Calcif Tissue Int (2002) 71:172–178
DOI: 10.1007/s00223-001-1087-x

Calcified Tissue International

Hydroxyapatite/Calcium Carbonate (HA/CC) vs. Plaster of Paris: A Histomorphometric and Radiographic Study in a Rabbit Tibial Defect Model

A. Jamali,[1] A. Hilpert,[2] J. Debes,[3] P. Afshar,[2] S. Rahban,[2] R. Holmes[2]

[1]Dept. of Orthopaedic Surgery, Massachusetts General Hospital, Boston, MA 02114, USA
[2]Division of Plastic Surgery, UC San Diego, San Diego, CA, USA
[3]Interpore-Cross International Inc., Irvine, CA, USA

Received: 25 July 2001 / Accepted: 16 January 2002 / Online publication: 5 June 2002

Abstract. The search for an ideal bone substitute is ongoing. Multiple osteoconductive bone substitutes are available today. Plaster of Paris (POP) (calcium sulfate) has been used for more than 100 years for treatment of skeletal defects. This implant is compared to a new material, hydroxyapatite/calcium carbonate (HA/CC), in a rabbit tibia model. HA/CC is made from partial conversion of coralline calcium carbonate to hydroxyapatite and has an outer hydroxyapatite layer and an inner calcium carbonate core, a combination that leads to faster resorption than that of pure hydroxyapatite. This study compares the histomorphometric and radiographic properties of POP and HA/CC in a rabbit tibial defect. Both implants preferentially restore bone to the cortex relative to the canal. Plaster of Paris was fully resorbed by 6 weeks both radiographically and histometrically and HA/CC was substantially resorbed by 42 weeks. No significant difference was noted in volume fraction of bone between the two implants at 42 weeks postimplantation. Hydroxyapatite/calcium carbonate is a biocompatible bone graft substitute with a rate of resorption significantly slower than plaster of Paris.

Key words: Bone graft — Calcium sulfate — Hydroxy apatite — Plaster of Paris — HA/CC

Bone defects are a common challenge in the clinical practice of orthopedics, plastic surgery, neurosurgery, and dentistry. The current "gold standard" has been autogenous bone grafting [1]. Complications of such procedures include infections, hematomae, sensory loss, pelvic instability, and fractures [2, 3]. Over 300,000 such procedures are performed per year making bone the second most commonly transplanted tissue after blood [1]. The search for exogenous compounds that can be used as bone graft substitutes has been ongoing.

Coralline hydroxyapatite implants have proven to be effective as osteoconductive implants [4, 5], however, CHA resorbs at approximately 2–5% per year and is therefore considered clinically permanent. This has diagnostic and biomechanical implications. Naturally occurring calcium carbonate has been demonstrated to be biocompatible, osteoconductive, and rapidly resorbable [6]. For large orthopedic defects, the rate of resorption of coralline calcium carbonate may be too rapid [7]. CHA is synthesized by a chemical conversion of the calcium carbonate (aragonite) skeleton of marine coral into calcium phosphate (hydroxyapatite) through a hydrothermal chemical exchange reaction [8], a conversion that initiates on the surface of the three-dimensional porous calcium carbonate matrix and progresses into the trabeculae. The thickness of the hydroxyapatite layer can therefore be controlled (Fig. 1). By converting only the surface of the calcium carbonate, a porous ceramic can be synthesized that is biocompatible, osteoconductive, and resorbable. This hybrid material is called hydroxyapatite/calcium carbonate (HA/CC) (ProOsteon 500R, Interpore, Irvine, CA) [9]. Controlling the thickness of hydroxyapatite on the calcium carbonate matrix controls the rate of resorption of the implant and its replacement by host bone [10]. Previous studies have indicated that the ideal calcium phosphate layer thickness is in the 2-10 μm range [9, 10].

Plaster of Paris (POP) (Osteoset®, Wright Medical, Arlington, TN) is composed of a medical grade calcium sulfate combined with a stearic acid-binding agent. This product is reported to degrade completely in 30–60 days in humans [11], and is implanted in its natural state as gypsum (calcium sulfate dihydrate). Calcium sulfate, shown to have osteoconductive properties, it has been used clinically craniofacial defects, long bone defects, as well as osseous cavities related to tumors and cysts [12, 13].

