Use of Demineralized Allogeneic Bone Implants for the Correction of Maxillocraniofacial Deformities

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Two major problems in maxillocraniofacial surgery are the limited amount of fresh autogenous bone, the standard material for bone grafting, and the resorption of the grafted bone. Experimental studies with demineralized, devitalized bone matrix have shown induction of endochondral ossification. Fifty-five demineralized allogeneic implants have been used in 44 patients over the past two years for a variety of congenital (n = 37) and acquired (n = 7) defects. The allogeneic bone was obtained from cadavers, prepared as powders, chips or blocks, and was demineralized. After having been sterilized by irradiation, they were used to augment contour, fill defects, or construct bone within soft tissue. Of implanted sites that could be evaluated by physical examination, 31 of 31 were solid by three months. By radiographic examination three of 19 were healed by three months, and an additional 11 were positive by six months. Induced bone was seen in four of four biopsy specimens. Infection occurred in four of 44 patients (9%), comparable with conventional grafts. Implant resorption occurred in four instances. Allogeneic demineralized implants offer several advantages over conventional bone grafting, such as avoidance of a harvesting operation, ease of manipulation, and potentially unlimited material in banked form. In addition, healing by induced osteogenesis may bypass the resorption seen with healing of mineral-containing grafts.

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CORRECTION OF MAXILLOCRANIOFACIAL deformities requires transfer of autogenous bone grafts in order to stabilize mobile skeletal segments, to augment skeletal contour, and to construct new skeletal architecture. The harvesting of ribs, iliac or tibial bone carries a risk of complication. The amount of available autogenous bone is limited, particularly in infants and young children. In some instances, the harvesting operation may be of greater magnitude than the primary surgical procedure, for example, the closure of a bony oronasal fistula. For these reasons (morbidity rate, limited source, and relative magnitude of the procedure) allogeneic and xenogeneic bone grafts have been used after freezing,[1,2] lyophilization,[3] deproteinization,[4] and irradiation.[5] However, banked bone does not heal as predictably as fresh autogenous bone grafts.[6]

Six years ago, the authors wondered whether or not fresh bone powder left over after fashioning fresh autogenous grafts would produce bone when placed in the craniofacial region. This query led us to review the studies of Urist[7] and Huggins,[8] which concerned the phenomenon of "induced osteogenesis." Urist discovered that demineralized cortical bone fragments stimulated osteogenesis within muscle in rabbits. Reddi and Huggins characterized the histologic sequence of ectopic osteogenesis with demineralized bone powder in the subcutaneous tissue of rats.[9] They showed that the geometry of this matrix[10] and its electric charge[11] are important determinants for bone induction. The authors developed animal models in the craniofacial region and demonstrated the 1) healing of cranial and mandibular defects that do not spontaneously heal, 2) bone construction within soft tissue and 3) that the phenomenon is not species-specific.[12,13] The demineralized, devitalized matrix induces an orderly sequence of endochondral osteogenesis throughout the implanted area. Powdered implants become amalgamated within the induced bone, whereas larger demineralized blocks induce bone on their surfaces and, more slowly, within cancellous spaces. Morphometric studies show demineralized implants do not undergo resorption during bone induction, in contrast to mineral-containing powders which are resorbed without bone production.[14]

Induced osteogenesis with demineralized implants is different from osseous healing that occurs with conventional bone grafts (Fig. 1). Fresh cancellous grafts are rapidly revascularized and survive to produce new bone from the transplanted living osteoblasts.[15–17] The predominant mechanism of healing with fresh or preserved cortical grafts is "creeping substitution," the

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concomitant resorption of the bone graft and its replacement via ingrowth of vascular and osteoblastic tissue from adjacent bone.[18–20] Induced osteogenesis, in contrast, is a phenotypic change of host pluripotential cells into osteoblasts. The process is one of local cellular transformation, in contrast to osseous transplantation with living cortical-cancellous grafts.

In 1889, Senn demineralized implants, acid-treated ox bone, as an antiseptic method of grafting tibial osteomyelitic defects.[21] Autogenous bone, demineralized with ethylene-diamine tetra-acetic acid (EDTA)[22] and, more recently, surface-demineralized allogeneic implants[23,24] have been used for spinal fusion.

