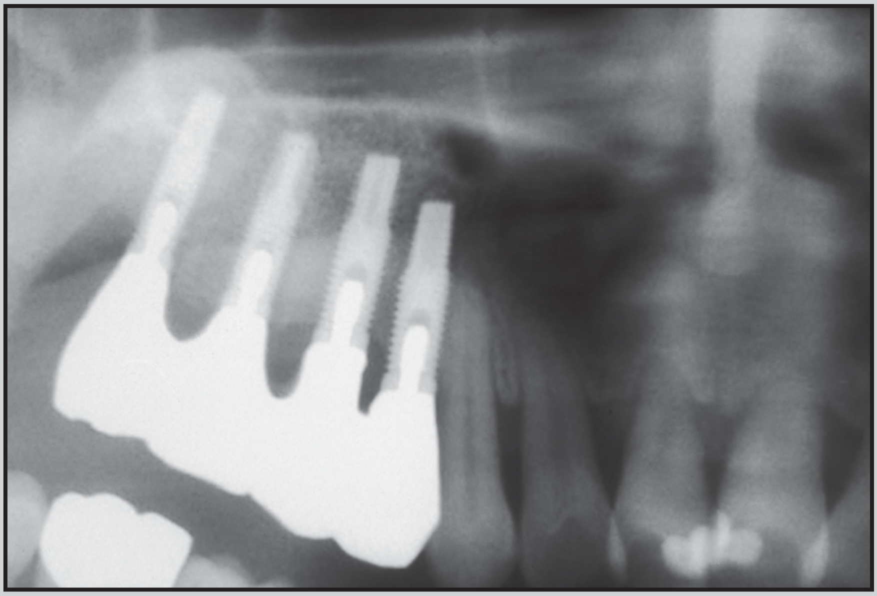
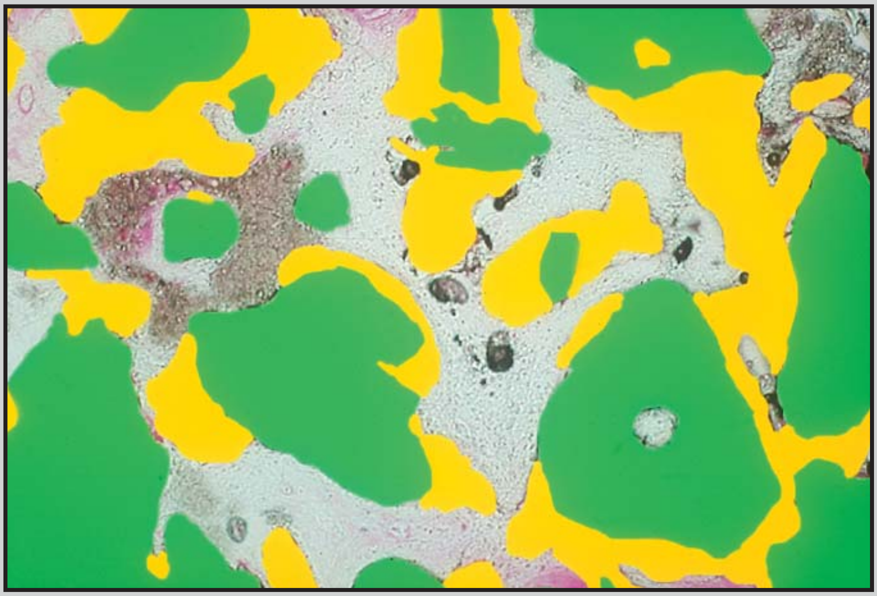
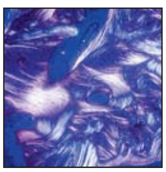




Bone Reaction to Bovine Hydroxyapatite for Maxillary Sinus Floor Augmentation: Histologic Results in Humans



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This study was designed to examine the sequential progress of healing, at two different time intervals, following delayed sinus augmentation using bovine hydroxyapatite (BHA) as the sole grafting material. Fourteen pairs of bone biopsies were taken from 10 patients after 6 and 12 months of healing, respectively. The biopsy specimens were examined histologically and histomorphometrically. The bone that was formed following sinus augmentation with BHA increased and matured over time up to 12 months after grafting; meanwhile, no overt signs of resorption of BHA were visible within the study period. (Int J Periodontics Restorative Dent 2006;26:471–481.)

