HUMAN HISTOLOGIC ANALYSIS OF MINERALIZED BONE ALLOGRAFT (PUROS) PLACEMENT BEFORE IMPLANT SURGERY

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KEY WORDS

Bone grafting
Osteoinduction
Augmentation
Allograft
Mineralized bone
Histology
Extraction

Because clinicians are placing more dental implants, it is becoming more important to maintain bone volume after tooth extraction. This article discusses the various bone-augmentation materials available to the clinician and illustrates a case report of particulate mineralized bone allograft (Puros) placement after extraction. Exposure of the grafted site after 5 months revealed a hard bony structure. Human histologic analysis at the light microscopic level revealed nonvital spicules of mature calcified bone having a highly organized matrix surrounded by viable noncalcified immature bone matrix, or osteoid. It was concluded that mineralized human allograft demonstrated the formation or remodeling of bone histologically and was clinically useful to maintain bone volume for implant placement after extraction. To the authors' knowledge, this is the first publication to demonstrate human histology of particulate mineralized bone allograft (Puros) after placement into an extraction site.

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INTRODUCTION

Growth in the dental implant industry is expected to continue from 2003 to 2007 at a compound annual rate of 9.4%.[1] As dental clinicians increase their placement and recommendation of dental implants, it becomes necessary to retain as much bone as possible after tooth removal. With the proliferation of practitioners utilizing bone-grafting augmentation techniques after extraction, there appears to be a search for the "ideal" bone-replacement material. In recent years, there has been an increased utilization of gamma-radiated human mineralized allograft, Puros (Tutogen Medical US Inc, Centerpulse, Carlsbad, Calif).[2] The purpose of this article is to demonstrate the use of human mineralized allograft as a grafting material within an extraction site before dental implant placement, which revealed the formation of osteoid as evidenced with human histology.

As the dental profession becomes more comfortable with the placement and recommendation of dental implants, clinicians will be increasingly evaluating

the longevity of natural teeth. Whether to treat an existing failing tooth depends on many factors, including the restorability of the structure remaining, endodontic status, periodontal status, condition of adjacent teeth, traumatic forces, occlusal considerations, opposing arch, and prosthetic treatment planning. Teeth with a prognosis of less than 5 years should be considered for extraction and grafted before placement with a dental implant.[3] With the increased utilization of dental implants as tooth replacements, it is imperative that the clinician maintain as much available bone as possible after tooth extraction. Even after a ''clean'' extraction, bone loss may be unpredictable, resulting in limitation of ideal implant placement.[4]

Tooth-extraction healing process

There are 4 stages of healing after tooth extraction. In the initial angiogenic stage, blood clotting occurs along with capillary formation within the first 5 days after extraction. The new-bone formation stage occurs next with the entire socket transforming into granulation tissue. In the bone-growth stage, immature woven bone forms within 4 to 5 weeks. The bone-reorganization stage occurs 6 weeks after extraction and continues for 6 months. Bone can take up to 52 weeks to fully mature.[3] The periosteum is an important structure vital to alveolar bone formation. Cells from the inner layer of the periosteum are responsible for bone remodeling, and cortical bone receives 80% of its arteriole blood supply from the periosteum. Careful atraumatic-extraction techniques and maintenance of the periosteum helps preserve alveolar bone.

After tooth extraction, facial bone loss may be significant.[4] In the anterior maxilla, up to 25% of the facial bone will resorb within the first year after extraction and continue to 40% to 60% over the next 3 years.[3] In the posterior region, the rate of bone loss is even greater, with up to 50% bone loss within the first year after extraction, particularly if a portion of the buccal plate is lost during extraction. The resulting limited buccal-lingual dimension often results in the formation of Division B or Division C bone, reducing the available bone for placement of root-form implants.[5]

Bone-grafting materials

Bone-replacement graft materials have played an important role in regenerative dentistry for many years.[6] Today's concept in tooth extraction should routinely consider maintenance of the existing extraction socket dimensions with some sort of bone-replacement material.[7] This procedure has been called *ridge preservation*.[8] The clinician essentially has 5 choices of bone-graft materials: autogenous grafts, alloplasts, natural hydroxyapatite (HA), xenografts, and allografts.

