Evaluation in cats of a new material for cranioplasty: a composite of plaster of Paris and hydroxylapatite

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The materials ordinarily used to reconstruct bone defects in the calvaria and facial bones either are difficult to shape, are partially resorbed by the body, or are likely to become infected if used near a contaminated area such as the frontal sinus. Calcium sulfate hemihydrate (plaster of Paris) has been known for years to have excellent reparative qualities in bone defects, but ordinarily it is quickly resorbed. Consequently, a new material, a composite of a dense form of plaster of Paris and hydroxylapatite, was devised to provide nonabsorbable hydroxylapatite particles for bone to form around and within during the phase of plaster absorption.

Two types of this material were evaluated in cranial defects in cats. Each of the plaster of Paris/hydroxylapatite mixtures was placed into a surgically unroofed frontal sinus and into a contralateral parietal trephine hole in a group of 32 cats. Two cats in each group succumbed to anesthesia, leaving two sets of 30 cats. During the entire follow-up period there was only one other death, with no evidence of wound infection, wound dehiscence, implant rejection, or cerebral dysfunction among the survivors. The cats in each group were sacrificed at 1, 2, 3, 5, 7, 8, 9, 10, or 12 months after operation. Following sacrifice, both the frontal and parietal defects were exposed and examined visually, histologically, and with histomorphometric analysis for new bone formation. New bone formation was present as early as 1 month after operation and continued to increase during the 12 months of the study. Based upon these osteogenic qualities, the ease of shaping the composite, and the lack of infection in the frontal sinus region, it is concluded that this substance could be a valuable new material for human cranioplasty.

KEY WORDS - cranioplasty □9 frontal sinus □9 hydroxylapatite □9 plaster of Paris □9 cranial defect □9 osteogenesis

O ver the years, many materials have been used to reconstruct cranial defects. At present, autogeneic rib and ilium grafts are occasionally used for cranioplasty, but these grafts may be difficult to shape to the exact contours of the skull, such as along the supraorbital ridge, and in time they are frequently absorbed to a significant extent.

Beginning in 1940, methyl methacrylate has been used extensively for cranioplasty. This substance is easy to mold and is not absorbed. However, it cannot be placed into a contaminated or potentially contaminated area because of the threat of infection. If a skull defect results from a contaminated penetrating or compound injury (including one that extends into the frontal sinus), or occurs as the result of a frank infection such as a bone flap infection that has led to the removal of the flap, the surgeon will usually delay cranioplasty with methyl methacrylate, often for a year.

An ideal substance for cranioplasty would be: 1) viable or capable of being vascularized, and therefore capable of growth and resistance to infection; 2) light in weight; 3) radiolucent; 4) thermally nonconductive, with a coefficient of expansion identical with that of the surrounding skull; 5) physically stable (nonionizing, noncorrosive, nonbiodegradable); 6) inert and biocompatible; 7) esthetically pleasing; 8) strong and protective, with the same biomechanical properties as skull; 9) malleable and easily contoured; 10) inexpensive; 11) readily available; and 12) sterilizable. Measured by these standards, the materials used for cranioplasty at
the present time do have limitations. Consequently, the search for better materials continues.

Plaster of Paris (calcium sulfate hemihydrate) and hydroxyapatite have each been used separately in animals and in human beings to fill bone defects. One of us (J.S.H.) has been involved in studies using plaster of Paris as a binder for hydroxyapatite particles in alveolar ridge augmentation and for filling periodontal bone defects,15,17-19,52 and the present investigation is a natural extension of that work.44 These previous studies of plaster of Paris/hydroxyapatite implants have shown that the plaster is absorbed over a few weeks or months, and is replaced simultaneously by fibrovascular tissue which maintains the form and integrity of the implant. Gradually, the fibrovascular tissue is replaced by bone. The goals of the present study were to determine whether a composite of plaster of Paris and hydroxyapatite could be used as a satisfactory long-term cranioplasty implant; whether and to what extent new bone formation would occur within the composite; whether porous hydroxyapatite would be incorporated into regenerating bone better than nonporous particles; and whether such a composite could be used in a presumably contaminated area (frontal sinus) without infection or extrusion.

Materials and Methods

A total of 64 adult cats (53 of which were female) with weights ranging from 1.5 to 4.5 kg (average 2.6 kg) were obtained from the Duke University Animal Procurement Services. The guidelines used in this research were those which have been formulated by the Institutional Animal Care and Use Committee of the Duke University Medical Center. All operative procedures were performed under carefully controlled, aseptic conditions with appropriate depth of surgical anesthesia. The operations and both preoperative and postoperative care were supervised by veterinarians on the staff of the Duke University Medical Center. This research and the animal procedures were reviewed annually by the Institutional Animal Care and Use Committee to insure humane treatment of the animals.