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Insufficient bone volume and poor bone density are common problems in edentulous patients with severely resorbed maxillae. One method that makes implant placement possible in such difficult situations is the augmentation of the maxillary sinus using various bone graft materials. The sinus floor augmentation procedure was pioneered and developed by Tatum in the mid-1970s, with his results reported in 1986.[1] However, Boyne and James[2] were the first to publish their clinical findings, in 1980. Modifications of the technique have been suggested by other authors.[3,4] The technique consists of preparation of a window in the buccal sinus wall, medial rotation of the bony wall in conjunction with elevation of the schneiderian membrane, and augmentation of the resulting cavity with autogenous bone and/or other grafting material(s). This procedure can provide increased bone volume and height to aid in primary stabilization of one or more endosseous implants.

Bovine hydroxyapatite (BHA) (Bio-Oss, Geistlich Biomaterials) is a bone substitute that has been investigated extensively as a bone graft material in

Table 1 Patient population data

Patient	Age/sex	Location(s) of implant*	Location(s) of biopsy*
CAS	52/M	26, 27	26
CSB	47/F	14, 15, 16, 17, 25, 26, 27	15, 16, 26
LMK	40/F	14, 15, 16, 17	15, 16
LJK	56/M	25, 26, 27	26
LNJ	44/M	16, 17	16
LSH	41/M	16, 17	16
LYH	62/F	16, 17	16
PJH	63/M	26, 27	27
PTK	49/M	15, 16, 17	16
SYD	62/M	15, 16, 17	16, 17

*FDI tooth numbering system. 14 = maxillary right first premolar; 15 = maxillary right second premolar; 16 = maxillary right first molar; 17 = maxillary right second molar; 25 = maxillary left second premolar; 26 = maxillary left first molar; 27 = maxillary left second molar.

animals[5–9] and humans.[10–14] This material has also shown promising results for augmentation of the maxillary sinus.[15–30] It has been proven to be osteoconductive and biocompatible. However, because ethical considerations have generally limited biopsy samples to one per implant, quantitative evaluation of the long-term development of new bone and resorption of BHA has not been done but has been restricted to the actual tissue conditions at the time of the biopsy. In the literature, the resorption of BHA is the subject of controversy. McAllister et al[27] reported that BHA grafted in maxillary sinuses of chimpanzees appeared to be resorbed and replaced by vital bone at the rate of regular bone remodeling for up to 1.5 years. Fugazzotto[30] reported that specimens examined 12 to 13 months postoperatively demonstrated almost complete resorption of BHA in cases of sinus grafting in humans. Meanwhile, other investigators[20,22–24] were unable to find any evidence of BHA resorption.

In an attempt to address questions regarding the resorption rate of BHA and concomitant changes in bone density, this study was designed to examine the sequential progress of healing, at 6 and 12 months after grafting, within individual patients after sinus augmentation with BHA as the sole graft material and implant placement 6 months later.

Method and materials

Ten partially edentulous patients, who ranged in age from 40 to 63 years (average 51.6 years), were free of systemic disorders, and required sinus augmentation, participated in this study (Table 1). Preoperative assessment by orthopantomography showed that all participants had insufficient residual height in the posterior maxillary alveolus (on average 2.25 mm) for simultaneous placement of implants. Sinus floor augmentation was carried out bilaterally in one patient and unilaterally in nine of the participants. Thirty implants were surgically placed in 11 grafted sinuses in 10 patients.

All procedures were fully explained, and patients gave written consent to treatment. The Clinical Research Institute of Seoul National University Hospital approved the study protocol.

Surgical procedure

All surgical procedures were completed by the same surgeon under local anesthesia. The techniques described by Tatum[1] and Boyne and James[2] were applied. Figures 1a to 1e demonstrate the steps of the procedure.

