0026$) and the PA-coated ($p=0.0001$). There was no difference in bone formation or bone apposition between the plain and PA-coated groups. Histology revealed greater depth of bone ingrowth in the BMP-7 augmented PA-coated group as compared to the plain ($p<0.0001$) and the PA-coated ($p<0.0001$) groups. There was also significantly greater bone apposition in the BMP-7 augmented PA coated groups as compared to the plain ($p=0.0014$) and PA-coated ($p=0.0067$) groups. There was no significant difference in depth of bone ingrowth or bone apposition between the plain and PA-coated groups. **Conclusion:** BMP-7 when used to augment PA-coated prostheses in a canine segmental defect model can significantly improve extracortical bone bridging and bone ingrowth. PA-coated implants may be considered to deliver the exogenous biological growth factors.

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The Use of Endothelial Progenitor Cells to Promote Bone Healing: A Defect Model in the Rat Femur

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Purpose: Endothelial Progenitor Cells (EPCs) have been proven to contribute to formation of new blood vessels. The objective of this study was to evaluate the effects of local EPC therapy on the stimulation of angiogenesis at a fracture site and the promotion of bone healing by increasing osteogenesis and callus formation. **Method:** Rat bone marrow EPCs were isolated and cultured. A segmental bone defect (4mm.) was created in the rat femur diaphysis and stabilized with a mini-plate. A gelfoam piece impregnated with a solution of EPCs (1×10^6) was placed into the fracture gap. Control animals received only saline-gelfoam with no cells. In total, 42 rats were studied: 21 in EPC and 21 in control groups. Seven animals were sacrificed from each group at one, two, and three weeks post-operatively. Plain radiographs of the operated femur were taken before sacrifice. Operated femurs were harvested and the specimens from the osteotomy site were collected for histological evaluation. The x-rays were scored in a scale from zero to five according to the percentage and the intensity of the bone filling at the osteotomy site. Hematoxylin-eosin stained slides were evaluated for new vessel formation and the amount of bone tissue. **Results:** Radiographically, at three weeks, the mean score for the EPC group was 4.5 with five out of seven animals having bridging callus; whereas for the control group, the mean score was 2.2 with no bridging callus formation. At two weeks, EPC treated animals had a mean score of 2.4, and the control group had a score of 1. Bone formation was insignificant at one week in either group, however, the scores tended to be higher in the EPC group animals than the control; 0.6 to 0.3 respectively. Histological evaluation revealed that the specimens from EPC treated animals had abundant spicules of trabecular bone containing predominantly bone cells, osteoid, and new vessels. Conversely, control animals had scarce trabecular bone with markedly less bone cells and vessels. **Conclusion:** Local EPC therapy stimulates angiogenesis and increases osteogenesis and callus formation post fracture. Our report encourages further investigation of the local use of EPCs as a potential therapy to promote bone regeneration.

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A Biodegradable Scaffold for the Treatment of a Diaphyseal Bone Defect of the Tibia

Paul R. T. Kuzyk, University of Toronto; Emil H. Schemitsch, University of Toronto; John E.D. Davies, University of Toronto

Purpose: The aim of our study was to evaluate bone formation and angiogenesis produced within a biodegradable poly-D, L-lactide-co-glycolide acid/calcium phosphate (PLGA/CaP) scaffold when used to treat a diaphyseal tibia defect and compare this to an iliac crest autograft or an empty defect. **Method:** An 8.0 mm diaphyseal defect was created in a canine tibia model. All tibiae were reamed to 7.0 mm and fixed with a 6.5 mm statically locked intramedullary nail. Eighteen canines were allotted into

three treatment groups: 1) empty (N=5), 2) iliac crest autograft (N=6), or 3) PLGA/CaP biodegradable scaffold Tissue Regeneration Therapeutics Inc., ON, Canada) (N=7). Fluorescent markers were given at different times: calcein green (six weeks), xylenol orange (nine weeks), and tetracycline (11 and 14 weeks). Animals were sacrificed at 15 weeks and perfused with a barium compound. Radiography, Micro CT, and brightfield and fluorescent microscopy were used for analysis. **Results:** Micro CT and brightfield images of scaffold samples displayed multiple vessels (10 to 100µm) within the scaffold. The bone volume and vasculature volume (measured with Micro CT) within the tibial defect site were reported as a percentage of the total volume of the defect site. The percent bone volume within the defect site was not different between treatment groups ($p=0.112$). There was greater percent vasculature volume in the scaffold group than the autograft group ($p<0.001$). Bone formation at the osteotomy sites was defined as the distance from the original osteotomy site to the tip of newly formed bone. Osteotomy bone formation was significantly greater in the scaffold group than the autograft group ($p=0.015$). Osteotomy sites associated with greater angiogenesis displayed greater bone formation. Bone formation rates were reported as the distance between the fluorescent bone labels. Autograft samples had the greatest bone formation rates within the periosteum. Autograft and scaffold samples had the greatest rate of bone formation within the cortex. **Conclusion:** Our canine tibial defect model provides a satisfactory facsimile of the traumatic tibia fracture with associated bone loss. The PLGA/CaP biodegradable scaffold we have employed promotes angiogenesis within a defect and could be used in conjunction with autografting.

