

## Comparison of Mineralized Cancellous Bone Allograft (Puros) and Anorganic Bovine Bone Matrix (Bio-Oss) for Sinus Augmentation: Histomorphometry at 26 to 32 Weeks After Grafting



Stuart J. Froum, DDS<sup>1</sup>/Stephen S. Wallace, DDS<sup>2</sup>/  
Nicolas Elian, DDS<sup>3</sup>/Sang Choon Cho, DDS<sup>4</sup>/  
Dennis P. Tarnow, DDS<sup>5</sup>

The present blinded, randomized, controlled investigation histomorphometrically evaluated the vital bone formed following bilateral grafting with two different materials—Puros, a mineralized cancellous bone allograft (MCBA), and Bio-Oss, an anorganic bovine bone matrix (ABBM)—at 26 to 32 weeks following graft placement. Thirteen patients were selected who required bilateral sinus augmentation. Following elevation of the lateral sinus walls, one material was placed in the right sinus and the other in the left sinus, as determined by randomized choice. Twenty-six to 32 weeks after grafting (the same time frame was used for each individual patient), a trephine core was taken from the previously elevated lateral wall area and sent for histomorphometric analysis. Cores were obtained from 22 healed sinus augmentations in 11 patients. Eight patients provided bilateral cores, two patients had intact MCBA cores but inadequate ABBM cores, and another patient had an intact ABBM core but an inadequate MCBA core. Histomorphometric analysis of 10 MCBA cores and 9 ABBM cores revealed average vital bone content of 28.25% and 12.44%, respectively. The average percentage of residual nonvital bone was 7.65% in the MCBA cores and 33.0% in the ABBM cores. Significantly more bone was formed in the MCBA sites ( $n = 8$  patients, paired  $t$  test). Histologically, both MCBA and ABBM particles were surrounded by new bone, osteoid, and osteoblasts. A higher average percentage of new vital bone was seen around the MCBA particles than around the ABBM particles. (*Int J Periodontics Restorative Dent* 2006;26:543–551.)

<sup>1</sup>Clinical Professor, Department of Periodontology and Implant Dentistry, College of Dentistry, New York University.

<sup>2</sup>Associate Clinical Professor, Department of Periodontology and Implant Dentistry, College of Dentistry, New York University.

<sup>3</sup>Assistant Professor and Director, Department of Periodontology and Implant Dentistry, College of Dentistry, New York University.

<sup>4</sup>Assistant Clinical Professor, Department of Periodontology and Implant Dentistry, College of Dentistry, New York University.

<sup>5</sup>Professor and Chair, Department of Periodontology and Implant Dentistry, College of Dentistry, New York University.

Correspondence to: Dr Stuart J. Froum, 17 West 54th Street, Suite 1 C/D, New York, NY 10019; fax: (212) 246-7599; e-mail: dr.froum@verizon.net.

The subantral augmentation (sinus lift) procedure is a well accepted and predictable method of increasing the volume of bone to facilitate the placement of implants in areas of the posterior maxilla with insufficient bone quantity and/or quality.<sup>1,2</sup> The original sinus augmentation protocol utilized autogenous bone from either intraoral or extraoral sources.<sup>3,4</sup> However, the use of autogenous bone requires a second surgical site and thus increases the duration of surgical intervention, surgical risk, and postsurgical morbidity, since healing is required at multiple sites.

Mineralized cancellous bone allograft (MCBA) has been used for grafting in multiple intraoral applications such as periodontics (infrabony defects),<sup>5,6</sup> oral surgery (extraction sites),<sup>7–9</sup> and implant dentistry (ridge augmentation).<sup>10,11</sup> MCBA is a human bone product obtained from cadavers and then processed and sterilized. A solvent-dehydrated form of this graft material, Puros (Zimmer Dental), produced with a new processing technique (Tutoplast, Tutogen Medical), is currently being used as a bone replacement for sinus augmentation procedures.<sup>12–14</sup>

Anorganic bovine bone allograft (Bio-Oss, Osteohealth) (ABBM) is a bone substitute that is manufactured from bovine bone mineral, which is then processed and sterilized for use in intra-oral grafting procedures. It is composed of only the mineral portion of bone. Two recently published evidence-based systematic reviews concluded that the results of sinus augmentations with xenografts are the most complete and well-documented in the published peer-reviewed literature.<sup>1,2</sup> Both of the grafting materials used in this study, MCBA and ABBM, have been approved by the Federal Drug Administration for use as sinus grafting materials.

The purpose of this prospective, blinded, randomized, controlled investigation was to compare the efficacy of solvent-dehydrated MCBA to ABBM in producing vital bone by 26 to 32 weeks following sinus augmentation.