The lateral osseous wall of the sinus was exposed with a vertical releasing incision by an extensive mucoperiosteal buccal flap in the edentulous posterior maxilla. A bony window into the sinus was created with a round carbide bur under copious irrigation with sterile saline solution. The bony window was rotated medially as the schneiderian membrane was detached. The technique was used to lift the sinus mucosa in a cranial direc-

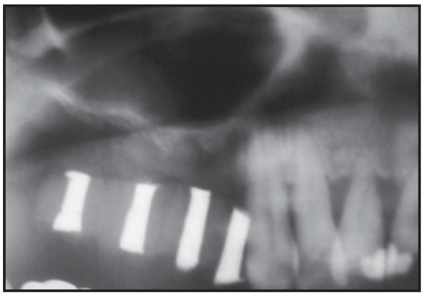


Fig 1a *Preoperative radiograph of a patient scheduled for sinus augmentation.*

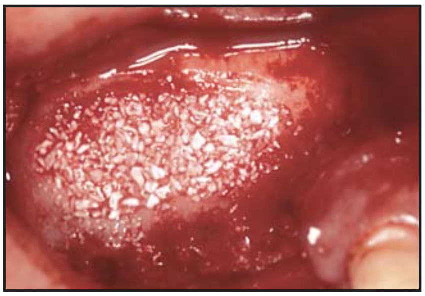


Fig 1b *Augmentation of the sinus floor with BHA.*

Fig 1c *Radiograph obtained after sinus augmentation procedure.*

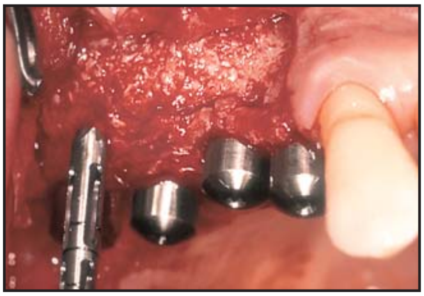
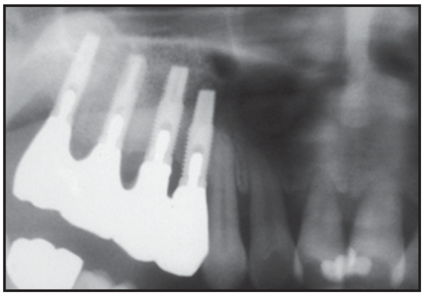


Fig 1d (left) *A second bone biopsy is taken at the time of implant exposure (12 months after BHA grafting).*

Fig 1e (right) *Radiograph obtained after prosthetic treatment.*



tion. The cavity thus created was filled with BHA (Bio-Oss). The augmentation material was compacted, and a resorbable collagen membrane (BioGide, Geistlich Biomaterials) was used to cover the buccal maxillary sinus wall defect and to prevent soft tissue from growing into the augmented region. The mucoperiosteal flap was repositioned and closed using 4-0 nylon sutures.

During the postoperative phase, patients were treated with systemic antibiotic therapy (amoxicillin/clavulanate potassium 375 mg three times daily) and anti-inflammatory analgesics (ibuprofen 400 mg three times daily) for 7 days. Patients were instructed to rinse twice daily with 0.1% chlorhexidine digluconate solution for 2 weeks. Patients were also instructed to avoid wearing their removable prosthesis and to refrain from blowing their noses for 2 weeks. Sutures were removed 14 days after surgery.

Following 6 months of healing, implants were surgically placed in the posterior maxilla in all patients. During this surgery, bone biopsies were obtained using a trephine bur (ACE Surgical Supply) with an inner diameter of 2 mm and an outer diameter of 2.8 mm. Implants (Restore RBM, Lifecore Biomedical) were placed into the osteotomy sites created by the biopsy sampling. The implants were submerged under primarily closed mucoperiosteal flaps. After an additional 6 months of healing (12 months after BHA grafting), the implants were uncovered. Mucoperiosteal flaps were raised to expose the window that had been prepared at the initial surgery. The second biopsy samples were obtained at sites parallel to the longitudinal axes of implants, about 2 to 3 mm lateral to the implants (see Fig 1d). The osteotomy defects created were filled with BHA. A total of 14 pairs of bone biopsies at 30 implant sites were obtained for histologic sectioning, which represented 6- and 12-month serial sections obtained extremely close to each other.

