Maxillary Sinus Augmentation Prior to Placement of Endosseous Implants: A Histomorphometric Analysis

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The aim of this prospective study was to histomorphometrically evaluate at various time intervals the mineralization stage and process of an allogeneic-xenogeneic bone graft used in sinus augmentation procedures. One biopsy was taken from 20 patients at either 6, 8, 10, or 12 months after sinus augmentation. Immediately following the biopsy, an endosseous implant was placed into the biopsy site. This protocol provided 4 groups of 5 patients each, based on healing time following sinus augmentation. Using backscattered electron image analysis, the specimens were histomorphometrically analyzed to determine the volume fractions of residual cancellous bone, newly formed bone, soft tissue, bovine hydroxyapatite, and "remineralized" freeze-dried demineralized bone allograft (rDFDBA). "Remineralization" of DFDBA particles was observed in a few areas in all specimens. Polarized light microscopy showed that only the 12-month biopsies had a predominance of lamellar bone formation. The area within the biopsies that represented the residual alveolar ridge consisted of 32.6% ± 8.6% (mean ± SD) of bone. In the grafted area of the biopsies the volume fraction of newly formed bone at 12 months (20.7% ± 8.3%) was significantly higher ($P < .05$, analysis of variance) than at 6 months (8.1% ± 3.0%). There was no statistically significant difference between newly formed bone in the inferior, central, and superior grafted areas in all 4 time intervals. This prospective study indicates that the mineralization process of an allogeneic-xenogeneic sinus graft is incomplete 6 months after the sinus augmentation procedure. New bone formation increased up to 12 months postaugmentation; however, it remained lower than the volume of residual bone.
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Key words: bone formation, bovine hydroxyapatite, demineralized freeze-dried bone allograft, dental implants, histomorphometry, remineralized DFDBA, sinus augmentation

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The utilization of dental implants in the rehabilitation of partially and completely edentulous patients is currently a widely accepted treatment modality.[1,2] Implant reconstruction in the posterior maxilla, however, presents a challenge, both surgically and restoratively. A 7-year life-table analysis revealed that posterior maxillary implants demonstrate significantly higher failure rates than implants in all other sites. Possible explanations for the lower survival rates include insufficient bone quality and quantity.[3]

Increasing the bone quantity available for implant placement may be accomplished by sinus augmentation. Various grafting materials have been used to augment the antral space, including autografts,[4–11] demineralized or mineralized allogeneic bone grafts,[10–12] hydroxyapatite,[9,11,13–16] and com-

posite graft materials.[9,11,15–17] Clinical follow-up studies of implants placed in augmented sinuses revealed success rates ranging between 63% and 100% with autogenous,[4,6,7,11] allogeneic,[11,12] alloplastic,[11,16] and composite[11,16,18] bone grafts.

Despite apparently acceptable short-term success rates, histomorphometric studies that determine the actual amount of new bone formation within bone grafts are essential to provide additional data on the long-term prognosis of implants in grafted sites. Histomorphometric analyses in humans revealed that new bone formation in antral grafts varies considerably depending on bone graft type and healing time.[9,13,15] However, these data are based predominantly on limited case reports. Hence, this study was designed prospectively to histomorphometrically evaluate at various time intervals the mineralization stage and process of an allogeneic-xenogeneic bone graft utilized in sinus augmentation procedures.

Materials and Methods

Patient Selection Criteria. Twenty partially or completely edentulous patients (11 females, 9 males, mean age 55.9 ± 7.9 years) with atrophic posterior maxillae were selected for this prospective clinical study. The patients were examined for medical contraindications, including acute or recurrent sinusitis, uncontrolled systemic diseases, and smoking. Using tomographic images, the bone height in the posterior maxilla was analyzed. The patients were included in the study if less than 5 mm of bone height existed inferior to the maxillary sinus floor and endosseous implant placement was indicated. All subjects had responded to an informed consent, which was approved by the Institutional Review Board for Human Studies at Loma Linda University, California.