The cats were separated into two groups of 32 each. The groups did not differ significantly in sex ratio or weight. Each cat underwent a standardized operative procedure. The cat was anesthetized with sodium pentoabarital (concentration 50 mg/ml) at a dosage of 40 mg/kg given intraperitoneally. Once anesthesia was obtained (in 5 to 10 minutes), the animal was placed into a stereotaxic head holder, and the scalp was clipped and shaved. A midline scalp incision was made from nasion to inion. The right temporalis muscle was reflected laterally after being sharply dissected from the skull, and the periosteum over the left frontal sinus was removed. A trephine hole, 1.2 cm in diameter, was placed in the right parietal region; the left frontal sinus was unroofed, and the mucosa was removed.

In the first group of 32 cats operated on between November, 1985, and August, 1986, the defects were filled with a mixture of 18- to 40-mesh dense sintered ceramic hydroxyapatite (Alveograf brand of durapatite), medical grade calcium sulfate hemihydrate (plaster of Paris) containing 0.85% K2SO4, and sterile water.* The relative amounts used were 3 gm durapatite particles, 1.5 gm USG plaster of Paris powder, and 0.5 ml water. In the second group of 32 cats operated on from July to September, 1986, the defects were filled with a mixture of porous hydroxyapatite particles with a particle diameter of about 425 to 1000 μ and a pore diameter of approximately 200 μ (Interpore 200 biomatrix granules), USG plaster of Paris powder with 0.85% K2SO4, and sterile water.† The proportions used were 3 gm Interpore particles, 1.5 gm USG plaster of Paris powder, and 1.2 ml water. Prior to mixing, the hydroxyapatite particles and plaster of Paris powder were sterilized separately or together, using dry heat at 120°C for 4 hours.

Both types of plaster/hydroxyapatite composite were easy to mix and to mold into the cranial defects to provide an excellent cosmetic effect. In each animal, the mixture hardened in the two bone defects within 2 to 5 minutes. The incision was closed in anatomical layers using interrupted 3-0 polyglactin sutures to approximate the temporalis muscle and fascia, and the galea aponeurotica, and 4-0 polyglycolic acid sutures to close the skin. The cat was allowed to recover from anesthesia, was returned to the Duke University Vivarium for postoperative care, and during the first 3 days postoperatively was given 100,000 U of procaine penicillin G and 125 mg of dihydrostreptomycin intramuscularly each day. Each cat was maintained at the Duke University Vivarium under a veterinarian’s care until sacrifice.


### TABLE 1

<table>
<thead>
<tr>
<th>Time to Sacrifice (mos)</th>
<th>Nonporous HA</th>
<th>Porous HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
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<td>4</td>
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<td>12</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>totals</td>
<td>30</td>
<td>29</td>
</tr>
</tbody>
</table>
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FIG. 1. Left: A cat skull showing the cranioplasty mixture in the left frontal and right parietal skull defects 1 month after insertion. Right: A cat skull showing bone ingrowth into the cranioplasty material, especially in the frontal region, 12 months postoperatively.

The animals were killed with a lethal dose (100 mg/kg) of intraperitoneally administered pentobarbital sodium at various time intervals between 1 and 12 months postoperatively, as listed in Table 1. The skin and mandible were removed from the head of each cat. The animal was decapitated and the nasal and occipital bones were removed with a saw. Each skull was placed into either a paraformaldehyde/glutaraldehyde fixative (phosphate-buffered to pH 7.4) or 70% ethyl alcohol.

The skulls were debrided of soft tissue, and the defects were examined grossly for evidence of implant softening, hydroxylapatite granule scattering, implant breakage, inflammation, and new bone formation (Fig. 1). The implants, along with a surrounding rim of normal bone, were removed for histological examination. The specimens containing nonporous hydroxylapatite that were examined histologically were obtained 1, 3, 5, 7, 10, and 12 months after implantation; the specimens containing porous hydroxylapatite that were examined histologically were obtained 1, 2, 3, 5, 7, 8, 9, 10, and 12 months postoperatively. The specimens were shaped into approximately 0.5 x 0.5 x 0.5-cm blocks and were stained and processed for histomorphometric quantitative analysis.