Histology

Histologic sections were prepared according to the technique described by Donath and Breuner.[31] The trephine burs with the intact bone cores were immersed in formalin, rinsed with

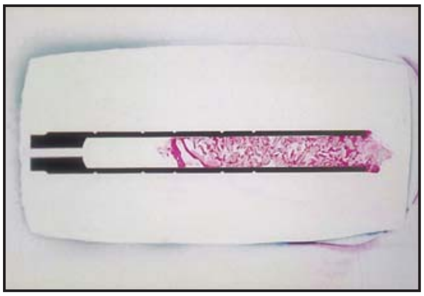


Fig 2 *Gross specimen: undecalcified ground section, including the trephine bur used to obtain the biopsy, with removed bone sample. The inner diameter of the trephine bur is 2 mm.*

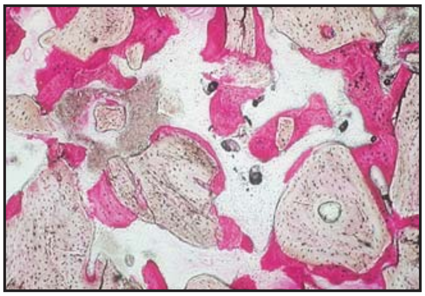


Fig 3a *Initial histologic finding (Multiple staining; original magnification × 25).*

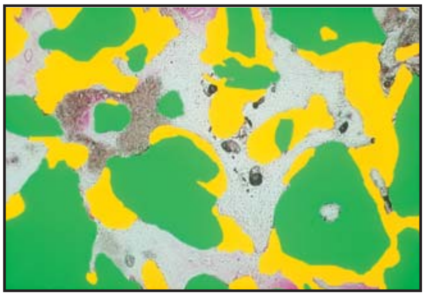


Fig 3b *Computerized image of Fig 3a, showing the histomorphometric markings of BHA particles* (green) *and newly formed bone* (yellow).

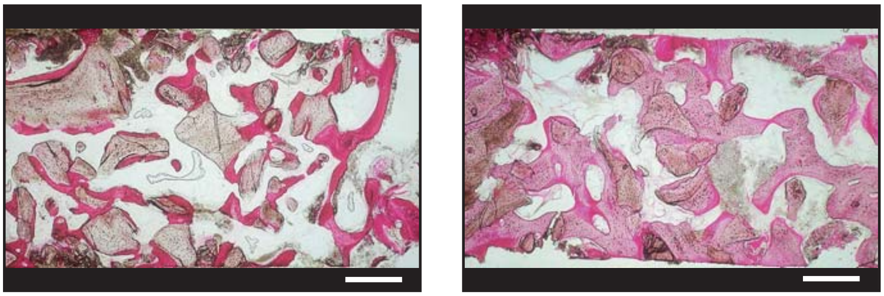
water, dehydrated, and embedded in super-low-viscosity embedding medium (Polysciences). Undecalcified ground sections were prepared using the Exakt cutting and grinding system (Exakt Apparatebau). The embedded specimens were mounted on acrylic glass slabs and sectioned longitudinally using a diamond saw. The sections were then ground and polished to a final thickness of 30 μm. Finally, the thin sections (Fig 2) were stained with Multiple stain (Multiple Stain Kit, Polysciences). The sections were examined under a light microscope with the aid of polarized light.

Histomorphometry

Conventional microscopic examination was followed up with computer-assisted histomorphometric analysis (Figs 3a and 3b). Measurements of newly formed bone, graft particles, and soft tissue were obtained using an automated image analysis system (Image Access Application, Bildanalyssystem) coupled with a video camera on a light microscope.

Because the visual field remained at a defined size of 1 mm^2, the software was able to calculate the proportions (%) of graft material and newly formed bone. Through differential calculus, the proportion of soft tissue over the entire surface was measured. The total perimeter of BHA particles and the portion of the perimeter of BHA particles in contact with bone tissue were also measured, and the degree (%) of bone-BHA contact was calculated. Two sections were obtained from each biopsy. Three visual fields were randomly selected from each section. Then, a total of six visual fields from each biopsy were measured. Regions in which residual bone was included in the sample core were excluded from histomorphometric analysis.

The Wilcoxon signed-rank test was used to compare the histomorphometric parameters. Statistical significance of the differences was confirmed with a P value below .01.