The objective of this study was to determine the resorption characteristics and biological properties of HA/CC granules with a hydroxyapatite thickness layer of

Correspondence to: A. Jamali, 77 Phillips St., #1, Boston, MA 02114, e-mail: jamaliamir@hotmail.com

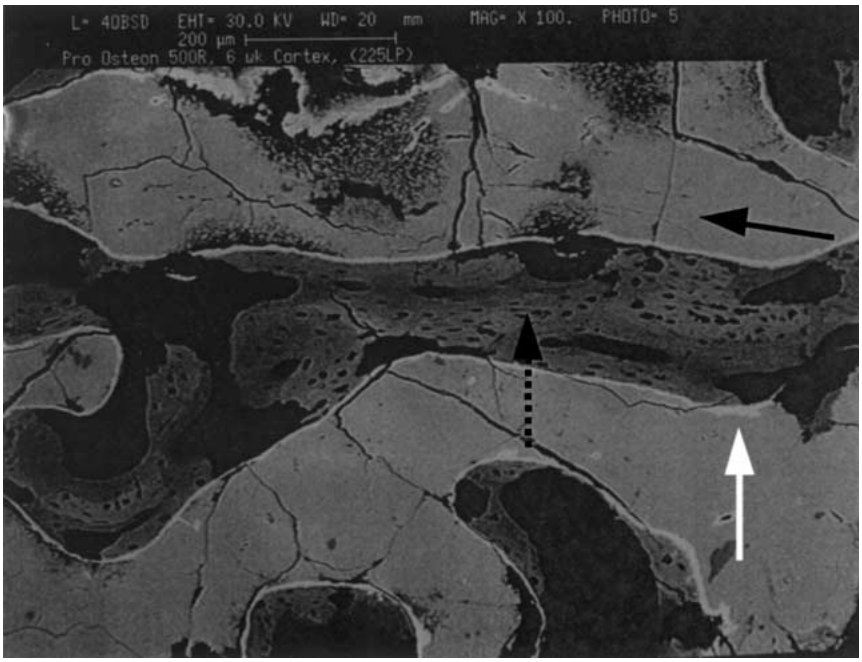


Fig. 1. Scanning electron micrograph. Hydroxyapatite/Calcium carbonate at 6 weeks showing hydroxyapatite outer layer (white arrow), calcium carbonate deep layer (*black arrow*), and bone ingrowth (*hatched black arrow*).

approximately 4 µm compared with POP pellets. All of the implant materials used in this study are currently cleared for use in humans by the United States Food and Drug Administration.

Materials and Methods

Twenty-five adult New Zealand White Rabbits were procured, numbered, and entered into the study after a minimum 3-day receiving period. The rabbits were female, skeletally mature (8–12 months) and weighed 4–6 kg. Three animals were examined radiographically immediately before surgery to confirm skeletal maturity. The animals were housed individually in stainless steel caging conforming to USDA regulations. Animals were cared for in strict compliance with institutional guidelines and the Guiding Principles in the Care and Use of Animals approved by the American Physiological Society.

Three rabbits died prior to initial analysis. One was lost on the day of surgery and the others at 2 and 4 weeks postsurgery. The perioperative death was attributed to hypersensitivity to the anesthetic agents. The two other cases were due to gastrointestinal complications. Postmortem analysis was not performed.

Six rabbits were complicated by infection and were not included in any of the experimental or control groups. One rabbit was analyzed by high-resolution faxitron radiographs at 24 weeks.

Fifteen rabbits were included in the experimental group. Analysis intervals were 6, 12, and 42 weeks postimplantation. All rabbits underwent placement of HA/CC or POP in the tibia. The side of each implant in each animal was selected preoperatively in a blinded, random fashion. Four rabbits were included in the 6-week interval, six in the 12 week group, and five in the 42 week group.

Surgery

The rabbits were anesthetized with an intramuscular cocktail of ketamine 5.0 mg/ml, xylazine 2.0 mg/kg, and acepromazine 1.0 mg/kg; this provided 20–40 min of anesthesia time. As needed, additional boluses of ketamine were used (0.1 mg/kg q 30–40 min IM). The operative sites were shaved and disinfected prior to incision. The implant sites were infiltrated with 0.25% bupivacaine (Marcaine®) subperiosteally for postoperative analgesia.