Encouraged by our laboratory results, we began clinical trials with allogeneic demineralized implants in December, 1978. Recently, evidence was shown for the successful induction of bone in humans implanted with demineralized material.[25] This report is a summary of experience with 42 patients with maxillocraniofacial defects, who received demineralized implants over the past two and one-half years. In addition, its use in two patients with long bone defects is reported.

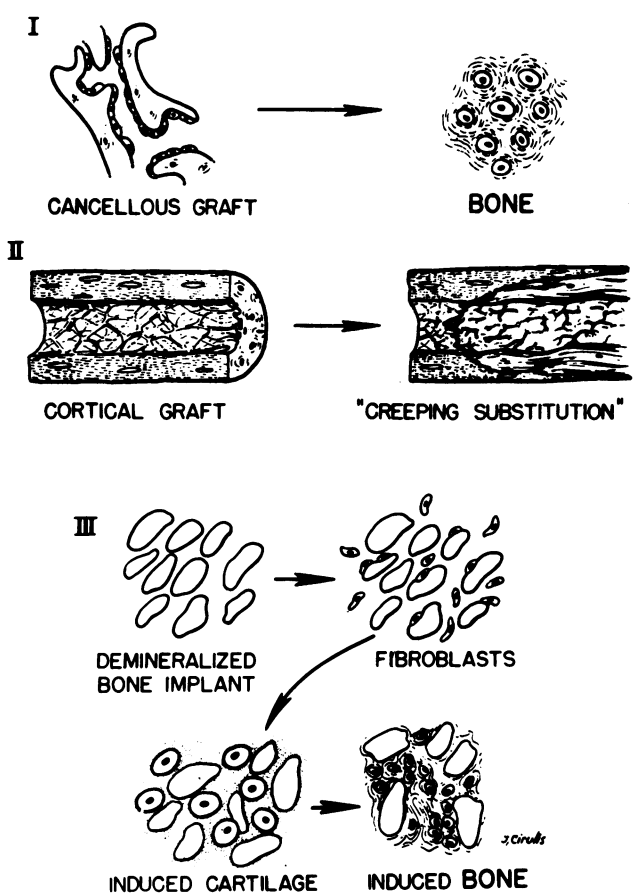


FIG. 1. Three mechanisms of osseous healing: I. Cancellous bone graft, II. Cortical bone graft, and III. Demineralized implant.

TABLE 1. *Patient Diagnoses*

Congenital	
craniosynostosis	2
hypertelorism	6
branchial arch deformity	2
cleft lip/palate	14
noncleft craniomaxillary hypoplasia	12
Acquired	
(post-trauma, tumor resection)	6
	42

* Two additional patients, one with congenital tibial pseudoarthrosis and another with posttraumatic femoral cyst, have been implanted with demineralized material.

Materials and Methods

Preparation of Implants

The implant material was processed from cadaver femurs procurred via the Inter-Hospital Organ Donor Program. The bone was prepared in three forms: powder (approximately 450μ in diameter), chips (3–5mm diameter), and corticocancellous blocks. The material was completely demineralized with 0.5 N HCl according to previously published procedures.[25] The double-wrapped implants were sterilized by cathode ray irradiation with 2×10^6 rads and stored at room temperature.

For use in the operating room, the implants were soaked in lactated Ringer's solution. The powder quickly rehydrated to a paste-like consistency whereas chips and corticocancellous blocks required a longer time (about 30 min) to rehydrate. The hydrated blocks were rubbery in consistency and could be carved easily.

Patient Selection

Volunteers were entered into this study according to the following criteria: 1) to avoid the harvesting pro-

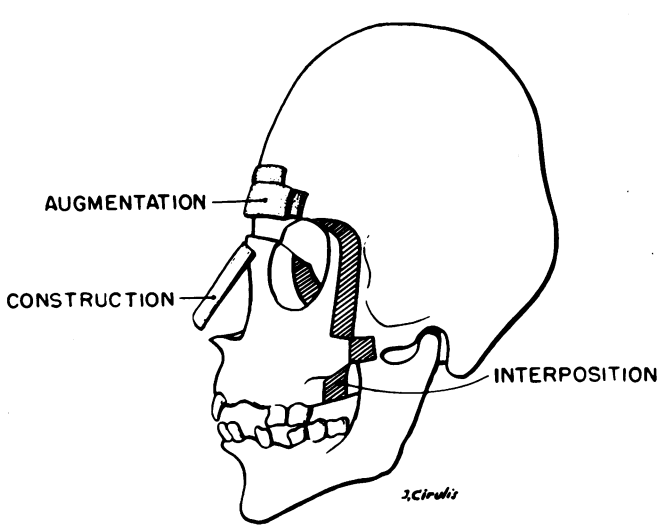


FIG. 2. Uses for demineralized implants: augmentation, interposition, and construction.