Figs 4a and 4b *Gross findings after 6 months* (a, left) *and 12 months* (b, right) *of graft healing. The 12-month specimen* (b, right) *shows a denser, thicker trabecular pattern than the 6-month specimen* (a, left). *Upper and lower black lines enclosing the tissue cores represent the trephine bur (undecalcified section, Multiple staining; bar = 500 µm).*

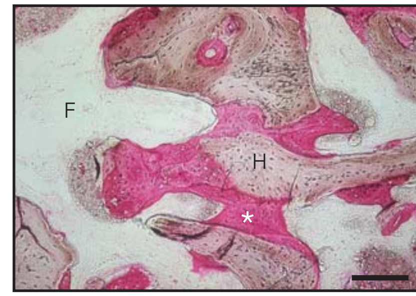
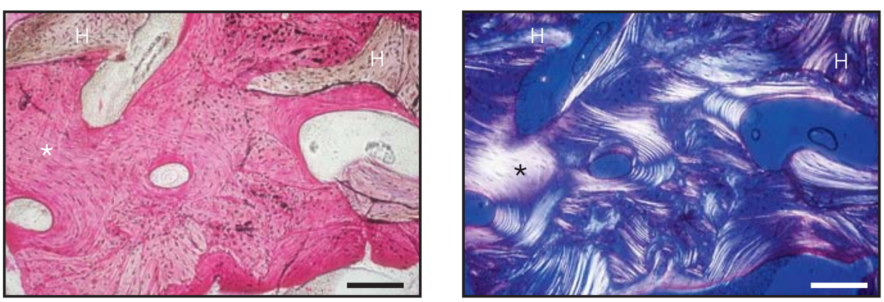


Fig 5 *Detail of a 6-month biopsy. BHA particles (H) were directly connected to the newly formed bone* (asterisk). *The bone was mainly woven, with some more mature lamellar bone apparent. The soft tissue between the trabeculae and the graft material resembled bone marrow tissue, which contained fat cells (F), various forms of fibroblasts, collagenous fibers, and blood vessels (undecalcified section, Multiple staining; bar = 200 µm).*



Figs 6a and 6b *Detail views of a 12-month biopsy.* (a, left) *The bone consisted mainly of lamellar bone. BHA particles* (H) *were fully embedded and integrated with the newly formed bone* (asterisk) *and had become interconnected through trabecula formation.* (b, right) *Photograph under polarized light. Characteristic birefringency demonstrates mature lamellar bone (undecalcified section, Multiple staining; bar = 200 µm).*

Fig 7 *Details of a 12-month biopsy. BHA particles (H) were fully embedded in mature lamellar bone* (asterisk). *No overt signs of resorption of the graft particles were visible (undecalcified section, Multiple staining; bar = 100 µm).*

Results

Clinical observations

Surgical outcomes were uneventful. There were three cases of nosebleeds following sinus grafting, but these did not result in lingering consequences. All 30 implants were stable and well integrated, both clinically and radiographically. Six months after they were placed, all implants were uncovered, restored, and loaded with fixed, implant-supported prostheses.

Histologic observations

Newly formed bone was evident in all augmented sites (Figs 4 to 7). The specimens consisted of newly formed bone tissue, remaining bovine bone particles, loose connective tissue, and occasional areas of fat cells. Graft particles were embedded in a mixture of woven and lamellar bone. Individual particles of BHA were easily identified, even in the 12-month specimens, because of their staining intensity and morphologic appearance, which included

Table 2 Histomorphometry of sinus biopsies following BHA grafting after 6 and 12 months of healing