The medial proximal tibial metaphysis was exposed bilaterally through a 2 cm skin incision and subperiosteal dissection. A 15×5 mm elliptical unicortical defect was made using a dental drill (Interpore International, Irvine, CA); this included removal of the underlying cancellous bone. The superior margin of the defect was initially marked approximately 5 mm distal to the medial tibial plateau. A second drill hole was then made 15 mm distally. Medial and lateral margins of the elliptical defect were made by drilling a medial and lateral hole 2.5 mm from the line connecting the proximal and distal drill holes. Multiple drill holes were then made to create the circumference of an ellipse. These were connected using the drill to make a smooth contoured tibial defect (Fig. 2). Sterile implant materials were packed into the defect, avoiding the surrounding soft tissue. The rabbits were randomly assigned to the procedure and retrieval interval prior to surgery.

One tibial defect was filled with POP tablets (approximate dimensions = 5 mm diameter × 3 mm height) and the other tibia with HA/CC granules (seive dimensions = 1–4 mm). The POP implants were placed in two rows of three tablets (six total tablets per tibia). The HA/CC granules were gently packed to fill the defects. The soft tissue was closed in layers with absorbable sutures. The implant site was not covered with the periosteum. The defect boundaries were tagged with two titanium dental microtacks (Interpore International, Irvine, CA) proximal and distal to the defect, to serve as a radiographic marker and as an aid to postmortem tissue processing.

Processing

To accomplish histologic processing, bone specimens including adjacent soft tissues were harvested *en bloc*, fixed in 10% buffered formaldehyde, and finally embedded in polyester resin. The specimens were processed into blocks and sectioned for histomorphometric analysis on a single plane perpendicular to the surface of the defects.

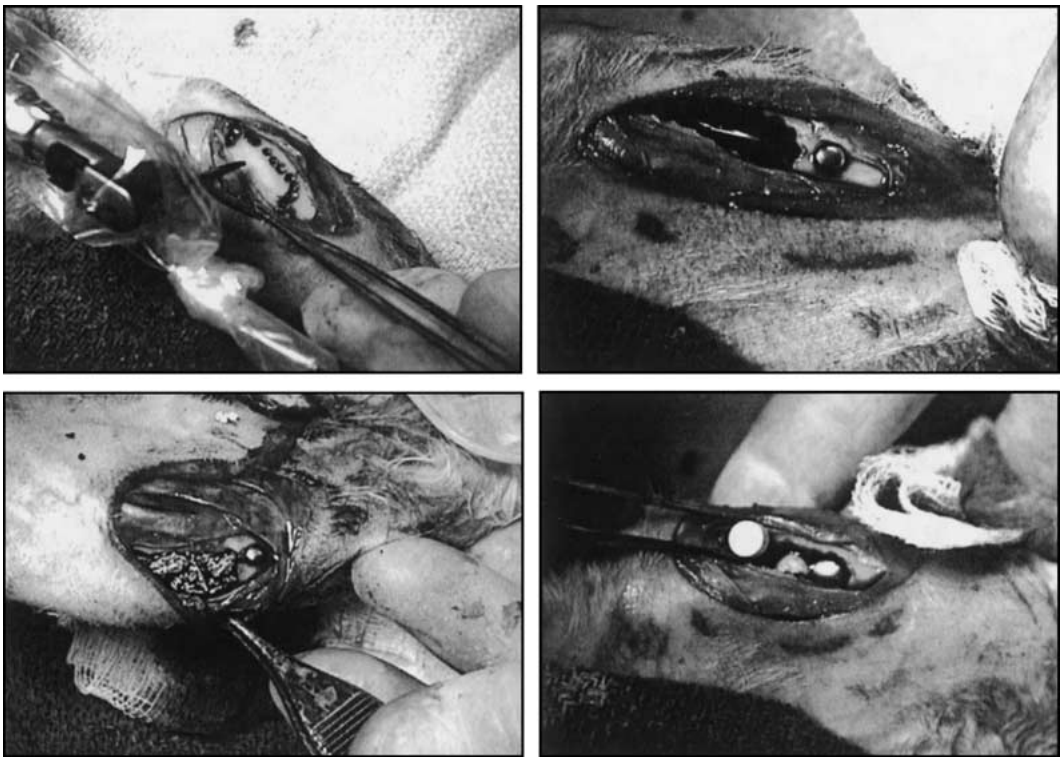


Fig. 2. Operative photographs. Top left: 5.0 × 15.0 mm elliptical defect is made on anterior surface of tibia with dental drill using sterile technique. Top right: A cortical window was removed and dental tacks were placed 2 mm away from edge of defect as marker. Bottom left and right: Defects are packed with HA/CC granules or plaster of Paris pellets.

