Histopathological Morphometric Evaluation of 2 Different Hydroxyapatite-Bone Derivatives in Sinus Augmentation Procedures: A Comparative Study in Humans

Zvi Artzi,* Carlos E. Nemcovsky,* Haim Tal,* and Dan Dayan†

**Background:** Xenografts to augment the maxillary sinus have been used extensively. The aim of the present study was to evaluate, qualitatively and quantitatively, two different HA derivatives of natural and synthetic sources on newly formed bone in the augmented sinus.

**Methods:** A bilateral sinus augmentation procedure with simultaneous (16 out of 20 sites) or subsequent implant placement was performed in 10 patients. The antrum was randomly filled with a deproteinized, bovine hydroxyapatite mineral (B-HA) on one side and a non-ceramic resorbable hydroxyapatite (NC-HA) on the other. Cylindrical specimens were harvested from the augmented core at 12 months. Decalcified specimens were sectioned at a cross-horizontal plane and stained with hematoxylin and eosin for histopathologic and histomorphometric examinations. Tissue area fractions of bone, marrow, and the grafted particles were calculated for each specimen from the lateral to the deep region, and changes in values were compared within each material and between them.

**Results:** New bone formation was evident. B-HA and NC-HA particles were observed in all specimens surrounded by newly formed bone in direct connection or by soft tissue marrow. Morphometrically in the B-HA sites, from the lateral to deeper area, bone area fraction increased from 29.8% to 54.2% (average 42.1%) and marrow area fraction decreased from 37.9% to 26.7% (average 33.3%). The mineral area fraction decreased from 32.3% to 19.1% (average 24.7%). All increasing/decreasing patterns were statistically significant ($P < 0.001$). In the NC-HA sites, from the lateral to deeper area, bone area fraction increased from 25% to 36.5% (average 32.3%) and marrow area fraction decreased from 51.6% to 41.9% (average 43.2%) ($P < 0.001$). The mineral area fraction decreased from 29% to 21.7% (average 24.6%) ($P = 0.038$). Comparison between the two HA derivative groups showed a significant difference between the bone area fraction averages ($P = 0.0053$) and between the increasing patterns along the core depth ($P = 0.0006$). There was also a significant difference between the decreasing marrow patterns ($P = 0.003$), but not between their averages. Comparison between the mineral area fractions showed no differences.

**Conclusions:** B-HA and NC-HA were proven to be biocompatible materials. Although the B-HA–augmented sites showed a higher percentage of bone formation at 12 months, both are suitable bone derivatives in sinus augmentation procedures and can accommodate osseointegrated implants. *J Periodontal* 2001;72:911-920.

**KEY WORDS**

Maxillary sinus augmentation; dental implants; hydroxyapatite/therapeutic use; bone regeneration; follow-up studies.

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Fixed maxillary implant reconstruction is a popular procedure in partial and/or total edentulous cases. At the post-extraction phase, the maxillary ridge resorbs physiologically. Since the average bone height in the posterior maxilla does not exceed 10 mm, subantral elevation techniques were introduced to enable fixed implant-supported restorations. This technique is a highly predictable procedure, which focuses on the quality and quantity outcome of the augmented materials and tissues. These serve as the implant tissue housing to which the implant should osseointegrate and by which it is supported. Intraoral autogenous bone, as well as extraoral sources, can be an appropriate donor site to successfully fill the sinus floor recipient sites. However, increasing patient morbidity and cost implicate the need for other sources.

Various osseous graft materials have been extensively used in oral surgery. Hydroxyapatite (HA) derivatives have been used with clinical success. Both bovine porous HA and synthetically produced HA claim to be suitable bone filler materials. A synthetic non-ceramic, slow-resorbing HA (NC-HA) has been reported as a biocompatible material, which successfully serves as a proper scaffold in bone augmentation procedures. These porous crystalline clusters act as a slow-resorbing matrix that allows infiltration of bone-forming cells and the subsequent deposition of host bone.

A different HA source of natural bovine bone (B-HA) was introduced to serve as an appropriate bone substitute in humans. This material has been extensively investigated in the literature and clinical trials have proven the material to be an osteoconductive and biocompatible material in osseous regenerative procedures.

