INTRODUCTION - Calcium sulfate (CS) has been used as a synthetic bone void filler material in various clinical indications to support new bone growth and remodeling. Conforming the filler material to osseous defects while providing osteoconductivity and implant stability are important clinical factors. A new synthetic advanced bone void filler made from shaped high purity CS granules with a carboxymethyl cellulose (CMC) based hydrogel addresses these issues. The granules have a unique interlocking six-arm shape that provides intergranule stability with interconnected porosity for bony ingrowth. The CMC based hydrogel is used as a handling agent when mixed with granules to produce a conformable filler. This gel used alone in a rabbit defect has been shown to be nontoxic and biocompatible [1]. The aim of this study was to compare new bone formation and mechanical properties in rabbit femoral defects filled with CS granules and CMC-hydrogel against CS granules alone and defects without test materials.

METHODS - A 6.4mm-diameter by 10mm-depth defect was made in the right lateral femoral condyle under anesthesia in 54 white New Zealand rabbits. This research protocol (#1145) was approved by the Animal Care and Use Committee at the University of Tennessee. Test groups consisted of: CS - two granules alone; CS+Gel - two granules mixed in a 1:1 ratio with CMC-hydrogel (JAX® granules and JAX® Gel, Smith and Nephew, Inc., Memphis, TN); Control - no filler. The surrounding tissue was sutured, and the rabbits were allowed to move freely after surgery. Radiographs were taken 24 hours post-operatively and at sacrifice. Six animals from each group were sacrificed after 2, 4 or 12 weeks and tissue prepared for histological analysis. Histological analysis was performed to assess the degree of new bone formation and resorption of the implant. Tissue samples were stained with toluidine blue, safranin O, hematoxylin and eosin or Masson trichrome (MT).

Nanoindentation was used to compare the mechanical properties of newly formed trabeculae around the filler material between the CS and CS+Gel groups (one bone sample per group, nine indents per sample). Nanoindentation experiments followed previously published protocols [2] to generate Young's modulus values (mean±stdev) for each group.

RESULTS - Analyses of blood taken at sacrifice indicated no problems specific to CS or CS+Gel groups. Radiographs indicated that the CS materials, although visible (radiopaque) in the post-op radiographs, were not discernable in the 2-week rabbits, indicating that the CS granules were decreasing in density and were resorbing. The defect itself, however, was still noticeable by 12 weeks in all three groups. Independent assessment determined that the CS and CS+Gel groups had better radiographic evidence of healing than the control group.

The 2- and 4-week non-treated control defects contained marrow with some evidence of collagen fibrils and bone around the outer edge of the defect (Fig.1A). The 2-week defects with either CS or CS+Gel material contained sizeable amounts of residual material surrounded by collagen fibrils with an outer layer of thin bony trabeculae (Fig.1B & C). The 4-week defects with either CS or CS+Gel material contained smaller amounts of residual material also surrounded by collagen fibrils with an outer layer of thin bony trabeculae. The amount of CS material decreased between 2 and 4 weeks but was still evident. There was no evidence of inflammatory tissues or cells around the CS or CS+Gel implants. All 12-week defects were filled with marrow tissue in the deep regions. However, in the superficial (cortical) region the 12 week CS and CS+Gel filled defects contained greater amounts of deposited collagen and larger bony trabeculae compared to controls receiving no implants. The growing bony trabeculae and osteoid in the CS and CS+Gel filled defects were evident at every timepoint by the presence of many osteoblasts lining the trabeculae with collagen fibrils around the forming trabeculae (Fig.2).

Nanoindentation showed an increase in Young’s modulus of the newly formed trabeculae bone in the CS groups from 2- to 4-weeks (2.39±1.07 GPa to 4.59±1.03 GPa; p<0.05). No statistical difference was found in the CS+Gel groups from 2- to 4-weeks (5.35±2.21 GPa to 5.35±2.09 GPa; p=0.99). Statistical difference was found between the CS and CS+Gel groups at 2-weeks (p<0.05) but not at 4-weeks (p=0.34).

DISCUSSION - The histological data indicated better than normal healing directly around the CS or CS+Gel material as evidenced by the collagen fibrils immediately surrounding the implant with peripheral intramembranous bone formation. The amount of collagen and bony trabeculae was visually much greater in the CS and CS+Gel filled defects compared to the non-treated controls in the 2 and 4-week groups, demonstrating the osteoconductivity of the materials. Osteogenesis in the 2- and 4-week CS and CS+Gel animals was seen especially around the filler material.

At 12-weeks the non-treated control defects showed very little histological evidence of bone healing, suggesting the defects were of critical size. In all 12-week specimens there was a noticeable lack of trabeculae deep in the defects but large trabeculae and osteoid were present in the superficial (near the cortex) regions of the CS and CS+Gel filled specimens. The CS and CS+Gel materials were fully resorbed between 4- and 12-weeks. Nanoindentation showed newly formed bone mechanical properties were similar between CS and CS+Gel groups.

CONCLUSION - This study showed that the use of the CMC-hydrogel handling agent with shaped CS granules has no biological adverse effects on the rabbits, produces bony healing and bone mechanical properties comparable to the granules alone, and produces enhanced bone formation compared to the non-treated controls.

REFERENCES

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