# CLINICAL, HISTOLOGIC, AND HISTOMORPHOMETRIC EVALUATION OF MINERALIZED SOLVENT-DEHYDRATED BONE ALLOGRAFT (PUROS) IN HUMAN MAXILLARY SINUS GRAFTS

*Sammy S. Noumbissi, DDS*
*Jaime L. Lozada, DDS*
*Philip J. Boyne, DMD*
*Michael D. Rohrer, DDS, MS*
*Donald Clem, DDS, MS*
*Jay S. Kim, PhD*
*Hari Prasad, MDT*

KEY WORDS

**Bone graft**
**Autograft**
**Allograft**
**Xenograft**
**Sinus augmentation**
**Mineralized bone allograft**

***Sammy S. Noumbissi, DDS,*** *is a former graduate student in the Graduate Program in Implant Dentistry, Loma Linda University, Loma Linda, Calif, and is in private practice in Chevy Chase, Md. Address correspondence to Dr Noumbissi at 4701 Willard Avenue, Suite 106, Chevy Chase, MD 20815 (e-mail: sammynoumbissi@comcast.net).*

***Jaime L. Lozada, DDS,*** *is professor and director in the Graduate Program in Implant Dentistry, Loma Linda University, Loma Linda, Calif.*

***Philip J. Boyne, DMD,*** *is in the Department of Oral Surgery, Division of Plastic Surgery, Loma Linda University, Loma Linda, Calif.*

***Michael D. Rohrer, DDS, MS,*** *is professor and director and* ***Hari Prasad, MDT,*** *is a senior research scientist at the Hard Tissue Research Laboratory, University of Minnesota School of Dentistry, Minneapolis, Minn.*

***Donald Clem, DDS, MS,*** *is in private practice in periodontics in Fullerton, Calif.*

***Jay S. Kim, PhD,*** *is a biostatistician in the Dental Education Services, Loma Linda University, Loma Linda, Calif.*

Demineralized freeze-dried bone allografts (DFDBA) have been successfully used alone or in composite grafts for many decades. Little research has been done on the effect of retaining the mineral content of bone allografts. This study histologically and histomorphometrically evaluated a new mineralized bone allograft material placed in human atrophic maxillary sinuses. Seven partially edentulous patients requiring sinus grafts before implant placement were selected for this study. Their age range was 56 to 81 years (mean 67.7 years). Test grafts consisted of a mineralized solvent-dehydrated cancellous bone allograft, and control grafts were a composite of DFDBA and deproteinized bovine bone xenograft (1:1). Bilateral cases ($n = 3$) received both test and control grafts on opposite sides, and unilateral cases received either a test ($n = 3$) or control ($n = 1$) graft only. At 10 months, core biopsies were taken from each graft site, and dental implants were placed into the augmented bone. All bone grafts resulted in new bone formation and all implants osseointegrated. Test grafts resorbed and were replaced by newly formed bone significantly faster and in greater quantities than were control grafts. No complications with grafts or implants were noted. Both test and control grafts achieved excellent results. The faster bone formation observed with the test graft may be due, in part, to its smaller particle size compared with the bovine portion of the control graft. Test grafts were either replaced by new bone or displayed new bone-to-particle surface contact in higher percentages than did control grafts. No differences in osseointegration or graft stability were noted 2 years after the study.

## INTRODUCTION

Jaw atrophy resulting from tooth loss, periodontal disease, use of a removable prosthesis, or pneumatization of the sinus can reduce or eliminate the residual maxillary ridge and significantly compromise a person's ability to function.[1–4] Although prosthetic rehabilitation alone can sometimes help improve function, facial support, lip competence, and facial esthetics,[5] the development of sufficient bone volume for implant placement[6] can often be addressed only by reconstructing the hard tissue anatomy through bone grafting. Autogenous bone meets all necessary physicochemical and biological requirements of a graft and can synthesize new bone at the implantation site (osteogenesis), form new bone by recruiting host mesenchymal stem cells that differentiate into osteoblasts (osteoinduction), and serve as a scaffold for new bone ingrowth and vascularization from the surrounding tissues (osteoconduction).[7,8] Inherent limitations in the use of autogenous bone grafts include the dimensions, quality, and quantity of obtainable bone[9]; increased operating time and cost for graft harvesting; and donor-site morbidity.[10]