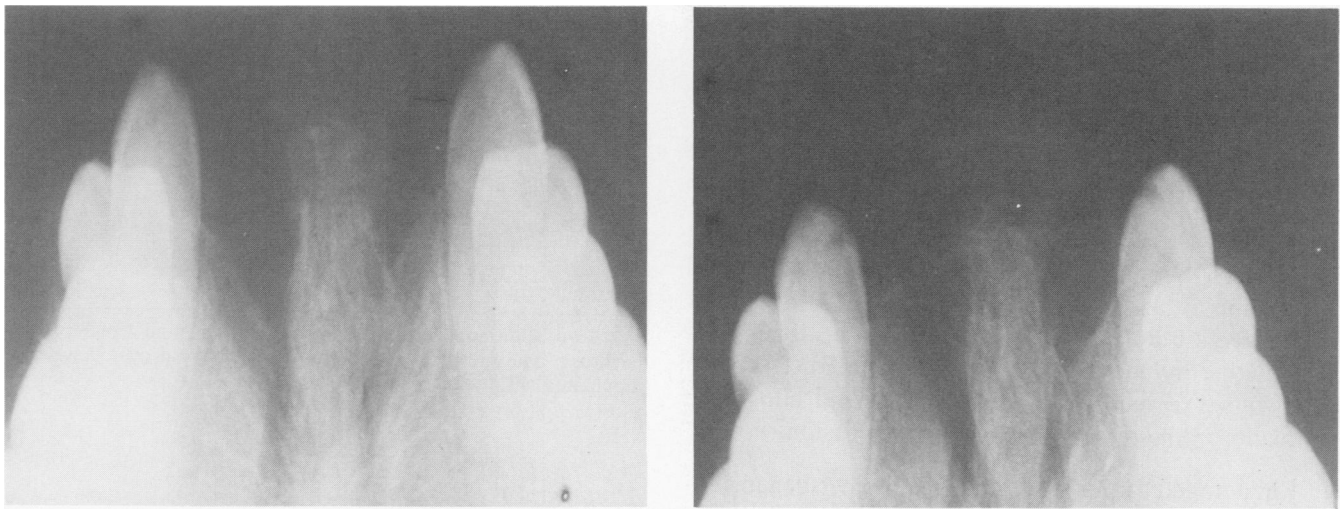


FIG. 3a and b. Occlusal radiograph of a 13 year old patient with bilateral cleft lip/palate, before (a, left) and 2 months after (b, right) implantation with demineralized powder, showing early ossification in the clefts.

cedure in an infant or frail person, 2) to fill a small defect, or 3) to supplement conventional grafting when insufficient autogenous bone was available. The implants were used in both congenital (n = 37) and acquired (n = 7) deformities. In addition, they were used to repair a posttraumatic femoral cyst, which had failed to heal with three previous conventional grafts, and a congenital pseudoarthrosis of the tibia. Allogeneic implants were used in 43 patients; in one patient, the implant was prepared from the patient's own calvaria. The patients ranged in age from 1–60 years (mean: 16 years) (Table 1).

The implants were used for 1) *interposition* (n = 28), within osteotomy gaps or cystic defects; 2) *augmentation* (n = 19), over intact bone surfaces; and 3) *construction* of new bone within soft tissue (n = 8) (Fig. 2).

Evaluation of Healing

Bone healing was documented by clinical and roentgenographic examination; one patient was studied by CT scan and ultrasonographic examination; biopsy specimens were obtained in four patients. Because of the various locations of implanted sites, healing in each patient could not be evaluated by all methods. For example, successful closure of an osseous fistula could be seen only by radiographic examination. Some implanted sites, *e.g.* the pterygomaxillary osteotomy gaps, could be evaluated only indirectly by the stability of the maxilla. Ethical considerations precluded biopsies unless a later operation was indicated.