The gold standard of bone-grafting materials is autogenous bone. This material forms bone by the processes of osteogenesis, osteoinduction, and osteoconduction. Osteogenesis is the mechanism of bone growth from viable bone cells known as osteoblasts, osteoinducton involves materials that are capable of inducing mesenchymal cells to differentiate into osteoblasts, and osteoconduction is the process that permits bone apposition from existing bone.[6] Autogenous bone is obtained from the same patient in whom the graft is placed. The advantage of autogenous bone is that it maintains bone structures such as minerals and collagen, as well as viable osteoblasts and bone-morphogenic proteins (BMPs). Autogenous bone is available intraorally from edentulous areas, the tuberosity, the mandibular symphysis, and the mandibular ramus. Extraoral auto-genous bone is available in larger quantities from the iliac crest, rib, tibia, and calvarium. The main deterrent to the use of autogenous bone is the morbidity of a second surgical site.

Alloplasts are synthetic materials that have been developed to replace human bone. They are biocompatible and are the most common type of graft materials utilized. The varying nature of commercially available pure graft materials, such as porosity, geometries, different solubilities, and densities, determines the resorption of these calcium phosphate–based graft materials. The alloplasts are osteoconductive materials.[6] Calcium phosphates, bioactive glasses, and biocompatible composite polymers comprise most of the alloplasts.

The advantage of alloplasts is the lack of potential to transmit diseases such as human immunodeficiency virus (HIV), bovine spongiform encephelitis (BSE), or hepatitis. Other advantages include low expense and unlimited volume of the materials. The disadvantage of alloplasts is that they are unpredictable in allowing bone formation[9]; therefore, particles can be encountered within the grafted site when the clinician returns for implant placement.

The natural biocorals are calcium carbonate materials, which mimic natural bone's hydroxyapatite structure. These materials are cost effective and pose no risk in transmission of communicable diseases. Again, the calcium carbonates are only osteoconductive.

Xenografts are derived from other species. They are processed to remove their antigenicity by

various chemical and preparation techniques. These materials are fabricated from the inorganic portion of bone from animals; the most common source is bovine. With the removal of the organic component, concerns about immunological reactions become nonexistent. The remaining inorganic structure provides a natural architectural matrix as well as an excellent source of calcium.[10] The advantage of the xenografts is that they maintain the physical dimension of the extraction socket grafted. The disadvantage of the xenografts is that they are only osteoconductive and the resorption rate of bovine cortical bone is slow, with the bovine cortical bone often present after 18 months in situ.[11] Patients may also have anxiety regarding BSE or "mad cow disease." BSE is a condition believed to be caused by a protein known as a prion.[12] Xenografts are processed and deproteinated at high temperatures, which prevent the transmission of BSE. Although no human cases of BSE have been reported to date,[13] some researchers are concerned about the long-term effects and the transmission of yet-unknown pathogenic proteins.[14]

Allografts are tissues taken from individuals of the same species as the host.[15] They provide type I collagen, which comprises most of the organic component of bone and must be processed carefully to guarantee safety. The most common allograft used is demineralized freeze-dried bone allograft (DFDBA). DFDBA is derived from human-cadaver bone whose donors have been screened, selected, and tested to be free of HIV and hepatitis. It is thoroughly processed to eliminate any diseases that might threaten the health of the recipient.

The bone is ground then immersed in 100% ethanol to remove fat, is frozen in nitrogen, and is then freeze dried and ground to smaller particles (250–750 μm). The desiccating step allows for long-term storage and decreases antigenicity. One of the processing steps is the use of 0.6 N hydrochloric acid or nitric acid, which tends to ensure its disease-free state.[3] This also removes the calcium and phosphate salts (all the mineral components) but retains collagen and more readily exposes the BMPs. After washing and dehydration, the material is either irradiated or sterilized in ethylene oxide. The use of irradiation is controversial because doses above 2.5 megarads are destructive to bone formation,[16] and studies indicate cytotoxic-compound formation within the graft in the presence of lipids.[17]

Allografts contain BMPs, which help stimulate osteoinduction. Thirteen proteins have been identified (BMP1–BMP13) that are osteoinductive compounds and encourage new-bone formation.[6] BMPs act as a signal in initiating and regulating specific tissue formation. This activity leads to a series of developmental processes that result in the differentiation of mesynchemal cells into osteoblasts. The amount of BMPs in any single allograft has shown dramatic variability. Osteoinductive properties of DFBBA can be demonstrated by bioassay analysis.