Allogenic (human) and xenogenic (animal, eg, bovine) bone grafts are the most common alternatives to autogenous bone, but both harbor slight risk of adverse immunologic reactions and infection,[8] and neither heal as predictably as fresh, autogenous bone.[11–14] Demineralized freeze-dried bone allografts (DFDBA) have been clinically used for over 40 years. The process of demineralization exposes the bone morphogenetic protein (BMP) present in the tissue, which has the capacity to induce a phenotypic change of host pluripotential cells into osteoblasts and cause an orderly sequence of endochondral osteogenesis throughout the implanted area.[12,15,16] However, several variables can negatively affect the osteoinductive capacity of the BMP, including donor age[17] and factors in tissue processing (eg, retrieval time and temperature,[18] sterilization method[19]). Consequently, clinical results with DFDBA have been mixed. The influence of mineralization (calcium) on the clinical performance of allogenic bone grafts still remains unclear.[13,15,20]

More recently, deproteinized mineralized bovine bone xenografts have been used for grafting. To prevent antigenicity, the bone tissue is chemically treated to remove its organic components (calcium-deficient carbonate apatite).[21] When processed under low heat (300°C), the exact trabecular architecture, porosity, and apatite crystalline content of the natural bone are maintained,[8,21] but the mineral particles are doubled in size.[22] Although this material appears to lack osteoinductive properties,[21,23,24] it still undergoes physiologic remodeling and becomes incorporated into bone over time.[21,23] Mixed clinical results with this bovine bone product have prompted some clinicians to recommend its use only as a composite graft with autogenous or allogenic bone when augmenting the alveolar ridge.[21]

This article reports on the results of a prospective clinical study that analyzed the quantity and quality of new bone formed in the maxillary sinuses of human subjects grafted with a new mineralized solvent-dehydrated cancellous bone allograft compared with a composite graft of DFDBA and deproteinized mineralized bovine bone.

## MATERIALS AND METHODS

This prospective study was conducted according to the research standards for human subjects established by the Graduate Program in Implant Dentistry and Institutional Review Board (IRB) of Loma Linda University, Loma Linda, Calif (IRB approval 51122).

### *Patients*

Study candidates were consecutive patients from the Graduate Program in Implant Dentistry at Loma Linda University School of Dentistry who presented with less than 5 mm of residual bone inferior to the maxillary sinus floor unilaterally or bilaterally and who met the study's selection criteria (Table 1). A comprehensive diagnostic workup was performed to thoroughly evaluate each candidate. This included a review of the patient's medical and dental histories, complete oral and radiographic evaluations, and fabrication of mounted study casts. A surgical template to guide placement of the implants relative to the planned restoration was created from a prosthetic wax-up. The treatment plan, study requirements, and alternative options were reviewed, and each patient signed an informed consent form before admission into the study. Presurgical intraoral photographs (Fugichrome Sensica 100 ASA color film) were taken of the maxillary and mandibular ridges and dentition, and pre- and postoperative instructions were provided orally and in writing to each patient.

### *Medication regimen*

Before surgery, each patient was prescribed 2 g of amoxicillin (or erythromycin if sensitive to penicillin derivatives) (Novopharm, Toronto, Canada) 1 tablet 4 times daily beginning the day before surgery for a total of 10 days. On the day of surgery, each patient also received 800 mg of ibuprofen. Postoperative instructions included rinsing 3 times daily for 2 weeks with 0.12% chlorhexidine gluconate (Peridex, Procter and Gamble, Cincinnati, Ohio). Patients were instructed to try not to blow their noses for at least 3 days after surgery and to cough or sneeze with an open mouth to prevent dislodging the graft. In addition, the application of pressure and ice at the surgical site, elevation of the head, and rest were recommended. Analgesics were prescribed to control pain and discomfort.

