

Increased Bone Formation Using a Calcium Sulfate and Calcium Phosphate Composite Graft

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Calcium phosphates (CaPO_4) and faster-resorbing calcium sulfate (CaSO_4) are successfully employed as synthetic bone grafts for treatment of contained defects. We used a canine critical-sized bone defect model to study an injectable $\text{CaSO}_4/\text{CaPO}_4$ composite graft that incorporated a matrix of CaSO_4 and dicalcium phosphate dihydrate into which β -tricalcium phosphate granules were distributed. The area fraction, ultimate compressive stress, and elastic modulus of restored bone and the relative rates of material resorption were compared between the $\text{CaSO}_4/\text{CaPO}_4$ composite graft and pure CaSO_4 pellets and to normal canine bone. The area fraction of bone in stained sections and the ultimate compressive stress of the regenerated bone were greater using the $\text{CaSO}_4/\text{CaPO}_4$ composite graft compared to pure CaSO_4 pellets after 13 and 26 weeks and were greater than normal bone. The elastic modulus of restored bone in defects treated with $\text{CaSO}_4/\text{CaPO}_4$ composite graft was greater than in defects treated with CaSO_4 pellets after 26 weeks, but similar to specimens of normal bone. A small amount of $\text{CaSO}_4/\text{CaPO}_4$ composite graft and no CaSO_4 pellets remained after 13 or 26 weeks. This novel $\text{CaSO}_4/\text{CaPO}_4$ composite holds promise for clinical applications where a strong, injectable, slower-resorbing, and biocompatible bone graft substitute would be advantageous.

Calcium sulfate has been in use as a bone void filler for several decades.¹³ Medical-grade calcium sulfate (CaSO_4) in the form of pellets or putty repairs bone comparably to autogenous bone graft when tested in a critical-sized, contained cancellous defect in dogs.^{15,16} Clinically, CaSO_4

has been used for repair of contained osseous defects secondary to benign bone tumors, trauma, or periprosthetic bone loss.^{4,5,8,11,15} In these settings, CaSO_4 alone or in combination with bone marrow aspirate, autograft bone, or demineralized bone matrix has positive clinical outcomes, reducing or eliminating the need for autogenous bone graft.^{4,5,8,11,15}

The exact mechanism of action of CaSO_4 is not well understood.^{1,21} The biomaterial resorbs over a period of 6 weeks in a contained osseous defect.^{4,5,8,11,15} During the process of resorption, there is vascular infiltration, osteoid deposition, and ultimately restoration of the defect with new mineralized bone trabeculae.^{15,18,19,21} In animal models, new bone is seen layering on the microscopic residual of CaSO_4 as it is substantially resorbed over the 6-week period.^{15,18,19,21} As with other calcium-based bone graft substitutes, CaSO_4 is well tolerated, evoking minimal inflammatory or foreign body reaction.^{1,15–21} The relatively rapid 6-week resorption period appears ideal for early bone repair.^{15,18,19} However, the rapid resorption may be responsible for the development in some clinical applications of serous drainage, which usually resolves spontaneously.⁸

Calcium hydroxyapatite and β -tricalcium phosphate also exhibit excellent biocompatibility and osteoconductive properties^{9,10,14} but are much slower resorbing than CaSO_4 .⁷ The rates of resorption of these synthetic calcium phosphate materials depend on many factors, including physical form, particle size, porosity, crystallinity, and surface characteristics.^{2,6,10,14} Although slower degradation may be desirable to potentially diminish serum production, resorption of a solid bolus of some of these materials can proceed very slowly, and they may not be fully replaced by bone, even after months or years, depending on site, loading, and vascularity.^{3,7,12}

One concept of an ideal synthetic bone void filler might combine the features of relatively rapid resorption of CaSO_4 with the slower resorption of calcium phosphates (CaPO_4). A $\text{CaSO}_4/\text{CaPO}_4$ composite graft material might

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enhance vascular infiltration and replacement of the graft by new bone, thus promoting improved restoration of a bone defect. Such a hybrid bone graft substitute has become available in the form of a triphasic CaSO_4 -based injectable composite which incorporates a matrix of calcium sulfate dihydrate and dicalcium phosphate dihydrate with β -tricalcium phosphate granules.

We hypothesized the amount, compressive strength and stiffness of restored bone would be greater when defects were treated with the new $\text{CaSO}_4/\text{CaPO}_4$ composite graft compared to conventional CaSO_4 pellets in a canine bilateral defect model after 13 and 26 weeks. We also hypothesized the $\text{CaSO}_4/\text{CaPO}_4$ composite graft would resorb more slowly than the pure CaSO_4 pellets.