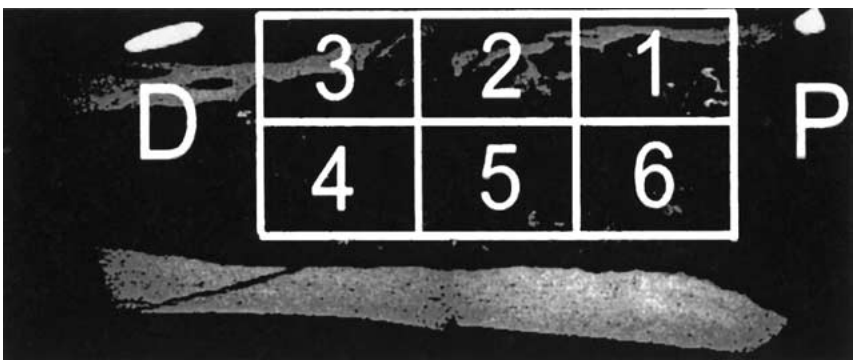


Fig. 3. Sampling protocol for scanning electron microscopy-back scatter emission.

Quantitative histomorphometry at the 6, 12, and 42-week intervals was performed using scanning electron microscopy-back scatter emission (SEM-BSE) to assess each implant material based on density. The volume fraction of implant and bone was measured using well-established bone histomorphometric methods [14].

To determine the time = 0 proportions of the implants, rabbit tibiae were obtained from animals previously sacrificed for other experiments not involving the musculoskeletal system. HA/CC granules and POP tablets were packed into these specimens which were then processed and subjected to the SEM-BSE-based histomorphometry as described above.

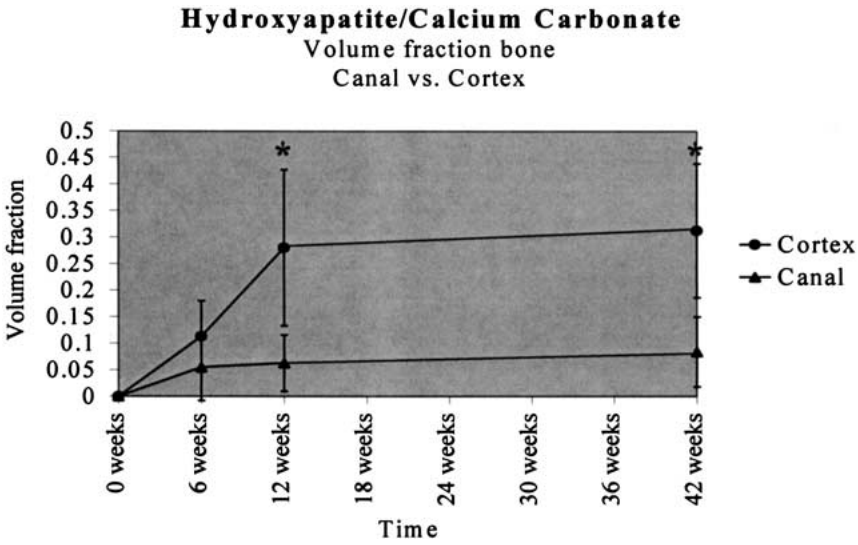
Due to the potential for varying rates of bone formation in the cortex compared with intramedullary canal, these two locations were analyzed separately.

Radiographic Examination

After disarticulation, at time equals 0, 6, 12, 24, and 42 weeks, radiographic examination was performed. High-resolution Faxitron (HP Model 4385N, Hewlett-Packard, Palo Alto, CA) radiographs were obtained at a setting of 45 kVp with a 40-second exposure time on radiographic film (XTL2, Kodak, Rochester, NY).

