Healing of Large Defects Treated With Calcium Sulfate Pellets Containing Demineralized Bone Matrix Particles

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Abstract

Calcium sulfate (OsteoSet, Wright Medical Technology, Inc, Arlington, Tenn) and calcium sulfate/demineralized bone matrix (DBM) pellets (OsteoSet DBM, Wright Medical Technology, Inc) have been evaluated preclinically in a bilateral medullary defect model of a canine humerus. In this model, both short (6 week) and long (26 week) time points have been evaluated. An analysis of bone response to the pellets was conducted using radiological, histological, mechanical, and quantification techniques. The calcium sulfat/DBM pellets exhibited more rapid trabecular bone remodeling as demonstrated by the absence of the ringlet bone structure typically seen with calcium sulfate pellets. We concluded that calcium sulfate and calcium sulfate/DBM pellets are both effective bone graft substitutes.

Demineralized bone matrix (DBM) has been used to reconstruct bone tissue in maxillofacial surgery, periodontics, and orthopedics. The first cited use of DBM was by Senn1 in 1889 for the successful repair of skull and long bone defects in dogs, and femoral and tibial defects in humans.

Over the next 75 years, few clinical studies were conducted due to the conflicting reports of ossification resulting from the variations in demineralization techniques.2

In 1965, Urist3 recognized the osteoinductive ability of DBM by using an animal model to show that DBM induced bone formation in a muscle site. At this same time, Urist implanted demineralized bone into human long bone defects and lumbar vertebrae and achieved positive results. Since 1965, numerous animal and clinical models have demonstrated the effectiveness of DBM in a variety of osseous defects.4,5

Demineralized bone matrix has been used in combination with surgical-grade calcium sulfate hemihydrate in periodontal and orthopedic applications to produce successful outcomes.6,9

Sottosanti9 summarized the purposes of calcium sulfate: to act as a binder to improve the handling characteristics of DBM, to limit DBM loss, and to improve the new bone yield by enhancing the effect of the bone morphogenetic protein.

With a proven history as a bone graft substitute, DBM can be used to promote bone growth in surgically created defects. Calcium sulfate pellets (OsteoSet, Wright Medical Technology, Inc) have a proven clinical history of osteoconduction and controlled resorption.10 When DBM is incorporated into a calcium sulfate/DBM pellet (OsteoSet DBM, Wright Medical Technology, Inc), it can be implanted and uniformly distributed in the defect.

Three studies were conducted at the same institution using the same canine model to study the effectiveness of calcium sulfate/DBM pellets (OsteoSet DBM) as a bone graft substitute. These studies compared the osteoconductivity and resorbability of calcium sulfate pellets impregnated with DBM (calcium sulfate/DBM pellets) to calcium sulfate pellets alone.

Materials and Methods

Study Overview

Three series of canine implantations were performed to compare calcium sulfate pellets to calcium sulfate/DBM pellets. The series included: (1) five dogs treated with calcium sulfate pellets for 6 weeks, (2) seven dogs treated with calcium sulfate/DBM pellets for 6 weeks, and (3) six dogs treated with calcium sulfate/DBM pellets for 26 weeks.

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pellets for 6 weeks, and (3) 11 dogs treated bilaterally for 26 weeks with calcium sulfate pellets in one humerus and calcium sulfate/DBM pellets in the other.

**Surgical Procedure**

Under general anesthesia and using an aseptic technique, a cranial approach was performed to the greater tubercle of the left and right humerus of each canine.

A cylindrical cavity was created bilaterally in the humerus by drilling axially through the greater tubercle into the medullary canal. The cavity measured 13 mm in diameter \( \times \) 50 mm in length. The prepared cavity was filled with 50 of the test or control pellets (either calcium sulfate or calcium sulfate/DBM). The pellet type was assigned to the left or right humerus by a computer-generated randomization schedule.

After implantation of the pellets, 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*, and regulations of the United States Department of Agricultural Animal Welfare Act.

**Implant Preparation**

All of the pellets measured 4.8 mm in diameter and 3.3 mm in height and were composed of surgical-grade calcium sulfate hemihydrate, stearic acid, and sterile water. The calcium sulfate/DBM pellets also contained 10% by weight canine DBM. The calcium sulfate pellets were gamma sterilized at 25-32 kGy, and the calcium sulfate/DBM pellets were sterilized with electronic beam radiation.

Canine DBM was purchased from a veterinarian tissue supply company (Veterinary Transplant Services, Kent, Wash). Canine bones were cleaned of soft tissue and ground to particle size \( <1250 \) mm (sieved). Bone particles were treated in a 0.6-N HCl solution until the pH dropped below 1.0 and then treated with antibiotics. The bone particles were rinsed with sterile water and phosphate buffer solution, packaged into plastic syringes, and frozen (-80° C). This process resulted in canine DBM that was "wet frozen." Prior to putty formulation, the bone was dried to remove free moisture.