Other human allografts include freeze-dried allografts (FDBA), demineralized allograft putties, and irradiated cancellous bone. The advantage of allografts is that they are readily available without a second surgical site and are osteoinductive. The disadvantage is that patients have certain fears attributed to religious beliefs or to possible transmission of diseases from a cadaver. Accredited bone banks require screening and testing before donor selection. With stringent sterilization and processing, there is only a 1 in 2.8 billion chance of contracting HIV, and no known occurrences have been reported to date.[18]

Puros human allograft

According to the manufacturer, Puros (Centerpulse Dental Division, Carlsbad, Calif) is a mineralized human allograft indicated for the replacement of osseous bone structures in maxillofacial oral surgery. It promotes rapid healing and remodels completely.[19] Puros is available in 0.5-mL and 1-mL amounts in the 250- to 1000-μm particle size and 1-mL or 2-mL amounts in the 1000- to 2000-μm particle size. Tutogen Medical has done extensive research with the Puros Accugraft, which is a 2-piece graft composed of cortical and cancellous bone to assist fusion in the lumbar spine from an anterior approach.[20] Tutoplast processed tissues have been in use for over 25 years in more than 500 000 surgical cases without a single documented case of disease transmission. All tissues are fully traceable to its original donor, and individual reference samples are stored for 15 years.[20]

MATERIALS AND METHODS

Case report

A 61-year-old woman presented to our office for consultation regarding treatment of her lower-right jaw. She occasionally had discomfort in the lower molar area. Her medical history was negative for cardiovascular disease, renal disease, endocrine disorders, and hematologic disorders. She did not smoke.

A periapical radiograph revealed the mandibular right

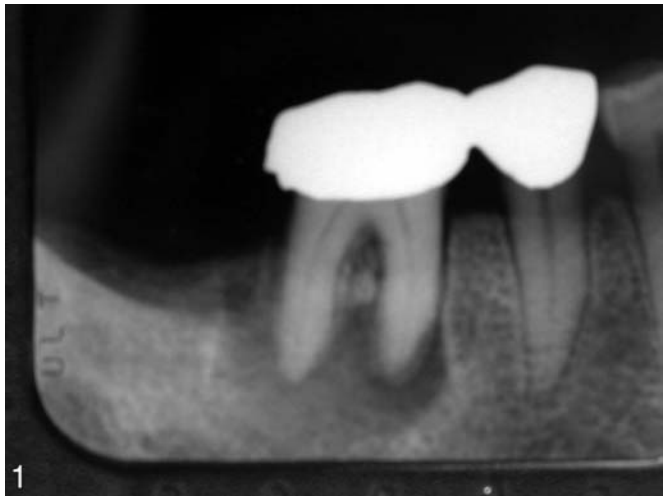


FIGURE 1. The patient's lower-right second molar exhibits a large radiolucency. Periodontal probing indicated a endodontic-periodontic lesion with a hopeless prognosis.

second molar (tooth #31) missing and mandibular right first molar (tooth #30) present with a large endodontic-periodontal lesion (Figure 1). Treatment was planned for extraction of tooth #30 and curettage of any existing soft-tissue lesion. At the time of extraction, bone grafting with Puros would be performed and allowed to heal for at least 5 months, followed by the placement of 2 root-form taper screw vent implants (Centerpulse Dental Division, Carlsbad, Calif) in the area of tooth #30 and #31. A prosthetic-treatment plan of 2 fixed porcelain fused to gold cementable crowns were to be inserted 3 months after implant placement.