Both B-HA and NC-HA use have been reported in numerous studies in sinus augmentation procedures. However, the functional long-lasting outcome of using B-HA and NC-HA as bone substitute materials still lacks critical histopathological observations.

To our knowledge, there is no histomorphometric study comparing synthetic HA versus natural HA in sinus augmentation in humans. The purpose of this study was to investigate the influence of 2 different porous HA materials, i.e., NC-HA and B-HA, on the formation of osseous tissue at the subantral augmented area, histologically and histomorphometrically. Both the characteristics of the grafted materials and the newly formed bone were evaluated.

MATERIALS AND METHODS
Ten adult patients (5 females and 5 males), ranging in age from 36 to 68 years (average 51 ± 15), with no systemic disorders who required bilateral sinus floor augmentation participated in the study. All patients had bilateral, large, pneumatized sinuses with moderate to severe atrophic posterior maxillae (6 mm or less in height) and inadequate bony housing for implant placement (Fig. 1). A panoramic and computerized tomography (CT) scan in 3 mm serial sections disclosed no pathological signs at the antral spaces.

In 16 sites in which the residual bone was at least 4 mm in height, an immediate approach of implant placement and sinus augmentation was performed simultaneously. In the remaining 4 sites, a staged approach was adopted, where sinus augmentation was carried out first followed by implant placement after 6 months. In each patient, one side was grafted with NC-HA, while B-HA was grafted in the contralateral sinus site.

Hard tissue core specimens were harvested at 1 year post-augmentation at the implant cover screw surgical exposure phase from all 10 pair sites. All procedures were explained, and patients signed consent forms. The study was approved by the Ethics Committee of Tel Aviv University.

Surgical Procedure
The procedures were performed in a sequential manner and randomly (by flipping a coin) on the left or right side of the maxilla, one month apart. Premedication with ketoprofen 100 mg was given 1 hour before surgery. Local anesthesia by buccal and palatal infiltration of 3% lidocaine HCl and 0.04 mg base norepinephrine was administered. Surgical procedures followed the technique described by Boyne and James and Tatum.

The lateral bony antral pneumatized wall was exposed with vertical releasing incisions by an extensive mucoperiosteal buccal flap at the edentulous posterior maxillary region. The contours of the lateral window were demarcated by a 2 mm diameter round
diamond bur, averaging 12 mm (H) by 20 mm (W). The distance between the alveolar crest and the inferior border of the window was measured for subsequent second stage for orientation at the reentry surgical phase. As the Schneiderian membrane was exposed, a broad flat curet was pushed gently between the membrane and the inner bony wall to separate, release, and loosely reflect the membrane. The fractured bony wall was pushed inward and upward, where the superior margin served as a hinge “green stick” or partial fracture of the window. This also eased the membrane reflection.

Once the Schneiderian membrane was elevated, a space was created underneath. This established void was filled with porous hydroxyapatite with either NC-HA in crystalline microporous form, 300 to 400 μ in size, or B-HA, 250 to 1,000 μ in size.

In the 16 sinus sites where implants were simultaneously placed, the grafted material was applied in two portions. The osteotomy implant site was prepared at the residual ridge. Thus, the first portion of the grafted material was applied through the fractured wall orifice. Then, the implants were placed, followed by application of the second portion of the HA particles. Upon passive fill of the site in all cases, an occlusive bioabsorbable membrane made from porcine collagen was applied to cover the entire external augmented site. Before the barrier membrane was applied, dimensions of the lateral window were measured, while the rim of the crestal alveolar ridge served as a reference.

Primary soft tissue closure was achieved using a non-resorbable 4-0 polyviolene suture.** Postoperative systemic antibiotics of 500 mg amoxicillin†† (TID) for 1 week and 275 mg naproxen sodium‡‡ (2 tablets initial dose, thereafter 1 tablet every 6 to 8 hours as needed) were prescribed for analgesia. A solution containing 0.2% chlorhexidine gluconate§§ was used as an antiseptic mouthwash for 45 seconds, twice daily for 2 weeks. Sutures were removed after 14 days. Soft tissue healing was uneventful. Radiographically (Fig. 2), radiopaque mineral particles were observed constantly during follow-up. These filled the site and were dominant by their clear radiopacity. At 12 months, upon surgical exposure of implant cover screws, all implants were stable with no visible crestal bone resorption.

