Resorption Evaluation of a Large Bolus of Calcium Sulfate in a Canine Medullary Defect

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Abstract

New bone formation and resorption of a calcium sulfate bone graft substitute implanted in five canines were evaluated in this study. Healing was assessed radiographically at 2, 6, and 13 weeks. At 13 weeks, the dogs were sacrificed, and the humeri were retrieved. High-resolution contact radiographs of the isolated humeri were obtained and the bones were sectioned for histology. Radiographically, the calcium sulfate appeared to be completely resorbed and replaced by bone at 13 weeks. Histological findings suggest that a residual amount of calcium sulfate remained, which may continue to act as an osteoconductive scaffolding. No adverse inflammatory response was observed.

Synthetic materials to replace or expand bone graft are of clinical interest because of the morbidity and procurement issues related to harvesting autogenous bone graft. Calcium sulfate has been proposed as a bone graft substitute. As early as 1892, use of calcium sulfate as a bone graft substitute was documented by Dreesman. Subsequent healing of bone defects treated with calcium sulfate was summarized by Peltier, who conducted a thorough literature review. Although successful outcomes were sporadic, he found that studies demonstrated that calcium sulfate resorbs and is well tolerated by tissues. By acting primarily as a space filler, calcium sulfate restores morphological contour and prevents the ingrowth of soft tissue into defects.

Peltier and Speer confirmed that calcium sulfate is an osteoconductive material that allows ingrowth of blood vessels and osteogenic cells. Calcium sulfate has demonstrated clinical efficacy in numerous bone grafting applications. Injection of calcium sulfate has gained increasing acceptance for closed treatment applications, particularly in the management of compression fractures.

The resorption rate of calcium sulfate is dependent upon many factors, including contaminants, impurities, and crystalline structure of the material. By creating a surgical-grade calcium sulfate hemihydrate (OsteoSet RBK, Wright Medical Technology, Inc, Arlington, Tenn) with crystalline structure of specific size and shape, the resorption rate of the material can be controlled at a rate that is consistent with that of new bone formation.

This study presents a radiological and histological evaluation of resorption and bone regeneration characteristics of surgical-grade calcium sulfate hemihydrate when mixed with saline and injected as a large bolus into surgically created defects in canine humeri.

MATERIALS AND METHODS

Study Overview

New bone formation and resorption of a calcium sulfate bone graft substitute implanted in five canines were evaluated in this study. Radiographs were obtained and assessed for healing immediately postoperatively and at 2, 6, and 13 weeks. The dogs were sacrificed at 13 weeks, and the humeri were retrieved. High-resolution contact radiographs of the isolated humeri were obtained and the bones were sectioned for histology.

Surgical Procedure

Under general anesthesia and using an aseptic technique, a cranial approach to the greater tubercle of the left and right humerus was performed. A cylindrical...
The cavity measuring 13 mm in diameter × 50 mm in length was created bilaterally in the humerus by drilling axially through the greater tubercle into the medullary canal.

The calcium sulfate powder was mixed with saline according to the manufacturer’s instructions, and 6 cc were injected through a 20-cc syringe into the prepared cavity. The supraspinatus tendon was closed over the defect in the greater tubercle, and the wound was closed in layers in a routine fashion.

All surgeries and animal care were performed in accordance with Institutional Animal Care and Use Committee-approved guidelines, the Guide for Care and Use of Laboratory Animals,11 and the regulations of the United States Department of Agriculture Animal Welfare Act.12

**Implant Preparation**

Surgical-grade calcium sulfate hemihydrate powder and a separate vial of saline were packaged as a kit and sterilized using gamma radiation at 25-32 kGy.

**Analytical Methods**

Radiographs were obtained and assessed for healing immediately postoperatively (Figure 1) and at 2, 6, and 13 weeks. High-resolution radiographs were made of the isolated humeri. The bones were sectioned serially in the transverse plane to produce sections for histological analysis. High-resolution contact radiographs were obtained of the sections. Later, the sections were stained with fuchsin and toluidine blue for undecalcified histology and studied by light microscopy.

Qualitative assessments of the postmortem contact radiographs of intact bones and cross sections and the stained histologic sections were performed to characterize the nature of the new bone and the residual graft material in the defects.

**RESULTS**

No intraoperative or postoperative complications were observed, wounds healed in a routine manner, and all dogs were weight bearing within 3 days following the procedure.
Sequential radiographs at 2, 6, and 13 weeks demonstrated progressive resorption of the bolus of calcium sulfate within the defect and apparent replacement by bone.

At 13 weeks, the calcium sulfate graft appeared to have completely resorbed, and the defect was filled with newly formed bone (Figure 2). These findings were confirmed by transverse section contact radiographs (Figure 3).

Histologically, at 13 weeks, all of the medullary defects treated with calcium sulfate demonstrated prominent osteoblastic rimming of the newly woven bone. The stained sections demonstrated thickened, interconnected trabeculae and bone marrow resembling the adjacent medullary bone.

At low magnification, trabecular bone and marrow filled the area of the defect and concentric layering indicating creeping substitution was noted (Figure 4).

A higher magnification showed residual calcium sulfate incorporated into the newly woven bone and in the immediate area, which continues to provide an osteoconductive scaffolding (Figure 5). There was no evidence of foreign body granulomas in any of the dogs.

**DISCUSSION**

Calcium sulfate in a large bolus used as a synthetic bone graft material in this study demonstrated biocompatibility. Resorption and bone replacement were characteristic of reported findings with calcium sulfate pellets, where the radiographic rate of resorption closely corresponded to the rate of new trabecular bone growth.

Newly formed woven bone was found between the edges of the surgically created defect and the resorbing implant, which is consistent with a creeping substitution mechanism of action. The apparent concentric layering pattern of bone formation noted in this study at 13 weeks is unique to calcium sulfate and implies guided formation.

Radiographically, the calcium sulfate implant appeared to be completely resorbed and replaced by bone by the 13-week time point. However, histological findings suggest that a residual amount of calcium sulfate remained that might continue to act as a physical three-dimensional scaffolding for new bone growth. No adverse inflammatory response was observed. Sequential contact radiographs exhibited normal cancellous architecture of the new bone, indicating that bone remodeling had occurred.

**CONCLUSION**

The results of this study indicate that calcium sulfate injected as a large bolus performs as a bone regenerative material in a similar manner as calcium sulfate in a pellet form. This has particular implications as it relates to an evolving treatment modality in which calcium sulfate is used as a closed treatment option for managing compression fractures (MIIG, Minimally Invasive Injectable Graft, Wright Medical Technology, Inc), particularly those of the distal radius (Figure 6). This study demonstrates that a large bolus of calcium sulfate is resorbed at a predictable rate and is consistently replaced by new bone.

**REFERENCES**