The patient was given 2 g of amoxicillin preoperatively. An inferior alveolar and buccal nerve block was administered. One milliliter of 250- to 1000-μm sized Puros human allograft was hydrated with sterile saline. The splinted crowns were sectioned, and a surgical curette was used to dissect the attachment from the tooth. A straight elevator and forceps were used in the removal of the tooth, without elevation of a periosteal flap. The soft-tissue lesion was curetted, and the socket was completely enucleated. A high-speed handpiece was used to create bleeding points within the lateral walls of the extraction site. A human mineralized (Puros) graft material was carried and packed lightly into the extraction socket with a periosteal elevator. The dental assistant placed wet gauze over the graft material to extrude excess fluid after each increment of graft placement. Care was made to place the material to the base of the socket.

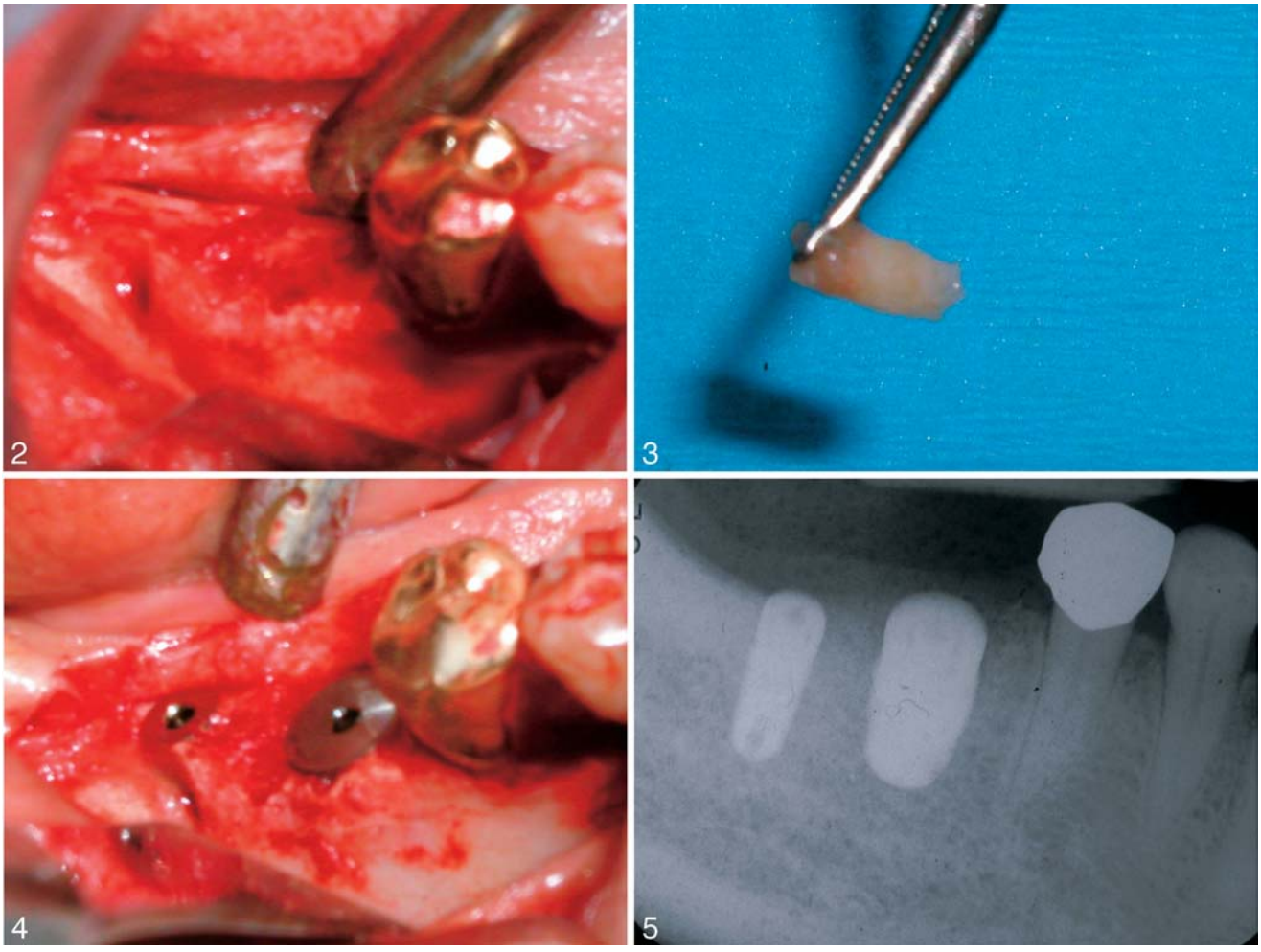
The socket was covered with Collatape (Centerpulse Dental Division, Carlsbad, Calif) and closure was made with 3-0 silk sutures criss-crossed over the extraction site. The patient was given postoperative instructions, which included eating on the opposite side of the mouth to avoid disrupting the graft. Prescriptions for amoxicillin 500 mg 1 tablet 3 times per day for 10 days postoperatively and oxycodone 5 mg every 4 hours as necessary for pain were given to the patient. The tissue-utilization record for the graft material was completed with an operative report and forwarded to Tutogen Medical in Alachua, Fla.

