Purified Chitosan Bone Fillers Increase Bone Formation Rates in Bone Defects

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Statement of Purpose: Autograft is the bone filler of choice in Orthopaedic procedures because it is osteoinductive. However, a second surgical wound must be made to collect bone leading to potential morbidity. Synthetic fillers have been used as bone repair materials. They must be combined with proteins to be osteoinductive. Although proteins can significantly increase bone formation rates they are difficult to sterilize and can be expensive. A non-protein based filler which substantially accelerates bone formation would be of great value for filling defects. Preliminary testing of two new chitosan based bone repair materials (Genis Inc. Reykjavik, Iceland) indicates that these materials accelerate bone formation. The purpose of this study was to characterize and quantify the extent to which the new materials accelerate bone formation.

Methods: A total of 18 rats used in this study were divided into 3 groups of 6. Group 1 was a drilled unfilled defect group. Group 2 was drilled and implanted with a chitosan formulation labeled GC1 and Group 3 was drilled and implanted with a purified chitosan labeled GC2. Both fillers contain calcium phosphate particles.

During implantation surgery, a standard lateral approach used in earlier studies of calcium ceramic materials and approved by the I.A.C.U.C. was used. Briefly, a skin incision was made along the lateral aspect of the hind limb from the hip to the stifle. A 2 mm hole was drilled in the mid-diaphysis of the femur. The defect was either left empty or filled with one of the two fillers. Test animals received two calcine injections 7 days apart to label their bones for quantitative assessment of mineral apposition rate (MAR) and bone formation rate (BFR).

At 3 weeks the test animals were euthanized and the femora were dehydrated in ethanol and embedded in polymethylmethacrylate. Slices were cut from the defect site and from the corresponding region on the contralateral control femur. One section was stained using a mineralized bone stain (MIBS) and the other with hematoxylin and eosin (H&E). Sections were evaluated quantitatively with both transmitted light for new bone growth and with fluorescence to calculate MAR and BFR. Data were analyzed using an ANOVA with a Tukey HSD post-hoc test utilizing a p value <0.05 for significance.

Results/Discussion: Test animals all tolerated surgery well and healing was uneventful. Back scatter electron microscopy of drilled unfilled bone sections showed only partial healing at the defect site. GC1 filled bones also showed bone formation, although, it was primarily periosteal with a small amount of bone in the endosteal canal. GC2 filled bones also showed extensive bone formation along the periosteal surface with some bone formation noted in the endosteal canal. No calcified tissue was observed inside the bone filler material in sections through the implant site. These observations suggest that early in the healing process the bone filler material accelerates bone formation along the perimeter of the filler material and in regions of rapidly growing bone. H & E stained sections showed regions of uncalcified tissue containing osteoblasts forming new bone in pores of the bone filler. In some sections from the GC2 group these tissues were apparent around the perimeter of the bone filler but were not noted around the perimeter of the GC1 material in the bone sections of group 2 test animals.

There was significantly more bone in the GC2 filled group than the GBRM filled group and the drilled unfilled group (3.444 ± 0.811 mm^2 vs. 2.045 ± 1.010 mm^2 vs. 1.230 ± 0.558 mm^2). The GC2 material also showed a more consistent increase in new bone than the GC1 group as demonstrated by the smaller standard deviation. In all limbs implanted with a filler there was significantly more bone than was measured from internal control limbs. Control femurs had a range of new bone formation from 0.2386 to 0.5036 mm^2. MAR for the drilled unfilled femurs was not significantly different from control bones. The MAR for both the GC1 and GC2 groups were different from their controls but were not significantly different from each other. BFR/BS for treated groups was not significantly different.

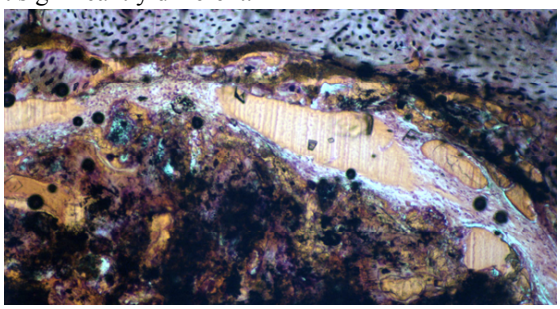

Figure 1 : H&E stained section shows pre-existing cortical bone (top) and new bone forming along surface of the bone filler (bottom) within the endosteal canal.

Conclusions: These studies demonstrate accelerated bone formation soon after filler placement with extensive new bone formation. The GC2 filler caused the most dramatic results providing nearly 200% more new bone than the drilled unfilled group. Additional studies to evaluate the best configuration for these bone filler materials will provide a technique to guide the rapid bone formation induced by these fillers to specific locations.

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