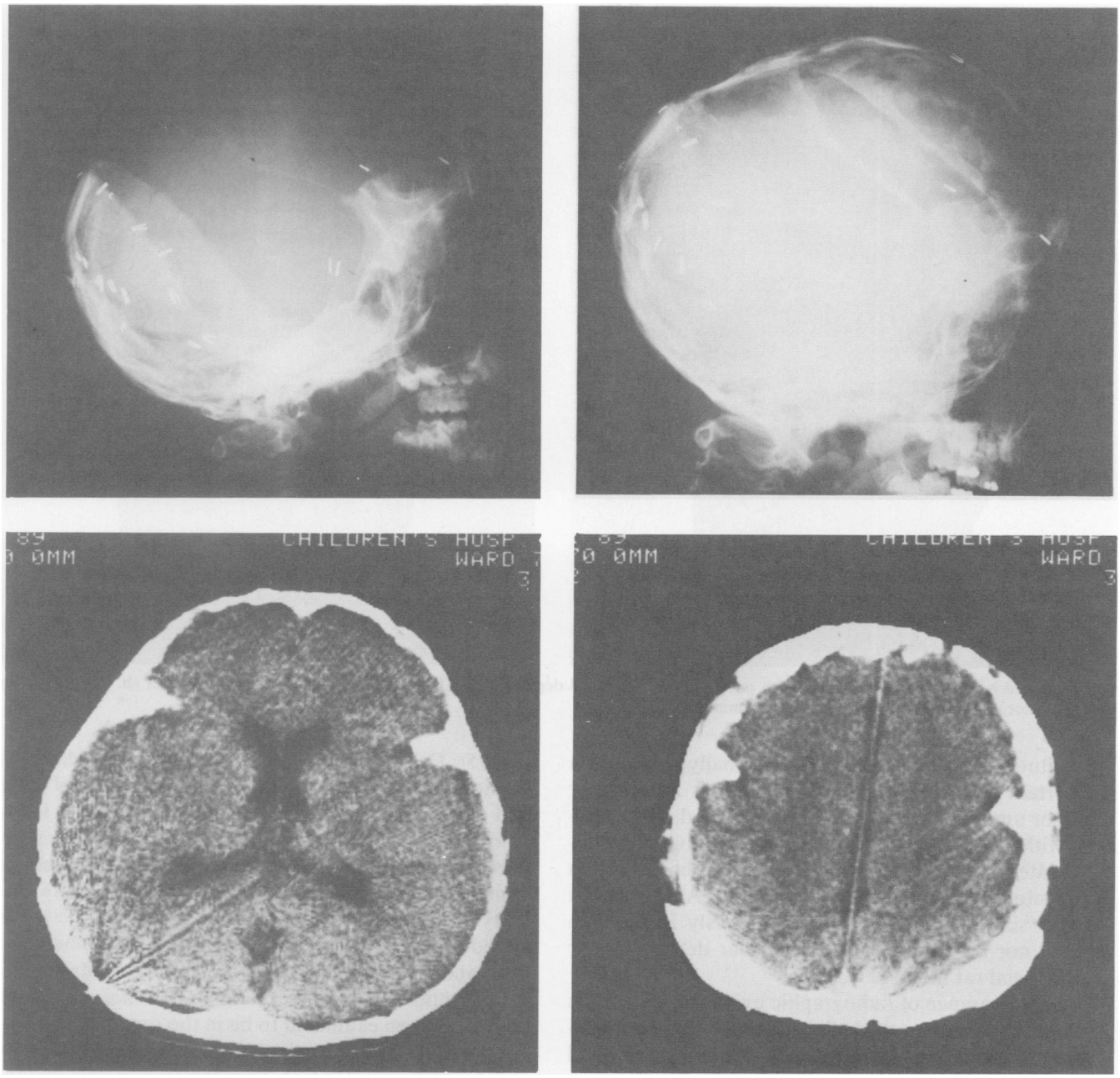
Results

Clinical healing was defined as "hard" by palpation for onlay construction and as stability of the segments for osteotomy gaps. Radiographic healing was defined by the appearance of mineralization within the defect (Fig. 3).

Of those sites suitable for evaluation, 31 of 35 were clinically healed within three months. By radiographic examination, three of 19 sites were positive before three months; another 11 were healed by six months. The five of 19 sites that were negative for osseous healing at six months were all "hard" or stable by physical examination. Bone and implanted matrix were present in four of four biopsy specimens. Ultrasound and CT scan documented healing of a large calvarial defect implanted with autogenous demineralized paste and chips (Fig. 4). A posttraumatic femoral cyst failed to heal with three attempts with fresh autografts. However, after implantation with demineralized chips, the cyst was healed by three months, as demonstrated by radiographic examination. The case of congenital pseudoarthrosis is too recent to evaluate.