The patient was seen 1 week postoperative for suture removal. Healing was uneventful over the next 4 months, with full soft-tissue closure. A radiograph was taken at the 4-month period, which demonstrated fill and radiopacity.

At 5-months postgrafting, the patient was scheduled for dental implant surgery. Two grams of amoxicillin was administered preoperatively, and anesthesia was administered as previously described. An incision was made over the crest of the ridge from the area of tooth #32 to #29, with a vertical releasing incision on the mesial of tooth #28. A full-thickness mucoperiosteal flap was elevated to reveal the underlying ridge. The area of the grafted site appeared similar to cortical bone but more vascular. Outlines of the root sockets could be differentiated, but the ''fill'' in the sockets was bone hard. Probing the site with surgical curette could not penetrate the fill, and there were no loose particles that could be removed (Figure 2).

A trephine drill with sterile irrigation was used to obtain a bone core before preparing the implant osteotomy site. The core was a hard structure and appeared as normal bone (Figure 3). The specimen was placed in formalin solution and submitted for microscopic histology.

The osteotomy sites were completed. A 10-mm long × 6-mm wide taper screw vent implant was placed in the area of tooth #30, and a 10-mm long × 3.5-mm wide taper screw vent implant was placed in the area of tooth #31. It was noted that greater buccal lingual width was available in the extraction socket grafted with human allograft as



FIGURES 2–5. FIGURE 2. Exposure of the grafted site shows ridge quality consistent with natural bone appearance. No graft particles are noted within the extraction site, and the site has a "bone hard" feel. FIGURE 3. Core specimen, which appeared like a bone core, was submitted for light microscopy. FIGURE 4. Large 6-mm wide diameter taper screw vent implant was placed in the area of tooth #30, whereas only a 3.5-mm wide diameter could be placed in the previously edentulous area of tooth #31. FIGURE 5. Postoperative periapical radiograph demonstrates maximum surface area implant in the Puros-grafted site.

compared with the existing edentulous area (Figure 4).