		6 months				12 months			
Patient	Site†	Bone (%)	BHA (%)	Soft tissue (%)	Bone-BHA contact (%)	Bone (%)	BHA (%)	Soft tissue (%)	Bone-BHA contact (%)
CAS	26	18.0 ± 4.3	32.8 ± 12.9	49.2 ± 8.8	31.3 ± 8.1	31.5 ± 3.5	30.5 ± 9.6	38.0 ± 9.9	45.3 ± 4.4
CSB	15	14.2 ± 3.1	18.7 ± 4.2	67.1 ± 4.6	24.7 ± 3.6	23.7 ± 2.6	18.8 ± 7.7	57.5 ± 7.4	32.8 ± 2.6
	16	17.2 ± 2.9	29.3 ± 3.0	53.5 ± 4.6	31.4 ± 8.4	24.5 ± 2.7	28.3 ± 3.2	47.2 ± 3.3	38.3 ± 4.9
	26	18.7 ± 2.8	32.1 ± 6.3	49.2 ± 6.4	39.9 ± 4.5	27.1 ± 1.7	31.1 ± 6.1	41.8 ± 6.1	47.1 ± 7.2
LMK	15	16.7 ± 2.5	36.2 ± 11.0	47.1 ± 11.7	34.0 ± 6.9	23.9 ± 5.8	36.1 ± 7.0	40.0 ± 7.0	36.7 ± 6.2
	16	18.1 ± 6.7	39.4 ± 4.5	42.5 ± 6.9	38.8 ± 3.1	27.8 ± 2.4	38.9 ± 8.9	33.3 ± 8.4	43.7 ± 3.5
LJK	26	15.3 ± 2.5	31.3 ± 14.2	53.4 ± 11.9	26.3 ± 3.1	22.2 ± 3.6	29.0 ± 4.2	48.8 ± 7.6	40.7 ± 7.8
LNJ	26	14.9 ± 4.4	35.3 ± 10.3	49.9 ± 13.3	25.7 ± 4.6	17.9 ± 4.0	31.3 ± 2.9	50.8 ± 5.7	40.0 ± 2.3
LSH	26	31.3 ± 4.9	24.1 ± 9.8	44.6 ± 9.6	40.1 ± 2.9	41.9 ± 2.7	22.8 ± 6.6	35.3 ± 7.8	57.9 ± 5.2
LYH	16	17.7 ± 3.7	22.6 ± 5.3	59.7 ± 7.8	30.7 ± 6.4	23.0 ± 2.3	22.4 ± 6.8	54.6 ± 4.8	47.3 ± 5.5
PJH	27	12.3 ± 2.4	32.2 ± 2.0	55.5 ± 4.1	22.1 ± 3.3	18.8 ± 4.1	32.2 ± 2.3	49.0 ± 5.3	33.3 ± 6.3
PTK	16	29.1 ± 3.9	24.2 ± 5.6	46.7 ± 7.5	43.2 ± 3.1	36.6 ± 7.6	25.7 ± 4.8	37.7 ± 10.7	53.0 ± 6.7
SYD	16	17.6 ± 3.3	31.9 ± 4.0	50.4 ± 5.9	31.8 ± 2.2	26.8 ± 3.2	28.8 ± 4.0	44.4 ± 5.4	32.3 ± 8.3
	17	14.4 ± 3.3	27.0 ± 4.0	58.6 ± 3.2	24.5 ± 4.3	27.1 ± 6.9	25.5 ± 3.1	47.5 ± 4.6	42.0 ± 5.4
Mean ± SD		18.3 ± 5.4	29.8 ± 5.8	52.0 ± 6.6	31.8 ± 6.7	26.6 ± 6.5*	28.7 ± 5.4^{NS}	44.7 ± 7.3*	42.2 ± 7.6*

All figures are expressed as mean ± standard deviation (n = 6).
*$P < .01$ = significantly different from 6 months (Wilcoxon signed rank test); $^{NS}P > .01$ = no significant difference between 6 and 12 months.
†FDI tooth numbering system. 15 = maxillary right second premolar; 16 = maxillary right first molar; 17 = maxillary right second molar; 26 = maxillary left first molar; 27 = maxillary left second molar.

sharp edges and an apparent lack of resorption. Neither resorption lacunae nor active osteoclasts were found in specimens at 6 and 12 months.

Specimens at 6 months

At 6 months, some of the BHA particles were surrounded by soft tissue, consistent with morphology of bone marrow tissue, while other BHA particles were directly connected to newly formed bone (see Fig 5). New bone was easily distinguished from BHA particles. Where present, the bone was primarily woven, but more mature lamellar bone was also occasionally observed. New bone apposition seemed to be directly over the surfaces of BHA particles, and the cancellous trabecular pattern of the BHA particles was thought to be serving as a scaffold for new bone growth. However, no specimens showed evidence of resorption, such as osteoclasts or resorption lacunae. The soft tissue between the trabeculae and the graft material was composed of connective tissue (fibroblasts, collagenous fibers, and blood vessels). This tissue showed no signs of inflammation.