The reflection of the buccal mucoperiosteal flap was apically extended to the location of the lateral sinus access window. The previously fractured elevated window boundaries were located according to previous measurements at first-stage surgery. All specimen cores were taken in the same manner, i.e., from the center of the previous lateral window, which was augmented by the grafted material. By using a 2.5 mm internal diameter trephine bur, a cylindrical bone sample 5 to 6 mm in length was harvested in an inward and upward direction toward the new location of the Schneiderian membrane (Fig. 3). An orthoradial periapical radiograph and the implant cover screws determined the exact position of the fixtures. This would avoid overapproximation of the trephine bur during the harvesting procedures.

Tissue samples were marked with black ink to identify the external end. Before the histological processing, all specimens were trimmed to the same length. Specimens were fixed in 10% neutral buffered formalin for 1 week and then decalcified with 5% formic acid for 2 weeks. The decalcified cylindrical specimens were then embedded in paraffin and transversely cut into serial sec-
tions, 5 µ in width, by a microtome. Each core was cut uniformly from the lateral to deep region. Twelve section cuts were mounted on each histological slide. Histopathologic examination was performed on every fifth slide, which was stained with hematoxylin-eosin (H&E).

**Histomorphometry**

Histomorphometric measurements were carried out on all H&E-stained slides, i.e., every 250 µ along each core specimen. Only preserved round sections were submitted for these examinations. Sections that were partly torn, folded, or ruined (approximately 25% of the sections) were excluded from the study. At least 14 H&E-stained slides were examined from each core specimen.

Analysis was conducted on the 3 tissue area fractions – bone, grafted mineral, and soft tissue marrow. Each tissue area fraction was examined from the most external section cut to the deepest section cut (i.e., section cuts 5 to 70). In addition, the 3 tissue area fractions were also observed by eliminating the overlying guided tissue regeneration membrane environment, thus observing section cuts 20 to 70, i.e., discarding the most externally augmented area of approximately 1.0 mm.

The histomorphometric method is an adaptation of the point-counting procedure. In practice, each section was examined using a projection microscope at ×40 magnification. A 64-square (1.5 cm by 1.5 cm) graticule was superimposed on the screen. Point counting was performed on 3 components in each section: bone, marrow, and the grafted particles (B-HA or NC-HA). Whenever the graticule-square center (marked by a “+”) hit one of the 3 components, the specified component scored 1 point. The sum of the points overlaying each of the specified components (PI) was calculated. Area fraction percentage of each component in each section was evaluated as a part of the whole section area, PI/∑, where ∑ represents the total number of points superimposed on each section.

Statistical analysis was carried out using ANOVA with repeated measures. The level of significance was examined in relation to 3 questions: 1) whether there was a significant difference between the averages of NC-HA and B-HA; 2) whether there was a significant pattern along the depth; and 3) whether this pattern in NC-HA–grafted sites was significantly different from B-HA sites. These questions were examined for each tissue area fraction. Tail probability significant value was considered. A P value of <0.05 was selected for statistical significance.

**RESULTS**

At 12 months post-augmentation, before the prosthetic phase, all sinus-augmented sites healed and all 72 implants were stable and radiographically and clinically integrated. Panoramic radiographs revealed (Fig. 2), by the radiopacity, that the augmented material encircled the implant bodies. There were no complaints of any functional side effects and/or any other inconveniences.

Histologically, new bone formation was evident in all augmented sites. B-HA and NC-HA–grafted particles were observed in all specimens, partly surrounded by connective tissue, while other parts presented a direct connection to the newly formed bone. Osseous tissue was mainly woven bone, highly enriched with osteocytes. Lamellar bone could be identified and was more prominent in the deep area of the core specimens.