MATERIALS AND METHODS

Ten skeletally mature, male, mixed-breed dogs (mean body weight, 28 kg; range, 24–32 kg) had a large critical-sized, axial medullary defect¹⁵ created surgically in both proximal humeri. In one humerus of each dog, the defect was injected with 6 ml of the experimental material $\text{CaSO}_4/\text{CaPO}_4$ composite graft (PRO-DENSE™, Wright Medical Technology, Inc, Arlington, TN). An identical defect in the contralateral humerus received an equal volume of the control material consisting of 50 (4.8-mm-diameter \times 3.3-mm) pellets of medical-grade CaSO_4 (OSTEOSET®, Wright Medical). Five of the dogs were studied for 13 weeks and five for 26 weeks, after which restoration of the defects by new bone formation was evaluated using histomorphometric methods and a mechanical test. We had prior protocol approval from our Institutional Animal Care and Use Committee.

The outcome variables were: (1) the area fractions of new bone and residual implanted materials in the defects quantified from stained histologic sections; and (2) the ultimate compressive stress and elastic modulus of postmortem samples cored from the treated defects. These observations were compared between defects that had received the $\text{CaSO}_4/\text{CaPO}_4$ injectable graft and those treated with CaSO_4 pellets and to similar data for normal bone of 10 normal proximal humeri determined using the same histomorphometric and mechanical methods from an additional five large male dogs (mean body weight, 32 kg; range, 30–34 kg). Inclusion of five dogs per group ensured a 78% power for detecting a difference between the study periods or between the treated and normal humeri with respect to the histomorphometric and mechanical variables if the probability that an observation in one period is less than an observation in another period is 0.999, based on the Mann-Whitney test with a 0.05 significance level. In addition, an *in vitro* accelerated dissolution study was conducted to estimate the dissolution rate of the $\text{CaSO}_4/\text{CaPO}_4$ composite graft relative to the pure CaSO_4 control material.

The $\text{CaSO}_4/\text{CaPO}_4$ composite graft was an injectable, CaSO_4 -based material that set *in situ*. The composite graft consisted of a powder component primarily of calcium sulfate hemihydrate with a moderate amount of calcium phosphate salts and a neutral aqueous diluent, both of which were terminally sterilized with

ionizing radiation. The powder and liquid components were combined in the operating theater, resulting in a viscous and cohesive paste that was injected into the prepared defect, where it set within 20 minutes after mixing. The hardened composite graft was triphasic, with a matrix consisting of calcium sulfate dihydrate (CaSO_4) and dicalcium phosphate dihydrate (DCPD) throughout which β -tricalcium phosphate (β -TCP) granules were distributed. (Supplemental materials are available via the Article Plus feature at www.corronline.com. You may locate this article then click on the Article Plus link on the right.)

For the surgical procedure, the dogs were given intravenous thiobarbiturate (8–16 mg/kg) preoperatively as induction for anesthesia and were operated on under general inhalation anesthesia using isoflurane to maintain the surgical plane. Under general aseptic technique, a cranial approach to the greater tubercle of each humerus was performed. A cylindrical cavity (13 mm in diameter \times 50 mm in length) was drilled axially through the greater tubercle into the medullary canal of each humerus using a custom guide. $\text{CaSO}_4/\text{CaPO}_4$ composite graft was delivered into the cavity in one humerus, and CaSO_4 pellets were implanted into the cavity in the contralateral humerus, randomized by side. The liquid and powder components of the $\text{CaSO}_4/\text{CaPO}_4$ composite were combined in a vacuum bone cement mixing apparatus (Summit Medical, Gloucestershire, UK). After mixing for 30 seconds under approximately 550mm Hg vacuum, the material was transferred to a 20-cc syringe, and a 6-cc bolus was delivered to the defect through an 11-gauge, 6-cm-long, Jamshidi-type needle, using a backfilling technique. In the contralateral humerus, the CaSO_4 pellets were distributed into the defect with the use of forceps. After delivery of the experimental or control material, the supraspinatus tendon was closed over the defect in the greater tubercle, and the fascia, subcutaneous tissues, and skin were closed in layers in a routine fashion. The $\text{CaSO}_4/\text{CaPO}_4$ composite material mixed and injected easily, and the CaSO_4 pellets were appropriately placed so both materials uniformly filled their defects as evidenced on immediate postoperative radiographs. In three of the humeri that received $\text{CaSO}_4/\text{CaPO}_4$ composite graft and in three that received CaSO_4 pellets, a small amount of the implanted material extended distal to the created defect for approximately 1 cm, following the track of a pilot drill hole within the medullary canal.

Postoperatively, the dogs were bearing weight on the second day, all wounds healed routinely, and the animals completed their assigned study periods without incident.

The dogs were allowed unrestricted weightbearing and pain was managed with analgesics. Immediate postoperative analgesia was provided using a fentanyl transdermal patch (100 mcg/hour) placed the day of surgery during preparation of the skin and left in place for 3 to 4 days and by intramuscular administration of buprenorphine (10–30 $\mu\text{g}/\text{kg}$) for 2 days and afterward on an as needed basis. Acetaminophen (10–15 mg/kg) was administered for 2 days thereafter. Cephalosporin antibiotics were given intraoperatively (1 g) and for 5 postoperative days (22 mg/kg).