TABLE 1

Patient-selection criteria

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| 1. Ability to read, comprehend, and sign written informed consent | 1. History of bruxism |
| 2. Age range 40 to 80 years | 2. Previously grafted sinuses needing regrafting |
| 3. Medical history that will fall within ASA* II or I classification | 3. Acute or chronic sinusitis |
| 4. Have a complete or partially edentulous posterior maxilla with <5 mm of residual bone (SA-4) bilaterally or unilaterally, as measured through tomographic and panoramic radiographs | 4. Inability for the patient to perform proper or acceptable oral hygiene |
| 5. Availability for monitoring during the entire course of the study | 5. Sinus membrane perforation involving more than half the surgically exposed membrane |
| 6. Any active periodontal disease must be treated before surgical intervention | 6. Current steroid therapy in excess of 5 mg prednisone per day |
| | 7. Pulmonary disease |
| | 8. Pregnancy or planned pregnancy or nursing during the course of the study |
| | 9. Mental or psychiatric disorders that will impair understanding and compliance with necessary procedures |
| | 10. Patients unwilling to follow a smoking-cessation protocol as defined by the standards of the Loma Linda University graduate program in implant dentistry |

*ASA = American Association of Anesthesiologists.

### *Graft materials*

Test grafts consisted of a 100% large particle mineralized cancellous allograft (Puros Cancellous Particulate, Zimmer Dental Inc, Carlsbad, Calif), which was prepared from cancellous donor bone treated for biological safety through a 5-step proprietary process (Tutoplast Process, Tutogen Medical GmbH, Neunkirchen am Brand, Germany): (1) delipidization, (2) osmotic contrast treatment, (3) oxidation treatment with hydrogen peroxide, (4) solvent dehydration, and (5) limited-dose gamma irradiation (17.8 GY).[8,25] Control grafts consisted of a 1:1 combination of DFDBA (Musculoskeletal Transplant Foundation, Holmdel, NJ; particle size 750–1000 μm) and deproteinized mineralized bovine bone (Bio-Oss, Geistlich AG, Wolhusen, Switzerland) (Table 2). In cases requiring bilateral sinus grafts (n = 3), test and control grafts were placed on opposite sides in the same patient. In cases requiring unilateral sinus grafts, patients received either a test (n = 3) or control (n = 1) graft only.

### *Dental implants*

Multithreaded tapered screw-type implants with microtextured surfaces (Tapered Screw-Vent MTX, Zimmer Dental Inc) were placed. Implant lengths and diameters were determined according to the needs of each patient.

### *Surgical procedures*

#### *Graft Placement*

Immediately before surgery, patients were asked to rinse with 0.12% chlorhexidine for 2 minutes. Anesthesia was administered by local infiltration with mepivacaine hydrochloride 2% (Polocaine, AstraZenica Pharmaceuticals LP, Wilmington, Del) with 1:20 000 epinephrine (Astra USA Inc, Westborough, Mass). An open-sinus grafting procedure with a hinged-window osteotomy technique as described by Tatum[26] and Smiler et al[27] was used. In the event that a tear in the Schneiderian membrane occurred during surgery, a bioabsorbable collagen membrane (BioMend, Zimmer Dental Inc) was placed over the perforation with a 2- to 3-mm overlap beyond the tear before graft placement. After grafting, the soft tissues were approximated and sutured (3-0, Vicryl, Ethicon, Sommerfield, NJ). The sutures were removed 2 weeks later after soft tissue healing, and the graft was allowed to heal for 10 months with monthly patient recall during that time.

TABLE 2

Patient demographics

| | Patient | | Type of Sinus Graft | |
|---|---|---|---|---|
| No. | Gender | Edentulism | Unilateral | Contralateral |
| 1 | Female | Partial | Test sample* | Control sample† |
| 2 | Male | Partial | Test sample | Control sample |
| 3 | Male | Complete | Test sample | Control sample |
| 4 | Female | Partial | Control sample | — |
| 5 | Female | Partial | Test sample | — |
| 6 | Female | Partial | Test sample | — |
| 7 | Female | Partial | Test sample | — |

*Test sample = 100% Puros.

†Control sample = 50% Bio-Oss + 50% demineralized freeze-dried bone allografts.