## Method and materials

Thirteen subjects (five men and eight women) were selected from those presenting to the Department of Periodontology and Implant Dentistry at New York University Kriser Dental Center who desired maxillary posterior implants and who did not have sufficient bone for the procedure. Each of these subjects required bilateral sub-antral sinus grafting to be eligible for this study. Less than 5 mm of crestal bone had to be present below the sinus floor, as determined by an axial computerized tomographic (CT) scan, for the patient to be considered for inclusion in this study. The age range was 46 to 75 years (mean, 59 years).

There were no limitations to enrollment by gender, race, ethnicity, or health status of the subject except those listed under the exclusion criteria. Subject exclusion criteria included patients who could not undergo standard oral surgery procedures for any reason, patients who smoked more than 10 cigarettes per day, and women who were pregnant or nursing a child.

## Diagnosis and surgery

The diagnostic and surgical procedures were as follows:

1. The diagnosis included the need for implants and a sinus augmentation procedure with the aid of panoramic radiographs and CT imagery.
2. The study was presented to the subjects, and both verbal and written informed consent were provided and accepted by the New York University School of Medicine Institutional Board of Research.
3. Each subject was required to take 500 mg amoxicillin (Teva Pharmaceuticals) 1 hour prior to surgery. For patients allergic to amoxicillin, 300 mg of clindamycin (Watson Laboratories) 1 hour prior to surgery was substituted.
4. Local anesthesia was administered (lidocaine hydrochloride 2% with 1:100,000 epinephrine or mepivacaine/carbocaine 3% without epinephrine [Abbott Laboratories]).

5. A full-thickness flap was reflected, exposing the lateral wall of the sinus.
6. A hinge or complete osteotomy of the lateral sinus wall was performed, as circumstances dictated.
7. The osseous wall and sinus membrane were elevated. If the bony window was removed to facilitate elevation of the membrane, it was not added to the grafted bone.
8. MCBA was placed in one sub-antral compartment and ABBM was placed in the contralateral sub-antral compartment. The mixture for each material was composed of 50% 0.25- to 1.0-mm particles and 50% 1.0- to 2.0-mm particles. A computer-generated randomized code was used to determine test and control sites. Depending on the sinus anatomy, 3 to 10 g of material were placed into each sinus. Depending on the patient and complexity of surgery, the sinus augmentations were performed simultaneously or at two separate appointments. The second surgery had to be performed no later than 6 to 8 weeks after the first surgery.
9. A synthetic bioabsorbable collagen membrane (BioMend Extend, Zimmer Dental) was hydrated for 1 to 5 minutes in sterile saline prior to insertion and placed over the lateral window. The membrane was extended at least 3 mm beyond the limits of the prepared window and pressed against the bone.

10. Primary closure of the flap with silk (Ethicon), polyglactin 910 (Vicryl, Ethicon), or expanded polytetrafluoroethylene (Gore-Tex, W.L. Gore and Associates) sutures was performed as part of each procedure.
11. Provisional fixed or removable appliances were relieved over the edentulous area prior to reinsertion.
12. Following surgery, subjects were placed on antibiotic coverage (amoxicillin 500 mg three times daily or clindamycin 150 mg four times daily, for 10 days), depending on the subject's history of drug allergy, and analgesics for pain relief (acetaminophen with codeine #3 [30 mg codeine phosphate] or #4 [60 mg codeine phosphate]), OMP Division, Ortho-McNeil Pharmaceutical, or ibuprofen 600 mg every 6 hours, McNeil-PPC). Rinses with 0.12% chlorhexidine digluconate (Peridex) twice daily for 2 weeks were also prescribed.
13. At stage 1 surgery, when implants were being placed (following a 26- to 32-week healing phase), a trephine core sample (10 mm in length and 3 mm in diameter) was retrieved near the superior position of the original lateral window osteotomy. This technique ensured that the cores were taken from the most central area of the graft, where the healing bone is considered to be the least mature. The cores were obtained from both study and control sites by one of the investigators in such a manner as to avoid compromise of future implant placement. Regardless of the time of sinus lift surgery, whether both lifts were done on the same day or as two separate procedures, the postsurgical timing of core harvesting was the same for both sinuses. Antibiotics and analgesics were prescribed with the same regimen that had been prescribed for the sinus lift surgery.
14. Sutures were removed 7 to 10 days postsurgery.
15. Blinded histomorphometric analysis was performed on the bone core samples to determine the vital bone content, connective tissue content, and residual graft material content. Specimen preparation and histologic and histomorphometric evaluation followed procedures that have been previously described.<sup>15-17</sup>

#### Statistical analysis

Statistical analysis consisted of averages and ranges for percent total bone volume, percent vital bone, percent connective tissue, and percent marrow. Thirty sections (three from each core) from the Puros (test/MCBA group) and 27 sections from the Bio-Oss (control/ABBM group) were evaluated. The analysis also included a paired *t* test to evaluate each of the above parameters in the eight bilateral cases in which the grafts were allowed to heal for the same time prior to core extraction.