Sampling

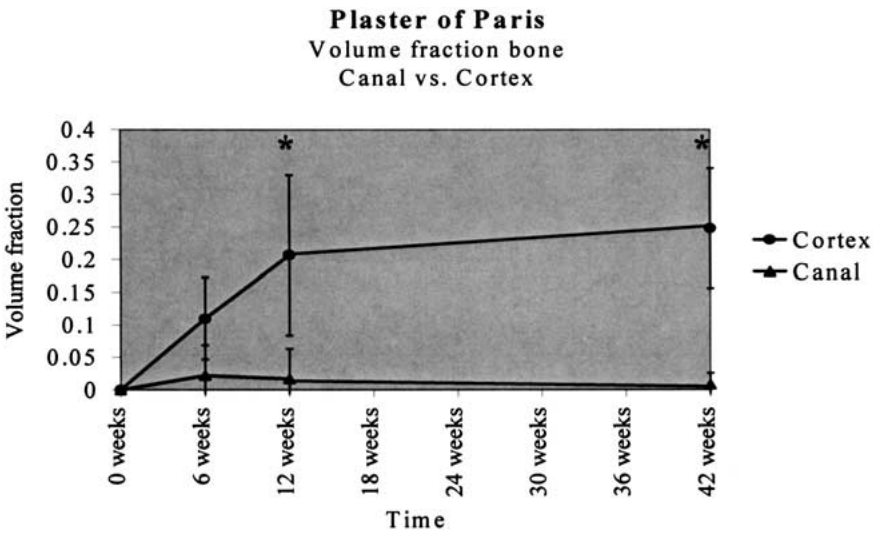
Each specimen was examined with eight scanning electron micrographs: three from the cortex, three from the canal at 40x magnification, and two lower power overviews at approximately 10x magnification. The cortex and medullary canals were quantified separately. The outer fields were aligned with the edge of the cortex, while the inner fields were positioned in the medullary canal. Proximal, middle, and distal fields of view were imaged from each cortical and canal region. These high power images were digitized and then analyzed with histometric image processing software [14] (Fig. 3). The software quantifies the relative proportion of soft tissue (or void space), bone, and implant based on gray-scale thresholds.



Time	Z-Value	P-Value
6 wks	-1.826	0.0679
12 wks	-2.023	0.0431*
42 wks	-2.023	0.0431*

* statistically significant

Fig. 4. HA/CC volume fraction of bone in cortex vs. canal regions by time period (volume fraction ± standard deviation).



Time	Z-Value	P-Value
6 wks	-1.826	0.0679
12 wks	-2.201	0.0277*
42 wks	-2.023	0.0431*

* statistically significant

Fig. 5. Plaster of Paris: Volume fraction of bone in cortex vs. canal regions by time period (volume fraction ± standard deviation).

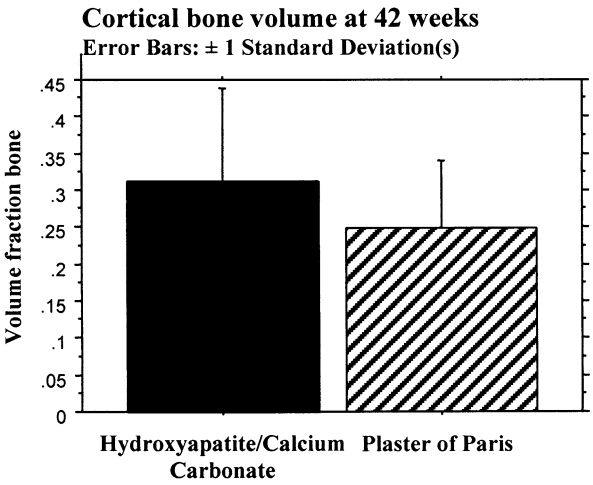
Statistical Analysis

Volume fraction of bone, implant, and soft tissue for each specimen was averaged by implant and time period. Data were analyzed by standard statistical software (StatView, Abacus Concepts, Berkeley, CA). HA/CC was compared with POP by evaluating the volume fractions of bone and of implant by time period with the Wilcoxon signed rank test. Statistical significance was set at $P < 0.05$.

Results

Histometry Results

The volume fraction of bone in the cortex and canal is shown for HA/CC and POP in Figures 4 and 5, respectively. Both implants preferentially restore bone to the cortex relative to the canal. The greatest rate of bone formation appears to be during the first 12 weeks for both implants. Final cortical bone volume at 42 weeks is represented in Figure 6. Cortical bone volume is greater at 42 weeks for HA/CC compared to POP (0.312 ± 0.126 vs 0.248 ± 0.092). This difference is not statistically significant ($p = 0.22$). Implant resorption is complete by 6 weeks in every POP specimen (Fig. 7). At the latest time point in the study, the volume fraction of HA/CC is 0.059 ± 0.049. This compares with 0.324 ± 0.029 at time zero, representing a residual of 0.059/0.324 = 18% at 42 weeks. HA/CC resorbs more slowly than POP at the 6, 12, and 42 week time periods. This difference is statistically significant at the 12 and 42 week time periods.