Dehydration of the specimens was accomplished using sequential immersion in 70% ethyl alcohol for 1 hour, 95% ethyl alcohol for 1½ hours, 100% ethyl alcohol for 1½ hours, and acetone for 1½ hours. The specimens were then exposed to equal parts of acetone and methyl methacrylate monomer for 24 hours. In glass vials 3 cm in diameter and 10 cm in height, the specimens were embedded in methyl methacrylate monomer which was polymerized in a vacuum oven at 37° to 40°C with 15 to 20 lb negative pressure until hardening occurred. The specimens were removed from the oven and allowed to harden completely over 5 to 10 days. Sections were cut 100 μ thick in a coronal plane using the Buehler Isomet low-speed saw, and then ground to 40-μ fragments using two roughened glass lens-grinding stones. Sections were mounted on slides with Pro-Exx mounting medium and allowed to dry for 48 hours using lead weights to promote the egress of air.

The above-mentioned staining method leaves mineralized bone unstained. Fibrous connective tissue is purplish blue, osteoid is light pink to red to reddish-purple, the nuclei of osteoblasts and osteoclasts stain purple, and their cytoplasm stains silver-gray.

In order to analyze quantitatively the amount of bone, osteoid, and fibrous tissue present at various time intervals after implantation, histological sections 40-μ thick from specimens obtained 1, 3, 5, 7, 10, and 12 months postoperatively were examined with a Merz-Schenk reticle at ×160 magnification with a Leitz Dialux microscope. The reliability of analyzing a field 40-μ thick in a single plane of focus has been previously established by Felsenfeld, et al. Fifty fields per slide were examined, and measurements detailing the percentage of the surface occupied by bone, osteoid, and fibrous tissue were made.

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† Bone stain supplied by Polyscience, Inc., Warrington, Pennsylvania.

‡ Buehler isomet saw, Model 11-1180, manufactured by Buehler, Ltd., Evansville, Indiana.

§ Pro-Exx mounting medium supplied by American Scientific Products, Inc., Charlotte, North Carolina.

* Merz-Schenk reticle manufactured by Wild Heerbrugg, Ltd., Heerbrugg, Switzerland; Leitz Dialux microscope manufactured by E. Leitz, Inc., Wetzlar, West Germany.
Results

In each group of 32 cats, there were two deaths within 12 hours of the procedure (three of the four were within 5 minutes of the induction of anesthesia), leaving two groups of 30 animals each. These 60 animals were followed for various periods up to 1 year after the operative procedure, and during this time there was only one death. This single death occurred 7 months postoperatively; its cause was not determined. During the follow-up interval there was no evidence of infection, implant rejection, or neurological deficits among the other 59 cats, which appeared and acted like normal cats.

At the time of sacrifice, the implanted skull defects were found to be well healed. The cosmetic aspects of the cranioplasties were excellent. The material was solid upon palpation, with no evidence of implant breakage or softening, infection, or displacement of material. There was no difference between the two groups of cats with respect to these observations.

Upon closer inspection and probing with a dental pick, there was evidence of new bone growth in the frontal sinus and in the parietal defect (primarily on its dural side) (Table 2). There was an obvious difference between the two groups of cats in this regard. Those that had received the porous hydroxylapatite showed earlier (2 to 3 months) and more extensive new bone formation. The group with the nonporous material had little new bone formation until 8 to 10 months after surgery as assessed by gross inspection and probing.

The results of the histomorphometric quantitative analysis are presented in Table 3. In both groups of cats, the amount of bone present increased gradually over 12 months while the amount of fibrous tissue gradually decreased over the same time period. The percentage of bone at 12 months varied from 65% to 69% and the percentage of fibrous tissue varied from 31% to 35%. The amount of osteoid present gradually decreased until it was almost absent at 7 months postoperatively.

Discussion

Bone is a collagen matrix that has become mineralized with hydroxylapatite. Hydroxylapatite, having a chemical composition of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH}_2)$, is the major constituent of bone and teeth. Various forms of processed hydroxyapatite (termed "hydroxyapatite") have been used in animals and in humans to fill bone defects.5,7,14,24,25,48 These materials are extremely well tolerated by the host and have the capability of becoming chemically bonded to bone.24 Hydroxylapatite is an available, sterilizable, relatively nonabsorbable, and strong substance.

If one is attempting not only to fill a bone defect with an implant, but also to promote osteogenesis, a porous material should have been more successful than a nonporous material because it should facilitate the ingrowth of fibrovascular tissue that will then ossify.3,11,12,22,23,26,27,42 Porous hydroxyapatite implants have been tested in animals and used in humans to fill bone defects.9,13,20,21,24,29,39,40,51 Tissue ingrowth and subsequent bone formation do occur.