Preoperative and Postoperative Medication. Before surgery, patients received 800 mg of ibuprofen (Ohm Laboratories, Franklin Park, NJ) and 2 g of amoxicillin (Novopharm, Toronto, Canada). Following surgery, the patients were prescribed ibuprofen (800 mg 3 times a day for at least 3 days) and amoxicillin (500 mg 3 times a day for 1 week). They were also instructed to rinse with 0.12% chlorhexidine gluconate (Peridex, Procter & Gamble, Cincinnati, OH) 3 times a day for 2 weeks.

Surgical Procedure. The patients were prepared and draped for a standard aseptic procedure, and local anesthesia was administered (Polocaine 2% with 1:20,000 levonordefrin, Astra USA, Westborough, MA). The sinus augmentation procedure followed the technique described by Boyne and James.[4] Briefly, a supracrestal incision was made from the canine or first premolar area and extended posteriorly to the ipsilateral maxillary tuberosity region. Vertical releasing incisions were made in the canine and tuberosity region. Mucoperiosteal flaps were raised to expose the lateral wall of the sinus. A rectangular osteotomy was initially outlined with a #4 round bur, ensuring that the inferior osteotomy was 5 mm above the sinus floor. The osteotomy was completed with hand instrumentation. The superior osteotomy was left intact to allow infracture of the lateral sinus wall. The Schneiderian membrane was carefully elevated within the sinus cavity so that it was completely free inferiorly, anteriorly, posteriorly, and medially. Simultaneously, the lateral sinus graft wall was fractured inwardly. A portion of the antral space was filled with a composite graft consisting of demineralized freeze-dried cortical bone powder (DFDBA) (Musculoskeletal Transplant Foundation, Holmdel, NJ; particle size of 250 to 420 µm) and bovine hydroxyapatite (HA) (Osteograf N, CeraMed, Lakewood, CO; particle size of 225 to 400 µm) mixed in a ratio of 1:1 by volume. The mucoperiosteal flaps were repositioned and sutured with horizontal mattress and single interrupted sutures (Gore-Tex Suture CV-5, WL Gore, Flagstaff, AZ).

Bone Labeling. After 6, 8, 10, and 12 months of healing, the patients were prescribed bone-marking fluorochromes according to the following protocol[19]:

1. Demeclocycline (Declomycin, Lederele Laboratories, Pearl River, NY) 300 mg 4 times a day for 1 day
2. Ten days of unlabeled bone formation
3. Tetracycline hydrochloride (Apothecon, Princeton, NJ) 250 mg 4 times a day for 2 days
4. Five days of unlabeled bone formation
5. Biopsy procedure

By means of the bone labels, the newly formed bone was distinguishable from graft material during the histomorphometric analysis.

Biopsy Procedure. Each patient was randomly assigned to have a single biopsy taken at either 6, 8, 10, or 12 months after sinus augmentation. This protocol provided 4 groups of 5 patients each, based on healing time following sinus augmentation. Prior to the biopsy, tomographic images were taken to determine the bone height between the ridge crest and the newly created sinus floor, as well as the buccopalatal direction of the osteotomy

(Fig 1). The resulting information helped to accomplish a standardized biopsy technique, with the biopsies obtained from the center of the graft buccopalatally and mesiodistally. The biopsy sample (2 mm in diameter) was harvested with a standardized trephine drill, starting from the alveolar crest and ending at the most superior part of the graft. Subsequently, a root-form implant was placed into the biopsy site according to the manufacturer's protocol.