### *Biopsy and Implantation Procedures*

Before the biopsy, the bone height between the residual crestal ridge and the newly created sinus floor, the buccopalatal direction of the osteotomy, and the radiographic appearance of the grafted sinuses were radiographically assessed with panoramic and tomographic X rays. Biopsies were collected at the projected implantation site and, where applicable, with the residual ridge bone. Attempts were made to obtain the biopsy core from the center of the graft buccopalatally and mesiodistally. The 2-mm diameter biopsy was harvested with a standardized trephine drill from the alveolar crest and ended at the predetermined depth of implant placement. The collected core was kept in the trephine drill and sent to the laboratory for processing. Immediately after the biopsy, the site was obliterated through surgical placement of a root form dental implant according to the implant manufacturer's protocol.

## *Histologic evaluation*

### *Histologic Processing*

The specimens were placed in 10% neutral buffered formalin and transported to the Hard Tissue Research Laboratory at the University of Minnesota School of Dentistry. Immediately after the specimens were received, the bone cores were dehydrated with a graded series of alcohols for 9 days. After dehydration, the specimens were infiltrated for 20 days with a light-curing embedding resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany) and constant shaking at normal atmospheric pressure. The specimens were then embedded and polymerized by 450 nm light at a specimen temperature that never exceeded 40°C. By using a cutting and grinding method described by Donath and Breuner[28] and Rohrer and Schubert,[29] the specimens were cut to a thickness of 150 µm (Exakt cutting/grinding system, Exakt Technologies, Oklahoma City, Okla) and then polished to a thickness of 45 µm with a series of polishing sandpaper discs from 800 to 2400 grit (Exakt microgrinding system) followed by a final polish with 0.3-µm alumina polishing paste. The slides were stained with Stevenel's blue and Van Gieson's picro fuchsin.

### *Histomorphometric Analysis*

Photomicrographs were obtained with a Zeiss Axiolab photomicroscope (Carl Zeiss, Jena, Germany) and a Nikon Coolpix 4500 digital camera (Nikon Corp, Japan). All core specimens were photographed at a fixed focal point and ×25 magnification for histomorphometric evaluation. Histomorphometric measurements were completed with a Macintosh G4 computer (Apple, Cupertino, Calif) and a public-domain image program (NIH Image, US National Institutes of Health, and available on the Internet at http://rsb.info.nih.gov/nih-image/) in combination with Adobe Photoshop (Adobe, San Jose, Calif). Identifying the new bone formation and differentiation from the residual graft particles was accomplished by evaluating the different maturity levels between newly formed bone and graft particles by using differential staining qualities, evaluating the different polarization patterns, as well as evaluating the presence or absence of osteocytes in lacunae. The following parameters were measured: (1) percentage of newly formed bone, (2) percentage of residual graft material, (3) percentage of residual graft material directly in contact with bone, and (4) percentage of fibrous tissue. These parameters were evaluated in both control and experimental sites.

### *Statistical Analyses*

If the response variables were to be normally distributed, a paired $t$ test at significant level $\alpha = 5\%$ was performed. If the response variables were not to be normally distributed, a Wilcoxon Man-Whitney rank test was performed at significant level $\alpha = 5\%$. A paired $t$ test was used to compare bone density in the experimental and control sites from

TABLE 3

Summary of histomorphometric findings (%)*

| Graft | Newly Formed Bone | Residual Graft Material | Residual Graft in Contact With Bone |
|---|---|---|---|
| Test | 40.33 | 4.67 | 54.33 |
| Control | 38.75 | 15.00 | 34.75 |

*Statistical significance of clinical differences could not be determined because of small sample size.

data obtained by computerized tomography.

## RESULTS

No clinical complications were noted at any time during this study. All dental implants osseointegrated and were successfully restored. Histomorphometric results of this analysis are summarized in Table 3, and histologic results are presented as follows:

### *Patient 1*

*Test Sample*

Low-power photomicrographs showed a core composed of very thick, dense trabeculae. Cancellous bone was not uniformly distributed throughout the core, but most of the bone was quite mature. High-power photomicrographs showed new bone had formed and was in intimate contact with the surface of the residual Puros particles (Figure 1A).

*Control Sample*

Low-power photomicrographs showed a core with fairly thick trabeculae in which numerous Bio-Oss particles were observed, which were concentrated in several areas rather than uniformly distributed throughout the core. The core itself appeared solid with good integrity. High-power photomicrographs showed the formation of new trabeculae around and bridging large Bio-Oss particles to form a cancellous network, whereas DFDBA particles were incorporated and resorbing in newly forming bone (Figure 1B).

