In Vitro and *In Vivo* Evaluation of a Slower Resorbing Calcium Sulfate Cement

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Introduction: Calcium sulfate (CS) cements have a long history of use as bone graft substitutes. Modern surgical grade CS cements offer high initial strength, good handling, and are consistently replaced by bone in many applications. However, there are indications in which slower resorption is desired. In the present work a CS cement has been modified to slow the resorption rate by inclusion of a biphasic calcium phosphate cement component.

Materials & Methods: The experimental group for all experiments was the composite, slower resorbing cement (SR). MIIG®X3 Bone Graft Substitute (X3) (Wright Medical, Arlington, TN) calcium sulfate was used as a control. The SR material was formulated to set in 15-19 minutes, whereas the X3 material sets in 7-10 minutes.

Diametral tensile strength (DTS) was measured on vacuum mixed specimens cast to 16mm OD X 25.4mm cylinders in closed-cell polyurethane foam. The sides of the foam blocks were removed prior to testing. Specimens (n=4) were cured for 1 and 24hrs in ambient air at room conditions or in bovine calf serum at 37ºC; additional specimens (n=4) of SR were cured for 3hrs in bovine calf serum at 37ºC to further investigate its setting kinetics. The specimens were transversely loaded to failure in compression using a MTS 858 Bionix® test system at a constant rate of 5mm/min. DTS was calculated from the formula $DTS = (2*P_{max})/(\Pi*L*H)$.

Dissolution tests were performed on 4.8mm OD X 3.3mm cylindrical pellets (n=5). Specimens were placed in 275mL of distilled water at 37ºC. Solutions were changed daily. Specimens were dried and massed daily for first 30 days and every 5 days thereafter until a residual mass of <5% was achieved. X-Ray diffraction (XRD) was used to identify the residual material.

A 6-week *in vivo* pilot study was conducted under an IACUC-approved protocol at Rush University. In each of 3 dogs, two defects measuring 9mm X 15mm were created in each proximal humerus. Previous studies have shown this to be a critical size defect. Each site was filled with either an injected bolus of SR (1-1.5cc), 4.8mm OD X 3.3mm pellets of SR, 4.8mm OD X 3.3mm pellets of X3, or an injected bolus of an intermediate resorbing CS cement. Each dog received one implant of each material. Healing of the defects and resorption of the materials were assessed from radiographs obtained after 0, 2, and 4 weeks and contact radiographs after 6 weeks. New bone formation and residual implanted material in the defects were evaluated using light microscopy of undecalcified, plastic embedded histological sections stained with basic fuchsin and toluidine blue. Area fraction of new bone and residual material in the defects were determined using histomorphometry.

Results and Discussion: The results of DTS testing are shown in Fig. 1. Under dry conditions the SR material was not completely cured at 1hr, as evidenced by a significant increase in strength after 24hrs ($p<0.001$). The X3 cement, designed to set more quickly, did not show a significant difference under the same conditions ($p=0.508$). In contrast, 1hr specimens cured *in vitro* exhibited an increase compared to air cured 1hr specimens, with a 27% higher strength for SR ($p=0.015$) and a 9% increase for X3 ($p=0.050$). Statistical analysis of strength versus time showed no significant difference for the SR material ($p=0.590$) and a 15% decrease for the X3 material ($p=0.008$) between 1 and 24hrs *in vitro*.

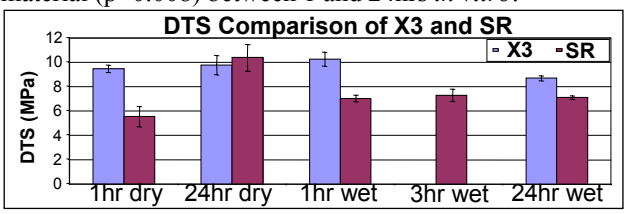

Figure 1. Diametral Tensile Strength

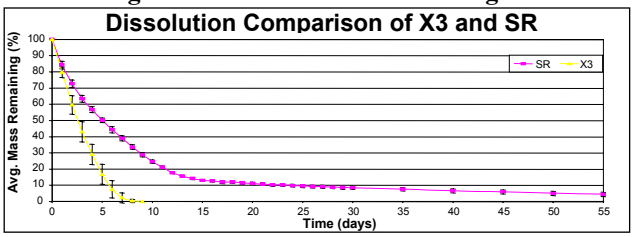

Figure 2. *In Vitro* Dissolution

Dissolution results are shown in Fig. 2. Linear regression of the initial part of the curves (100 to ≥20% remaining) was used to estimate the dissolution rates. The SR and X3 rates were 8.2%/day and 18.5%/day, respectively. XRD of residual SR material showed it to be beta tricalcium phosphate, a known bioresorbable and osteoconductive material.

In the *in vivo* study, the radiographic and histologic data indicated that both types of pellets and boluses were replaced with newly formed osteoid, woven, and lamellar bone that had formed in concentric lamellae at the previous implant sites. At 6 weeks area fraction of new bone formation was 35.9±6.1% for defects implanted with SR pellets and 26.7±10.0% for defects implanted with X3 pellets. At 6 weeks, the majority of the implanted pellet materials had resorbed, but there was slightly more residual implant material in SR pellet defects compared to the X3 pellet defects. For the SR bolus implants new bone formation was 15.6±5.6% with 29.9±11.9% residual implant material. Smaller fractions of new bone formation are expected for bolus materials at early time points due to larger percentages of residual material and smaller surface area to implant volume ratios when compared to those of pellets.

Conclusions: The composite CS cement demonstrated consistent setting and strength characteristics similar to those of the control. The dissolution rate of the SR material was 56% slower than that of the control, with equivalent or superior early *in vivo* bone growth.