**Analytical Methods**

Radiographs were taken of the humeri preoperatively, immediately postoperatively, and at 2, 6, 13, and 26 weeks.

Additionally, explanted humeri were sectioned transversely and processed for plastic embedded undecalcified histology. The histologic sections were stained with basic fuchsin and toluidine blue and were examined by light microscopy. Contact radiographs of the cut sections were taken.

Mechanical testing was performed on the 26-week specimens. From each animal, a 17-mm thick section was obtained from between the middle and distal section of each humerus. At the time of testing, a cylindrical trabecular specimen (8-mm diameter \( \times \) 16-mm long) was machined from the specimen block. The prepared specimen was mounted on an Instron 1321 servohydraulic mechanical test system (Instron Corp, Canton, Mass) and subjected to a uniaxial compressive strain at a rate of 0.5 mm/min until failure. A stress-strain curve was generated, and the elastic modulus and strength of bone were calculated.

On the 26-week specimens, the area fraction of new bone was measured using computer analysis of backscattered electron scanning images of unstained sections. Areas of identifiable calcium sulfate and DBM were excluded.

**RESULTS**

Postoperative radiographs revealed all test materials to be well contained in the prepared cavities. All animals completed the required interval of experimentation with normal wound healing and no surgical complications, infections, or adverse reactions.
Eleven canines were treated in the 26-week study with radiographs taken preoperatively, immediately postoperatively, and at 2, 6, 13, and 26 weeks. Additionally, contact radiographs of the entire humerus were taken after sacrifice.

The radiographs illustrated that the defects were filled with pellets at the initial postoperative period, followed by a significant decrease (25% for calcium sulfate pellets; 50% for calcium sulfate/DBM pellets) in pellet dimension and density at 2 weeks. Also at 2 weeks, an increase in bone density along the original bone defect demonstrated concurrent bone formation with the resorption of the pellets.

By 6 weeks, there was no evidence of either type of pellet. At 13 and 26 weeks, there was, overall, an equivalent radiographic appearance of defects treated with the two pellet types where density of the bone in the defect was similar to that of the surrounding medullary bone. The serial radiographs showed progressive resorption of the pellets and apparent restoration of normal radiographic medullary density.

At 6 weeks, typical contact radiographs of transverse humeral sections from defects treated with calcium sulfate pellets illustrated pellet remnants with concentric lamellae of newly formed bone (Figure 1).

At the same time, contact radiographs from a defect treated with calcium sulfate/DBM pellets (Figure 2) showed slightly visible remnants of pellets with concentric lamellae of newly formed bone surrounding the individual pellets. Throughout the void, there was consistent new bone formation around the pellets in an organized trabecular pattern.

Contact radiographs at 26 weeks for both calcium sulfate (Figure 3) and calcium sulfate/DBM pellets (Figure 4) revealed a well-developed network of fine and coarse trabeculae with interconnected struts fully occupying the entire defect and continuous with adjacent medullary bone.

Figure 3: Contact radiograph at 26 weeks of a defect filled with calcium sulfate pellets. A well-developed network of fine and coarse trabeculae with interconnecting struts occupies the entire defect. Remodeling of the concentric lamellae has occurred (A). Histological section at 6 weeks of a defect filled with calcium sulfate pellets. Concentric pattern of bone formation surrounding residual pellets (black) is evident (B).

Figure 4: Contact radiograph at 26 weeks of a defect filled with calcium sulfate/DBM pellets. A well-developed network of fine and coarse trabeculae with interconnecting struts occupies the entire defect (A). Histological section at 6 weeks of a defect filled with calcium sulfate/DBM pellets. New bony trabeculae incorporate DBM particles and residual CaSO₄. Demineralized bone matrix particles and residual CaSO₄ are scarce (B).

In summary, contact radiographs illustrated new bone formation surrounding the calcium sulfate and calcium sulfate/DBM pellets. From 6 to 26 weeks, the new bone matured and filled the defects.

Typical histological sections from a defect treated with calcium sulfate pellets at 6 weeks illustrated bone formation on the surface of a residual pellet that formed in a series of concentric lamellar structures with no evidence of foreign body granulomas. Residual traces of pellet material were totally incorporated by the new bone formation. Thin trabeculae of bone also developed independently in the drilled cavity and in the resorbed pellets.

Histologic sections of a calcium sulfate/DBM pellet-treated defect at 6 weeks showed abundant new trabecular bone formation throughout all of the defects with no evidence of foreign body granulomas. New bony trabeculae incorporated DBM particles and residual calcium sulfate. The trabeculae were thickened and their surfaces primarily showed osteoblastic activity. Overall, DBM particles and residual CaSO₄ were scarce. The ringlet structure found when using calcium sulfate pellets was absent, as the bone formation was more consistent with normal architecture.