### *Patient 2*

*Test Sample*

Low-power photomicrographs showed that the trabeculae were not well connected and that the core did not have much integrity. High-power photomicrographs showed new bone had formed of the surfaces of the residual Puros particles (Figure 2A).

*Control Sample*

Low-power photomicrographs showed that the residual Bio-Oss was present as 1 large piece. Many very small control-graft particles were present and not incorporated into new bone formation (Figure 2B). High-power photomicrographs showed that bone had grown in contact with the large Bio-Oss particle and that

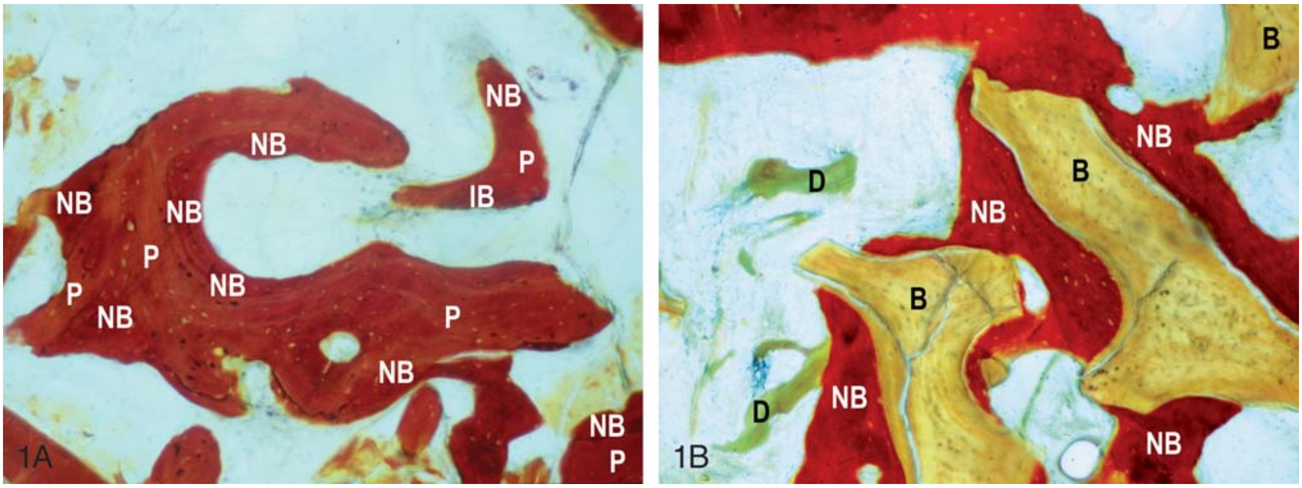
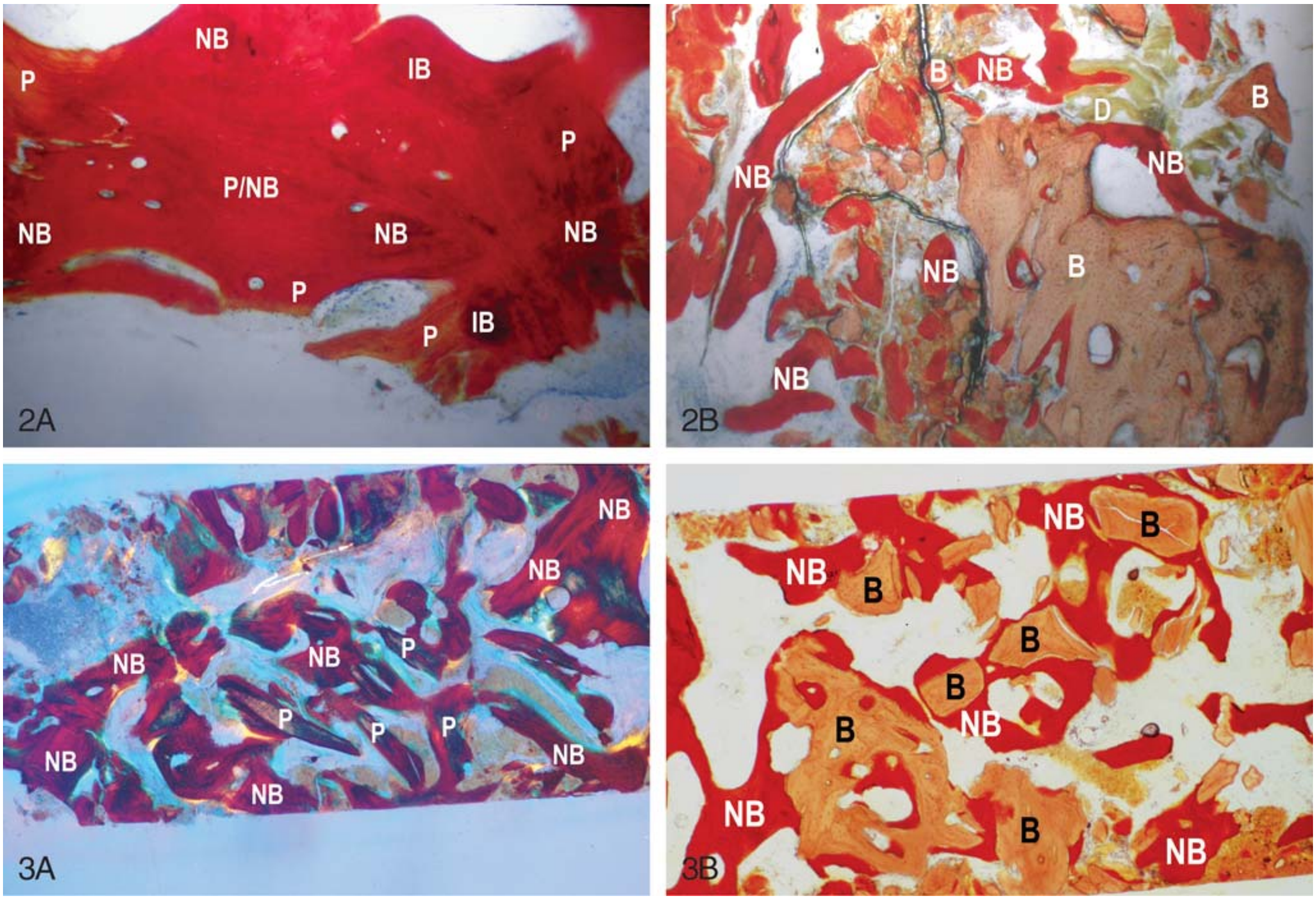