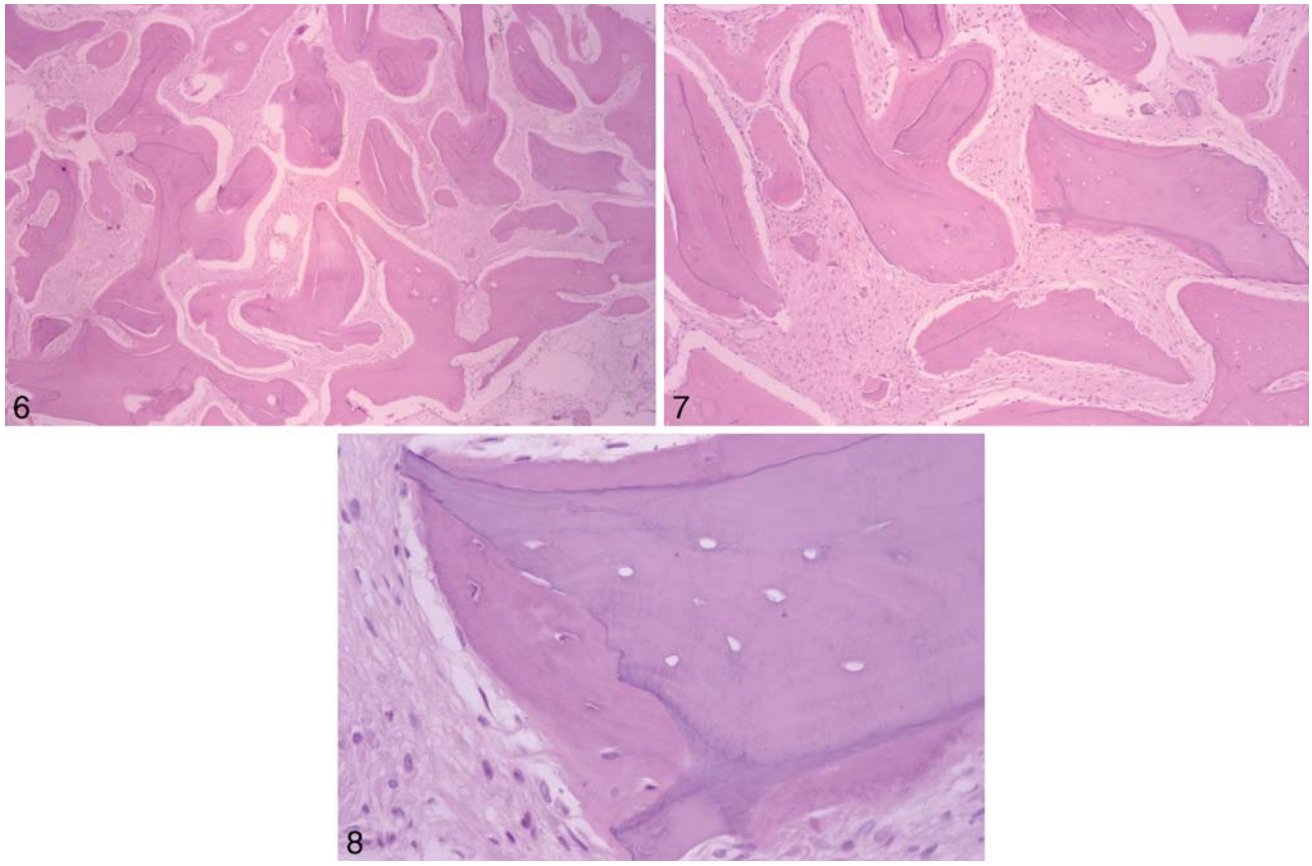
A postoperative periapical radiograph was taken 2 months after the implant surgery. The increased surface area of the implant in the area of tooth #30 was observed as compared with the smaller-width implant in the area of tooth #31. The radiograph showed no radiolucency surrounding the implant or the graft (Figure 5). The patient was restored with 2 cementable porcelain fused to gold crowns 2 months later.

RESULTS

Histologic description

The specimen consisted of a 0.8-cm × 0.4-cm × 0.4-cm cylinder-shaped piece of hard tissue, which was fixed in a solution of 10% buffered formalin and decalcified in a 5% hydrochloric acid solution. Sections of 3-μm to 4-μm thickness were stained with hematoxylin and eosin, and the specimen was reviewed in multiple sections. Light microscopic evaluation showed the specimen to consist of anastomosing, curvilinear segments of osseous tissue exhibiting intramembranous and appositional bone growth that were situated in a vascular and cellular fibrous connective tissue.

