Anorganic Bovine-Derived Hydroxyapatite vs β-Tricalcium Phosphate in Sinus Augmentation: A Comparative Histomorphometric Study

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The choice of augmentation material is a crucial factor in sinus augmentation surgery. Bovine-derived hydroxyapatite (BHA) and beta-tricalcium phosphate (β-TCP) have been used successfully in sinus augmentation procedures. Choosing one of these materials for sinus augmentation is still controversial. The aim of this clinical study was to compare the biological performance of the new BHA graft material and the well-known synthetic β-TCP material in the sinus augmentation procedure. The study consisted of 23 patients (12 male and 11 female) who were either edentulous or partially edentulous in the posterior maxilla and required implant placement. A total of 23 two-step sinus-grafting procedures were performed. BHA was used in 13 patients, and β-TCP was used in 10 patients. After an average of 6.5 months of healing, bone biopsies were taken from the grafted areas. Undecalcified sections were prepared for histomorphometric analysis. The mean new bone formation was 30.13% ± 3.45% in the BHA group and 21.09% ± 2.86% in the β-TCP group (P = .001). The mean percentage of residual graft particle area was 31.88% ± 6.05% and 34.05% ± 3.01% for the BHA group and β-TCP group, respectively (P = .047). The mean percentage of soft-tissue area was 37.99% ± 5.92% in the BHA group and 44.86% ± 4.28% in the β-TCP group (P = .011). Both graft materials demonstrated successful biocompatibility and osteoconductivity in the sinus augmentation procedure. However, BHA appears to be more efficient in osteoconduction when compared with β-TCP.

Key Words: anorganic bovine-derived hydroxyapatite, beta-tricalcium phosphate, sinus augmentation, histomorphometry

INTRODUCTION

Implant placement in the posterior maxilla often requires surgical involvement of the subantral area because of insufficient bone volume. Maxillary sinus floor augmentation performed for inserting the implants in this region was first described by Boyne and James and Tatum. This surgical procedure makes the implant placement possible by enhancing the alveolar bone height in this region.

A wide variety of graft materials have been used to augment the maxillary sinus floor. Autogenous bone grafts are considered to be the gold standard since they are not immunogenic and they have osteogenic, osteoinductive, and osteoconductive properties. However, there are several disadvantages including donor site morbidity, limping when the graft is taken from the iliac crest, prolonged healing time, second surgical intervention, requirement of general anesthesia and hospi-
nalization, increased cost of treatment, and unpredictable resorption of the graft. These disadvantages have led to a search for suitable graft materials that are biocompatible and osteoinductive or at least osteoconductive alternative to autogenous bone substitute in sinus floor augmentation procedure.

Various bone-grafting materials such as alloplasts (hydroxyapatite, β-tricalcium phosphate, bioactive glass), xenografts (bovine or coralline hydroxyapatite), or allografts (freeze-dried de-mineralized bone) are currently being used as alternatives or supplements to autogenous bone. These biomaterials act as a scaffold for further bone formation. However, the process of bone rebuilding is slower when compared with autogenous bone grafts.

Anorganic bovine-derived hydroxyapatite (BHA), one type of xenograft, has been shown to be a safe and biocompatible bone graft material with osteoconductive properties. Also, several experimental and clinical studies have shown successful results of BHA graft materials when used for maxillary sinus floor augmentation. BHA and β-TCP have been used successfully in sinus augmentation procedures. Choosing one of these materials for sinus augmentation is still controversial, and no consensus has yet been reached. Also, the BHA graft material used in the present study is a new product.

The aim of this clinical study was to compare the biological performances of the new BHA graft material and the well-known synthetic β-TCP material in the sinus augmentation procedure by using histomorphometry.

**Materials and Methods**

**Patient selection**

The study consisted of 23 patients (12 male and 11 female) who were either edentulous or partially edentulous in the posterior maxilla and required implant placement. The mean age of the patients was 48.65 years (range, 25–63 years). The main criteria for inclusion in the study group was a residual alveolar ridge height of <5 mm and width of ≥5 mm. Panoramic radiographs were taken from the patients, and these radiographs were used to evaluate the residual alveolar ridge height. The surgical phase was planned as a sinus augmentation procedure followed by placement of implants. The exclusion criteria of the patients were unsatisfactory oral hygiene, heavy smoking (>15 cigarettes/d), uncontrolled diabetes mellitus or other systemic diseases, and acute maxillary sinusitis. All of the patients were healthy with no disease that might influence the treatment outcome. The patients were fully informed about the procedures including the surgery, graft materials, and implants. The written informed consents were collected. The research protocol was approved by the Faculty of Dentistry Ethics Committee.