**Morphometric Observations**

**B-HA–augmented sites.** Average bone area fraction of the 10 sites was 42.1% (SD = 10.0). From the external to the deeper area (Figs. 4 and 5), bone area frac-
tion ranged from 29.8% to 54.2%. Average marrow area fraction was 33.3% (SD = 14.7). The marrow area fraction of the 10 sites decreased from 37.9% at the external area to 26.7% at the deep side. The average area fraction of the grafted mineral particles was 24.7% (SD = 9.99). It decreased from 32.3% at the external side to 19.1% at the deep side (Table 1). All 3 tissue area fraction increasing/decreasing patterns were statistically significant (P < 0.001) (Fig. 6). The increasing/decreasing pattern for bone and marrow area fractions from section cuts 20 to 70 was statistically significant (P < 0.001 for bone and P < 0.01 for marrow). The decreasing pattern of B-HA particles from section cuts 20 to 70 was statistically significant (P < 0.001).

NC-HA–augmented sites. Average bone area fraction of the 10 sites was 32.2% (SD = 8.15). It increased in an outward-inward direction from 25% to 36.5% (Figs. 7 and 8) (P < 0.01). Average marrow area fraction was 43.2% (SD = 6.38). It decreased in an outward-inward direction from 51.6% to 41.9% (P < 0.001). The average area fraction of grafted mineral NC-HA particles was 24.6% (SD = 8.37). It ranged from 29% to 21.7% (Table 2), which was statistically significant (P = 0.038) (Fig. 9). Further examination of section cuts 20 to 70 revealed that the bone-increasing pattern in this area was statistically significant (P = 0.001); the marrow-decreasing pattern was not statistically significant (P = 0.38); and the decreasing grafted mineral particle pattern was statistically significant (P = 0.01).

B-HA–augmented sites versus NC-HA–augmented sites. Bone area fractions of B-HA and NC-HA augmented sinuses increased from external to deeper section cut areas. However, their averages increased differently (B-HA > NC-HA), with a statistical significance of P = 0.0053. In addition, when the increasing pattern of each bone area fraction was compared, there was a statistically significant difference between the two groups (P = 0.0006) (Fig. 10).

In B-HA and NC-HA grafted sites, both marrow area fractions showed a decreasing pattern from external to deeper section cuts. The B-HA average marrow area fraction (33.3%) was lower than that of NC-HA (43.2%). However, a value of 0.058 showed only a close to significant level. When the decreasing pattern of marrow area fraction of B-HA was compared to that of NC-HA, the differences were statistically significant (P = 0.003) (Fig. 11).

There was no statistically significant difference between the averages (P = 0.98) or their pattern along the core depth (P = 0.08) when the area fraction of B-HA particles (24.7%) was compared to NC-HA particles (24.6%) (Fig. 12).

DISCUSSION

The present study is a critical morphometric evaluation of NC-HA and B-HA as augmen-

Table 1.

Percentage of Tissue Area Fraction by Depth of the 10 B-HA–Augmented Sites

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Furthermore, to the best of our knowledge, it is the first report comparing morphometric analysis of 10 NC-HA sites versus 10 B-HA sites, which were performed bilaterally in humans.

Allografts, xenografts, and alloplasts have been used extensively in sinus augmentation procedures. Several authors54,57,59-62,64,69 morphometrically examined B-HA in the sinus area. In animal studies, McAllister et al.62 observed 47% vital bone area fraction and 19% B-HA in chimpanzees. Hürzeler et al. 57 observed a range of 20% to 32% area fraction of new bone and 9% to 16% of the grafted mineral in 3 rhesus monkey specimens from B-HA-augmented sinuses. Yildirim et al.69 examined the efficacy of B-HA in combination with venous blood in humans. In a 6-month period, newly formed bone averaged 14.7% and the average B-HA area fraction was 29.7%. Piattelli et al.64 reported 30% bone, 40% marrow, and 30% B-HA area fraction during the same examination period. Valentini et al.60 observed 27.5% new bone area fraction and 27% B-HA at 12 months. While the present data relating to the B-HA mineral area fraction were in agreement with other reports,62,70 bone area fraction was higher compared to several human studies.59,60,69 When a different B-HA material, produced by a higher temperature sterilization process, was examined histomorphometrically in the sinus area,71,72 similar results were reported. Hanisch et al.72 reported 20.7% newly formed bone, and Froum et al.71 reported 16% to 45% (mean 29%) area fraction of newly formed bone. The present data disagree with Hanisch et al.,72 who found no difference of bone fraction between superficial and deep sections.