Specimens at 12 months

In most specimens, individual particles of BHA were still clearly identifiable and embedded in mature bone. At 12 months, the bone consisted mainly of lamellar bone, which is very well organized (see Figs 6a and 6b). The graft particles were integrated into the newly formed bone, which was observed interconnecting through the particles in a trabecular form. Upon light microscopic examination, no overt signs of resorption of the graft particles were apparent (see Fig 7).

Histomorphometric observations

Table 2 and Fig 8 show the results of the histomorphometric measurements. The average percentage of newly formed bone at 12 months (26.6% ± 6.5%) was significantly higher ($P < .01$) than that seen at 6 months (18.3% ± 5.4%). The proportion of newly formed bone in the sites studied ranged from 12.3% to 31.3% at 6 months and from 17.9% to 41.9% at 12 months. The degree of bone-BHA

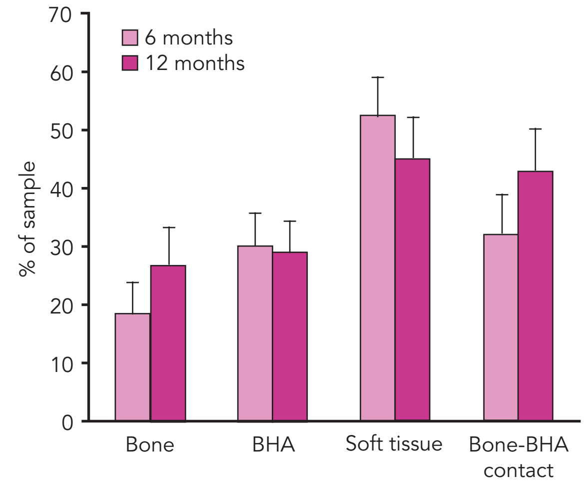


Fig 8 *Histomorphometric findings after grafting with BHA.*

contact at 12 months (42.2% ± 7.6%) was also significantly increased (*P* < .01) over that seen at 6 months (31.8% ± 6.7%). Meanwhile, the average proportion of the sample occupied by BHA particles did not show significant changes between 6 months (29.8% ± 5.8%) and 12 months (28.7% ± 5.4%) (*P* > .01).

Discussion

Histologically, newly formed bone following sinus augmentation with BHA had increased in volume and matured over time up to 12 months after grafting in the present study. However, neither resorption lacunae nor osteoclasts were positively identified during the 12 months of the study period. In addition, histomorphometrically, there was no significant difference in proportion of BHA particles between the 6-month and the 12-month groups. These results suggest that BHA, when used in human maxillary sinus grafting, is not fully resorbed within 12 months.

In the literature, the resorption of BHA has been a controversial subject. Some investigations[6,8,27] have detected evidence indicating resorption of BHA in animal models. McAllister et al[27] observed that the area of new bone increased—from 62% at 7.5 months to 70% at 18 months—whereas the proportion of BHA decreased from 19% to 6%. Based on their results, they suggested that BHA is resorbed and replaced by vital bone. Meanwhile, Schlegel[32] could identify BHA granules, even after a healing period of up to 7 years. Skoglund et al[33] observed remaining BHA histologically 44 months after augmentation of a maxillary alveolar ridge. In human histologic studies following sinus augmentation with BHA, Yildirim and coworkers[22,23] were unable to find any evidence of BHA resorption. Moreover, they suggested that slow resorption—ie, at a rate similar to that of physiologic remodeling—appears to be appropriate when BHA is used in sinus floor augmentation, because rapidly progressing degradation would endanger the stability of the implant site. Valentini et al[20] pointed out that the absence of BHA resorption would not jeopardize osseointegration of implants, since no contact between the graft particles and the implant surface were observed in any of their sections obtained from humans.

In addition to questions regarding resorption of BHA, there are also conflicting reports as to whether bone density following sinus augmentation with BHA increases over time. Haas et al[29] observed an increase in the percentage of bone following sinus augmentation with BHA in a sheep model.

