ULNA DEFECT HEALING WITH A BONE GRAFT SUBSTITUTE AND LOW INTENSITY PULSED ULTRASOUND

++Orthopaedic Research Laboratories, University of New South Wales, Prince of Wales Hospital, Sydney, Australia
w.walsh@unsw.edu.au

INTRODUCTION:
Healing of bone defects continues to be a challenge in orthopaedic surgery. Osteoconductive bone graft substitutes provide an “off the shelf” option to treat bone defects alone or in combination with local autograft. While many of these materials have demonstrated some new bone formation in vivo, the new bone is often limited to areas where the bone graft substitute is in close proximity to a bony bed.

Low intensity pulsed ultrasound (LIPUS) has been shown to augment bone healing in a variety of animal and clinical settings. The potential synergistic effect of low intensity pulsed ultrasound and bone graft substitutes to improve bone defect healing was explored in this study. An established critical defect in the adult rabbit ulna was used to compare defect healing with a tricalcium phosphate bone graft and low intensity pulsed ultrasound.

METHODS:
A 1.5 cm defect was made in the right ulna of 18 adult NZ White Rabbits following ethical approval. The defects were filled with equal amounts of a tricalcium phosphate bone graft substitute (JAX TCP, Smith & Nephew, Memphis TN). In half the rabbits the ulna defect site was treated with low intensity pulsed ultrasound (Exogen, Smith & Nephew, Memphis TN) for 20 minutes per day (n = 9). The ultrasound signal provided 30 mW/cm² with a pulse width of 200 μseconds at 1.5 MHz.

Control and LIPUS treated animals were killed at 4 weeks (n = 3 per group) and 12 weeks (n = 6 per group) after surgery. The harvested forelimbs were faxitroned in the anteroposterior and lateral planes using an HP Faxitron and high-resolution mammography film. The faxitron radiographs were graded for evidence of implant resorption and new bone formation by three blinded observers. The samples were then fixed in phosphate buffered formalin and bone mineral density (BMD) was calculated for a defined region of interest within the defect site; measured using a Norland pDEXA Sabre scanner (Norland Medical Systems, Inc, NY), DEXA data was analysed with a 2-way analysis of variance using SPSS for windows.

The samples were finally decalcified in formic acid-formalin, paraffin embedded and sectioned for routine histology using H&E and Trichrome stains. Immunohistochemistry was also performed for Type I collagen, VEGF, PCNA and CBFA-1 using standard techniques. Three blinded observers assessed histology and immunohistochemistry for new bone formation, tissue reaction and protein expression.

RESULTS:
No adverse events were encountered post-operatively or during ultrasound treatment.

Faxitron radiographs revealed new bone formation at the defect margins with evidence of subtle implant resorption by 12 weeks. For the defects filled with JAX TCP with no LIPUS treatment.

In contrast, defects filled with JAX TCP and treated with LIPUS demonstrated significant amounts of new bone formation compared to controls at 4 and 12 weeks; at the margins, as well as within the defect. Radiographic evidence of new bone was found between the JAX TCP particles as well as those that were in direct contact with a bony bed at 4 and 12 weeks. LIPUS did not appear to alter the resorption rate of the JAX TCP based on radiographs.

DEXA data revealed a significant increase in bone mineral density with ultrasound treatment at 4 weeks (p<0.05) (Table 1) while no difference was observed at 12 weeks.

Histological analysis at 4 weeks confirmed the radiographic findings. LIPUS treated animals demonstrated a significant increase in bone formation at 4 and 12 weeks compared to controls. New woven bone formation was observed at the defect margins in all animals at 4 and 12 weeks. New woven bone was found between the TCP JAX particles in the LIPUS treated defects (figure 1).

New bone formation was noted in the untreated group when the JAX TCP was in direct contact with a bony bed. At 12 weeks, bone formation between the JAX TCP particles was significantly advanced with LIPUS treatment. Bone formation between the JAX TCP particles was noted throughout the sections. Woven bone was abundant especially when the JAX TCP was in contact with a bony bed. Immunohistochemistry confirmed a type I collagenous matrix between the JAX TCP particles in the control and LIPUS treated samples at 4 and 12 weeks. VEGF, CBFA-1 and PCNA levels were elevated in the LIPUS treated animals compared to no treatment. CBFA-1 expression was most noted in active osteoblasts adjacent to new bone formation.

DISCUSSION:
Osteoconductive bone graft substitutes are clinically successful in the treatment of bone defects. However, new bone formation is often limited to areas where the bone graft substitute is in contact with native bone. This study examined if low intensity pulsed ultrasound could improve bone formation in a critical size ulna defect filled with a tricalcium phosphate bone graft. As expected, the resorption rate of this bone graft substitute by 12 weeks was limited, and LIPUS treatment did not increase the in vivo resorption rate of the bone graft substitute. Radiographic, DEXA and histological analysis demonstrated a significant improvement in bone formation with LIPUS. The increased new bone formation demonstrates similar results to those we have observed at the tendon-bone interface following LIPUS treatment. Protein expression for VEGF, CBFA-1 and PCNA was upregulated with LIPUS treatment. The use of low intensity pulsed ultrasound in combination with bone graft substitutes may offer a viable treatment option in the repair of bony defects.

**Smith & Nephew Inc., Memphis, TN, USA

Table 1. DEXA data at 4 weeks.