The osseous tissue component consisted of nonvital spicules of more darkly staining, mature calcified bone having a highly organized matrix characterized by a lamellar structure, lacunae absent of osteocyte nuclei, and canaliculi radiating from the lacunae. Situated on the peripheral aspects of these nonvital spicules of bone were layers of a more



FIGURES 6–8. FIGURE 6. Low-power photomicrograph shows numerous curvilinear and interconnected segments of osseous tissue (grafting substrate) in a cellular and vascular fibrous connective tissue exhibiting intramembranous bone growth (hematoxylin and eosin, original magnification ×4). FIGURE 7. Intermediate-power photomicrograph shows several curvilinear segments of osseous tissue (grafting substrate) in a cellular and vascular fibrous connective tissue exhibiting intramembranous bone growth (hematoxylin and eosin, original magnification ×10). FIGURE 8. High-power photomicrograph shows appositional deposition of osteoid matrix having osteocyte nuclei within lacunae against nonvital mature bone (hematoxylin and eosin, original magnification ×40).

lightly staining, viable, noncalcified immature bone matrix, or osteoid. The osteoid contained osteocyte nuclei housed within lacunae but lacked a lamellar structure and canaliculi. Osteoblasts were observed to rim the osteoid deposits in some areas, and an occasional osteoclast was observed.

The nonosseous component was composed of a cellular fibrous connective tissue containing plump, spindle-shaped mesenchymal cells interspersed in a network of collagen fibers with numerous small vascular channels. A few chronic inflamed cells, mostly lymphocytes and an occasional plasma cell, were observed in some areas (Figures 6 through 8).

Histology demonstrated viable bone formation around the mineralized human allograft particles. There were no foreign-body reactions to the material or the alloplastic particles within the bone.

DISCUSSION

Patients often need to be evaluated for both short- and long-term prognosis for a tooth. The clinician has a responsibility to recommend the best long-term treatment available for the patient after extraction, which is often a dental implant. Because of the increased recommendation of dental implants as a tooth replacement, it is important for the dentist to consider ridge preservation at the time of tooth extraction.[8]

Unpredictable bone loss can occur after tooth extraction, particularly if there is an existing bony defect or radiolucency present.[4] Today, there is an abundant amount of bone-replacement graft materials available to the clinician.[6] Although alloplastic materials are plentiful and inexpensive, concerns arise as to their predictability in achieving bone replacement before implant place-

ment. Often a fibrous connective tissue encapsulation of the residual graft particles occurs, which can delay or complicate dental implant placement.[9] The use of human allograft has been long established as a good alternative to patient autogenous grafting because it avoids the need for bone grafting from the other sites.

Puros is a mineralized human bone allograft with an excellent history of efficacy and safety. Mineralized human bone allograft has the advantage of providing both the BMP and the minerals necessary to achieve osteoinductive properties. It is often preferred to use a bone-augmentation material with mineral content instead of DMFBA, especially when lateral-ridge augmentation is required before dental implant placement.[21]

Tutogen Medical has derived a very complete and rigorous process in obtaining donors that makes Puros allograft consistently pure, sterile, and extremely safe. Physicians complete donor selection, and next-of-kin interviews are used to collect relevant information about donor's medical, demographic, and social history. All donor blood is tested and found to be negative or nonreactive to syphilis, hepatitis B surface antigen, hepatitis C antibody, HIV-1 and -2, and human T-cell leukemia virus (HTLV)-I and -II. The material is also processed as a unit and kept separate from materials and from other donors to avoid potential cross-contamination and to increase tissue safety (C. Schopt, written communication, July 28, 2003).

Puros is obtained from human cancellous bone that can be found mainly at the ends of the long bones such as the humerus head, femoral head, femoral condyles, and the plateau of the tibia. Secondary sources include the illium and vertebral bodies. The natural collagen architecture is maintained, which provides an excellent bone matrix very similar to natural bone. The process involves 27 washes of various fluids and solutions that remove fats, cellular material, and noncollagenous proteins. These washes deactivate and destroy any remaining proteins that may be pathogenic and preserve BMPs and the natural trabecular pattern.

The first processing step removes lipids and inactivates viruses and prepares the tissue for other steps to penetrate the graft more effectively. Studies have shown that lipids may alter bone allograft incorporation in several ways.[17] Because of their high fractional volume, they represent a barrier to cellular invasion. Lipids can also induce giant cell reactions, which, through the inflammatory process, can increase bone resorption and encapsulating fibrosis with a release of various cytokines and prostaglandins. Their peroxidation products can impair bone formation by inducing osteoblast death. Delipidization appears to be an essential technique in the management of bone banks, especially when radiation is used.[17]

The Puros graft receives low-dose gamma irradiation (17.8 kGy) in a narrow rotating sterilization system. This irradiation ensures biomechanical integrity, preserves protein structure, and inactivates all remaining viruses.