**Sinus floor augmentation**

A total of 23 two-step sinus grafting procedures were performed under local anesthesia. The patients received prophylactic antibiotics: 1 g amoxicillin orally for prophylaxis 1 hour prior to sinus floor augmentation surgery. A crestal incision positioned slightly toward the palatal aspect was performed throughout the entire length of the edentulous area. Vertical releasing incisions were made anteriorly and posteriorly. A mucoperiosteal flap was then elevated, allowing good access to the lateral sinus wall. A sinus window was prepared by a round diamond burr under continuous cooling using sterile saline solution. Following exposure of the Schneiderian membrane, the buccal window was gently mobilized toward the medial aspect of the sinus. Using curettes of different shapes and sizes, the sinus membrane was gradually separated.

The space created between the maxillary alveolar process and the sinus floor was filled with either 2.5–3 g deproteinized BHA (BonePlus-xs, Integros Ltd, Adana, Turkey; particle size 1000–2000 μm) or 2 g β-TCP (Kasios TCP, Launaguet, France; particle size 1000–2000 μm; Figures 1 and 2). The choice of whether the sinus would contain the test substance Boneplus-xs or Kasios TCP filling material was determined randomly. At the beginning, the study population was planned as 26 patients (13 patients for each group). The patients...
were randomly allocated to 1 of 2 groups according to admission order. However, in the β-TCP group, 1 patient was lost to follow-up and 2 patients did not accept taking of the biopsy. For the 13 patients (7 men, 6 women) in group 1, BHA was used, and for the 10 patients (5 men, 5 women) in group 2, β-TCP was used as graft materials for sinus augmentation. All graft materials were mixed with blood from the operation site before placement into the opened sinus cavity. No membranes were used to cover the lateral wall. Complete wound closure was performed with 3/0 resorbable sutures (Absorbex, Steril Sağlık Malz. A.Ş., İstanbul, Turkey). Amoxicillin (Alfoxil, Fako İlaç A.Ş., İstanbul, Turkey) 1000 mg 3 times a day for 1 week, flurbiprofen (Majezik, Sanovel İlaç San. Tic. A.Ş., İstanbul, Turkey) 100 mg twice a day for 3 days, and chlorhexidine gluconate 0.12% and benzidamine HCl 0.15% mouthwash (Kloroben, Drogsan İlaçları San. Tic. A.Ş., Ankara, Turkey) twice a day for 1 week were given to all patients. The patients were examined 1 week later, and sutures were removed.

**Implant placement and biopsy retrieval**

After an average of 6.5 months of healing (range, 6–8 months), a total of 51 implants were placed: 30 implants in the BHA site and 21 implants in the β-TCP site. Bone biopsies were taken from the future implant bed in the grafted posterior maxilla by using a trephine bur of 2 mm inner diameter and 3 mm outer diameter under copious irrigation with cool saline. The deep of the bone biopsy was the same as the length of the implant. Only a single bone biopsy specimen per grafted site was harvested.

**Histomorphometry**

Harvested cylindrical specimens were fixed in 4% neutral buffered formaldehyde for histomorphometric evaluation. The specimens were dehydrated in a graded series of ethanol and embedded in methyl methacrylate–based resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). Undecalcified ground sections from the bone biopsies were prepared according to the method described by Donath and Breuner. Sections were taken through the longitudinal axis of each cylindrical core and reduced to a thickness of 40 μm. Two sections were prepared from each block. The sections were stained with toluidine blue. All sections were used for histomorphometric evaluation. Images of the sections were obtained with a digital camera (Olympus DP 70, Tokyo, Japan) attached to a microscope (Olympus BX50). The obtained images were transferred to a computer, and image analysis software (TAS V 1.2.9; Steve Paxton, University of Leeds, West Yorkshire, United Kingdom) was used for histomorphometric analysis. The bone biopsy samples contained both the grafted area and the residual bone, but the residual bone was not included in the histomorphometric measurements. The following histomorphometric measurements were performed: (1) new bone formation in the grafted area (percentage of newly formed bone area to total measured area), (2) area of graft particles (percentage of graft particle area to total...
measured area), and (3) soft-tissue area in the grafted zone (percentage of soft-tissue area to total measured area).

**Statistical analysis**

SPSS 18.0 was used for statistical analysis. The chi-square test was performed to compare the categorical measurements between the 2 groups. The Mann-Whitney U test was performed to compare the numerical measurements between the 2 groups. Differences were considered significant when $P$ values were less than .05.