#### Results

Thirteen bilateral sinus augmentations were performed on 13 patients. Two patients were withdrawn from the study because of failure to obtain the required core specimens within the 26- to 32-week postsurgical time frame, as was specified in the protocol. Cores were obtained 26 to 32 weeks postsurgery from 22 healed sinus augmentations in 11 patients. Eight patients provided bilateral cores. Two other patients had intact MCBA cores but inadequate ABBM cores. Another patient had an intact ABBM core but an inadequate MCBA core. In these patients these cores were obtained but could not be processed because they broke up into pieces when attempting to remove them from the trephine. One patient had bilateral cores obtained after 26 weeks. All other cores were taken 29 to 32 weeks after sinus augmentation surgery, at the time of implant placement. Small schneiderian membrane perforations that occurred during surgery were reported in 29% of the treated sinuses (six sinuses in the MCBA group and one sinus in the ABBM group). All perforations were repaired with collagen membranes (BioGide, Osteohealth, or BioMend, Zimmer Dental).

Histomorphometric analysis of the 10 MCBA cores revealed a 35.90% average total bone volume, of which an average of 76.90% was vital. This resulted in an average vital bone content of 28.25% (range, 8% to 51%). Similar analysis of the nine cores taken from the ABBM-grafted sinuses revealed an average of 12.44% bone content (range, 5% to 24%), all of

**Table 1** Histomorphometric results of core samples taken from sinuses augmented with MCBA (Puros) and ABBA (Bio-Oss)

Sample type/no.	Total % bone volume	% vitality	% vital bone	% marrow	% residual graft	Time of core removal (wk)
Puros						
1	51	100	51	49	0	31
2	39	100	39	61	0	30
3	38	94	36	62	2	32
4	NP	—	—	—	—	—
5	29	83	24	71	5	32
6	29	29	8	71	21	26
7	32	73	23	68	9	30
8	46	96	44	54	2	30
9	21	94	20	79	1	30
10	37	69	26	63	11	30
11	37	31	11	63	26	29
Mean	35.90	76.90	28.25	64.10	7.65	—
Bio-Oss						
1	NP	—	—	—	—	—
2	5	100	5	45	50	30
3	NP	—	—	—	—	—
4	24	100	24	46	30	32
5	12	100	12	51	37	32
6	5	100	5	70	25	26
7	9	100	9	57	34	30
8	25	100	25	57	18	30
9	5	100	5	69	26	30
10	21	100	21	40	39	30
11	6	100	6	56	38	29
Mean	12.44	100	12.44	54.56	33.00	—

NP = core taken but could not be processed.

which was vital. The average percentages of marrow and connective tissue were 64.10% and 54.56%, respectively, for the MCBA- and ABBM-treated sinuses. Analysis of the cores from ABBM-treated sinuses revealed an average residual xenograft material of 33.0%. Analysis of the cores from the MCBA-treated sinuses revealed an average of 7.65% residual nonvital bone (Table 1). A paired *t* test of the eight bilateral sinus lifts with each material (ABBM and MCBA) allowed to heal for identical periods of time prior

to core extraction revealed the following statistics:

1. Total % bone volume:  $t(7) = 9.465, P < .001$
2. % vital bone:  $t(7) = 3.757, P = .007$
3. % marrow:  $t(7) = 3.354, P = .012$
4. % residual graft:  $t(7) = -4.859, P = .002$

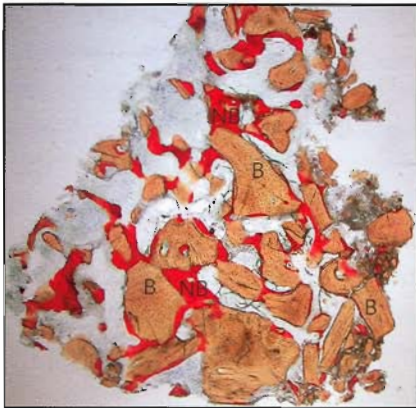
All are significant.