Histologic Processing. The specimens were fixed in 10% buffered formalin, dehydrated in alcohol, and embedded in specialized resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). Initial midaxial sections of 200 µm were made by means of the cutting-grinding system (Exact Medical Instruments, Oklahoma City, OK). The sections were then ground to 40 to 50 µm and used unstained for histomorphometric analysis and light fluorescence microscopy. Subsequently, the sections were reground to 10 to 20 µm and stained with 1% toluidine blue for histologic analysis, including brightfield and polarized microscopic evaluation. Procedures followed routines described by Donath.[20]

Histomorphometric Analysis. The undecalcified sections were analyzed histomorphometrically using backscattered electron image analysis.[21] Before being placed in the scanning electron microscope chamber, the specimen surfaces were plated with gold palladium. Backscattered electron images were obtained in field sizes of 2 mm × 2 mm, digitized as a 256 × 256 array of 8-bit density values, and transferred to a microcomputer. Volume fractions of the following tissue components were computed based on differences in optical density (tissue components are listed in decreasing order of density):

- Bovine HA
- Residual alveolar bone
- "Remineralized" DFDBA (rDFDBA)
- Newly formed bone
- Soft tissue

It should be noted that soft tissue includes DFDBA, connective tissue, and vascular and bone marrow spaces (Fig 2). "Remineralization" of DFDBA was confirmed by backscattered imaging and light microscopy. Particles were considered to be rDFDBA if their optical density was between the density of bovine HA and newly formed bone and if brightfield and/or fluorescence microscopy revealed empty lacunae and no osteogenic activity within the particles.

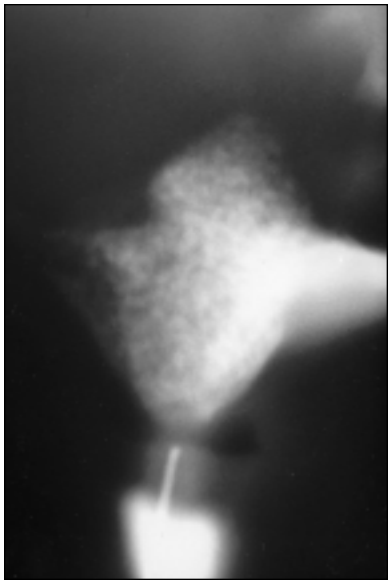

Fig 1 Tomographic image of the posterior maxilla following sinus augmentation; bone height and buccopalatal biopsy direction are determined using a guttapercha point.

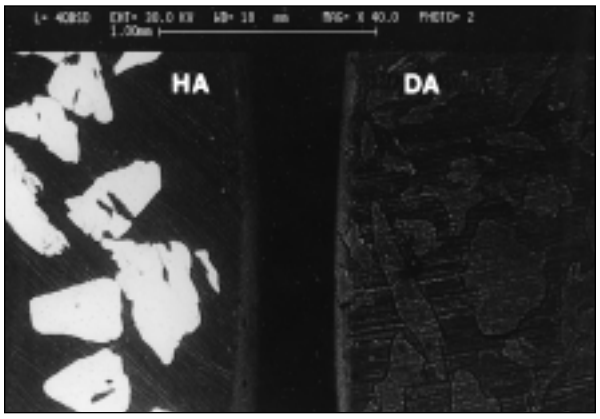

Fig 2 Backscattered electron image of bovine hydroxyapatite (HA) and original unimplanted DFDBA (DA). The particles of DFDBA appear black, indicating the lack of mineralized tissue, whereas bovine HA particles are represented by white zones, revealing highly mineralized tissue (original magnification ×40).

To evaluate the mineralization process of the sinus graft, the grafted region of each specimen was divided into 3 equally sized areas to determine tissue components at the inferior, center, and superior levels of the biopsies.

Statistical Methods. Group means and standard deviations were calculated for each measured parameter. Differences between evaluation groups and between different biopsy areas were

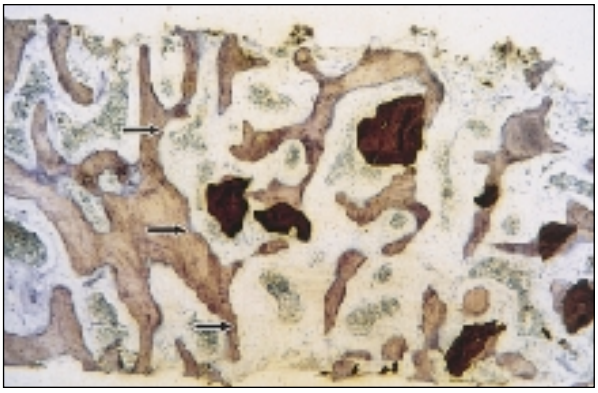

Fig 3a Photomicrograph of 10-month biopsy. Arrows indicate the original maxillary sinus floor. The grafted area of the biopsy *(right)* consists of newly formed bone, bovine HA particles, remineralized DFDBA particles, and bone marrow spaces (toluidine blue stain, original magnification ×10).