For example, Roser, et al.,46 investigated the use of implants of porous hydroxyapatite with a pore size of

### TABLE 2

<table>
<thead>
<tr>
<th>Type of HA in Mixture</th>
<th>No. of Cranio-</th>
<th>No. With</th>
<th>% With</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>plasties</td>
<td>New Bone</td>
<td>New Bone</td>
</tr>
<tr>
<td>nonporous</td>
<td>F</td>
<td>P</td>
<td>Total</td>
</tr>
<tr>
<td>porous</td>
<td>29</td>
<td>29</td>
<td>58</td>
</tr>
</tbody>
</table>

* Data collected by gross inspection and probing. HA = hydroxyapatite.
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| TABLE 3 |
|------------------|------------------|------------------|
| **Percentages of bone, fibrous tissue, and osteoid present at various times after cranioplasty** |
| **Time Interval (mos)** | **Frontal Defect (%)** | **Parietal Defect (%)** |
|                  | Bone | Fibrous Tissue | Osteoid | Bone | Fibrous Tissue | Osteoid |
| nonporous hydroxylapatite |
| 1                 | 22   | 63           | 15      | 12   | 74           | 14      |
| 3                 | 45   | 48           | 7       | 38   | 56           | 6       |
| 5                 | 47   | 49           | 4       | 45   | 52           | 3       |
| 7                 | 53   | 45           | 2       | 55   | 43           | 2       |
| 10                | 58   | 42           | 0       | 60   | 39           | 1       |
| 12                | 69   | 31           | 0       | 68   | 32           | 0       |
| porous hydroxylapatite |
| 1                 | 6    | 86           | 8       | 10   | 73           | 16      |
| 3                 | 36   | 60           | 4       | 32   | 63           | 5       |
| 5                 | 44   | 50           | 5       | 43   | 53           | 4       |
| 7                 | 65   | 34           | 2       | 60   | 38           | 2       |
| 10                | 62   | 37           | 1       | 64   | 35           | 1       |
| 12                | 68   | 32           | 0       | 65   | 35           | 0       |

100 to 150 μ to reconstitute mandibular defects and also to reconstruct the frontal sinus in dogs. By 1 week the frontal implants were quite firm, but minimal migration of the implants had occurred. Granulation-type tissue had proliferated throughout all the implants by 1 month, and bone ingrowth occurred progressively from the periphery. Koyama and Handa have employed porous hydroxylapatite in their patients as a wedge for fixation of a bone flap at craniotomy, and Kumakawa, et al., have used it for frontal osteoplasty.

In 1986, Holmes reported to the Plastic Surgery Research Council his investigation of porous hydroxyapatite as a material for cranioplasty (RE Holmes, unpublished data). The material he used, Interpore 200, was the same as that tested in our experiment, but in block rather than particulate form. It is made by the conversion of the calcium carbonate exoskeleton of the marine coral Poritidae porites to hydroxylapatite with a nominal pore size of 200 μ by a hydrothermal reaction. Holmes placed a 5 × 15 × 20-mm block of steam-sterilized porous hydroxyapatite, shaped without regard to the orientation of the pores, into a parietal craniectomy defect in each of 17 dogs. The implant areas were studied at 3, 6, 24, and 48 months. Wound healing was normal in all animals. The hydroxyapatite matrix ranged from 35.0% to 42.2% with no resorption over time. Connective tissue was noted within the implant pores near the pericranial and dural surfaces. Ingrowth of bone was present to some extent in all of the implant specimens. The amount of bone ranged from 11.2% to 23.8% and increased over time, but only at a rate of 1.6% per year. The bone extended across the interior of the implant from one cranial shelf to the other. The percentage of the hydroxyapatite matrix surface covered by bone ingrowth ranged from 29.7% to 52.3% and increased 3.0% per year. For each 0.5-mm incremental distance from either the cortical or periosteal surface, the amount of bone ingrowth decreased 2.3%. The bone was usually in direct apposition to the hydroxyapatite matrix without intervening soft tissue. Both osteons with Haversian canals and interosteonic Volkmann's canals were identified in the regenerating bone. There was no evidence of inflammation or foreign-body reaction.

Both solid and porous hydroxyapatite particles have been used for reconstruction of the alveolar ridge. One of the problems with such usage has been the displacement and migration of the particles after their insertion. One of us (J.H.S) and his associates tested various substances such as blood, albumin, and collagen in animal models to bind the particles together and to make the alveolar implant easier to shape. Of the substances tested in animals (and subsequently used in more than 70 patients with periodontal disease), Hanker and his colleagues found that plaster of Paris gave the best results. Furthermore, during these investigations, they discovered that the porous hydroxyapatite/plaster of Paris mixture stimulated more bone formation in mandibular defects in rats than did the nonporous hydroxyapatite/plaster of Paris mixture.