13 –

Effect of the hVEGF Transfer on Endogenous VEGF mRNA Expression in a Rat Osteoblast or Fibroblast Culture Model

Claire Li, University of Toronto; **Ru Li**, University of Toronto; Michael D. McKee, University of Toronto; Emil H. Schemitsch, University of Toronto

Purpose: Vascular Endothelial Growth Factor (VEGF) plays an important role in promoting angiogenesis and osteogenesis during fracture repair. Our previous studies have shown that cell-based VEGF gene therapy accelerates bone healing of a rabbit tibia segmental bone defect in-vivo, and increases osteoblast proliferation and mineralization in-vitro. The aim of this project was to examine the effect of exogenous human VEGF (hVEGF) on the endogenous rat VEGF messenger RNA (mRNA) expression in a cell-based gene transfer model. **Method:** The osteoblasts were obtained from the rat periosteum. The fibroblasts were obtained from the rat dermal tissue. The cells were then cultured to reach 60% confluence and transfected with hVEGF using Superfect. Four groups were: 1) osteoblast-hVEGF, 2) fibroblast-hVEGF, 3) Osteoblasts alone, and 4) Fibroblasts only. The cultured cells were harvested at 1, 3 and 7 days after the transfection. The total mRNA was extracted (TRIZOL); both hVEGF and rat VEGF mRNA were measured by reverse transcriptase- polymerase chain reaction (RT-PCR) and quantified by VisionWorksLS. **Results:** The hVEGF mRNA was

detected by RT-PCR from transfected osteoblasts after three days of gene transfection. The hVEGF mRNA expression in transfected fibroblasts increased exponentially at days 1, 3 and 7 after the transfection. We compared the endogenous rat VEGF mRNA expression level of the osteoblasts or fibroblasts that were transfected with hVEGF with the cells without the transfection. The hVEGF transfected osteoblasts had a greater rat VEGF mRNA expression than the non-transfected osteoblasts. Furthermore, when hVEGF was transfected to the rat fibroblasts, the endogenous mRNA expression level measured was also greater than that from the non-transfected fibroblasts. Rat VEGF mRNA expression increased in the first three days of the hVEGF transfection, but the expression level was reduced at Day 7. **Conclusion:** These results suggest that cell-based hVEGF gene therapy enhances endogenous rat VEGF mRNA expression in both osteoblasts and fibroblasts.

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Vascular Endothelial Growth Factor Regulates Osteoblast Cell Death in Osteoporotic Vertebral Fracture

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Purpose: Apoptosis of osteoblasts and osteoclasts regulates bone homeostasis. Vertebral osteoporotic insufficiency fractures are characterised by pathological rates of osteoblast apoptosis. Skeletal injury in humans results in 'angiogenic' responses primarily mediated by vascular endothelial growth factor(VEGF), a protein essential for bone repair in animal models. Osteoblasts release VEGF in response to a number of stimuli and express receptors for VEGF in a differentiation dependent manner. This study investigates the putative role of VEGF in regulating the lifespan of primary human vertebral osteoblasts (PHVO) in-vitro. **Method:** PHVO were cultured from biopsies taken at time of therapeutic vertebroplasty and were examined for VEGF receptors. Cultures were supplemented with VEGF(0–50ng/mL), a neutralising antibody to VEGF, mAB VEGF(0.3ug/mL) and Placental Growth Factor (PIGF), an Flt-1 receptor-specific VEGF ligand(0–100 ng/mL) to examine their effects on mineralised nodule assay, alkaline phosphatase assay and apoptosis. The role of the VEGF specific antiapoptotic gene target BCI2 in apoptosis was determined. **Results:** PHVO expressed functional VEGF receptors. VEGF 10 and 25 ng/mL increased nodule formation 2.3- and 3.16-fold and alkaline phosphatase release 2.6 and 4.1-fold respectively while 0.3ug/mL of mAB VEGF resulted in approx 40% reductions in both. PIGF 50ng/mL had greater effects on alkaline phosphatase release (103% increase) than on nodule formation (57% increase). 10ng/mL of VEGF inhibited spontaneous and pathological apoptosis by 83.6% and 71% respectively, while PIGF had no significant effect. Pretreatment with mAB VEGF, in the absence of exogenous VEGF resulted in a significant increase in apoptosis (14 versus 3%). BCI2 transfection gave a 0.9% apoptotic rate. VEGF 10 ng/mL increased BCI2 expression four fold while mAB VEGF decreased it by over 50%. **Conclusion:** VEGF is a potent regulator of

