

Effect of Adding Resorbable Calcium Sulfate to Grafting Materials on Early Bone Regeneration in Osseous Defects in Rabbits

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This experiment was designed to study the osteogenic potential of adding medical-grade resorbable calcium sulfate mixture to grafting materials for filling osseous defects. Twelve New Zealand rabbits were divided into an active group of 10 animals and a control group of 2 animals. The median condyle of each femur was drilled to create 8-mm-deep cavities. Active osseous defects consisted of 20 cavities and were filled with Osteograft, BOP, or Capset (calcium sulfate) alone; a mixture of Osteograft and Capset; or a mixture of BOP and Capset. Osteograft and BOP were each mixed with Capset in a 4:1 ratio. Each grafting material filled 4 osseous cavities, and 4 osseous cavities were left unfilled to act as controls. The observation period was 8 weeks. Block sections of the femoral heads were prepared for decalcified histologic assessment. It appeared that mixing grafting materials with calcium sulfate powder in a 4:1 ratio, respectively, facilitated the process of osteogenesis and increased new bone bonding to remnants of the grafting materials, in spite of the poor osteoconductive property of BOP and moderate osteoconductivity of Osteograft. However, calcium sulfate material alone is not recommended for use as a bone filler. (INT J ORAL MAXILLOFAC IMPLANTS 2000;15:859-864)

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The literature has presented numerous research projects attempting to find or improve bone substitute materials. Most of these bone substitutes fall into the category of osteoconductive grafting materials.¹ These act as a scaffold for the deposition of bone-forming cells and the ingrowth of blood vessels and cellular matrix, which are necessary for bone remodeling and the regeneration process. The materials in this category include various forms of collagen, ceramics, bioglasses, polymers, xenografts, allografts, and synthetic hydroxyapatites.

Sixty-five percent of bone matrix contents are calcium and phosphate. Based on the understanding of the physiology of bone formation, calcium is essential for bone uptake and formation during childhood. Calcium is also given as a supplementary source to elderly individuals, especially women, in

whom the hormones responsible for calcium deposition and absorption are depleted.

Plaster of paris is the hemihydrate form of calcium sulfate. It has been used in medicine for over a century. In 1892 Dreesmann² used plaster of paris as a filler material in osseous bone defects. Following his investigations, researchers carried out several experiments to evaluate the use of calcium sulfate in animal models³ and in humans.⁴ Several investigations have since been carried out in both orthopedics and dentistry.⁵⁻¹² Bell⁵ showed that calcium sulfate takes 3 to 6 weeks to resorb. Peltier⁶ concluded that the material could be used safely and did not lead to complications. Peltier⁷ also noticed that the serum calcium level was not raised by the gradual resorption of calcium sulfate material after implantation. He was one of the first orthopedic surgeons to report the surgical use of calcium sulfate in the modern era.⁶⁻⁹ In dentistry, various forms of calcium sulfate mixture have been tried, with promising results.¹⁰⁻¹² Recently, calcium sulfate has become commercially available in a sterile, resorbable form.

This experiment was designed to study the osteogenic potential value of adding medical-grade resorbable calcium sulfate to grafting materials in

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filling osseous defects. BOP and Osteograf were selected as the grafting materials. BOP (Biocompatible Osteoconductive Polymer) (Diversified Tech International SA, Brussels, Belgium) is a resorbable, implantable biopolymer composed of 100% copolymer methyl methacrylate resin 1-vinyl-2-pyrrolidone and is supplied as a powder in crystal form, ranging in size from 30 to 100 μm . Osteograf LD (CeraMed, Lakewood, CO) is a resorbable, synthetic hydroxyapatite (HA). It is a pure, porous, non-ceramic grafting material and is manufactured in a form between 250 and 420 μm in size. Its chemical formula is $\text{Ca}_{10}(\text{P}_{10})_6(\text{OH})_2$. It contains no alpha or beta tricalcium phosphates, as are found in ceramic HA. Capset (Lifecore, Chaska, MN) is a composition of plaster of paris and is available as a biocompatible, sterile, medical-grade resorbable calcium sulfate hemihydrate powder.