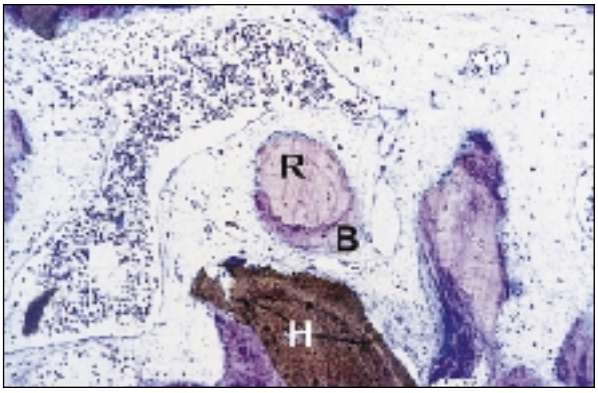

Fig 3b Higher magnification of the specimens shown in Fig 3a. Newly formed bone (B) surrounds bovine HA (H) and remineralized DFDBA particles (R) (toluidine blue stain, original magnification ×20).

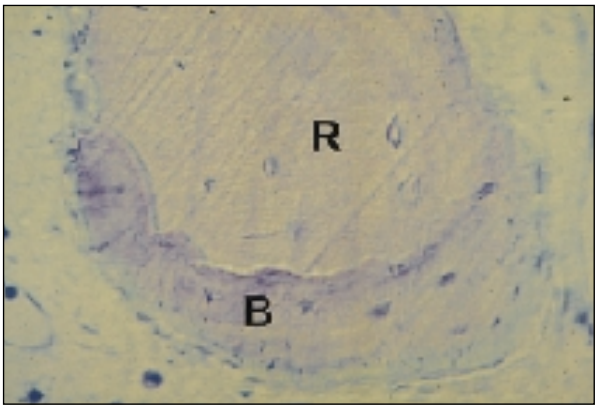

Fig 3c High-power photomicrograph of the biopsy shown in Fig 3a. The newly formed bone (B) partially surrounding the remineralized DFDBA particle (R) reveals nuclei containing lacunae, whereas the lacunae within the remineralized DFDBA particle are empty (toluidine blue stain, original magnification ×100).

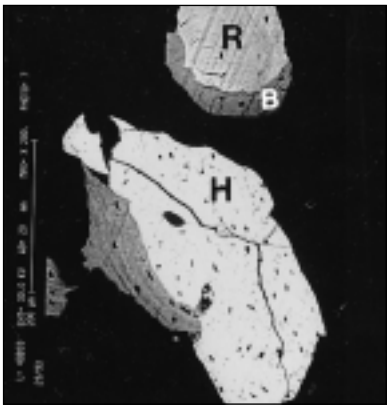

Fig 3d Backscattered electron image of the specimen shown in Fig 3a. The remineralized DFDBA particle (R) reveals a density that is higher than that of newly formed bone (B) but lower than the density of bovine HA (H) (original magnification ×200).

analyzed using 2-way analysis of variance (ANOVA) with post-hoc Tukey test for paired comparisons. A P value < .05 was chosen for statistical significance.

Results

Histologic Observations. In the grafted area of the biopsies, new bone formation was seen against bovine HA particles at all 4 time intervals. With increased healing time, the new bone formed a mosaic of bovine HA and remineralized DFDBA particles connected by cancellous bone (Fig 3a). The remaining spaces between the bovine HA, newly formed bone, and rDFDBA consisted of loose connective tissue with a low cellular content, marrow, and vascular spaces.