Hanisch et al[15] also reported that new bone formation increased up to 12 months postaugmentation in humans. However, Yildirim et al[22,23] failed to confirm an increase in bone proportion over time in humans. They suggested that bony healing of BHA is mainly influenced by the healing response of the individual patient and is less dependent on the healing time of the augmentation material. These investigators interposed results from different individuals in different time intervals. The present study showed an increase in the percentage of bone in the grafted area and the degree of bone-BHA contact over a 6-month period (from 6 to 12 months) in the same patients, without a concomitant decrease in the proportion of BHA.

Although there was no evidence of BHA resorption, the results of the present study still show that BHA is a suitable grafting material for human sinus augmentation procedures. Uncomplicated integration of BHA particles with newly forming bone was confirmed histologically, together with an impressive 100% survival rate at the time of implant uncovering, supporting its use for human sinus augmentation. Every specimen in this study showed newly formed bone surrounding the BHA particles. The grafting material showed high biocompatibility and a certain amount of osteoconductivity. Graft particles were directly connected to the newly formed bone via a conduction channel. Osteoconductivity, by definition, is when new bone growth is promoted in the environment because of the nature of the graft material. In the histologic sections in this study, most of the mineral particles were surrounded by bone in different levels of remodeling, rather than soft tissue marrow, suggesting new bone ingrowth immediately adjacent to the particles. After 6 months of healing, osseous tissue was mainly woven bone, highly enriched with osteocytes. On the other hand, lamellar bone could be identified and was more prominent in the 12-month specimens.

Many authors have examined the fraction of new bone following grafting of BHA in the sinus area morphometrically. In animal studies, McAllister et al[34] observed that 47% of the studied area was bone in chimpanzees, while Hürzeler et al[28] observed a range of 20% to 30% of area fraction of new bone in rhesus monkeys. Artzi et al[17,21] reported an average of 42.1% bone proportion using BHA particles[17] and an average of 34.2% of bone area using BHA block grafts[21] at 12 months after grafting in human sinuses. The present study observed a lower percentage of bone area than the cited investigations. The average proportion of newly formed bone obtained in this study, by percentage, was 18.3% at 6 months and 26.6% at 12 months. These values were comparable with the 6-month results of Yildirim and coworkers (14.7%)[22] and the 12-month results of Valentini et al (27.5%).[20] These differences may be explained by differences in species (human versus nonhuman primate) and by the differing processing methods of the specimen (decalcified versus undecalcified). In the present study, the bone core was undisturbed after harvesting, and undecalcified sections were prepared with a trephine bur and kept intact with the bone to minimize the dispersion of bone tissue and therefore any possible dimensional changes. Yildirim and coworkers[22,23] used the same technique.

In this study, the authors used BHA alone without any autogenous bone in sinus augmentation. The biggest advantage of using only a bone substitute is obvious—no additional donor site is needed for harvesting of autogenous bone. However, the question still remains as to what role autogenous bone plays in the healing process and whether it can be completely replaced with a substitute. Recently, to address this question, Hallman et al[24] evaluated implant integration in the posterior maxilla after sinus floor augmentation with autogenous bone, BHA, or a 20:80 mixture of the two. Their histomorphometric analysis showed no differences in both new bone area and bone-implant contact between three groups, indicating that autogenous bone graft can be substituted with BHA—up to 80% or even 100%—for sinus floor augmentation. Their result suggests that the effect of adding autogenous bone remains unclear. Artzi et al[17,21] reported that all implants placed in sinuses augmented with 100% BHA particles[17] or BHA block only[21] were clinically successful. The present study also indicated that all implants placed in sinuses augmented with BHA alone were 100% clinically successful to the time of prosthetic loading. This result may suggest that BHA alone can be used as a substitute for autogenous bone for sinus augmentation.

In summary, within the limits of this study, the results demonstrate that: *(1)* clinically, implant placement in 100% BHA in sinus augmentation procedures resulted in predictable integration; *(2)* histologically, newly formed bone following sinus augmentation with BHA had increased in volume and matured over time up to 12 months after grafting; and *(3)* no overt signs of resorption of BHA were visible during the 12 months of the study period.

Acknowledgments

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