FIGURE 1. (A) Patient 1, test graft: Puros (P) particles undergoing resorption exhibited intimate surface contact with new bone (NB) (original magnification ×10). (B) Patient 1, control graft: New bone connected large, intact Bio-Oss (B) particles, and demineralized freeze-dried bone allografts (D) particles underwent resorption (original magnification ×10).



FIGURES 2 and 3. FIGURE 2. (A) Patient 2, test graft: Puros (P) particles underwent resorption. Puros particles were so well incorporated in new bone (NB and P/NB) and immature bone (IB) (in dark red) that their delineation was barely perceptible (original magnification ×4). (B) Patient 2, control graft: New bone (NB) grew on the outer edges and inside a former Haversian canal of a large Bio-Oss (B) particle. Numerous smaller pieces of Bio-Oss were not incorporated in new bone in contrast to incorporated demineralized freeze-dried bone allografts (D) particles (original magnification ×4). FIGURE 3. (A) Patient 3, test graft: Puros (P) particles were well incorporated and often difficult to differentiate from new bone (NB). The entire bone core demonstrated excellent integrity with dense, thick trabeculae (original magnification ×4). (B) Patient 3, control graft: Bio-Oss (B) particles made up a substantial portion of the cancellous bone pattern and were surrounded by new bone (NB) (original magnification ×4).

new bone formation was present in the former Haversian canal of the particle.

### Patient 3

*Test Sample*

Low-power photomicrographs showed a core with good integrity and dense, thick trabeculae. Graft particles were so well integrated that it was nearly impossible to differentiate them from the new bone (Figure 3A). High-power photomicrographs showed new bone formation surrounding some Puros particles.

*Control Sample*

Low-power photomicrographs showed a core with very good integrity and a good cancellous pattern. Bio-Oss particles made up a substantial portion of the cancellous bone pattern (Figure 3B). High-power photomicrographs showed various areas of bone surrounding Bio-Oss particles.