The phenomena of "remineralization" of DFDBA particles was observed in a few areas in all specimens. These rDFDBA particles were partially or completely surrounded by osseous tissue, either with intact surfaces or well-integrated into the newly formed bone with indistinct borders (Fig 3b). Higher levels of magnification revealed empty lacunae within rDFDBA particles, but lacunae containing nuclei were evident within the newly formed bone adjacent to the allograft particles, indicating viable bone (Fig 3c). These findings

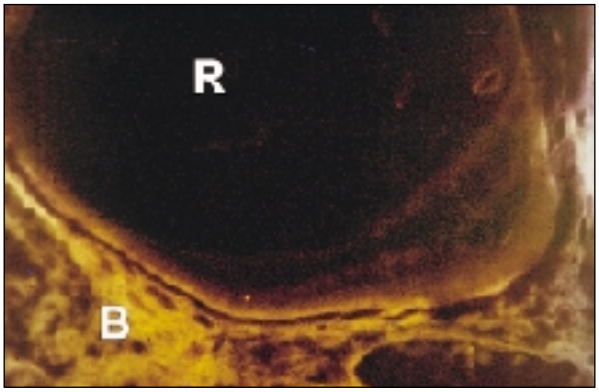

Fig 4 Fluorescent photomicrograph. Distinct and diffuse demeclocycline and tetracycline labels are seen within newly formed bone (B) but not within the remineralized DFDBA particle (R) that it surrounds (original magnification ×100).

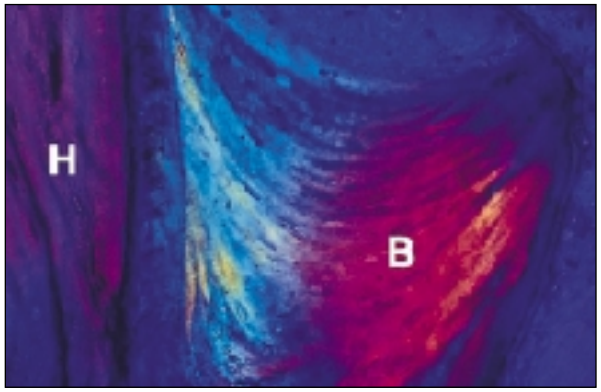

Fig 5 Polarized light photomicrograph of 12-month biopsy. New bone formation (B) attached to the bovine HA particle (H) shows concentric rings characteristic of lamellar bone (original magnification ×100).

were confirmed by fluorescence microscopy. Fluorescent photomicrographs showed diffuse or distinct demeclocycline and/or tetracycline labels only within the newly formed bone. No such evidence of osteogenic activity was observed within the rDFDBA (Fig 4). Backscattered imaging revealed the density of rDFDBA particles to be between the density values of bovine HA and newly formed bone, thus further indicating remineralization of the original DFDBA (Fig 3d). Since backscattered imaging did not reveal any mineralized tissue within the original, unimplanted DFDBA particles (Fig 2), implanted non-remineralized DFDBA was indistinguishable from the surrounding connective tissue during histologic examination.

Polarized light microscopy showed that the 6-, 8-, and 10-month biopsies consisted of mixed woven and lamellar bone adjacent to the bovine HA and rDFDBA particles. In the 12-month biopsies, however, predominantly lamellar bone was seen (Fig 5). The area within the biopsies representing the residual alveolar ridge consisted of lamellar bone in a loose trabecular structure.

Histomorphometric Observations. The biopsy area representing the residual alveolar process was analyzed in 17 of the 20 biopsies. The remaining 3 biopsies were devoid of residual alveolar ridge and thus were not included in this analysis. The residual ridge consisted of 32.6% ± 8.6% (mean ± SD) bone and 67.4% ± 8.6% soft tissue. The grafted area of the biopsies was analyzed in all 20 biopsies. The volume fraction of newly formed bone at 12 months (20.7% ± 8.3%) was significantly higher ($P < .05$, ANOVA) than at both 6 and 8 months (8.1% ± 3.0% and 9.0% ± 3.8%, respectively) (Table 1; Figs 6 and 7). Values for bovine HA, rDFDBA, and soft tissue at different time intervals varied but did not reveal any significant differences.