Resorption of the experimental and control bone graft substitute materials and their replacement with new bone was monitored from anterior-posterior and lateral radiographs taken pre-

operative, immediately postoperative, and at 2, 6, 13, and 26 weeks postimplantation under intravenous thiobarbiturate (8–16 mg/kg) anesthesia. Resorption of both bone graft substitutes was apparent beginning at 2 weeks. Resorption of CaSO₄ pellets was essentially complete by 6 weeks, while the CaSO₄/CaPO₄ composite graft was still evident at 6 and 13 weeks, and some composite graft persisted, even at 26 weeks. As the implanted materials resorbed, they were replaced with mineralized bone within the defects. In defects treated with CaSO₄/CaPO₄ composite graft, there was a radiodense zone in the adjacent medullary bone at 6 and 13 weeks. This phenomenon was not observed surrounding defects that had received CaSO₄ pellets. (Supplemental materials are available via the Article Plus feature at www.corronline.com. You may locate this article then click on the Article Plus link on the right.)

Upon completion of their assigned study periods, the animals were euthanized using intravenous sodium pentobarbital. Contact radiographs were obtained of the isolated right and left humeri. The bones retrieved from the 10 dogs with treated humeri, and the normal bones from the other five dogs were processed and analyzed histologically and mechanically in a similar manner. The humeri were serially sectioned in the transverse plane using a diamond-blade cutoff saw and a custom alignment jig to obtain comparable sections of the right and left humeri. A 20-mm-long section from the middle level of each defect site was obtained and frozen in saline-soaked gauze at –20°C for subsequent use in the mechanical test. The remaining sections were fixed in 10% neutral-buffered formalin for histologic preparations. High-resolution radiographs were made of the cut sections. Methylmethacrylate-embedded, undecalcified histologic slides at the level of the defect in each humerus were ground and stained with basic fuchsin and toluidine blue and studied by three of the investigators (RMU, DJH, TMT) using light microscopy. On two stained slides from each defect, one just proximal and the other just distal to the mechanical test specimen, the amounts of new bone and residual implanted materials within the defects were quantified using standard point counting techniques by superimposing a rectangular grid on digital images of the central 12-mm diameter of the defect area. The data were expressed as the percent area fraction ([points positive for the feature of interest/total possible points] × 100). Qualitative assessments of the stained slides and contact radiographs of specimen bones and cross-sections were also conducted to characterize the nature of new bone and residual material in the defects.

A mechanical compression test was performed to determine the strength and stiffness of specimens of new bone in the restored defects and in similar specimens of bone from the normal humeri. A cylinder (8 mm diameter × 20 mm long) was cored from the frozen specimens in the long axis of the defect using a diamond core drill and thawed in saline to room temperature. The specimens were tested using a Model 8874 servohydraulic mechanical testing system, a 1000 N Dynacell Dynamic Load Cell, Bluehill Mechanical Testing Software (all Instron Corp; Canton, MA), and a modified compression suppress (ASTM D 695; Wyoming Test Fixtures, Laramie, WY). Unconfined, uniaxial compression tests were performed at a crosshead speed of 0.5 mm/minute until obvious specimen failure was observed as

indicated by a substantial drop in the load curve or 30% strain of the specimen was achieved. Data for the axial deformation and the applied load were acquired at 10 Hz. Specimen ultimate compressive stress and elastic modulus were calculated from the resulting stress-strain curves using the Bluehill Mechanical Testing Software.

In the *in vitro* accelerated dissolution study, test samples of CaSO₄/CaPO₄ composite graft and samples of pure CaSO₄, each measuring 4.8 mm in diameter by 3.3 mm, were cast in a silicone elastomer mold. The samples were allowed to cure for a minimum of 4 hours and then dried to constant mass at 40°C and the values recorded. Five sets of the CaSO₄/CaPO₄ composite and of the CaSO₄ samples were studied. Individual samples were placed in 50-mL fritted glass extraction thimbles and weighed to an accuracy of 0.01 mg. The thimbles were then placed in polyethylene bottles, filled with 275 mL deionized water, and positioned in a water bath at 37°C. The thimbles were removed from the bath after each 24-hour period and drained, dried, and cooled before weighing. After determining the remaining mass, the samples were placed in fresh water and returned to the bath. The experiment was discontinued after 9 days for the CaSO₄ samples when there was no measurable mass remaining. After 30 days, the measurement frequency for the CaSO₄/CaPO₄ composite samples was modified to once every 5 days, although the water was still changed daily. The experiment was discontinued after 55 days when the residual mass of the CaSO₄/CaPO₄ composite samples reached less than 5%.