Plaster of Paris is produced by heating gypsum (CaSO₄ • 2H₂O) so that it loses 75% of its water and becomes the hemihydrate of calcium sulfate. When it is mixed with water, a paste is formed that rapidly solidifies with a slight exothermic reaction. However, the setting of a mixture of plaster of Paris and hydroxyapatite particles is slightly endothermic (JS Hanker, unpublished observations). The time required for setting can be shortened by the addition of potassium sulfate and can be lengthened by adding water. The hemihydrate of calcium sulfate is readily available and inexpensive. It can be sterilized easily by dry heat (for example, 300°F or 160°C for 4 hours). The hydrated paste can be molded to a bone defect, and after it has set, the plaster of Paris is easily shaped. The resulting material is light in weight and strong, with a measured compression strength of 245 kg/sq cm.

Plaster of Paris has been used as a bone substitute in human patients since at least 1892. Its properties have also been tested in several animal models. It is inert in that it does not incite a local inflammatory or foreign-body response but, in a publication in 1980, Coetzee noted an elevation of the erythrocyte sedimentation rate to approximately twice the original rate in each of his 100 patients, with a return to normal within 10 days. Coetzee also noted a mild temporary increase in polymorphonuclear leukocyte counts within the same time frame. However, he was using the calcium sulfate primarily to fill bone defects in the mastoid, and it is of note that 50 of his 100 patients were being treated for mastoiditis and another 20 for a cholesteatoma. These circumstances may also account for the observation that 5% of his patients complained of painful cervical lymph glands postoperatively.
In 1961, Peltier,37 who had extensive experience with the use of plaster of Paris to fill defects in bone, both in dogs and in humans, reported an experience with 20 patients. Nineteen had lesions of bones in the limbs and one had a lesion of the sacrum. He concluded that: "The plaster of Paris appears to act primarily as a space-occupying material or 'filler' whose most important property is its natural rate of absorption from the bone. The rate of absorption coincides closely with the rate at which new bone can grow into a defect. The absorption of the plaster of Paris and the subsequent regeneration of bone occur rapidly over a period of weeks or months. The use of plaster of Paris in infected cavities does not give rise to extra hazards or complications."37

In 1981, Beeson3 published the results of an investigation of the use of plaster of Paris in dogs as an alloplastic implant in the frontal sinus. After it had been placed into the frontal sinus of six dogs, there was complete bone regeneration within 4 to 6 months. Beeson concluded that plaster of Paris seems to stimulate osteoneogenesis when implanted in contact with bone or periosteum. Coetzee4 used calcium sulfate to obliterate the frontal sinus in three of his patients. The lesions treated were massive mucoceles, and the calcium sulfate was used to restore the patients' features. There were no complications.

The cat was chosen as a model for the present study because it has a frontal sinus, has been used in previous cranioplasty experiments, and is less expensive than a dog.34,35,43 Montgomery34 has shown that, during periods of up to 1 year after the insertion of autogeneic fat into the denuded frontal sinus of the cat (that is, after the mucous membrane and inner cortical bone lining of the sinus have been removed), there is no regeneration of the mucous membrane and only an occasional and slight amount of new bone growth. Pudenz46 has shown, in a study of feline cranioplasty using tantalum plates in calvarial trephine openings, that by 27 days after implantation the proliferating bone at the edge of the defect had approximated the edge of the metal and by 317 days there was complete closure of the cranial defect by new bone formation. This new bone formation was in the form of a thin lamina of bone beneath the tantalum plate; no proliferation of bone occurred over the external surfaces of the plate.

Based on the body of information discussed above, it is not surprising that, in the present study, bone regenerated into the implanted plaster of Paris/hydroxyapatite mixtures, presumably from the remaining dura mater and pericranium. However, the completeness of the new bone formation was greater than we had expected. The ease of insertion and molding of the plaster of Paris/hydroxyapatite mixture, the firmness of the hardened mixture throughout the periods of observation, the extent of the osteoneogenesis, the excellent cosmetic qualities of the implanted material, and the absence of loosening or infection (even in the obliterated frontal sinus) indicate that it has the potential of being a better material for orbitofrontal cranioplasty than either autogeneic bone grafts or methyl methacrylate. It is also possible that the use of this material might permit earlier filling of cranial defects resulting from a compound injury or the removal of an infected bone flap. Finally, it might be of value for cranioplasty in children in whom continuing skull growth and some degree of spontaneous bone regeneration are expected.10

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