Z-Value	P-Value
-1.214	0.2249

Fig. 6. Histometric percentage cortical bone at 42 weeks by implant. Wilcoxon signed rank test (volume fraction bone HACC vs. plaster of Paris).

Radiographic Evaluation

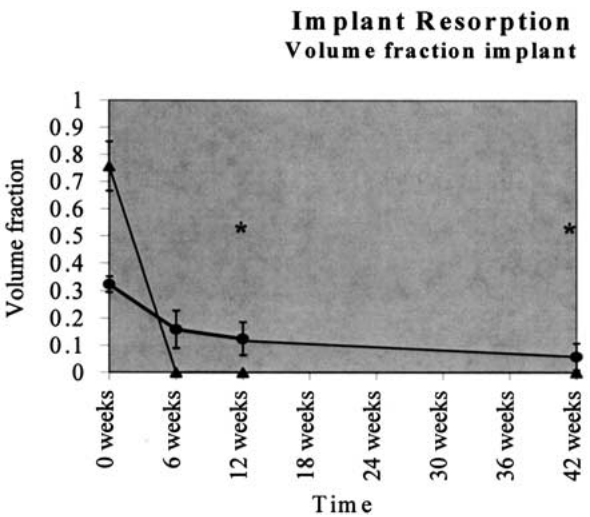
Postmortem Faxitron high resolution radiographs demonstrated complete resorption of POP by 6 weeks, corresponding to the histomorphometric data. During the remainder of the study, POP specimens gradually demonstrated increased radiodensity, corresponding to new bone formation seen by SEM (Fig. 8). In the HA/CC group, granules persisted until the 42 week study endpoint, confirming the slow resorption of this implant. At 42 weeks, by visual inspection of Faxitron radiographs, no difference in radiodensity was identified between the POP treated and HA/CC treated group.

Discussion

The need for bone graft substitutes is an ongoing challenge in the field of clinical medicine. Plaster of Paris [11, 13, 15, 16, 17] and coralline hydroxyapatite [4, 12, 14, 15, 18] are two commonly used osteoconductive biomaterials. This study demonstrates the variable rate of implant resorption between POP and a new hybrid material, hydroxyapatite/calcium carbonate, both radiographically and by SEM.

Plaster of Paris resorbs within 6 weeks in this rabbit tibia model. Turner et al. [13], in a canine humerus model treated with a 13 × 50 mm cylindrical defect, compared bone formation after treatment with plaster of Paris, autologous bone graft, and an empty defect. At 6 weeks, there was residual implant at the central tablet sites histologically as well as radiographically. At 24 weeks, defects treated with plaster of Paris had similar amounts of bone to those treated with autograft. The plaster of Paris in that study was maintained radiographically and histologically for a longer period compared with the rabbit model in this study. This may be a reflection of the smaller number of pellets used in our defect (6 vs. 50) as well as differences in bone metabolism between the rabbit and the dog.

Coralline hydroxyapatite has been characterized as a relatively permanent implant. It has been used extensively, particularly as a substitute for cancellous bone graft [4, 8, 12, 14, 15, 18]. It has a very slow resorption rate [18, 19]. The need for a bone graft substitute with intermediate resorption characteristics led to the development of HA/CC [9, 10]. We performed pilot experiments using coralline hydroxyapatite (CHA) in defects identical to the ones in this study. Extrapolating from the initial percentage filling for granules, at 42 weeks, 69% of the CHA implant remained compared to 18% of HA/CC. The hybrid compound, HA/CC, benefits



Time	Z-Value	P-Value
6 wks	-1.826	0.0679
12 wks	-2.201	0.0277 *
42 wks	-2.023	0.0431 *

* statistically significant

Fig. 7. Implant resorption vs. time. Cortical volume fraction of implant at 6, 12, and 42 weeks for HA/CC vs. plaster of Paris.