### Patient 4

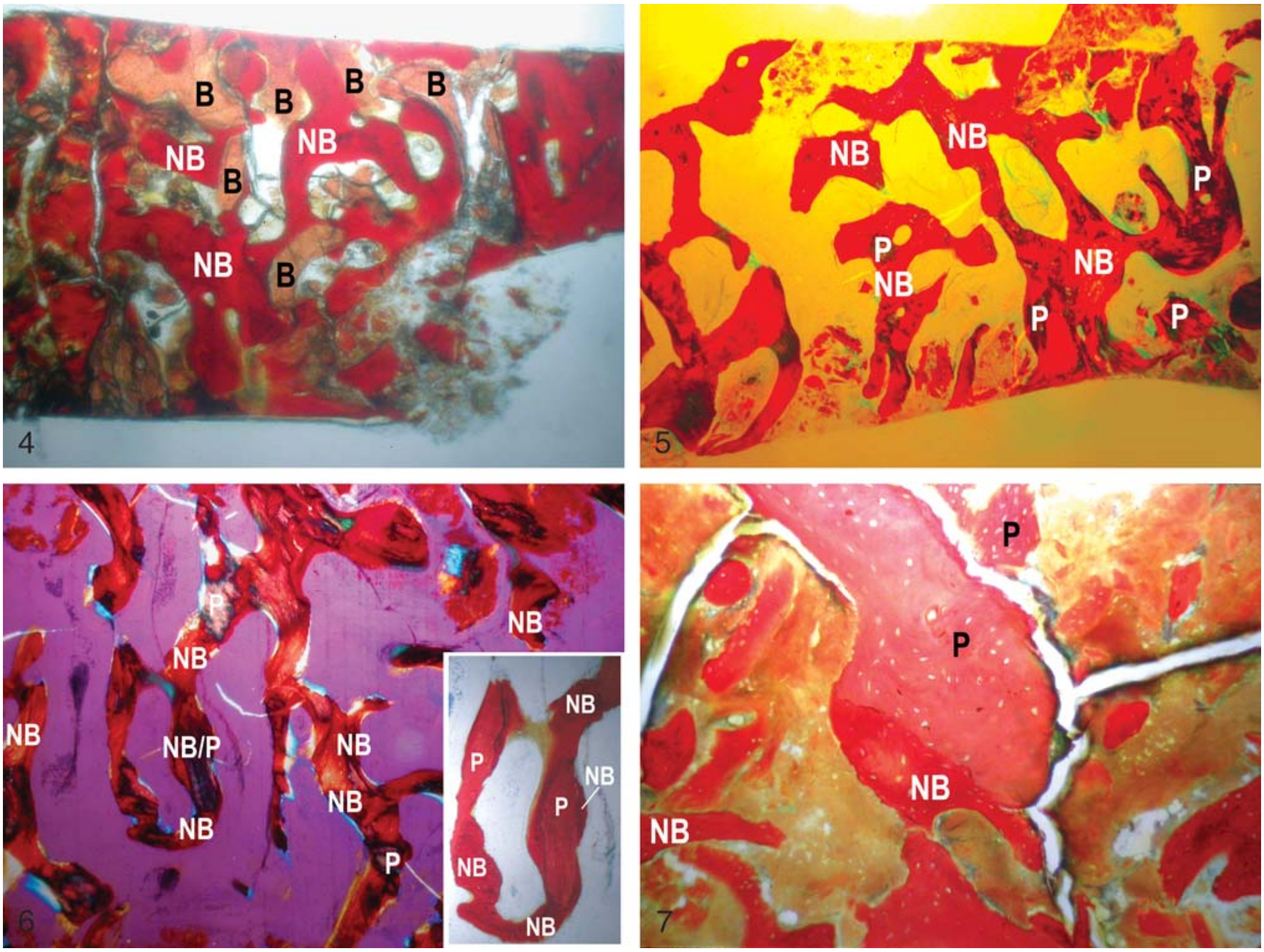
*Control Sample Only*

Low-power photomicrographs showed a fairly solid core with good bone formation around Bio-Oss particles. The cancellous network was well formed with good, thick trabeculae bridging among the Bio-Oss particles (Figure 4). High-power photomicrographs showed new bone formation around Bio-Oss particles.