We used the Friedman test to analyze the histomorphometric (area fractions of bone and residual implanted materials) and the mechanical variables (ultimate compressive stress and elastic modulus) when comparing the CaSO₄/CaPO₄ composite graft-treated defects to the defects treated with CaSO₄ pellets. The Mann-Whitney test was used to compare the histomorphometric (area fractions of bone and residual implanted materials) and mechanical data (ultimate compressive stress and elastic modulus) between the 13- and 26-week study periods and to compare the data between the CaSO₄/CaPO₄ composite graft-treated defects and the values obtained for normal bone of the proximal humerus. Data are presented as the mean and standard deviation. A 0.05 significance level was used for all statistical tests. We used SPSS for Windows (Version 13) for data management and statistical analysis. The data were analyzed using nonparametric statistical methods.

RESULTS

There was more ($p = 0.025$) bone in the stained sections of defects treated with CaSO₄/CaPO₄ composite graft after both 13 and 26 weeks compared to defects treated with CaSO₄ pellets; and there was more ($p = 0.008$) bone in the 13-week CaSO₄/CaPO₄ composite-treated defects compared to bone in the normal humeri (Figs 1–4). At 13 weeks, the area fraction of new mineralized bone was twofold greater in defects treated with the composite graft material ($39.4\% \pm 4.7\%$) than in defects treated with conventional CaSO₄ pellets ($17.3\% \pm 4.3\%$) ($p = 0.025$) and

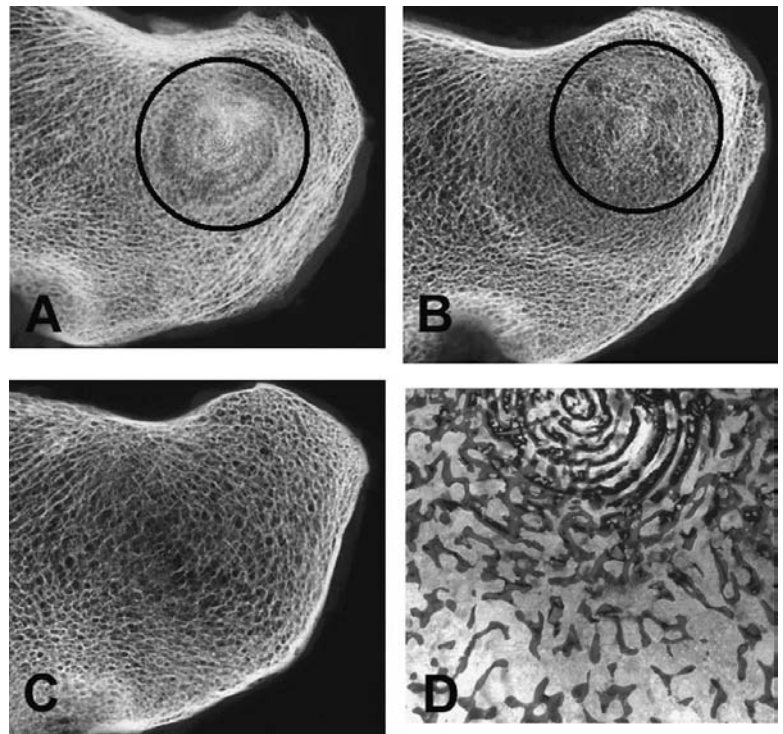


Fig 1A–D. Transverse section radiographs of the 13-week specimen (A) and the 26-week specimen (B) revealed all of the defect sites (black circles) treated with $\text{CaSO}_4/\text{CaPO}_4$ composite graft contained bone trabeculae that blended with the adjacent medullary bone. A normal humerus is shown for comparison (C). Highly radiodense DCPD matrix and β -TCP material, often in a pattern of concentric rings, remained at 13 weeks (A) and to a much lesser extent at 26 weeks (B). The concentric ring pattern in the contact radiographs corresponded in the 13-week stained histologic sections (D) to bands of new bone layered on the surfaces of residual CaPO_4 material (Stain, basic fuchsin and toluidine blue; original magnification, $\times 20$).

in normal bone ($14.5\% \pm 2.4\%$) ($p = 0.008$) (Fig 2). The new bone layered on the surfaces of residual DCPD matrix and β -TCP material, forming a concentric ringlike pattern (Figs 1A, 1D, 3A–B). At 26 weeks, the bone had remodeled to a more normal architecture (Figs 1B, 1C, 4), but there was still more ($p = 0.025$) bone in defects treated

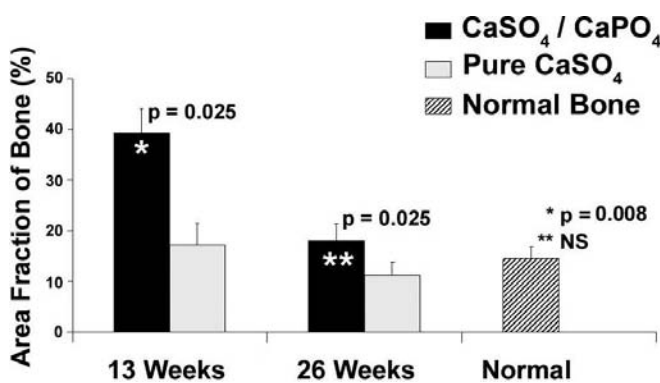


Fig 2. A bar graph shows the area fraction of mineralized bone was greater at both 13 weeks and 26 weeks in defects treated with the $\text{CaSO}_4/\text{CaPO}_4$ composite graft than in defects treated with conventional CaSO_4 pellets ($p = 0.025$). The area fraction of bone at 13 weeks in defects treated with the composite graft was also greater compared to normal bone ($p = 0.008$). The data represent mean \pm standard deviation. NS = not significant.