METHODS AND MATERIALS

Twelve male New Zealand rabbits weighing a mean of 3.5 kg (\pm 0.25 kg) were used in this experiment and were divided into an active group of 10 animals and a control group of 2 animals. Animals were anesthetized preoperatively with an intramuscular injection of Ketamine (10 mg/kg, Tekam, Al Hikam Pharmaceuticals, Amman, Jordan) and xylazine (0.15 mg/kg, Seton 2%, Laboratorios Calier SA, Barcelona, Spain). In addition, 1.0 mL of local anesthesia (2% lidocaine/epinephrine 1:80,000; Astra, Sodertalje, Sweden) was injected at the surgical sites prior to the start of surgery. Postoperatively, all animals were given an intramuscular injection of long-acting antibiotics (0.2 mL/kg, 12,500,000 IU benzyl penicillin benzathine and 5 g streptomycin per 100 mL; Duphaphen Strep BP, Solvay, Italy) and an analgesic dose of Analgin (0.5 mL Pharmalgin, Arab Drug Co, Cairo, Egypt).

Animals were fed a standard rabbit diet and maintained separately in cages at the animal research facility of King Saud University.

Surgical Procedure

The femoral condyles were chosen for the surgical procedures. The surgical fields of both femurs were shaved and washed with 0.2% chlorhexidine gluconate. The skin incision and subperiosteal dissection were carried out on both femoral condyles. In the control group, the median condyle of each femur was drilled with a 5-mm-diameter round bur, using copious saline irrigation, to create an 8-mm-deep cavity. Four cavities were washed with sterile saline and left unfilled to act as a control. The

periosteum and skin were closed in layers with resorbable 4-0 sutures (Vicryl, Ethicon, Johnson & Johnson International, Brussels, Belgium).

In the active group, the femoral condyle was drilled in the same manner on each side. Each of the 5 investigated graft materials, either alone or in combination, filled 4 osseous cavities, making a total of 20 active osseous cavities. The examined grafting materials were: Osteograf alone, BOP alone, Capset (calcium sulfate) alone, a mixture of Osteograf and Capset, and a mixture of BOP and Capset. The BOP or Osteograf and Capset were mixed in a ratio of 4:1, respectively. The tested animals were observed for 8 weeks. Examination for proper wound healing was conducted daily for the first operative week and once a week thereafter.

Histologic Processing

After the animals were sacrificed, both femoral heads were dissected from the soft tissues and sectioned en bloc. Each bloc was labeled and placed in 10% neutral buffered formalin solution. Specimens were fixed in a formalin solution for 24 hours and then placed in Cal-Ex solution (Fisher Diagnostics, Fairlawn, NJ) until fully decalcified. Specimens were rinsed in running tap water for 5 hours to remove excess acid from the tissue. Specimens were dehydrated in increasing percentages of ethyl alcohol (70 to 90%); chloroform was then used for 1 hour to clear the specimens, which were then impregnated in melted paraffin wax.

The embedded samples were sectioned with a rotatory microtome in 5- μm slices. Sections were mounted on acrylic glass slides using photo-polymerizing glue. The final sections were stained with hematoxylin-eosin, washed with water, and finally dried. A precision adhesive press affixed parallel acrylic glass slides with photo-polymerizing glue onto the stained sections.

Histometric Analysis

All sections were evaluated by the same examiner using an image analysis system. This technique measured percentages of newly deposited bone, soft tissue (loose connective tissue, blood vessels, open pores, and empty spaces), and graft remnants. The system used for analysis consisted of the following: (1) a light optic microscope (Polyvar, Reichert-Jung, Vienna, Austria); (2) an image analysis device (Leica, Cambridge, United Kingdom); (3) a video-camera (Donphisha, Sony, Tokyo, Japan); and (4) a computer-based image processor based on the Qwin software program (Leica) for histometry. Qwin is a Windows-based image analysis tool that provides measurement in a fully automated system.