With regard to the mineralization process of the graft, the newly formed bone did not differ significantly between the inferior, center, and superior parts of the grafted area of the biopsies at all 4 time intervals (Table 2).

Discussion

This study evaluated bone formation at various intervals following sinus augmentation with a composite allogeneic-xenogeneic graft material. In the grafted area of the biopsies, new bone formation at 12 months after sinus augmentation was significantly greater than at 6 and 8 months after augmentation. The area representing the residual alveolar ridge, however, consisted of more bone than the grafted area at any time interval.

To date, the ideal bone graft for alveolar reconstruction procedures has not been determined. The autograft remains the most effective material, because it provides key processes for bone regeneration: osteoconduction, osteoinduction, and osteogenesis. However, only extraoral donor sites offer the volume of bone that is required for sinus augmentation procedures. Hence, the benefits of autografts are restricted because of limited donor sources and associated morbidity. As a result, it would be desirable to use a readily available, safe, and effective substitute for autografts. The results of this study of allogeneic-xenogeneic bone graft material showed a volume fraction of 21% of newly formed bone 12 months after sinus augmentation.

Table 1 Mean Volume Fractions of Bovine Hydroxyapatite, Newly Formed Bone, Remineralized DFDBA, and Soft Tissue Within Grafted Area of Biopsies at Various Time Intervals Following Sinus Augmentation

Time	bHA (%)	NB (%)	rDFDBA (%)	ST (%)
6 mo	21.6 ± 4.2	8.1 ± 3.0	6.4 ± 4.7	63.9 ± 5.9
8 mo	19.3 ± 8.6	9.0 ± 3.8	3.0 ± 1.5	68.6 ± 8.9
10 mo	12.6 ± 7.3	11.8 ± 2.4	3.5 ± 1.9	72.2 ± 4.9
12 mo	20.9 ± 9.1	20.7 ± 8.3*	2.9 ± 1.7	55.5 ± 11.1

n = 20.
bHA = bovine hydroxyapatite; NB = newly formed bone; rDFDBA = remineralized DFDBA; ST = soft tissue.
* Significantly higher than at 6 and 8 months following sinus augmentation ($P < .05$).

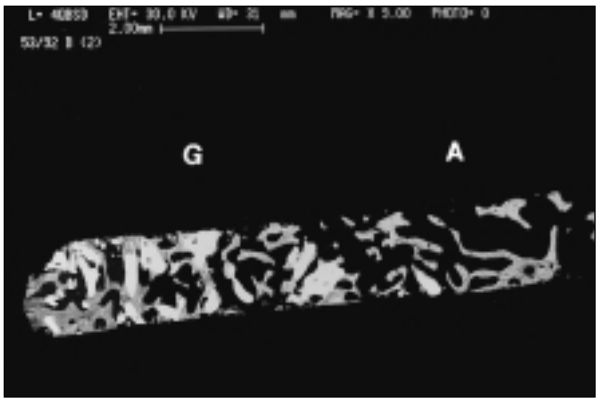

Fig 6 Backscattered electron image of 12-month biopsy. In the grafted area of the biopsy (G), the grey zones represent newly formed bone, whereas white zones are bovine HA particles. The grey zones within the residual alveolar ridge (A) represent original bone (original magnification ×9).