with the $\text{CaSO}_4/\text{CaPO}_4$ composite graft ($18.0\% \pm 3.4\%$) than in defects treated with CaSO_4 pellets ($11.2 \pm 2.6\%$) (Fig 2). Regardless of the area fraction of bone, the defects were restored with new bone trabeculae and marrow with only scattered foci of fibrous tissue in all of the stained sections (Figs 3A–B, 4A–B).

The cored bone samples from defects treated with the $\text{CaSO}_4/\text{CaPO}_4$ composite graft were several fold stronger ($p \leq 0.046$) than those treated with CaSO_4 pellets after both 13 and 26 weeks and were also stronger ($p \leq 0.047$) than the specimens of normal bone (Fig 5). At 13 weeks, the ultimate compressive stress was greater ($p = 0.025$) for $\text{CaSO}_4/\text{CaPO}_4$ composite-treated defects (5.29 ± 2.61 MPa) compared to defects treated with CaSO_4 pellets (0.90 ± 0.44 MPa). At 26 weeks, the ultimate compressive stress was also greater ($p = 0.046$) for $\text{CaSO}_4/\text{CaPO}_4$ composite-treated defects (2.19 ± 0.41 MPa) compared to the CaSO_4 pellet-treated defects (0.44 ± 0.49 MPa). The ultimate compressive stress of the bone cores from defects treated with $\text{CaSO}_4/\text{CaPO}_4$ composite graft after both 13 and 26 weeks was also greater ($p = 0.009$ and $p = 0.047$, respectively) than similar cored specimens of trabecular bone from the 10 normal proximal humeri (1.36 ± 0.59 MPa). The elastic modulus of defects treated with $\text{CaSO}_4/\text{CaPO}_4$ composite graft material also was several fold greater ($p = 0.025$) than defects treated with CaSO_4 pellets after 26 weeks (150 ± 73 MPa versus 16 ± 24

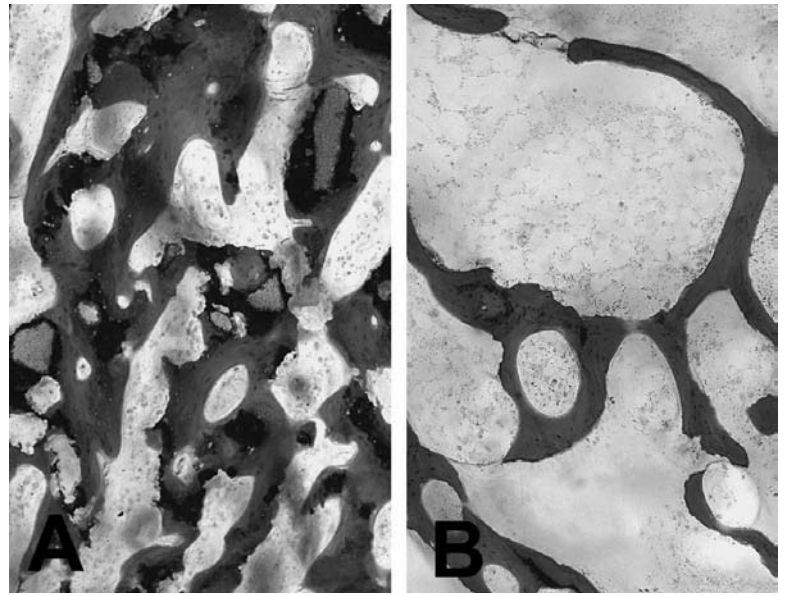


Fig 3A–B. Photomicrographs of stained histologic slides show the typical nature of bone in a defect treated with CaSO₄/CaPO₄ composite graft at 13 weeks with incorporated matrix (dark-stained) and β-TCP granules (gray) (A), compared to a defect treated with conventional CaSO₄ pellets at 13 weeks without residual material (B) (Undecalcified ground sections; stain, basic fuchsin and toluidine blue; original magnification, ×100).

MPa); but there was no difference in the elastic modulus of the CaSO₄/CaPO₄ composite-treated defects at either 13 weeks or 26 weeks compared to normal bone (104 ± 76 MPa).

A small amount of residual implanted material remained in defects treated with CaSO₄/CaPO₄ composite graft, while no residual material was present after 13 or 26 weeks in the defects treated with CaSO₄ pellets (Figs 3A–B, 4A–B). The area fraction of residual DCPD matrix decreased ($p = 0.047$) with time from 2.9% ± 2.8% at 13 weeks to 0.6% ± 0.8% at 26 weeks. The area fraction of residual β-TCP granules also decreased ($p = 0.016$) from 3.6% ± 1.0% at 13 weeks to (0.8% ± 1.4%) at 26 weeks.