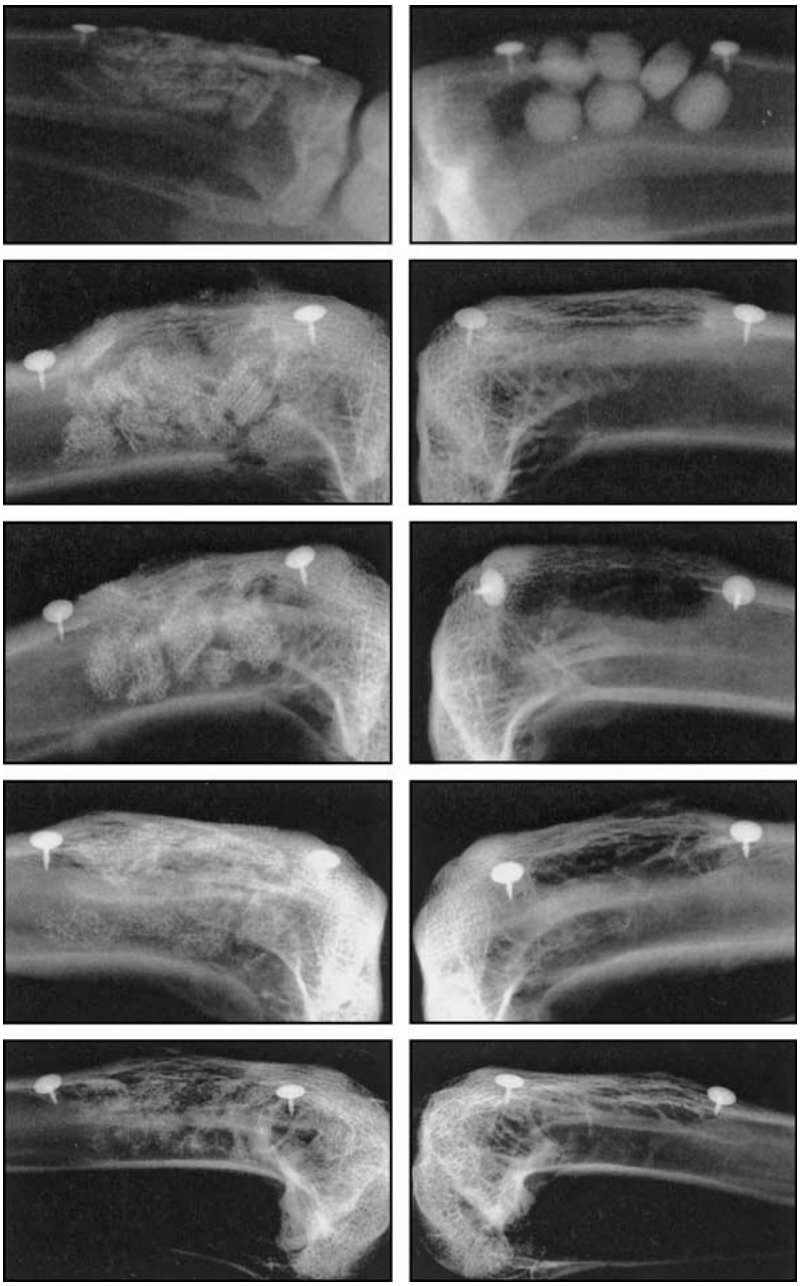


Fig. 8. Radiographic comparison of HA/CC to POP. Left column: HA/CC immediately postoperatively at 6 weeks, 12 weeks, 24 weeks, and 42 weeks, respectively. Right column: plaster of Paris immediately postoperatively at 6 weeks, 12 weeks, 24 weeks, and 42 weeks, respectively.

from a longer presence than calcium carbonate which is the main constituent of coralline implants. Additionally, it avoids the permanence of hydroxyapatite, which can act as a foreign body as well as compromising radiographic evaluation. The ability to control the thickness of the hydroxyapatite layer has clinical relevance by maximizing the degree of bone ingrowth and allowing modulation of the rate of implant resorption.

The macroscopic properties of POP and HA/CC are quite different. Plaster of Paris (POP) is currently marketed as a cylindrical solid tablet whereras HA/CC is manufactured as blocks or granules. The filling of cylindrical defects with pellets compared with granules leads to markedly different initial volume percentages of implant (32% for granules vs. 76% for pellets). The dense structure of the plaster of Paris does not interfere with its resorption.

By the end point of this study, there was a greater percentage of cortical bone in the HA/CC group compared with the POP group, however, this difference was not statistically significant. Potentially, greater numbers of specimens may have demonstrated this difference more definitively.

The volume fraction of bone at various time periods was similar for both types of implants in this study. The degree of bone formation was similar to data obtained in pilot experiments using the same rabbits with no implants placed in the defect. This may be explained by

the location of the defect in the well-vascularized tibial metaphysis. The periosteum was not repaired but may have healed at an early enough time period to contribute to a new cortex, independent of the metaphyseal bone graft substitute. Additionally, this 5 × 15 mm noncircumferential defect may be inadequate for demonstrating small differences among the various implants. Future studies may require full thickness diaphyseal defects with decreased endogenous healing potential to better delineate the effect of the bone graft.