From a histologic point of view, both ABBM and MCBA particles

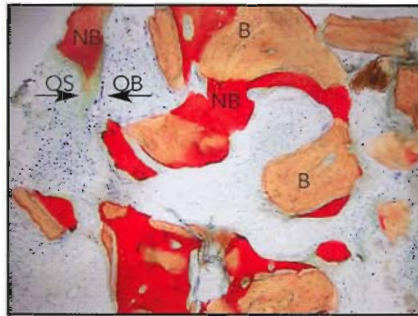
appear to be osteoconductive. Osteoblasts and osteoid were seen in conjunction with new bone formation around the ABBM particles (Figs 1a to 1c). The MCBA particles were surrounded by greater amounts of new bone and osteoid (Figs 2a to 2d).

## Discussion

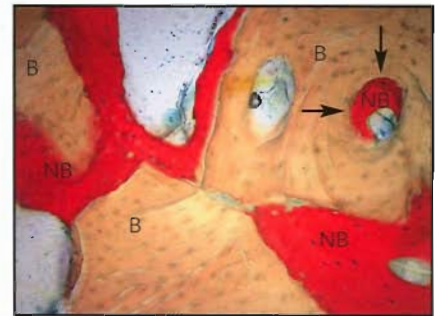
Bone replacement materials have been used in the sinus lift procedure to avoid the drawbacks inherent in the



**Fig 1a** Low-power view of numerous particles of Bio-Oss (B) of various sizes. Bridges of newly formed bone (NB) can be seen among the particles in areas where the particles are close to each other, forming a cancellous bone pattern. The Bio-Oss particles are partially surrounded by bone (Stevenel's blue and Van Gieson's picro fuchsin; ×25).



**Fig 1b** High-power view of new bone formation (NB) between particles of Bio-Oss (B). Osteoblasts (OB) line osteoid (OS) in an area of new bone formation (Stevenel's blue and Van Gieson's picro fuchsin; ×100).



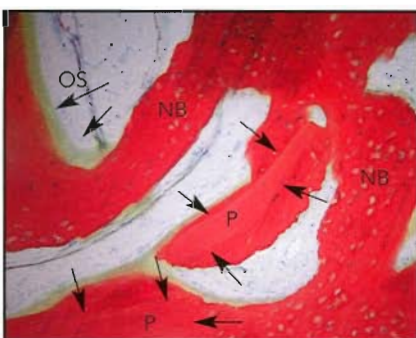
**Fig 1c** Very high-power view of newly formed bone (NB) bridges between particles of Bio-Oss (B). New bone is forming in the haversian canal area (arrows) of the Bio-Oss (Stevenel's blue and Van Gieson's picro fuchsin; ×200).



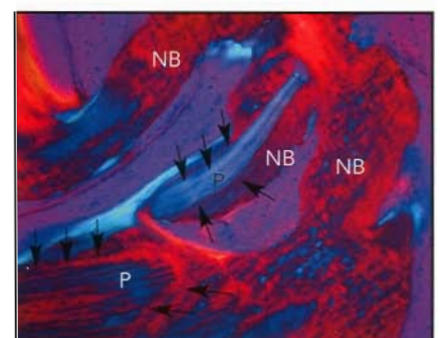
**Fig 2a (left)** Low-power view of a core showing trabeculae in which the Puros particles (P) are generally incorporated into the newly formed bone (NB). Arrows indicate interface between Puros particles and new bone (Stevenel's blue and Van Gieson's picro fuchsin; ×25).



**Fig 2b (right)** Medium-power view showing how well the Puros particles (P) are incorporated into the newly formed bone (NB) trabeculae. Arrows indicate interface between Puros particles and new bone (Stevenel's blue and Van Gieson's picro fuchsin; ×40).



**Fig 2c (left)** High-power view of new bone formation (NB) among particles of Puros (P). Osteoid material (OS) lines the new bone. Arrows indicate interface between Puros particles and new bone (Stevenel's blue and Van Gieson's picro fuchsin; ×100).



**Fig 2d (right)** Polarized view of the previous high-power image emphasizing the difference in pattern and maturity between Puros (P) and newly formed bone (NB). Arrows indicate interface between Puros particles and new bone (Stevenel's blue and Van Gieson's picro fuchsin; ×200).

harvesting of autogenous bone. They have been shown to be effective and have demonstrated high implant survival rates. These graft materials include: allografts<sup>18</sup> (both mineralized and demineralized), xenografts<sup>17,19,20</sup> (from different species, usually bovine), and alloplasts<sup>21,22</sup> (synthetic). The literature shows a wide range of results with different grafting materials.<sup>23-32</sup>

This is the first randomized controlled clinical trial to report on a direct comparison of an MCBA to an ABBM in sinus augmentation. The present study used a bilateral sinus protocol utilizing the lateral wall (hinged window or complete osteotomy) technique as described by Smiler,<sup>33</sup> with the same barrier membrane placed over the windows. The difference in the graft material was the only study variable. However, even in this model, factors such as difference in the size and morphology of the sinuses, the amount of residual crestal bone, and operator differences remain as potential variables. As established in previous studies, the cores were coded and sent to a histopathologist, who was blinded as to the augmentation material used.