The in vitro dissolution study indicated 90% of the implant material was resorbed after 7 days for the pure CaSO₄ controls and after 24 days for the CaSO₄/CaPO₄ composite graft samples (Fig. 6). Scanning electron imaging (Fig. 7) and xray diffraction analysis of CaSO₄/CaPO₄ composite samples at 15 days revealed depletion of the CaSO₄ phase with only the less soluble DCPD and β-TCP granules remaining, forming an open pore structure. After 55 days, only β-TCP granules remained.

DISCUSSION

A novel injectable bone graft substitute was studied that combined several calcium-based materials having differ-

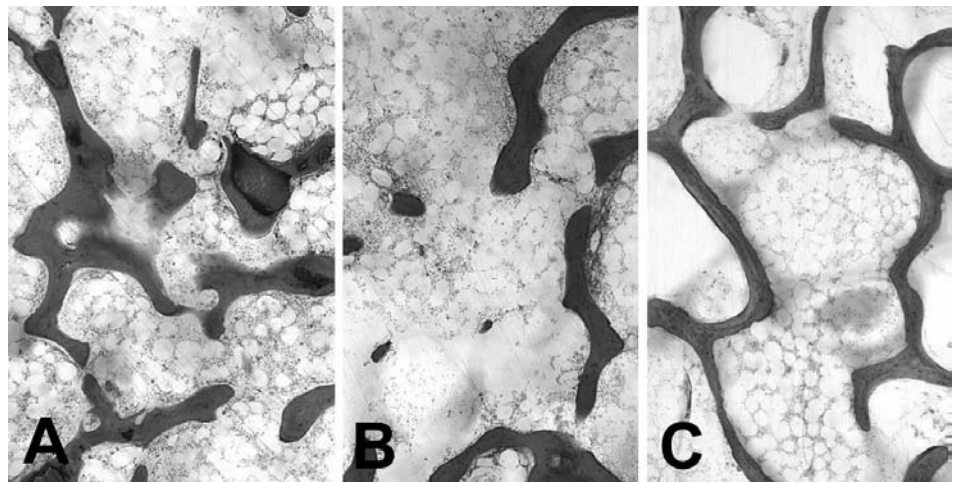


Fig 4A–C. Photomicrographs of stained histologic slides show the typical nature of bone in a defect treated with CaSO₄/CaPO₄ composite graft at 26 weeks with few β-TCP granules (gray) (A), compared to a defect treated with conventional CaSO₄ pellets at 26 weeks with no pellets remaining (B) and to normal bone of the canine proximal humerus (C) (Undecalcified ground sections; stain, basic fuchsin and toluidine blue; original magnification, ×100).

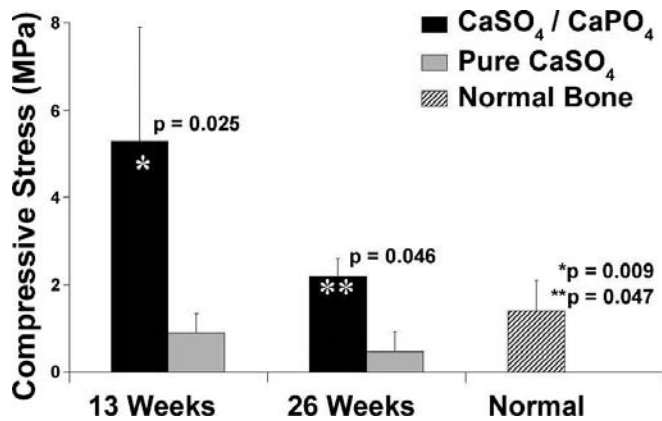


Fig 5. Bar graph shows the ultimate compressive stress of the cored bone samples was greater in defects treated with the CaSO₄/CaPO₄ composite graft than in defects treated with conventional CaSO₄ pellets at both 13 weeks ($p = 0.025$) and 26 weeks ($p = 0.046$). The ultimate compressive stress of bone in defects treated with the composite graft was also greater than normal bone at 13 weeks ($p = 0.009$) and 26 weeks ($p = 0.047$). The data represent mean \pm standard deviation.

ent resorption rates. The CaSO₄/CaPO₄ composite graft was formulated to provide a tailored resorption profile that would be slower resorbing than conventional CaSO₄ pellets but would resorb faster than has been observed with calcium phosphates.^{3,7,12} The composite graft was engineered so in vivo the majority of the CaSO₄-DCPD matrix would resorb relatively early, but more slowly than pure CaSO₄, to promote vascular infiltration deep into the bo-