**RESULTS**

Perforation of the Schneiderian membrane occurred in 2 patients in the BHA group and 1 patient in the $\beta$-TCP group during the sinus augmentation procedure. The difference between the 2 groups was not statistically significant ($P = .999$). The perforations were covered with a resorbable collagen membrane (Collagen AT, Padova, Italy). The postoperative course was uneventful, and all dental implants were shown to be osseointegrated clinically. The mean age at grafting was 48.31 ± 11.68 years (range, 25–62 years) in the BHA group and 49.10 ± 11.96 years (range, 31–63 years) in the $\beta$-TCP group. The difference between the 2 groups was not statistically significant ($P = .733$; Table 1). The mean healing time in the BHA group was 6.38 ± 0.65 months and in the $\beta$-TCP group was 6.60 ± 0.69 months. There was no statistically significant difference between the 2 groups ($P = .389$; Table 1).

**Histomorphometric analysis**

Table 2 shows the results of the histomorphometric measurements of BHA compared with $\beta$-TCP. The mean new bone formation was 30.13% ± 3.45% in the BHA group and 21.09% ± 2.86% in the $\beta$-TCP group. The difference between the 2 groups was statistically significant ($P = .001$). The mean percentages of residual graft particle area were 31.88% ± 6.05% and 34.05% ± 3.01% for the BHA group and $\beta$-TCP group, respectively. The difference between the 2 groups was statistically significant ($P = .047$). The mean percentage of soft-tissue area was 37.99% ± 5.92% in the BHA group and 44.86% ± 4.28% in the $\beta$-TCP group. The difference between the 2 groups was statistically significant ($P = .011$). Figure 3 (A and B) and Figure 4 (A and B) show the typical appearance of newly formed bone around BHA and $\beta$-TCP.

**DISCUSSION**

The chemical composition of bone graft material is not the only factor in determining the nature and extent of scaffold biodegradation. Besides physiological conditions, characteristics such as crystallinity, crystal size, particle size, size distribution, porosity, and surface roughness have been reported to influence biological performance.\(^{30,31}\) Bovine-derived hydroxyapatite is similar to human cancellous bone both in terms of its crystalline and morphological structure. It is also biocompatible and osteoconductive but has no osteoinductive property.$^{17,22}$ However, the rate and mechanism of its resorption are still unclear.$^{19,23,32,33}$ Sartori et al,$^{19}$ in their 10-year follow-up study, reported that resorption of BHA was a slow but continuous process. Also, they found that the resorption rate was 3.6% per year for the initial 2 years and then decreased consistently in the following 8 years, with a mean value of 0.58% per month. Schlegel and Donath$^{34}$ identified the presence of BHA 6 years after the grafting procedure. They reported BHA as a permanent implant.

Beta-tricalcium phosphate is a derivative of hydroxyapatite, which is the inorganic component

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**Table 1**

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<th>Age of the patients and healing period after augmentation*</th>
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<td>Age, Mean ± SD (Min/Max)</td>
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* BHA indicates bovine-derived hydroxyapatite; $\beta$-TCP, beta-tricalcium phosphate.
of bone. This alloplast is osteoconductive and biocompatible, but it is not an osteoinductive material. Osteoconductive properties are responsible for appositional bone growth on the surface or into pores, channels, or pipes without evidence of toxic reaction.\textsuperscript{13,22,35} Since it is ceramic in nature, there is no risk of transmission of certain infectious diseases, which is theoretically possible with xenograft materials.\textsuperscript{22}

The impact of the resorption rate on the amount of newly formed bone in augmented sites is still unclear. It has been demonstrated that, unlike BHA, $\beta$-TCP is extensively resorbed in 12 to 18 months and is replaced by bone that is similar both functionally and anatomically to the original bone.\textsuperscript{33,36–38} In an animal study, Artzi et al\textsuperscript{36} reported that $\beta$-TCP was completely resorbed in 24 months, whereas BHA particles still occupied a remarkable area fraction without significant resorption even after 6 months. In the present study, the mean percentage of residual graft particle area in the BHA group was $31.88\% \pm 6.05\%$ and in the $\beta$-TCP group was $34.05\% \pm 3.01\%$ after an average of 6.5 months of healing ($P = .047$). Irrespective of graft particle area fraction, new bone area fraction at the BHA sites ($30.13\% \pm 3.45\%$) was significantly greater than that at the $\beta$-TCP grafted sites ($21.09\% \pm 2.86\%; P = .001$). It can be assumed that the macro- and micro-porous configurations of BHA particles result in better osteoconductive properties.