Infection occurred in four of 44 patients (9%), a rate comparable with conventional grafts used over the same period of time. The infections were apparent within the first week after implantation. The organisms were *alpha Streptococcus*, in two patients and *Staphalococcus aureus* in two others. All infections occurred in association with an intraoral or nasal incision. Portions of the implants, cultured at time of insertion, were sterile.

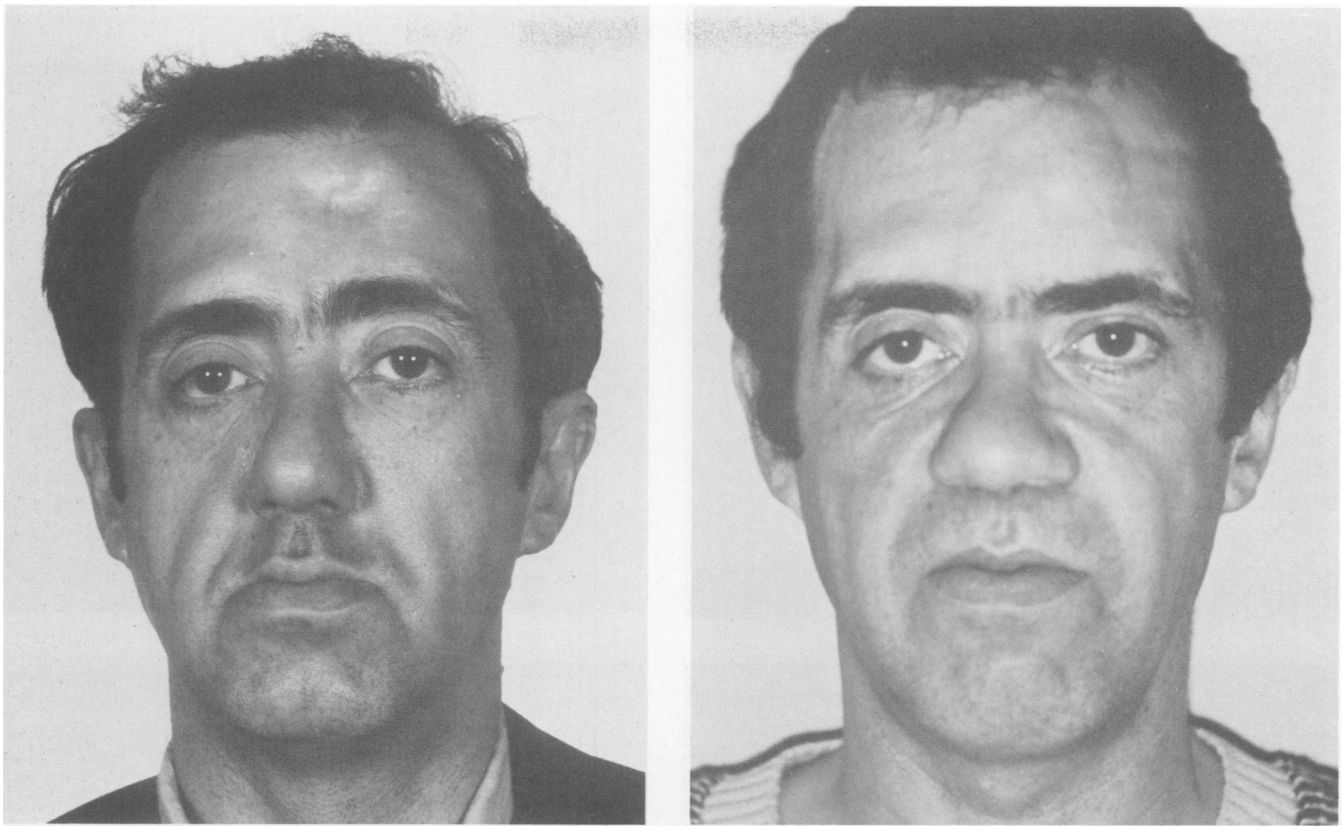
Resorption was evaluated by palpation for onlay implants, loss of stability with interpositional implants, or loss of architecture with soft tissue implants. Resorption of the implant occurred in four patients, powder, in one patient, corticocancellous in three patients. Two patients required reoperation and conventional bone grafting following resorption.