Images of newly formed bone, soft tissue, and graft remnants were identified by different given colors in each image. These were digitized and transferred to the computer software for image processing and analysis of the quantity fraction (relative percentage) for each tissue type. The mean percentage value for each tissue type was considered. All sections (active and control) were compared in the same manner.

RESULTS

All animals healed uneventfully during the study period.

Active Osseous Cavities

BOP. Crystals of the filling material occupied most of the osseous cavities (70%). Minimal osteogenesis (bone formation) was observed between the BOP crystals, with no signs of inflammation (13%). A sign of angiogenesis (blood vessels) between BOP crystals was seen in some locations embedded in a connective tissue stroma (17%) (Figs 1a and 1b, Table 1).

Osteograf. Despite a limited quantity of osteogenesis, the amount of newly formed bone deposited between graft particles was greater than that observed in the osseous cavities filled with BOP crystals (19%). Graft material occupied 19% of this field. Similar to the BOP-filled defect, signs of angiogenesis and absence of inflammation were seen in a connective tissue stroma (62%) (Figs 1c and 1d, Table 1).

Capset. Osseous cavities filled with calcium sulfate material showed lamellar bone formation in the center of the cavities. The regenerated bone (24%) was deposited in an active stroma. Several blood vessels without signs of inflammation were seen throughout the osteoid matrix. Large empty spaces surrounded the active stroma, separating it from the borders of the osseous cavities and resulting in incomplete bone fill (76%) (Figs 1e and 1f, Table 1).

BOP + Capset Mixture. Here, BOP crystals underwent more dissolution than in osseous cavities filled with BOP alone (39%). The spaces between the graft material were filled with dense bone. New bone was seen throughout the osseous cavity in a greater amount (29%) than in osseous cavities filled with BOP alone. Empty spaces, connective tissue, and blood vessels occupied 32% of the field. No inflammatory cells were seen (Figs 2a and 2b, Table 1).

Osteograf + Capset Mixture. New bone ingrowth was lamellar-like and predominantly filled spaces between implanted particles in a larger quantity (40%) than in osseous defects filled with Osteograf alone. The regenerated bone incorporated well with remnants of Osteograf particles, which led to bone attaching to residual implanted material without signs

of fibrous tissue encapsulating the Osteograf particles. Many blood vessels in an active stroma could be seen throughout the bone cavities without sign of inflammation (41%). Graft remnants (19%) were still undergoing hydrolysis (Figs 2c and 2d, Table 1).

Control Osseous Cavities

The control cavities were bordered with normal bone trabeculae surrounding largely empty space, fat, and bone dust (96%) (Fig 3, Table 1).

DISCUSSION

Plaster of paris has been used as a guided tissue regeneration barrier^{13,14} and as a vehicle for bone morphogenic protein.¹⁵ The present study showed that mixing calcium sulfate material with BOP or Osteograf in a ratio of 1:4, respectively, appeared to favorably affect the potential of osteogenesis and led to an increase in trabecular bone bonding with remnants of grafting materials, in spite of the poor osteoconductivity of BOP and moderate osteoconductivity of Osteograf. However, non-grafted osseous cavities failed to stimulate substantial new bone growth during this experiment.

Among the advantages of adding calcium powder to grafting materials is that it acts as a direct source of calcium supply during the osteogenesis process and binds directly to host bone.^{11,16,17} This concept was illustrated in the present study in osseous defects filled with a combination of graft material and calcium sulfate (Fig 2, Table 1).

Several researchers^{16,18} have mixed calcium sulfate powder with non-resorbable HA particles and implanted the composite in osseous defects. They concluded that plaster of paris assisted in the placement of grafting materials into recipient sites and inhibited the migration of HA particles. They have also noted that subsequent resorption of the calcium sulfate leaves controlled porosities for bone ingrowth and attachment to the non-resorbable HA, which results in a composite that is superior in handling properties to those of HA alone.

Yamazaki and coworkers¹⁵ implanted purified bone morphogenic protein (BMP) into femoral muscle, with and without calcium sulfate, and concluded that osteogenesis was significantly enhanced by adding calcium sulfate to BMP. The Yamazaki work could indicate that the calcium sulfate served as a vehicle for the BMP and as a potential mediator for accelerating calcification of the formed bone.