The search for a synthetic graft similar to autologous graft is challenging. Results with NC-HA have been reported in various defects, including sinus augmentation procedures.20-22,50-53 In the present study, all implants were successfully functional over a 3-year period where NC-HA was used as the augmentation material. When NC-HA was compared to a ceramic HA, the latter had a cultural growth delay and a lower viability percentage than NC-HA, which showed

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with the aid of fluorochrome markers showed comparable results. Implant-new bone formation contact was 25% on the NC-HA side and 27% on the B-HA side. However, new bone formation and its remodeling process were faster on the B-HA side.

In the present study, there was no difference between the grafted mineral area fractions (B-HA 24.7% versus NC-HA 24.6%) and no difference between the mineral materials in regard to the depth of the examined cores. Apparently, although these fillers resorb over time, they served as a mineral reservoir and as a scaffold for new bone formation. In the established void in the sinus floor area, it preserved the hard matrix for osseous housing around functional implants. However, when bone area fractions were examined, a higher bone area fraction was observed medially in the deep augmented area, i.e., close to the implants and to the new position of the Schneiderian membrane. A larger blood supply to the deep augmented area due to the proximity of the sinus membrane contributed to the increased new bone formation in this area compared to the lateral-external side, where the nourishment was temporarily reduced during the lateral fractured bony wall technique.

As measured morphometrically, a higher newly formed bone area fraction was noted on the B-HA side. This significant difference was constantly observed along the cores, and correlatively there was a decreasing pattern of the marrow area fraction, which can be explained on the structural particle level. While NC-HA particles are fine, microporous 200 to 400 µ grains, the interpores, i.e., macropores and micropores, of the B-HA particles are much more pronounced. This facilitates cell ingrowth at the recipient site by the host. This difference in porosity might explain, in part, the higher bone formation in the B-HA side compared with the NC-HA side, at least in short-term observations. It should be emphasized that the delicate crystallinity, especially of the B-HA material, prohibits any mechanical compression at the augmented site. Consequently, an augmentation procedure in non-defined borders, such as the sinus floor area, and allowance of only passive fill of the material could cause voids and blood-clotting islands. This could also explain the minor variations in mineral area fraction along the depth cores in several specimens. In a solid defined site, such as an extraction socket, equal material fill could be anticipated throughout the socket depth.

acceptable physical and biological properties of an implant material. The osteoconductive ability of NC-HA has been tested in an implantable chamber. At 12 weeks, NC-HA particles were incorporated with ingrowing bone without intervening soft tissue and were proven to be a biocompatible osteoconductive material that could act as a mineral reservoir which slowly resorbs over time.

B-HA and NC-HA have been tested in experimental sinus augmentation in dogs. Histological examination
When an autogenous intramembranous source is used as the sinus augmentation material,55 48% bone area fraction was shown histomorphometrically. It appears that in the present study, the grafted mineral probably replaced the bone marrow and to a lesser extent, the calcified hard tissue, i.e., newly formed bone. Although new bone formation increased up to 12 months post-augmentation, it remained lower than the volume of the residual bone.72

In all cases in the present study, the augmented area was covered by a bioabsorbable bilayer collagen membrane composed of types I and III porcine collagen.

The advantages of using an overlying barrier membrane in sinus augmentation procedures have been previously examined.75,76 Tarnow et al.76 in a human comparative study showed an increasing amount of vital bone formation when the barrier membrane was applied in sinus-augmented sites. A recent histochemical investigation in rat jaws77 showed new cortical compact bone formation along and under the GTR membrane. Therefore, the membrane promotes corticalization and maturation of the newly formed bone in the outlined augmentation procedures.

CONCLUSIONS

In 10 pair sites, NC-HA and B-HA showed biocompatibility. New bone formation was established around immediate and delayed implant placement. Histomorphometrically, a significantly higher new bone formation along the core sites was established on the B-HA-grafted sinuses versus the NC-HA sites as observed in a 12-month period. The deep augmented area showed an increasing amount of bone area fraction compared to the lateral area. Clinically, both B-HA and NC-HA grafted areas are capable of accommodating osseointegrated implants.

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