### Patient 5

*Test Sample Only*

Low-power photomicrographs showed a short core with fairly thick, connected trabeculae. Puros particles were very well



FIGURES 4–7. FIGURE 4. Patient 4, control graft: Large Bio-Oss (B) particles were bridged by new bone (NB) formation on their surfaces (original magnification ×4). FIGURE 5. Patient 5, test graft: Resorbing Puros (P) particles are visible as dark areas within significant new bone (NB) formation (red areas). Puros leaves new bone behind as it resorbs (original magnification ×4). FIGURE 6. Patient 6, test graft: Puros (P) graft particles were incorporated in new bone (NB) and underwent resorption as the new bone formed (original magnification ×4, polarized). Enlargement (insert) shows the pattern of allograft incorporation more clearly (original magnification ×10). FIGURE 7. Patient 7, test graft: Puros (P) particles are difficult to distinguish from new bone (NB) (original magnification ×10).

integrated into newly formed bone and were very hard to detect even in high-power photomicrographs (Figure 5).

### Patient 6

*Test Sample Only*

Low-power photomicrographs showed a small bone core of cancellous bone with connected trabeculae. Differentiation could be noted between new bone formation and small, incorporated graft particles (Figure 6a). High-power photomicrographs showed the Puros particles more clearly (Figure 6b).

### Patient 7

*Test Sample Only*

Low-power photomicrographs showed a fairly long bone core with good integrity consisting of thin, interconnected trabeculae. Puros particles were difficult to distinguish in newly formed bone. In high-power photomicrographs, a lamellar pattern of mature bone could be seen around the Puros particles (Figure 7).

## DISCUSSION

Histologic examination revealed that graft turnover (resorption and replacement by new bone) occurred more rapidly with the test grafts compared with the composite control grafts. This may be attributable, in part, to structural changes that occur in the mineral phase of deproteinized bovine bone xenograft during heat processing at 300°C, which enlarges the xenograft mineral particles to approximately twice the size of mineralized bone allograft particles.[22] In comparison, processing does not change the mineral particle size of mineralized bone allograft, which retains a bonelike structure with interconnecting porosity.[22]

The fear of bovine spongiform encephalopathy (BSE) (''mad cow disease'') transferring to humans (although no report has been made in the literature)[30,31] and the discovery of human immunodeficiency viruses surviving in allogenic bone after tissue processing[32] have underscored concerns about disease transmission from xenografts and allografts. The internationally accepted definition of sterility is the absence of any viable pathogen (eg, bacteria, viruses, fungi, protozoa).[33] Energy (eg, ultraviolet light, heat, irradiation) or chemicals (eg, formalin, betapropiolactone, alcohols) commonly applied during tissue processing are effective in killing most pathogens or rendering them incapable of infection or replication by changing their protein structure or deoxyribonucleic acid or ribonucleic acid sequences.[34]

Of greater concern are infectious protein particles, called prions, which lack the nucleic acids common to viruses, bacteria, fungi, and parasites. Consequently, prions are extremely resistant to conventional inactivation procedures.[30,35–46] Prion-related diseases are a group of fatal neurodegenerative disorders that cause a spongiform change in the gray matter of the brain and the accumulation of prion proteins within the central nervous systems of both humans (eg, Creutzfeld Jacob disease) and animals (eg, BSE, scrapie).[47] Several substances have been reported to effectively inactivate prions, including solvent-dehydration used in the processing of the mineralized bone allograft used in this study.[48–52]

It is important to note that modern tissue-processing techniques, adherence to good manufacturing practices, rigid screening of potential tissue donors, and sterility-validation studies minimize the risk of disease transmission from banked tissues.[31,53,54] All the heterogeneous tissues used in the present study thus offered a safe and effective alternative to autogenous bone for augmenting the maxillary sinus.

## CONCLUSIONS

Test and control grafts both resulted in successful new bone formation. Test-graft particles resorbed and were replaced by new bone significantly faster than were control-graft particles. These 2 findings confirm a more rapid resorption and replacement by new bone with Puros. Two years after the completion of the study, no differences in osseointegration or stability were noted among implants placed in test and control sites.

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