The value of applying or mixing calcium material with bone graft materials has also been recognized by some manufacturers. HTR beads (HTR Sciences,

Figs 1a to 1f Osseous defects filled with graft materials alone (low-power magnifications $\times 20$ to 30 ; high-power magnifications $\times 80$).

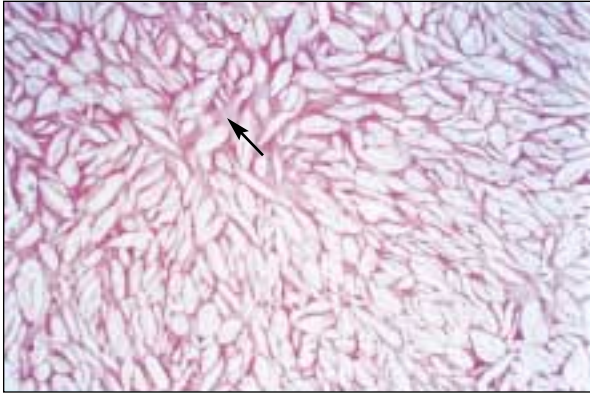


Fig 1a Defect filled with BOP. BOP crystals occupy the major portion of the osseous cavity, with minor hydrolysis. Note the minimal amount of osteogenesis and angiogenesis (arrow).



Fig 1b Same field seen in Fig 1a but with low-power magnification, showing the borders of the cavity.



Fig 1c Defect filled with Osteograft particles, which underwent moderate hydrolysis. Note osteogenesis (arrow) and angiogenesis (arrowhead) between particles, both of which are slightly more than what was found in osseous cavities filled with BOP.

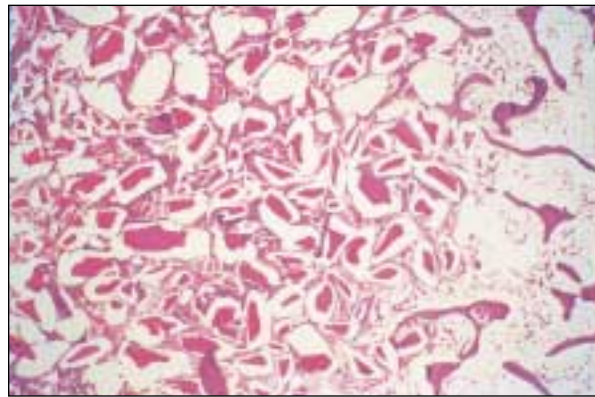


Fig 1d Same field seen in Fig 1c but with low-power magnification, showing borders of the cavity.

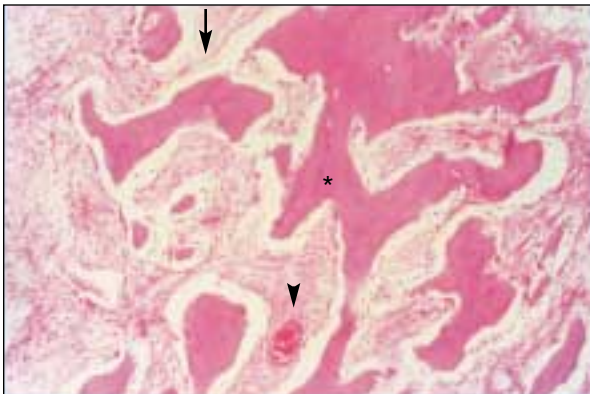


Fig 1e Cavity filled with Capset, which has resorbed completely. Note the lamellar bone formation (asterisk) at the center of the cavity formed in active stroma. Active osteogenesis has taken place, with osteoblast lining (arrow) laid down along the surfaces of newly formed bone and several blood vessels (arrowhead) located throughout the active stroma.