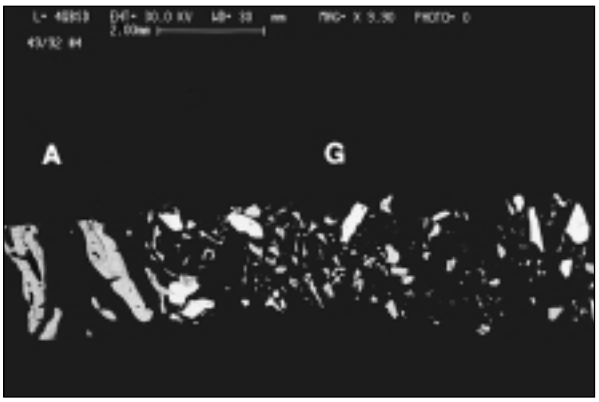

Fig 7 Backscattered electron image of 6-month biopsy. Compared to the 12-month biopsy in Fig 6, fewer grey zones representing new bone formation are visible between the bovine HA particles within the grafted area of the biopsy (G) (original magnification ×10).

Table 2 Mean Volume Fractions of Newly Formed Bone Within Grafted Area of Biopsies at Various Biopsy Levels Following Sinus Augmentation

	Biopsy level		
Time	Inferior (%)	Center (%)	Superior (%)
6 mo	9.7 ± 3.7	7.4 ± 1.2	4.5 ± 2.7
8 mo	6.5 ± 1.8	8.4 ± 1.9	12.2 ± 4.9
10 mo	12.4 ± 3.0	12.8 ± 2.7	10.4 ± 2.3
12 mo	18.0 ± 5.0	20.6 ± 5.2	22.0 ± 14.3

Other human histomorphometric studies on antral grafts have evaluated autogenous bone with or without HA and HA with or without DFDBA. New bone formation varied between 5% and 59%.[9,13,15] As a result of variations in bone graft type, healing time, and execution, direct comparisons with the present study are difficult to make. Instead, intraindividual comparisons should be emphasized, comparing bone formation within the augmented site to that of the residual ridge. In this study, less bone was observed in the grafted area than in the remaining alveolar process. Hence, the allogeneic-xenogeneic bone graft substitute used in this investigation appears to have limited potential. Further studies are required to ascertain the minimum bone ingrowth in grafted sites that is required to function as the bony bed for long-term successful dental implants.

The results of this study revealed the phenomenon of "remineralization" of implanted DFDBA. Because of processing, DFDBA consists of an organic matrix without any mineralized tissue.[22] The lack of mineralized tissue components with DFDBA was further confirmed by analyzing unimplanted DFDBA. Following the implantation of DFDBA into the maxillary sinus, all biopsies showed mineralized areas without any osteogenic activity adjacent to newly formed viable bone. These indicate zones of remineralized DFDBA, since analytic methods, including backscattered electron imaging and brightfield and fluorescence

microscopy, were applied to differentiate the remineralized DFDBA from bovine HA and newly formed bone. The mechanism of this remineralization process is unclear. The fact that remineralized DFDBA was always partially or completely surrounded by newly formed bone suggests that the attached osseous tissue might function as a mineral reservoir during remineralization. This finding is supported by other researchers.[23] Whether or not the remineralization of DFDBA has any clinical significance is uncertain, considering that rDFDBA is nonviable bone and its volume fraction within the augmented area 12 months after augmentation averaged only 3%.

The present study also investigated the mineralization process within the antral graft by analyzing the newly formed bone at different biopsy levels. The results indicated no significant difference between the inferior, center, and superior biopsy areas at all 4 time intervals. Within the limits of the study, these findings may indicate that mineralization occurs simultaneously from the original sinus floor as well as the sinus walls. Hence, newly formed bone is found in similar amounts in the areas of the new and original sinus floors. In contrast, others have suggested that most new bone was formed close to the original sinus floor.[24,25] Further controlled studies are needed to elucidate the mineralization process within the augmented maxillary sinus.

Conclusions

The present study provides evidence for bone formation in the maxillary sinus following implantation of a composite allogeneic-xenogeneic bone graft. However, there was less bone in the sinus graft 12 months after augmentation than in the residual alveolar ridge. Further studies are necessary to ascertain the minimum bone ingrowth in grafted sites that is required to function as a bony bed for the long-term success of dental implants.

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