FIGS. 4a–d. A 7 year old boy with craniosynostosis underwent calvariectomy (a, top left) and reconstruction 2 weeks later with autogenous demineralized chips and powder. Radiograph 1 year later (b, top right) shows good mineralization throughout with few patchy areas of demineralization. Extent of bony healing confirmed on CT scan (c, bottom left) at level of lateral ventricles, high sphenoid ridge and (d, bottom right) 3cm higher through convexity of skull.

Discussion

This patient series shows how demineralized implants can be used to produce osseous healing in the craniofacial region. There is evidence that healing occurred by transformation rather than by the mechanism of "creeping substitution," as with conventional osseous transplantation.[25] First, the experimental studies of Urist, Reddi and Huggins, and from our laboratory, demonstrate that demineralized implants induce bone in extra-skeletal sites. A new bone can be formed within facial soft tissue in the identical pattern of previously implanted demineralized powder.[12] Second, in the four patients who underwent biopsies, the histologic picture was identical to those seen with experimental craniofacial induction.[25] Third, osseous healing occurred throughout the defect, as a field phenomenon, not from the edges as would be seen with "creeping



FIGS. 5a and b. A 40-year-old patient with posttraumatic forehead depression (a, left) and one and one-half years later (b, right) showing maintenance of contour augmentation with demineralized implants.

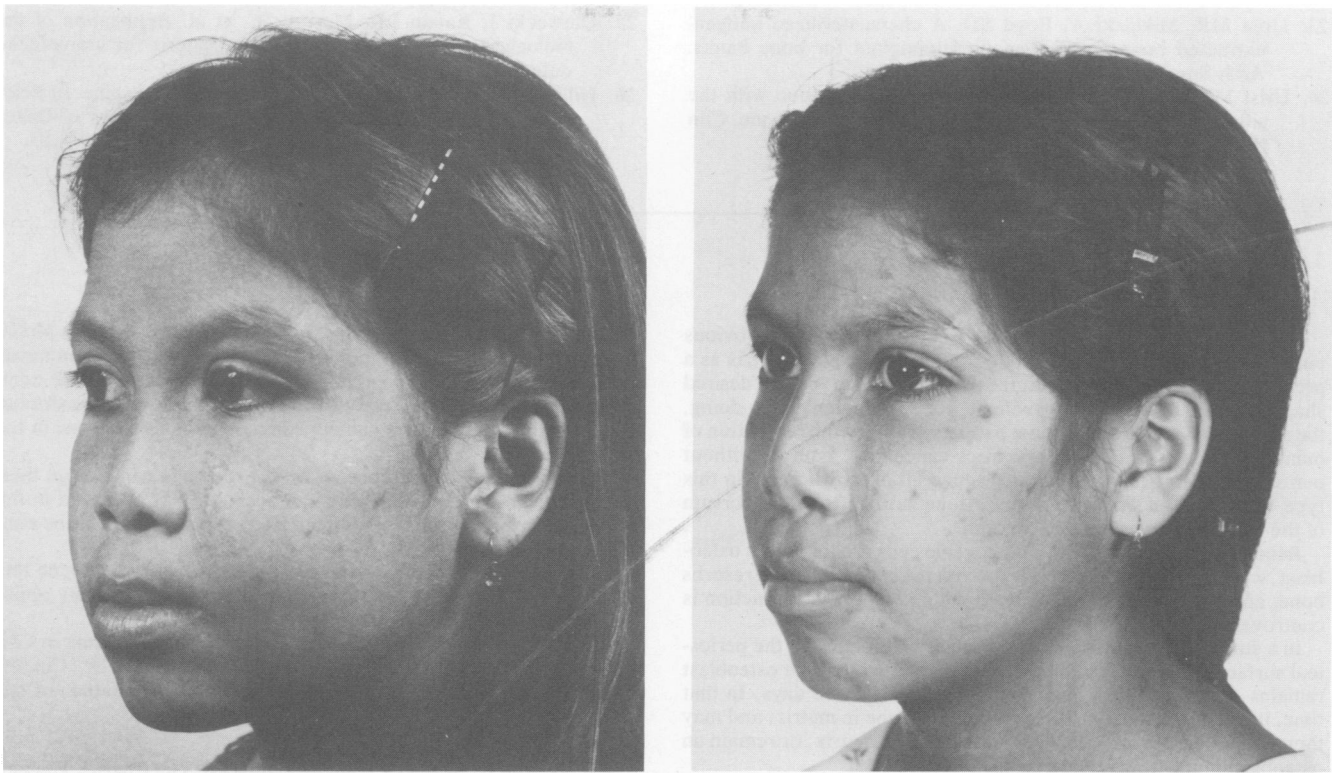
substitution" from adjacent bone. Finally, evaluation of certain patients, in whom healing was notoriously slow or unpredictable, showed unexpected rapid healing with demineralized implants. This point is well-illustrated in our case of total calvarial reconstruction with autogenous demineralized material. A skull defect of this size does not heal spontaneously in this age group, nor do trephine or mandibular defects in our craniofacial rat models.