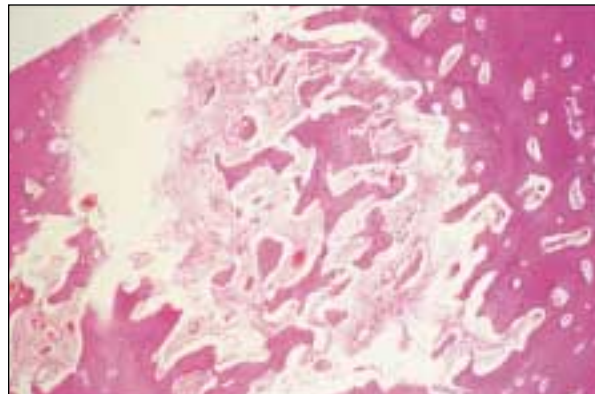


Fig 1f Same field seen in Fig 1e but with low-power magnification. Note the large empty spaces surrounding the active stroma located between the bone borders of the osseous cavity and newly formed bone.

Table 1 Calculated Percentages of New Bone, Soft Tissue, and Graft Remnants

Type of graft	Soft tissue and empty spaces (%)	Graft remnants (%)	New bone (%)
BOP	17	70	13
Osteograft	62	19	19
Capset	76	0	24
BOP + Capset	32	39	29
Osteograft + Capset	41	19	40
Control (no filler)	96	0	4

Figs 2a to 2d Osseous defects filled with a mixture of Capset and either BOP or Osteograft (low-power magnifications $\times 20$ to 32 ; high-power magnifications $\times 80$).

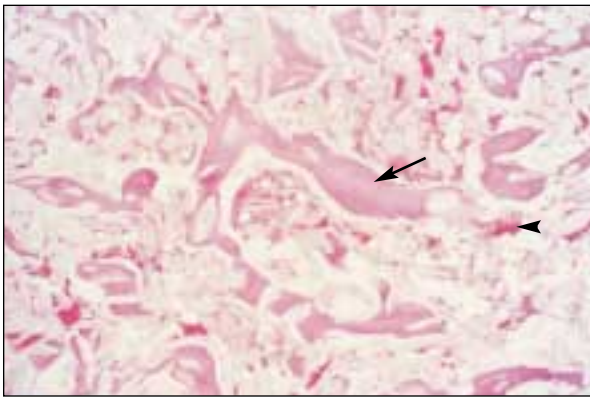


Fig 2a Defect filled with BOP and Capset. Note the increased amount of osteogenesis between the resorbed BOP crystals (*arrow*) and increased amount of angiogenesis (*arrowhead*).

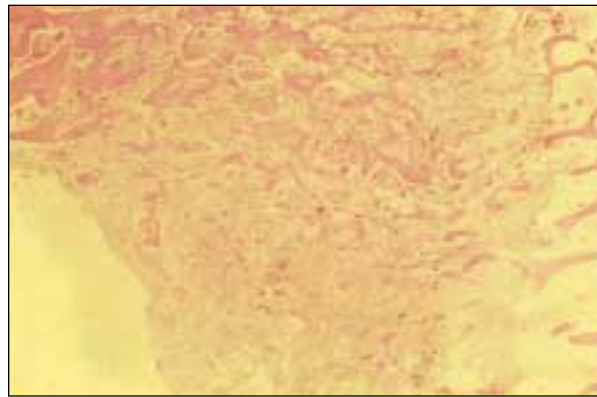


Fig 2b Low-power view of defect filled with BOP and Capset.

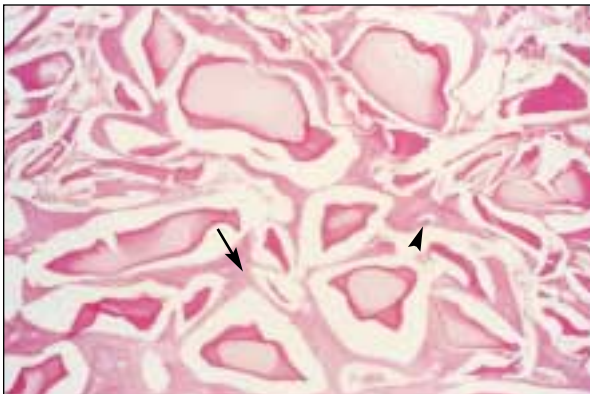


Fig 2c Defect filled with Osteograft and Capset. The field was filled with a larger amount of new lamellar trabeculae (*arrow*) interlocked with remnants of Osteograft particles. Blood vessels (*arrowhead*) can be seen throughout the defect.