Six procedures were complicated by draining or encapsulated abscesses. These wounds were treated with drainage, irrigation, and debridement. Four of these rabbits were sacrificed at 6 weeks and they were analyzed histometrically and compared with 6-week noninfected animals. There was no significant difference in implant resorption when compared with noninfected animals. Although analysis was not carried out to a further date, this initial data is reassuring that the implant material is not weakened or altered by an infectious environment.

This study demonstrated that HA/CC and POP have similar properties as bone graft substitutes. Although plaster of Paris resorbs more quickly than hydroxyapatite/calcium carbonate, by 42 weeks, both implants are almost completely metabolized. This new material provides the clinician with an alternative bone graft substitute with a macroscopic structure analogous to cancellous bone while avoiding the relative permanence of coralline hydroxyapatite.

References

1. Urist M (1980) Bone transplants and implants. In: Urist M (ed) Fundamental and clinical bone physiology. Lipincott, Philadelphia, pp 331–368
2. Kurz L, Garfin S, Booth R (1989) Harvesting autogenous iliac crest bone grafts: a review of complications and techniques. Spine 14:1324–1331
3. Younger E, Chapman M (1989) Morbidity at bone graft donor sites. J Orthop Trauma 3:192–195
4. Holmes R (1979) Bone regeneration within a coralline hydroxyapatite implant. Plast Reconstr Surg 63:626–633
5. Chiroff R, White E, Webber K, Roy D (1975) Tissue in growth of Replamineform implants. J Biomed Res 9(4):29–45
6. Guillemin G, Meunier A, Dallant P, Christel P, Pouliquen J-C, Sedel L (1989) Comparison of coral resorption and bone apposition with two natural corals of different porosities. J Biomed Mater Res 23:765–779
7. Hilpert A, Curran R, Debes J, Calhoun C, Shors E, Holmes R (1996) Resorption of calcium carbonate implants in the rabbit tibia: a comparison of calcite and aragonite. Annual meeting of the Biomedical Engineering Society, San Diego, CA
8. Bucholz R, Carlton R, Holmes R (1989) Interporous hydroxyapatite as a bone graft substitute in tibial plateau fractures. Clin Orthop Rel Res 240:53–62
9. Shors E, Kopchok G, Holmes R, Rosenstein D, Bumbalough T (1991) Biodegradation of porous hydroxyapatite/calcium carbonate implants in rabbits. Trans Orthop Res Soc 37:510
10. Shors E, Rosenstein D, Holmes R, Kopchok G, Bumbalough T (1991) The material and in vivo properties of a biodegradable bone graft substitute, 17th Annual Meeting of the Society for Biomaterials, Scottsdale, AZ
11. Peltier (1961) The use of plaster of Paris to fill defects in bone. Clin Orthop 21:1–31
12. Habal M, Reddi A (1992) Bone grafts and bone substitutes. WB Saunders, Philadelphia, pp 461
13. Turner TM, Urban RM, Gitelis S, Kuo KN, Andersson GB (2001) Radiographic and histologic assessment of calcium sulfate in experimental animal models and clinical use as a resorbable bone-graft substitute, a bone-graft expander, and a method for local antibiotic delivery. One institution's experience. J Bone Joint Surg Am (83-A suppl 2):8–18
14. Holmes R, Hagler H, Coletta C (1987) Thick section histometry of porous hydroxyapatite implants using backscattered electron imaging. J Biomed Mater Res 21:731–739
15. Holmes R, Mooney V, Bucholz R, Tencer A (1984) A coralline hydroxyapatite bone graft substitute: preliminary report. Clin Orthop Rel Res 188:252–262
16. Peltier, Speer (1993) Calcium sulfate. In: Habal M, Reddi A (ed) Bone grafts and bone substituties. WB Saunders, Philadelphia, pp 243–251
17. Robinson D, Alk D, Sandbank J, Farber R, Halperin N (1999) Inflammatory reactions associated with a calcium sulfate bone substitute. Ann Transplant 4:91–97
18. Holmes R, Hagler H (1988) Porous hydroxyapatite as a bone graft substitute in cranial reconstruction. A histometric study. Plast Reconstr Surg 81:662
19. Bucholz R, Carlton A, Holmes R (1987) Hydroxyapatite and tricalcium phosphate bone graft substitutes. Orthop Clin North Am 18:323