Piattelli et al[22] showed the main differences between FDBA and DFDBA. In FDBA, the resorption process is scarce and cells with acid phosphatase were not found, whereas with DFDBA the resorption process is present and cells were positive for acid phosphatase. In FDBA, the particles farthest from the host bone were lined with newly developed bone, whereas in the DFDBA the graft particles were located far from the host bone and composed of scarce connective tissue collagen fibers. In FDBA, all osteocytic lacunae were filled by osteocytes, and in some areas Haversian systems with a capillary center were found. In DFDBA, the osteocytic lacunae were mainly empty.

Although human mineralized allograft has been recommended for use, there is little human histology to verify normal healing. Histologic sections showed osteoid formation around nonvital human bone spicules. Osteoblasts were observed to rim the osteoid deposits.

The authors of this report have placed particular mineralized bone allograft (Puros) into the extraction sites of more than 65 patients. Extraction sites with acute infections (exudate, acute pain, or cellutitis) were excluded from graft placement at the time of extraction. Since August 2001 (over a 2-year period), approximately 280 mL of bone mineralized human allograft (Puros) was placed into extraction sites and sinus grafts and was used in conjunction with symphysis block autogenous onlay grafts (180 mL of 25-μm to 1000-μm particle size and 100 mL of 1000-μm to 2000-μm particle size).

Teeth were removed by an atraumatic extraction technique. Every attempt was made to extract teeth without a full-thickness periosteal flap in order to maintain the blood supply and buccal plate as best as possible. If the tooth required sectioning for extraction, all attempts were made to preserve as much bone as possible.

After tooth extraction, all soft-tissue remnants were removed with surgical curettes. Defect morphology was evaluated at

the time of extraction. If the buccal plate was missing (a 4-wall bone defect), a collagen membrane (Biomend, Centerpulse Dental Division, Carlsbad, Calif), which resorbs after 8 weeks, was placed beneath the periosteum. Rapid Acceleratory Phenomenon was induced with the creation of bleeding points within the extraction sockets by use of high rotary instrumentation.[23]

Closure was made with 3-0 silk, 4-0 chromic gut, or 4-0 vicryl sutures. If sufficient soft tissue was present, primary closure was obtained as best as possible with sutures alone over the graft material. In large molar extraction sites, a resorbable collagen membrane was placed over the graft material and slung by sutures. Immobilization of the graft was ascertained by soft diet for 1 to 2 weeks and relief of any removable prosthesis over the extraction site. Most extraction sites were allowed to heal for a period of 3 to 6 months before reentry and dental implant placement. After healing, the graft material clinically forms a dense bone within the extraction site, which allows the surgeon to place implants.

Conclusion

Given the abundant scientific literature of bone-grafting materials, clinicians who perform oral surgery are often recommending ridge preservation to their patients before extraction. Many different bone-grafting materials are available to the dentist. The authors of this report have used particular mineralized bone allograft in the extraction sites of more than 65 patients. Since August 2001, over 280 mL of bone mineralized human allograft (Puros) has been placed into extraction sites and sinus grafts and used in conjunction with symphysis block autogenous onlay grafts.

The purpose of this article was to present the human histologic analysis of a clinical case of the placement of mineralized bone allograft (Puros) into an extraction site. The mineralized allograft was easy to utilize after extraction and resulted in normal healing of the soft and hard tissues. Upon reentry for dental implant surgery, the material appeared hard and resistant to osteotomy preparation. Histologic evidence showed that normal bone replacement was evident without any foreign-body reaction. It was concluded that mineralized human allograft demonstrated the formation or remodeling of bone histologically and was clinically useful to maintain bone volume for implant placement after extraction.

Note

To the authors' knowledge, this is the first published report demonstrating human histology of mineralized allograft after placement into an extraction. The authors acknowledge that there was no financial support from any of the aforementioned product manufacturers. Further long-term studies with controlled sites should be performed on this material as evidence of its efficacy and safety.

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