At 26 weeks, the histological sections showed a similar appearance for
Figure 7: Calcium sulfate pellet — A 26-week light micrograph illustrates thickened trabeculae and bone marrow. Residual pellet material (arrows) is incorporated within the bone trabeculae (A). Calcium sulfate/DBM pellet — Remaining DBM particles (arrows) are incorporated within the bone trabeculae (B).

### TABLE 1

<table>
<thead>
<tr>
<th>Pellet Type</th>
<th>Mean Compressive Strength (mPa)</th>
<th>Mean Elastic Modulus (mPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium sulfate</td>
<td>0.87 ± 0.71</td>
<td>59.0 ± 60.7</td>
</tr>
<tr>
<td>Calcium sulfate/DBM</td>
<td>0.69 ± 0.34</td>
<td>44.7 ± 34.2</td>
</tr>
<tr>
<td>P value</td>
<td>0.132</td>
<td>0.366</td>
</tr>
</tbody>
</table>

Abbreviation: DBM = demineralized bone matrix.

### TABLE 2

<table>
<thead>
<tr>
<th>Pellet Type</th>
<th>Area Fraction of New Mineralized Bone</th>
<th>Total Area Fraction of Residual Calcium Sulfate</th>
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</thead>
<tbody>
<tr>
<td>Calcium sulfate</td>
<td>11.4 ± 2.3%</td>
<td>0.32 ± 0.16%</td>
</tr>
<tr>
<td>Calcium sulfate/DBM</td>
<td>11.2 ± 2.33%</td>
<td>0.36 ± 0.30%</td>
</tr>
<tr>
<td>P value</td>
<td>1.000</td>
<td>0.739</td>
</tr>
</tbody>
</table>

Abbreviation: DBM = demineralized bone matrix.

the sites treated with both types of pellets (Figure 7). The defects contained thickened, interconnected trabeculae and bone marrow resembling adjacent medullary bone. In summary, the histological sections revealed bone formation and defect filling throughout the defect with both pellet types. The bone matured from 6 to 26 weeks.

The mechanical testing yielded no significant difference in either the mean compressive strength or the mean elastic modulus of bone filling the defects treated with both types of pellets after 26 weeks (Table 1).

The bone quantification analysis yielded no significant difference in either the area fraction of new mineralized bone or the total area fraction of residual calcium sulfate in defects treated with both types of pellets after 26 weeks (Table 2).

### DISCUSSION

By 3 weeks, all defects treated with calcium sulfate pellets exhibited diminished density by approximately 50%. By 6 weeks, the pellets were only minimally detectable and were punctuated by multiple sites of circular bone densities corresponding to previous pellet sites.

By 6 weeks, all defects treated with calcium sulfate/DBM pellets exhibited new bone filling the cylindrical cavity. Few circular bone densities corresponding to previous pellet sites were observed.

At 13 and 26 weeks, there was, overall, a similar radiographic appearance of defects treated with the two pellet types where the density of bone in the defect was similar to that of the surrounding medullary bone. A slight increase in cortical and periosteal bone was present in humeri with both pellet types and remodeled to a normal appearance by 26 weeks.

Histology sections showed a similar appearance for the calcium sulfate and calcium sulfate/DBM treated defects consistent with complete bony restoration. The defects contained thickened, interconnected trabeculae and bone marrow resembling the adjacent medullary bone. Pellets or pellet fragments were absent.

In keeping with the analysis of scanning electron microscope images, dark-stained, granular material — apparently residual calcium sulfate — was only rarely observed and was completely incorporated into bony trabeculae. In addition, in the calcium sulfate/DBM treated defects, few residual particles of DBM were observed. These were also well incorporated into the matured trabecular bone. Two calcium sulfate and one calcium sulfate/DBM treated sites showed a small, central focus of fibrous tissue.

### CONCLUSION

Calcium sulfate and calcium sulfate/DBM pellets have been evaluated preclinically in a bilateral medullary defect model in the canine humerus. In this model, both short (6 week) and long (26 week) time points were evaluated. An analysis of the bone response to the pellets was conducted using radiological, histological, mechanical, and quantification techniques. In all analysis methods, the two pellet types behaved
similarly, with no significant differences detected. The calcium sulfate/DBM pellets (OsteoSet DBM) exhibited more rapid trabecular bone remodeling as demonstrated by the absence of the ringlet bone structure typically seen with calcium sulfate pellets. Calcium sulfate and calcium sulfate/DBM pellets are both effective bone graft substitutes.

REFERENCES