The appearance of radiographic evidence of osseous healing over six months belies the more rapid consolidation noticed on physical examination. It is well known that osseous healing in facial fractures is not accompanied by radiographic ossification, unlike the early calcification of the callus in long bone healing. Earlier evidence of bony healing by radiographic examination may be possible with CT scan. This technique delineates small amounts of bone and fine bony septae within a large soft tissue volume. The degree of discrimination is finer than with conventional tomography.[26]

The use of these implants, which can be stored at room temperature and are readily available, has several obvious advantages. Operating time is shortened, as is the postoperative recovery period and hospital stay. Demineralized implants were particularly useful in patients where only a small amount of bone was needed (Fig. 5). Demineralized bone is easy to manipulate and fashion. Hydrated powder has the consistency of paste and can be used to fill depressed areas or caulk irregularities following osteotomy and placement of standard cortical grafts. A hydrated corticocancellous demineralized implant provides some immediate stability, yet is malleable enough so that it can be introduced through a small incision (as for nasal construction) and soft enough so that it can be carved (Fig. 6).

Resorption of standard mineral-containing bone grafts has been estimated to be in the range of 30–70% of graft bulk. This is particularly noticeable when grafts are placed in an onlay fashion in the craniofacial region, where they are not subjected to physical forces, stress, motion and compression. A theoretical advantage of demineralized implants is to bypass obligatory resorption. Resorption did not occur in the majority of our patients. It did, however, occur in four patients. Two of these implants were soaked in povidone-iodine before operation; this has been shown to inhibit induced osteogenesis.[25] The other two instances of resorption were attributed to technical failure.

Xenogeneic demineralized bone appears to be effective in our laboratory model, and clinical studies have been initiated. Evaluation of the physical properties of induced bone, such as ability to withstand stress, particularly in a weight-bearing area, must be done.



FIGS. 6a and b. A 12-year-old girl with nasomaxillary hypoplasia (Binder's Syndrome), before (a, left) and 2 years after (b, right) insertion of a demineralized cortico-cancellous implant to construct the nasal dorsum.

Because induction is related to the surface area of exposed matrix,[14] particle size is critical. Powder is currently the best material, as it provides a maximum area of exposed inductive matrix.

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Discussion

Dr. M. J., Jurkiewicz (Atlanta, Georgia): There are obvious tangible advantages to being able to use allogeneic bone matrix as a prepared shelf substance, implanted as a paste or carved to a desired shape, and induce volume-for-volume bone formation. In so doing, the authors have thus far, in most patients, avoided the resorption of onlay bone grafts, whether cortical or cancellous, with or without periosteum. Graft resorption has plagued all of us who deal in this type of work in all sites except one. Bone autografts to the dorsum of the nose are consistently successful.

Recognized and well-defined osteogenic cells are three: the osteoblast, whose job it is to synthesize matrix; the osteoclast, that resorbs bone; and the osteocyte, a small cell within bone whose function is controversial.

In a study of the osteogenic cell population kinetics on the periosteal surface of growing rabbits, Owen has shown that the osteoblast remains on the bone surface for approximately three days. In that time, it will lay down three times its own volume in matrix, and may then either become an osteocyte, embedded in matrix, or remain an osteoblast lining a Haversion canal.

The fibroblast in osteogenic tissue lies outside the layer of osteoprogenitor cells, or preosteoblasts. There seems to be nothing to distinguish it from fibroblasts elsewhere. Under the conditions of this study, that fibroblast and others can be induced to proliferate and differentiate into a bone-producing cell.

