Introduction: Defects in bone are encountered following trauma, infection, tumor, or failure of a prosthetic device such as a joint replacement. Bony defects can be restored with the use of bone graft obtained from another bone of the same patient, but this involves additional surgery and the risk of additional pain and complications at the donor site. Bone graft obtained from cadavers is also available, but it is expensive and, despite elaborate precautions, may pose a risk for transmission of certain viral agents. Calceon 6® calcium sulfate pellets (Synthes USA) is a synthetic bone graft substitute for use as a general bone void filler. The purpose of this study was to evaluate new bone formation comparing treatment with Calceon 6® calcium sulfate pellets to autologous cancellous bone graft in a bilateral medullary critical-size defect model in the canine proximal humerus after 6 weeks and 26 weeks.

Materials and Methods: Under an IACUC-approved protocol, 14 skeletally mature, male hound-type dogs weighing 30 to 35 kg. were randomized into study periods of 6 weeks (N=7) or 26 weeks (N=7). Under general anesthesia, a 13 mm × 50 mm cylindrical cavity was created axially in each proximal humerus. The defect in one humerus was implanted with 50 (4.8 x 3.3 mm) pellets of the test material, Calceon 6® calcium sulfate. The contralateral humerus received a comparable volume (6 cc) of the control material, autologous cancellous bone obtained from reaming the medullary canal. The treatment groups were randomized by side. Clinical radiographs of the humeri were obtained immediately following the surgery and at 3, 6, 12 and 26 weeks postoperative.

After 6 and 26 weeks, the animals were euthanized and the humeri were removed and fixed in formalin. The specimens were sectioned transversely and processed for undecalcified, plastic-embedded histology. Image analysis of backscattered-electron scanning electron microscope images of unstained sections from proximal, middle, and distal levels of the defect were used to quantify the area fractions of new bone, residual calcium sulfate and particles of bone graft remaining in the defect. For each of the study groups, the amounts of new bone and residual graft materials on the side treated with Calceon 6® was compared to the contralateral side treated with autologous cancellous bone graft. Three additional sections were stained with basic fuchsin and toluidine blue and studied to determine the nature of the tissues and residual implant materials in the defect by light microscopy.

The quantitative data were analyzed using nonparametric statistics. The Friedman test was used to analyze treated and control side data within groups, and the Mann-Whitney test was used to compare data between the groups. Differences with p-values less than 0.05 were considered significant.

Results: There were no intraoperative complications. All 14 animals recovered uneventfully, resuming weight bearing within 2 days. There were no postoperative infections, wound healing complications or fractures. Post mortem gross observations indicated that all of the surgical sites were completely healed without evidence of inflammatory reaction. No extraosseous lesions were noted.

At 6 weeks, back scattered-electron SEM analysis of new bone formation in the defects showed that the mean area fraction of new mineralized bone was 3.6% (range, 2.1% to 5.4%) for the Calceon 6® treated defects and 8.7% (range, 4.3% to 11.9%) in the autograft-treated defects. The difference between the treatments was significant at p=0.008.

At 26 weeks, the mean area fraction of new mineralized bone was 8.8% (range, 7.5% to 10.2%) for the Calceon-treated defects and 10.0% (range, 8.3% to 11.7%) in the autograft-treated defects. Thus, the area fraction of bone in Calceon-treated defects decreased from 6 weeks to 26 weeks (p=0.001), so that by 26 weeks the area fraction of bone was equivalent between the Calceon and autograft treated defects (p=0.257).

For residual implant material, the analysis showed that the mean area fraction of Calceon 6® remaining in the defects decreased significantly from 3.6% (range, 3.0% to 5.2%) at 6 weeks to 0.06% (range, 0.0% to 0.2%) at 26 weeks (p<0.001). The mean area fraction of residual cancellous bone graft decreased from 1.3% (range, 0.3% to 1.8%) at 6 weeks to 0.09% (range, 0.0% to 0.3%) at 26 weeks (p=0.001).

The quantitative data revealed extensive formation of new bony trabeculae throughout the defects whether they had been treated with Calceon 6® or autologous cancellous bone graft. At 6 weeks, the defects contained predominantly mineralized woven and lamellar bony trabeculae, osteoid and marrow elements. The Calceon-treated defects were distinguished by intense new bone formation and remodeling and sites of circular trabeculae of bone, which had formed in association with largely resorbed pellets. The remaining Calceon 6® appeared as dark-staining, granular deposits most often incorporated into the new mineralized trabeculae. In other areas, residual Calceon 6® was covered by osteoid or was undergoing apparent resorption by multinucleated, osteoclast-like cells. In contrast, in the autograft-treated defects, there was less osteoid than in the Calceon-treated defects, and remodeling of new bone was less intense. Residual particles of bone graft were seen incorporated into new bone trabeculae. Two autograft-treated defects had rare, minute foci of cartilage formation associated with fragments of the cancellous bone graft. There was a greater increase in medullary density surrounding the defects treated with the Calceon 6® pellets compared to defects treated with autograft. Periosteal new bone was evident in 2 of 14 Calceon-treated humeri, but none of the autograft-treated humeri. At 6 weeks, the pellets were no longer visible on either the clinical or postmortem whole bone radiographs. Radiographs of specimen cross-sections demonstrated complete filling by trabecular bone. All defects treated with Calceon had a high and the absence of pellets and extensive new bone formation in defects treated with Calceon 6® pellets. Circular trabeculae of bone had formed at the previous sites of the resorbed pellets. The 12 and 26-week radiographs demonstrated few differences between the Calceon-treated and autograft-treated defects. The density of the defects was similar to the surrounding medullary bone and the slight periosteal reactions had subsided.

Conclusion: Calceon 6® calcium sulfate pellets were surgically implanted into a created defect in the canine proximal humerus and new bone formation in the defect was compared to a defect in the contralateral control humerus treated with autologous cancellous bone graft. The Calceon 6® pellets were as effective as autologous cancellous bone graft in promoting new bone formation in the defect. Radiographically, the Calceon 6® pellets were substantially resorbed and replaced with new bone by 3 weeks. Histologically, at 6 weeks the area fraction of new mineralized bone was greater in the Calceon-treated defects than in the autograft-treated defects. However, by 26 weeks the area fraction of new bone in the Calceon 6® pellet-treated defects was equivalent to defects treated with cancellous autograft bone. Calceon 6® pellets were largely resorbed by 6 weeks. By 26 weeks, the area fraction of residual Calceon 6® was less than 1%, and the remaining material was incorporated into new bone trabeculae without adverse inflammatory response.

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