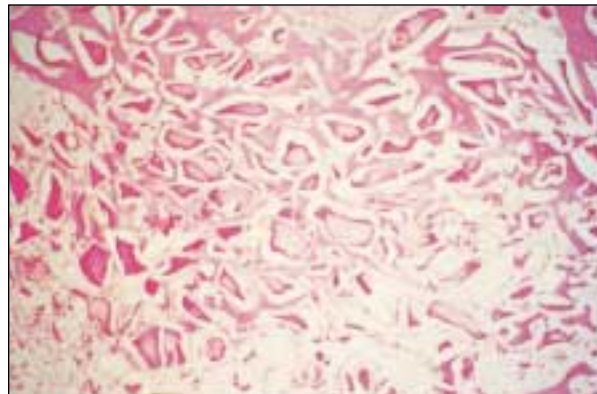


Fig 2d Low-power view of field seen in Fig 2c.

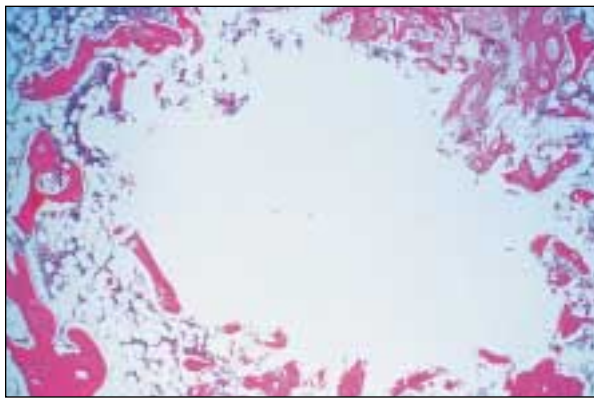


Fig 3 Control ungrafted osseous defect ($\times 40$). The empty osseous cavity is surrounded by normal bone borders, marrow spaces, and bone dust.

Norwalk, CT), which are mainly synthetic non-resorbable hydrophilic polymer, are covered with a layer of calcium hydroxide.¹⁹ Hapset (CeraMed) is also a mixture of resorbable calcium sulfate material and non-resorbable calcium phosphate particles.¹⁷

Calcium sulfate alone cannot be effectively used as a bone filler. By the time a calcium sulfate mixture starts to resorb, it allows bone ingrowth to fill the defect from the surrounding osteogenic cells. The dissolution rate of the material, however, empties the osseous cavity in a shorter time than that required for bone growth to occur. This results in inadequate new bone ingrowth in the spaces of the resorbed material. The lack of complete bone healing in osseous defects filled with calcium sulfate alone (Figs 1e and 1f) was a result of the absence of a scaffold that allows the bone remodeling process to take place with gradual physiologic timing. Shaffer and App¹⁷ have documented clinical observations confirming this conclusion. They implanted calcium sulfate material into human periodontal defects and found that defects filled with calcium sulfate alone did not induce more bone formation than non-implanted control defects. Peltier⁶ and Frame¹² have reported that, although calcium sulfate enhances bone formation, it appears that the rate of resorption is faster than the rate of bone growth.

Sottosanti²⁰ and Anson²¹ reported clinical observations showing that the addition of calcium sulfate to freeze-dried bone allografts reduced particle loss and accelerated the rate of bone regeneration in periodontal defects and on exposed dental implant surfaces. Earlier, Peltier and Orn⁹ added calcium sulfate to autogenous and homogenous bone grafts in dogs and observed accelerated bone healing when these grafts were mixed with calcium, compared with control sites of allogeneic bone grafts without calcium sulfate.

CONCLUSION

The results of this study suggest that mixing calcium sulfate hemihydrate with bone graft materials accelerates osteogenesis and increases calcification and the quantity of new bone in a shorter period of time. However, calcium sulfate material is not recommended for use alone as a bone filler.

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