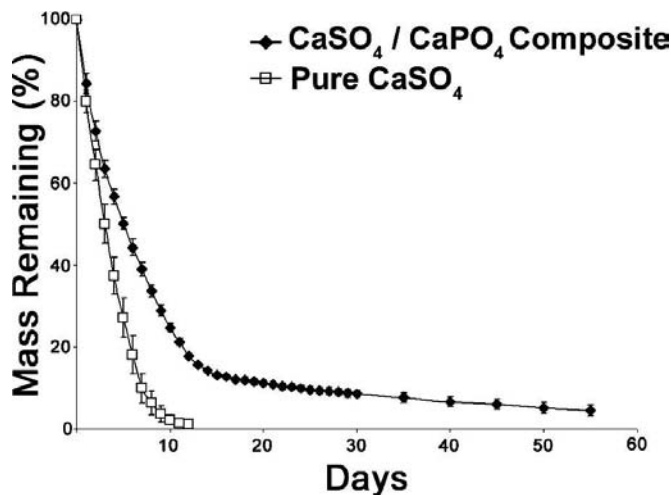


Fig 6. There was a markedly slower rate of resorption for CaSO₄/CaPO₄ composite graft compared to the CaSO₄ test samples in the in vitro comparative dissolution study. The graph shows the percent mass remaining versus time for CaSO₄/CaPO₄ composite graft and CaSO₄ samples in deionized water (mean \pm standard deviation).

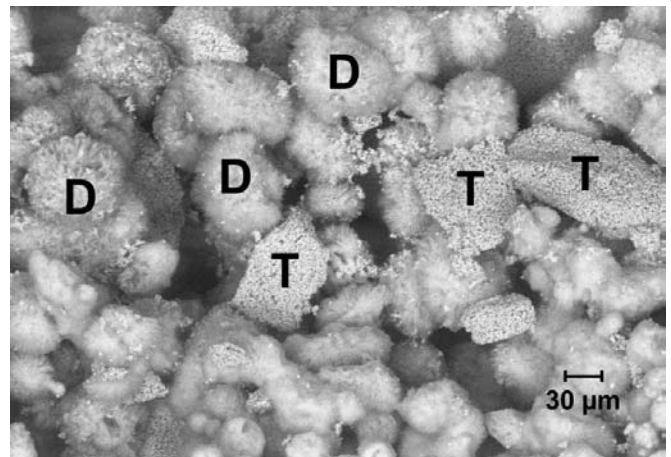


Fig 7. A scanning electron micrograph of a CaSO₄/CaPO₄ composite graft sample from the in vitro dissolution test shows the open pore nature of the composite graft after 15 days. The remaining material consisted primarily of the less soluble DCDP phase of the matrix (D) and β -TCP granules (T) (original magnification, $\times 500$).

lus, while the remaining matrix and the β -TCP granules distributed within the composite graft could provide a scaffold for new bone formation. We hypothesized the amount, compressive strength and stiffness of restored bone would be greater and the material resorption profile slower when bone defects were treated with the new CaSO₄/CaPO₄ composite graft than when treated with conventional CaSO₄ pellets in a bilateral critical-sized bone defect model in the canine proximal humerus.

In light of the remarkable increase in the amount and strength of regenerated bone using CaSO₄/CaPO₄ composite graft, one limitation of our study is the earliest events in vivo of material resorption and new bone formation were not examined. An original goal was to assess of the ability of the CaSO₄/CaPO₄ composite graft to restore a large contained defect after 13 weeks, when moderate bone healing and material resorption was expected, and at 26 weeks, by which time bone healing and remodeling to more normal trabecular architecture could occur. Observations in the vitro dissolution test did physically document the early and rapid opening of pores in the matrix of the CaSO₄/CaPO₄ composite graft, exposing the DCPD and β -TCP phases as an osteoconductive scaffold. This finding is in agreement with an unpublished pilot study of the CaSO₄/CaPO₄ composite graft in a transcortical proximal humerus defect model which demonstrated the majority of the CaSO₄-DCPD matrix had resorbed at 6 weeks, allowing deep infiltration of vessels and new bone formation on the surfaces of the remaining DCPD and β -TCP granules (Fig 8). However, a thorough histological interpretation of the initial mechanism of action of the

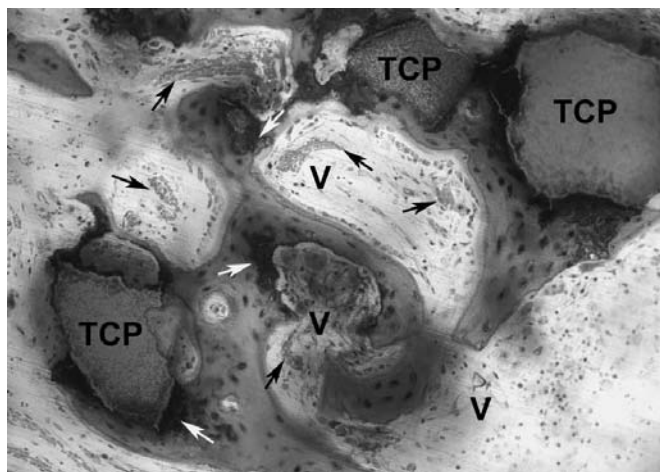


Fig 8. A previous pilot study of CaSO₄/CaPO₄ composite graft in a 9-mm-diameter transcortical proximal humerus defect demonstrated the majority of the CaSO₄-DCPD matrix had been resorbed at 6 weeks, allowing deep infiltration of capillaries (black arrows) and venules (V) and newly formed bone lined with osteoblasts on the surfaces of the remaining DCPD matrix (white arrows) and the β-TCP granules (TCP) (Undecalcified ground section; stain, basic fuch sine and toluidine blue; original magnification, ×154).