Some years ago Glimcher and his colleagues in Boston demonstrated that native type 640 Ångstrom axial repeat collagen fibrils, reconstituted from uncalcified tissues, such as rat tail tendon, calf skin, and guinea pig tendon, were able to nucleate apatite crystals from calcium phosphate solutions. Only the 640-Angstrom banding pattern collagen fibrils were able to do this. Other aggregation forms of tropocollagen were found to be inactive.

This preamble leads me to several questions. Why does collagen in sites other than bone and cartillage not calcify? Is it, in fact, an induced fibroblast that makes the bone under the conditions of this study, or could this represent a bypassing of matrix production with induction of apatite nucleation directly by the transplanted bone collagen?

Is the follow-up period long enough to be certain the resorption will not occur? Nicholas Senn, in 1898, as you alluded to in the manuscript, prepared demineralized ox bone, and used it to fill a tibial defect. There followed a number of papers on the subject with interest waxing and waning over the years.

Patients of Blair and Brown who had homograft cartilage implants that I had an opportunity to study did have late resorption, presumably because of rejection.

Dr. Joseph E. Murray (Boston, Massachusetts): Dr. Mulliken initiated this study to test my empiric use of autogenous bone mush as a contour filler in craniofacial reconstruction. He set up a laboratory model, and he found out that the bone that I had been using had no osteogenic potential at all.

Not daunted, and with Dr. Folkman's enthusiastic support, he, Dr. Glowacki and Dr. Kaban studied other laboratory models, and within two years the materials were suitable for use in humans.

Dr. Charles Huggins, Sr., described this "as a superb example of cooperation between a gifted biochemist and the imaginative surgeon."

My second point is the historic and conceptual. In 1962, at a meeting of this Association in Washington, D.C., I presented the first use of Imuran in human renal transplantation. Dr. Francis Moore at that time, in discussion, correctly predicted that the then newly coined term "immunosuppressive therapy" would become commonplace in the 1960's. Today I foresee that the term "transformation of cells" will become equally commonplace to surgeons in the 1980's.

The importance of the concept is that the cells can change their phenotype under the influence of an acellular matrix placed in the proper environment. Fibrocytes can become osteocytes, if the conditions are suitable.

It has been an added pleasure in my clinical work to notice this spinoff from craniofacial surgery toward studies potentially applicable to deformities of the trunk and extremities.

It is most appropriate that this presentation is being made in Chicago where Dr. Dallas Phemister was a stimulus to Dr. Charles Huggins and his work. Dr. Huggins is of course the father of our laboratory work.

Dr. David B. Skinner (Chicago, Illinois): Dr. Charles Huggins regrets that he is not able to be here today to discuss this paper. He asked me to extent his congratulations to Dr. Mulliken and his colleagues, and he had one question for them, as is his usual way.

Dr. Huggins points out that the preparation of this material involves treatment with strong acid, followed by ethanol and ether, all of which are bacteriocidal, so why do you irradiate it before you put it in? He believes the irradiation may weaken the preparation somewhat.

Dr. John B. Mulliken (Closing discussion): Dr. Jurkiewicz has obviously done his reading; I had some difficulty following his scholarly discussion. I believe he is asking, How does the demineralized matrix work? I do not know the basic answer to that, but I can say that there is something specific about material that was once bone and is now demineralized.

We know that the matrix is composed of highly cross-linked collagen and that particle size and surface charge are important. Dr. Huggins showed that the matrix charge is critical. He could reverse bone transformation, turn it off and turn it on again, by soaking his demineralized powder in various highly charged electrochemicals.

This brings us to Dr. Huggins' pointed question, relayed by Dr. Skinner, about the need to irradiate the demineralized implants. We have to use irradiation to implant the material into patients, as prescribed by our human studies protocol. There is no question, we have shown this experimentally, that once bone powder is irradiated its osteogenic potential is diminished. We are currently looking into better ways to sterilize and process the material to optimize its osteogenic capacity.

Further insight into mechanism comes from recent studies by Glowacki. She has shown that nonosseous tissue can become osteogenic. Glutaraldehyde-treated porcine cardiac valves induce osteogenesis when placed in our cranial defect experimental model. Untreated valves do not. This work is important in terms of the calcification of replaced valves that occurs clinically.

In summary, I do not know the answer to Dr. Jurkiewicz's question yet, but we must continue studies of the mechanism. I believe the process involves a change in phenotypic expression of the local cells, those cells that have the genetic potential to make other tissues as long as they are not too far along the lines of differentiation.