osteoblast life-span in-vitro. This autocrine feedback regulates survival of these cells, mediated via the KDR receptor and expression of BC12 antiapoptotic gene. This mechanism may represent a novel therapeutic model for the treatment of osteoporosis.

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Characterization of Rat and Mouse Forelimb Compression Models for Studies of Woven Bone Repair in Response to Fatigue Damage

Thomas Karakolis, McMaster University; **Gregory R. Wohl**, McMaster University

Purpose: Bone fatigue damage can lead to stress fractures and may play a role in fragility fractures. The rat forelimb compression model has been used to examine biological responses and gene expression associated with woven bone repair after fatigue damage. Development a similar mouse model would enable the use of genetically modified mice to study molecular mechanisms associated with bone repair. **Method:** Following approval from our Central Animal Facility, forelimbs of male retired breeder C57BL/6 mice and Sprague Dawley rats (n=31 each) were loaded in axial compression across the carpus and olecranon. First, both forelimbs (postmortem, n=6 each) were monotonically loaded to determine failure load. Next, both forelimbs of animals (postmortem, n=5 each) were loaded cyclically to sub-fracture load (67% of monotonic load for mice, 55% for rats) until fatigue failure. Following analysis of fatigue displacement histories, right forelimbs (post-mortem, n=10 each) were loaded cyclically to a set displacement short of the expected failure displacement (mice–30%; rats–55%). Non-loaded left forelimbs served as controls. Three-point bending tests were performed on the ulnae; mechanical properties were compared between fatigued and non-loaded limbs. Finally, right forelimbs (n=10 each) were cyclically loaded in anaesthetised (2.5% isoflurane) animals to 30% (mice) and 55% (rats) of failure displacement. Animals recovered for seven days; microCT imaging and three-point bend tests were performed on the ulnae. **Results:** Ultimate forelimb failure loads were 5.63 ± 0.47 N (mouse) and 57.1 ± 5.8 N (rat). Measured from the 10th cycle, fatigue failure occurred at displacements of 1.68 ± 0.21 mm (mouse) and 2.96 ± 0.22 mm (rat). In three-point bending, fatigue damaged ulnae failed at significantly lower loads versus control (mouse -51.6%; rat -32.1%). After seven days healing, bone cross-sectional area was significantly greater (microCT) and mechanical properties partially recovered (-13.8% versus control). **Conclusion:** Rat and mouse forelimb fatigue loading models have been developed to induce repeatable bone damage. Observed differences in fatigue behaviour necessitated different loading parameters between models. Following seven days of healing, recovery of mechanical strength accompanied woven bone formation (demonstrated by microCT). Further work will compare the biological, woven bone, response between the mouse and rat forelimb models.

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The Effect of Intramedullary Reaming on a Diaphyseal Bone Defect of the Tibia

Paul R. T. Kuzyk, University of Toronto; John E.D. Davies, University of Toronto; Emil H. Schemitsch, University of Toronto