For the clinician, the decision as to which material to use in sinus augmentations is based on the survival rate of implants placed in these grafted sinuses. Studies by Valentini et al,<sup>19</sup> Hising et al,<sup>34</sup> and Hallman et al<sup>35</sup> showed a higher survival rate of implants placed in sinuses grafted with 100% xenografts than in sinuses grafted with 100% autogenous bone or composite grafts of xenograft and autogenous bone. A recent retrospective study reported an overall survival rate of 94.5% after a mean func-

tioning period of  $6.5 \pm 1.9$  years. The implant survival rate for sinuses grafted with 100% ABBM was 96.8%, compared to a 90% implant survival rate for sinuses grafted with a mixture of ABBM and demineralized freeze-dried bone.<sup>36</sup> In two separate systematic reviews of the sinus augmentation procedure by Wallace and Froum<sup>1</sup> and Del Fabbro,<sup>2</sup> the survival rate for implants in sinuses augmented with xenograft material was statistically the same or higher than that for implants in sinuses grafted with xenograft and autogenous bone and for implants in sinuses grafted with 100% autogenous bone.

Although the aforementioned studies and reviews support the efficacy of ABBM because of its unlimited supply and safety, the clinician must remember that xenografts are osteoconductive rather than osteoinductive, and therefore the bone turnover rate is slower than that of an autogeneous graft. To achieve a similar amount of vital bone as that reported with autogenous bone grafts, a longer healing time is required.<sup>37,38</sup> Thus, from a biologic standpoint, the larger the sinus, the longer the maturation time that is required for a sinus grafted with a xenograft alone to achieve a similar level of vital bone as a sinus grafted with autogenous bone.

The results of the present histomorphometric study indicate that a greater average percentage of vital bone was obtained at 26 to 32 weeks postsurgery in the MCBA-treated sites. Although this may not directly correlate to the survival of implants placed in those treated sinuses, the percentage of vital bone formed is one indication of the performance of a bone

graft or bone replacement graft in an augmented sinus. The formation of a high percentage of vital bone in a reasonable time period following augmentation would indicate that a material is suitable for grafting. The literature shows a wide range of results when utilizing ABBM alone as a sinus graft material, with vital bone content ranging from 2% to 33%.<sup>17,19,28,35,38</sup> In the present study, the average percentage of vital bone at 26 to 32 months after sinus augmentation with MCBA (28.25%) exceeded that found in the ABBM-grafted sinuses (12.44%) by about 15%. Moreover, in a direct comparison of eight bilateral cases, the total percentage of vital bone formed was significantly greater in the MCBA-treated sinuses than in the contralateral ABBM-treated sinuses. All of the new bone that was formed around the ABBM particles showed 100% vitality. The ABBM particles were not considered "bone." This contrasts to the percent of vital bone reported in conjunction with the MCBA particles, which ranged from 29% to 100%. This discrepancy in vital bone reported with MCBA derives from the fact that the particles of nonresorbed MCBA were considered nonvital bone when calculating the total vitality of the combination of MCBA particles and new surrounding bone. The MCBA/new bone complex, for example, in patient #3 consisted of 38% total bone volume (mineralized complex) present. However, in this complex only 94% was vital (new bone), which equates to a total of 36% of vital new bone (in this measured area).

Although the number of cases included in the present study is too

small to draw any general conclusion as to the efficacy of one material compared to the other, it is interesting to note that in all bilateral cases the vital bone formed using MCBA exceeded that obtained with ABBM. In light of this it is interesting to further examine the MCBA used in the present study.