Prevention of unwanted early resorption of BHA graft material seems to be an added advantage of this graft material.\textsuperscript{39} A histological study indicated that the highest histological grades were achieved

\begin{figure}[h]
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\caption{Undecalcified sections of beta-tricalcium phosphate. Toluidine blue; original magnification $\times4$ (a) and $\times10$ (b). TCP indicates beta-tricalcium phosphate; NB, new bone; ST, soft tissue. Figure 4. Undecalcified sections of BHA. Toluidine blue; original magnification $\times4$ (a) and $\times10$ (b). BHA indicates anorganic bovine-derived hydroxyapatite; NB, new bone; ST, soft tissue.}
\end{figure}
with the use of cancellous bone autograft.\textsuperscript{40} However, bovine xenograft was the second best in the histological scale grading. The other substitute (TCP) had similar scores but was inferior to both autograft and xenograft.\textsuperscript{30} Similarly, Simunek et al.\textsuperscript{22} reported that deproteinized bovine bone was more efficient in osteoconduction when compared with \(\beta\)-TCP in the sinus augmentation procedure. Positive osteoconductive properties of BHA may be documented by close contact between the material and newly formed bone.\textsuperscript{41,42} The interconnected porous system of anorganic bovine bone has a size and structure conducive to vessel ingrowth.\textsuperscript{41,43} In a clinical study, Piattelli et al.\textsuperscript{41} used anorganic bovine bone in the sinus augmentation procedure. They found small capillaries, mesenchymal cells, and osteoblasts inside some Haversian canals in specimens harvested at 6 months, while at 18 months, all the Haversian canals had been filled by newly regenerated bone.

The healing period may affect the results of the present study. Zerbo et al.\textsuperscript{13} reported that bone formation was likely to continue increasing and the \(\beta\)-TCP was progressively replaced if longer times were used. Also, they described that \(\beta\)-TCP was an acceptable bone substitute material for augmentation of the maxillary sinus but the rate of bone formation was somewhat delayed in comparison to autologous bone. In the present study, one way to improve the new bone formation in the \(\beta\)-TCP group would be to extend the healing period to 9 to 12 months. However, a longer healing period is a disadvantage for this graft material. This means that prosthetic rehabilitation is delayed when \(\beta\)-TCP is used for the sinus augmentation procedure.

In the present study, 30.13\% \(\pm\) 3.45\% new bone formation was analyzed for the BHA group after an average healing period of 6.38 \(\pm\) 0.65 months. Sartori et al.\textsuperscript{19} reported 29.8\% new bone formation in sinus augmentation by using BHA after 8 months of healing. Similarly, Simunek et al.\textsuperscript{22} reported 34.2\% and Piattelli et al.\textsuperscript{41} reported 30\% new bone formation by using BHA in sinus augmentation procedures. On the other hand, this result was superior to the 14.7\% rate reported by Yildirim et al.\textsuperscript{17} and the 21\% rate reported by Valentini et al.\textsuperscript{16}

The mean new bone formation was 21.09\% \(\pm\) 2.86\% for the \(\beta\)-TCP group after an average healing period of 6.60 \(\pm\) 0.69 months. This result was comparable to the 21.4\% rate reported by Simunek et al.\textsuperscript{22} On the other hand, this result was superior to the 17\% rate reported by Zerbo et al.\textsuperscript{13} and Zijderveld et al.\textsuperscript{14} and inferior to the 36\% rate and the 29\% rate reported by Szabo et al.\textsuperscript{11,12}

There are only a few studies that compare the effect of \(\beta\)-TCP and BHA as sinus graft materials. In an experimental study, Artzi et al.\textsuperscript{36} used \(\beta\)-TCP and BHA to restore the mandibular bony defects in dogs and compared bone healing. They reported that the \(\beta\)-TCP bone area fraction was significantly greater than BHA sites at 6 months.

In a clinical study, Artzi et al.\textsuperscript{33} compared the osteoconductive capability of BHA and \(\beta\)-TCP in the sinus augmentation procedure. Both graft materials were mixed (1:1 ratio) with autogenous cortical bone chips harvested from the mandible. At 12 months postaugmentation, \(\beta\)-TCP and BHA promoted new bone formation in sinus grafting, but the amount of newly formed bone was significantly greater in BHA-grafted sites.

Simunek et al.\textsuperscript{22} compared the efficacy of BHA and \(\beta\)-TCP in sinus augmentation surgery in a prospective human study. They found that new bone formation in the BHA group was significantly greater than in the \(\beta\)-TCP group.

In this study, comparison of BHA and \(\beta\)-TCP was not performed in the same patients because the patients underwent unilateral sinus augmentation procedure. This is the limitation of this study. Another study can be conducted with patients who need a bilateral sinus augmentation procedure in order to compare these 2 graft materials in the same patients. Also, further studies should be done to evaluate the long-term success of the implants placed into these graft materials.

In this clinical study, both graft materials demonstrated successful biocompatibility and osteoconductivity in the sinus augmentation procedure. However, BHA appears to be more efficient in osteoconduction when compared with \(\beta\)-TCP.

**ABBREVIATIONS**

BHA: bovine-derived hydroxyapatite
\(\beta\)-TCP: beta-tricalcium phosphate

**REFERENCES**