Purpose: The purpose of this study was to relate the extent of reaming to bone formation occurring around a critical sized defect in the tibia. **Method:** Eleven canines were allocated into 2 groups: empty (N=5) or iliac crest autograft (N=6). All tibiae were reamed to 7.0 mm and fixed with a 6.5 mm statically locked intramedullary nail after creation of an 8.0 mm diaphyseal defect. The extent of reaming of the canal was dependent on the cross-sectional area of the tibia as all tibiae were reamed to 7.0 mm. Fluorescent markers were given at different times: calcein green (6 weeks), xylenol orange (9 weeks), and tetracycline (11 and 14 weeks). Animals were sacrificed at 15 weeks and perfused with a barium compound. Radiography, Micro CT, brightfield microscopy and fluorescent microscopy were used for analysis. **Results:** Bone and vasculature volume within the defect were reported as a percentage of the total volume of the defect. Linear regression analysis of percent bone volume (dependent variable) and canal area (independent variable) provided a Pearson correlation coefficient of 0.925 ($p=0.025$) for the empty group and 0.244 ($p=0.641$) for the autograft group. Linear regression analysis of percent vasculature volume (dependent variable) and canal area (independent variable) provided a Pearson correlation coefficient of 0.784 ($p=0.117$) for the empty group and -0.146 ($p=0.783$) for the autograft group. Bone formation at osteotomy sites was defined as the distance from the original osteotomy site to the tip of newly formed bone. Linear regression analysis of bone formation at the osteotomy sites (dependent variable) and canal area (independent variable) provided a Pearson correlation coefficient of 0.132 ($p=0.832$) for the empty group and -0.937 ($p=0.006$) for the autograft group. Bone formation rates were reported as the distance between the fluorescent labels. Bone formation rate was less within the endosteum, cortex and periosteum with extensive reaming in empty samples. **Conclusion:** Our results suggest that the acute management of tibia fractures with bone defects should involve limited reaming. This does not apply when the defect is autografted. Limited reaming may be defined by the cross-sectional area of the tibia in ratio to that of the reamer.

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Use of Co-Registered High-Resolution Computed Tomography Scans Before and After Screw Insertion as a Novel Technique for Bone Mineral Density Determination Along Screw Trajectory

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Purpose: Bone mineral density (BMD) is an important factor in the performance of orthopaedic instrumentation both in and ex-vivo, and until now, there has not existed a reliable technique for determining BMD at the precise location of such hardware. This paper describes such a technique

using cadaveric human sacra as a model. **Method:** Nine fresh-frozen sacra had solid and hollow titanium screw placed into the S1 pedicles from a posterior approach. High-resolution micro-computed tomography (CT) was performed on each specimen before and after screw placement. All images were reconstructed with an isotropic spatial resolution of 0.308 mm, reoriented, and the pre-screw and post-screw scans were registered and transformed using a six-degree rigid-body transformation matrix. Once registered, two points, corresponding to the center of the screw at the cortex and at the screw tip, were determined in each scan. These points were used to generate cylindrical regions of interest (ROI) with the same trajectory and dimensions as the screw. BMD measurements were obtained within each of the ROI in the pre-screw scan. To examine the effect of artefact on BMD measurements around the titanium screws, annular ROI of 1 mm thickness were created expanding from the surface of the screws, and BMD was measured within each in both the pre- and post-screw scans. **Results:** The registration process was accurate, with an error of 0.2 mm. Four specimens were scanned five times with repositioning, and error in BMD measurements was $\pm 2\%$. BMD values in the cylindrical ROI corresponding to screw trajectories were not statistically different from side to side of each specimen ($p = 0.23$). Artefact-related differences in BMD values followed an exponential decay curve as distance from the screws increased, approaching a low value of approximately 20 mg HA/cc, but not disappearing completely. **Conclusion:** CT in the presence of metal creates artefact, making measured BMD values near implants unreliable. This technique is accurate for determination of BMD, non-destructive, and eliminates the problem of this metal artefact through the use of co-registration of a pre- and post-screw scan. This technique has applications both in-vitro and in-vivo.

18 –

Treatment of Fracture Non-union Using Recombinant BMP-7; Single Centre Experience

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Purpose: To analyse the results of the use of Recombinant Bone Morphogenetic Protein (BMP-7) for treatment of fracture nonunions at our institution. **Method:** From 2001 to 2006, 23 patients with fracture non-union were treated with BMP-7 for bone healing. There were 14 male and nine females. The mean age of patients was 45 years (Range 21-76 yrs). There were 11 femoral, nine tibial and three humerus fractures. There were four open injuries. The average number of operations before BMP-7 insertion was 2.5 (Range 0-6). The mean time between the injury and BMP insertion was 52 months (Range 5-312). Nine (40%) patients had previous autologous bone graft inserted without union. 4 patients had BMP-7 insertion on its own. In another 4 patients it was mixed with allograft. In the rest of 15 patients BMP-7 was mixed with autologous bone graft. 2 patients needed BMP-7 insertion on 2 separate occasions. In all except 1 patient the original fixation of the fracture had to be revised using various appropriate methods.

Results: All the fracture went on to unite within an average of seven months (Range 4-16). There were no complications from the use of BMP-7.

Conclusion: Use of recombinant BMP-7, bone graft and stable fixation lead to fracture union in all our patients. We believe that the use of BMP-7 improved the chances of fracture healing in persistent non-unions and it is safe and easy to use.