The study material, (solvent-dehydrated) MCBA (Puros), is obtained from cadaver bone and processed according to American Association of Tissue Banks standards using the Tutoplast processing technique.<sup>13,14,39</sup> The latter includes cleaning and ultrasonic delipidization in acetone, osmotic treatment in alternating baths with varying concentrations of saline and distilled water, oxidative treatment in baths of hydrogen peroxide solutions, dehydration in sequential acetone baths, and treatment with limited-dose gamma irradiation (17.8 Gy).<sup>13,14</sup>

The Tutoplast process has been shown to inactivate HIV and the agent responsible for Creutzfeldt-Jakob disease.<sup>40</sup> In an experimental study of tissues from individuals who had died from AIDS and hepatitis C, no infectivity could be detected after this method of processing.<sup>14</sup>

When compared to the ABBM-treated sites, the greater presence of vital bone in the MCBA-treated sites may suggest a difference in osteoconduction capabilities.<sup>41-44</sup> The retained collagen in MCBA may be an important aspect of its osteoconductive potential.<sup>45-47</sup>

In the present study, the fate of the residual MCBA (7.65%) and ABBM (33.0%) is unknown. However, a recent clinical and histomorphometric study comparing MCBA (Puros) with a 1:1

combination of decalcified freeze-dried bone allograft (DFDBA) plus ABBM (Bio-Oss) as a human maxillary sinus graft material concluded that "test-graft (MCBA) particles resorbed and were replaced by new bone significantly faster than were the control (DFDBA + ABBM) graft particles."<sup>48p178</sup> In that study, "newly formed bone" was histomorphometrically calculated to be 40.33% in the MCBA cores and 38.75% in the combination DFDBA + ABBM cores. Residual graft material averaged 4.67% in the MCBA cores and 15.00% in the DFDBA + ABBM cores.<sup>48</sup> The presence of these mineralized particles in both materials may prevent the occurrence of repneumatization (slumping), which has been reported when 100% autogenous bone or a 2:1 mixture of autogenous bone and ABBM was used as the grafting material.<sup>49</sup> Long-term follow-up data following the placement of implants in sinuses grafted with the two materials used in the present study is necessary to determine whether and to what extent repneumatization occurs and the long-term survival rate of those implants.

In conclusion, based on this comparison of the histomorphometric healing response following the use of Tutoplast-processed MCBA and ABBM in sinus augmentation procedures, MCBA material should be considered a viable alternative to the use of 100% autogenous bone or 100% ABBM.

## Acknowledgments

The authors would like to thank Michael D. Rohrer, DDS, MS, Professor and Director, Division of Oral and Maxillofacial Pathology and Hard Tissue Research Laboratory, University of Minnesota School of Dentistry, Minneapolis, and Hari S. Prasad, BS, MDT, Senior Research Scientist, University of Minnesota School of Dentistry, Minneapolis, for their efforts and histomorphometric analysis of the specimens presented in the present study. This study was supported by Zimmer Dental, which donated the MCBA and membranes.

## References

1. Wallace SS, Froum SJ. Effect of maxillary sinus augmentation on the survival of endosseous dental implants. A systemic review. *Ann Periodontol* 2003;8:328-343.
2. Del Fabbro M, Testori T, Francetti L, Weinstein R. Systemic review of survival rates for implants placed in the grafted maxillary sinus. *Int J Periodontics Restorative Dent* 2004;24:565-578.
3. Boyne PJ, James RA. Grafting of the maxillary sinus floor with autogenous marrow and bone. *J Oral Surg* 1980;38:613-816.
4. Wood RM, Moore DL. Grafting of the maxillary sinus with intraorally harvested autogenous bone prior to implant placement. *Int J Oral Maxillofac Implants* 1988;3:209-214.
5. Becker W, Urist M, Becker BE, et al. Clinical and histologic observations of sites implanted with intraoral autologous bone grafts or allografts. 15 human case reports. *J Periodontol* 1996;67:1025-1033.
6. Wang H-L, Shotwell JL, Tatsuma I, Neiva RF. Multidisciplinary treatment approach for enhancement of implant esthetics. *Implant Dent* 2005;14:21-29.
7. Block MS, Finger I, Lytle R. Human mineralized bone in extraction sites before implant placement. Preliminary results. *J Am Dent Assoc* 2002;133:1631-1638.