CaSO₄/CaPO₄ composite graft would require time points over the first one to four weeks following implantation.

Another limitation of the study design was the choice of CaSO₄ pellets as the control material. The rationale for choosing conventional medical-grade CaSO₄ pellets as the material for comparison included their demonstrated effectiveness in experimental studies^{15,18,19,21} and in clinical usage^{4,5,8,11,15} and the considerable data that exist on CaSO₄ pellets from previous studies using this animal model at similar evaluation time points.^{15,16,18–20} Because autogenous bone graft is the “gold standard” of comparison for bone graft substitutes, further studies would be warranted to make a paired comparison between the CaSO₄/CaPO₄ composite graft and autogenous bone.

Although it was anticipated the amount and strength of regenerated bone might be somewhat greater using CaSO₄/CaPO₄ composite graft, the area fraction of new mineralized bone at 13 weeks was twofold greater and the compressive stress several fold greater compared to CaSO₄ pellets and similarly greater than normal bone. These magnitudes of new bone formation well exceed the area fraction of regenerated bone previously reported using the same animal model at 13 weeks to study other CaSO₄-based bone graft substitutes, including CaSO₄ pellets with a modified crystal structure,¹⁹ CaSO₄ paste,²⁰ CaSO₄-based putty,²⁰ CaSO₄-based putty with deminer-

alized bone matrix particles,¹⁶ or using autogenous cancellous bone graft¹⁶ (Fig 9).

By 26 weeks, the regenerated bone in the composite graft-treated defects had remodeled to a more normal architecture, area fraction, and compressive stress compared to the specimens of normal bone. The elastic modulus of the bone in defects treated with the composite graft was not different from normal bone, and histologically the regenerated trabeculae blended gradually with the surrounding bone. Because there was no modulus mismatch, the high strength of the restored defects would not adversely affect the surrounding medullary bone.

In conclusion, the injectable CaSO₄/CaPO₄ composite graft increased the amount and strength of restored bone when compared to conventional CaSO₄ pellets after 13 and 26 weeks in a canine critical-sized bone defect model and when compared to specimens of normal bone. The novel composite graft represents an advancement in the biologic performance of CaSO₄-based materials, providing a composite graft that is faster resorbing than reported for some calcium phosphate materials but slower resorbing than pure CaSO₄ pellets. This CaSO₄/CaPO₄ composite graft may be advantageous in the treatment of benign bone tumors and in trauma applications, including distal radius, tibial plateau, and vertebral compression fractures, or other settings where an enhanced amount and strength of restored bone using a highly biocompatible bone graft substitute would be desirable.

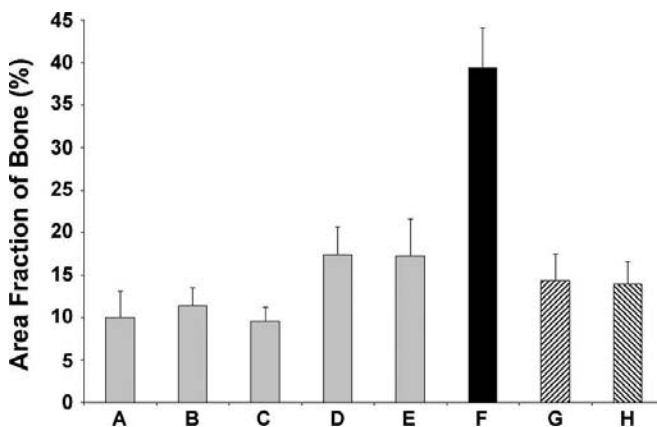


Fig 9. Bar graph compares data on the area fraction of restored bone from the present study with data from previous studies of other bone graft materials using the same critical-sized defect model at the 13-week evaluation time point. Data for other CaSO₄-based bone graft substitutes include α-calcium sulfate hemihydrate pellets (A),¹⁹ CaSO₄ putty (B),²⁰ CaSO₄ paste (C),²⁰ and CaSO₄ putty containing demineralized bone matrix particles (D).¹⁶ Data from the present study are shown for CaSO₄ pellets (E), CaSO₄/CaPO₄ composite graft (F), and normal bone (G). These are compared to treatment of defects with cancellous autograft bone (H).¹⁶ The data represent mean ± standard deviation.

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