8. Minichetti JC, D'Amore JC, Hong AYJ, Cleveland DB. Human histologic analysis of mineralized bone allograft (Puros) placement before implant surgery. *J Oral Implantol* 2004;30:74–82.
9. Wang H-L, Kiyonobu K, Neiva RF. Socket augmentation: Rationale and technique. *Implant Dent* 2004;13:286–296.
10. Feuille F, Knapp CL, Brunsvold MA, et al. Clinical and histologic evaluation of bone replacement grafts in the treatment of localized alveolar ridge defects. Part 1: Mineralized freeze-dried bone allograft. *Int J Periodontics Restorative Dent*. 2003;23:29–35.
11. Block MS, Degen M. Horizontal ridge augmentation using human mineralized particulate bone: Preliminary results. *Int J Oral Maxillofac Surg* 2004;62(suppl 2):67–72.
12. Froum SJ, Tarnow DP, Wallace SS, et al. The use of a mineralized allograft for sinus augmentation: An interim histological case report from a prospective clinical study. *Compend Contin Educ Dent* 2005;26:81–88.
13. Schöpf C, Diaber W, Tadiz D. Tutoplast processed allografts and xenografts. In: Jacotti M, Antonelli P (eds). *3D Block Technique from Image Diagnostics to Block Graft Bone Regeneration*. Milano, Italy: RC Libri SRL, 2005:53–75.
14. Günther KP, Scharf H-P, Pesch H-J, Puhl W. Osteointegration Lösungsmittel-konservierter Knochen-transplantate im Tiermodell. *Osteologie* 1996;5:4–12.
15. Donath K, Breuner G. A method for the study of undecalcified bones and teeth with the attached soft tissues: The Sage-Schliff (sawing and grinding) technique. *J Oral Pathol* 1982;11:318–326.
16. Rohrer MD, Schubert CC. The cutting-grinding technique for histological preparation of undecalcified bone and bone-anchored implants: Improvements in instrumentation and procedures. *Oral Surg Oral Med Oral Pathol Radiol Endod* 1992;74:73–78.
17. Froum SJ, Tarnow DP, Wallace SS, Rohrer MD, Cho SC. Sinus floor elevation using anorganic bovine bone matrix (OsteoGraf /N) with and without autogenous bone: A clinical, histologic, radiographic, and histomorphometric analysis—Part 2 of an ongoing prospective study. *Int J Periodontics Restorative Dent* 1998;18:528–543.
18. Van Den Bergh JP, ten Bruggenkate CM, Krekeler G, Tuinzing DB. Maxillary sinus floor elevation and grafting with human demineralized freeze dried bone. *Clin Oral Implants Res* 2000;11:487–493.
19. Valentini P, Abensur D, Wenz B, Peetz B, Schenk R. Sinus grafting with porous bone mineral (Bio-Oss) for implant placement: A 5-year study on 15 patients. *Int J Periodontics Restorative Dent* 2000;20:245–253.
20. Yildirim M, Spiekermann H, Handt S, Edelhoff D. Maxillary sinus augmentation with the xenograft Bio-Oss and autogenous intraoral bone for qualitative improvement of the implant site: A histologic and histomorphometric clinical study in humans. *Int J Oral Maxillofac Implants* 2001;16:23–33.
21. Szabo G, Huys L, Coulthard P, et al. A prospective multicenter randomized clinical trial of autogenous bone versus beta-tricalcium phosphate graft alone for bilateral sinus elevation: Histologic and histomorphologic evaluation. *Int J Oral Maxillofac Implants* 2005;20:375–381.
22. Artzi Z, Kozlovsky A, Nemcovsky CE, Weinreb M. The amount of newly formed bone in sinus grafting procedures depends on tissue depth as well as the type and residual amount of the grafted material. *J Clin Periodontol* 2005 Feb;32:193–199.
23. Tadjoeidin ES, De Lange GL, Holzmann PJ, Kuiper L, Burger EH. Histological observations on biopsies harvested following sinus floor elevation using a bioactive glass material of narrow size range. *Clin Oral Implants Res* 2000;11:334–344.
24. Tarnow DP, Wallace SS, Froum SJ, Rohrer MD, Cho SC. Histologic and clinical comparison of bilateral sinus floor elevations with and without barrier membrane placement in 12 patients: Part 3 of an ongoing prospective study. *Int J Periodontics Restorative Dent* 2000;20:116–125.
25. Van den Bergh JPA, ten Bruggenkate CM, Groeneveld HH, Burger EH, Tuinzing DB. Recombinant human bone morphogenetic protein-7 in maxillary sinus floor elevation surgery in 3 patients compared to autogenous bone graft. *J Clin Periodontol* 2000;27:627–636.
26. Yildirim M, Spiekermann H, Biesterfeld S, Edelhoff D. Maxillary sinus augmentation using xenogenic bone substitute material Bio-Oss in combination with venous blood: a histologic and histomorphometric study in humans. *Clin Oral Implants Res* 2000;11:217–229.
27. Groeneveld Eh, Van den Bergh JP, Holzmann P, ten Bruggenkate CM, Tuinzing DB, Burger EH. Histomorphometrical analysis of bone formed in human maxillary sinus floor elevations grafted with OP-1 device, demineralized bone matrix or autogenous bone. Comparison with non-grafted sites in a series of case reports. *Clin Oral Implants Res* 1999;10:499–509.
28. Piatelli M, Favero A, Scarano A, Orsini G, Piatelli A. Bone reaction to anorganic bovine bone (Bio-Oss) used in sinus augmentation procedures: A histologic long-term report of 20 cases in humans. *Int J Oral Maxillofac Implants* 1999;14:835–840.
29. Jensen OT, Shulman LB, Block MS, Iacono VJ. Report of the Sinus Consensus Conference of 1996. *Int J Oral Maxillofac Implants* 1998;13(suppl):11–45.
30. Valentini P, Abensur D, Densari D, Graziani JN, Hammerle C. Histological evaluation of Bio-Oss in a sinus floor elevation and implantation procedure: A human case report. *Clin Oral Implants Res* 1998;9:59–64.
31. Avera SP, Stampley WA, McAllister S. Histologic and clinical observations of resorbable and nonresorbable barrier membranes used in maxillary sinus graft containment. *Int J Oral Maxillofac Implants* 1997;12:88–94.
32. Wheeler SL, Homes R, Calhoun. Six-year clinical and histologic study of sinus-lift grafts. *Int J Oral Maxillofac Implants* 1996;11:26–34.
33. Smiler DG. The sinus lift graft: Basic technique and variations. *Pract Periodontics Esthet Dent* 1997;9:885–893.

34. Hising P, Bolin A, Branting C. Reconstruction of severely resorbed alveolar crests with dental implants using a bovine mineral for augmentation. *Int J Oral Maxillofac Implants* 2001;16:90-97.
35. Hallman M, Sennerby L, Lundgren S. A clinical and histologic evaluation of implant integration in the posterior maxilla after sinus floor augmentation with autogenous bone, bovine hydroxyapatite, or a 20:80 mixture. *Int J Oral Maxillofac Implants* 2002;17:635-643.
36. Valentini P, Abensur DJ. Maxillary sinus grafting with anorganic bovine bone: A clinical report of long-term results. *Int J Oral Maxillofac Implants* 2003;18:556-560.
37. Merckx MAW, Maltha JC, Stoelinga PJW. Assessment of the value of anorganic bone additives in sinus floor augmentation: A review of clinical reports. *Int J Oral Maxillofac Surg* 2003;32:1-6.
38. John H-D, Wenz B. Histomorphometric analysis of natural mineral for maxillary sinus augmentation. *Int J Oral Maxillofac Implants* 2004;19:199-207.
39. Mould DL, Dawson AM, Smith W. Scrapie in mice. The stability of the agent to various suspending media, pH and solvent extraction. *Res Vet Sci* 1965;36:151-154.
40. Keith JD Jr, Petrunaro P, Elwell CW Jr, Caputo C, Schöpf C. Clinical and histologic evaluation of a mineralized block allograft: Results from the developmental period (2001-2004). *Int J Periodontics Restorative Dent* 2006;26:321-327.
41. Rogers KD, Daniels P. An x-ray diffraction study of the effects of heat treatment on bone mineral microstructure. *Biomaterials* 2002;23:2577-2585.
42. Hislop WS, Finlay PM, Moos KF. A preliminary study into the uses of anorganic bone in oral and maxillofacial surgery. *Br J Oral Maxillofac Surg* 1993;31:149-153.
43. Pinholt EM, Bang G, Haanaes HR. Alveolar ridge augmentation in rats by Bio-Oss. *Scand J Dent Res* 1991;99:154-161.
44. Committee on Research, Science and Therapy of the American Academy of Periodontology. Tissue banking of bone allografts used in periodontal regeneration. *J Periodontol* 2001;72:834-838.
45. Bunyaratavej P, Wang H-L. Collagen membranes: A review. *J Periodontol* 2001;72:215-229.
46. Pachence JM, Berg RA, Silver FH. Collagen: Its place in the medical device industry. *Med Device Diag Ind* 1987;9:49-55.
47. Doillon, CJ, Silver FH. Collagen-based wound dressing: Effects of hyaluronic acid and fibronectin on wound healing. *Biomaterials* 1986;7:3-8.
48. Noubissi SS, Lozada JL, Boyne PJ, et al. Clinical, histologic, and histomorphometric evaluation of mineralized solvent-dehydrated bone allograft (Puros) in human maxillary sinus grafts. *J Oral Implantology* 2005;31:171-179.
49. Hatano N, Shimizu Y, Ooya K. A clinical long-term radiographic evaluation of graft height changes after maxillary sinus floor augmentation with a 2:1 autogenous bone/xenograft mixture and simultaneous placement of dental implants. *Clin Oral Implants Res* 2004;15:339-345.