ID

Two Composite Bone Graft Substitutes for Maxillary Sinus Floor Augmentation: Histological, Histomorphometric, and Radiographic Analyses

Safak Ahmet, DDS,* Bahattin Alper Gultekin, DDS, PhD,* Zihni Cuneyt Karabuda, DDS, PhD,† and Vakur Olgac, DDS, PhD‡

Insufficient bone volume is a common problem in the rehabilitation of the edentulous posterior maxilla with implant-supported prostheses. Bone volume is limited by the pneumatized maxillary sinus and loss of alveolar bone height. To resolve this problem, maxillary sinus floor augmentation (MSFA) was developed.[1,2] This technique is based on the elevation of the sinus membrane from the sinus floor and grafting in the intermediate space. Tatum[3] first performed this procedure in the 1970s. Boyne and James published the first article on "sinus lift procedure" in 1980,[4] and the technique has been modified several times over the years.

Since the early 1980s, numerous graft materials have been used for MSFA, including autogenous bone, allografts, xenografts, alloplasts, and mixtures of various materials.[4–6] Autogenous bone is the gold standard of graft materials because of its

Objective: *To histologically, histomorphometrically, and radiographically compare clinical performance of 2 composite bone graft substitutes for maxillary sinus floor augmentation (MSFA).*

Materials and Methods: *Partially or totally edentulous patients requiring MSFA underwent grafting procedures using a 2:1 mixture of biphasic calcium sulfate (CS) and deproteinized bovine bone (group CB) or biphasic CS and alloplast (group CA). Grafts were allowed to heal for 5 months before placing the implants. During implant surgery, bone samples were collected from grafted areas for histology and histomorphometry. Graft height was analyzed using cone beam computed tomography.*

Results: *Sixteen patients completed the study. Mean percentages of new bone were 34.40% ± 18.91% and 36.71% ± 15.32% for the CA and CB groups, respectively; percentages of residual graft particles were 6.98% ± 5.09% and 5.52% ± 4.12%, respectively. The only significant finding was a greater graft height loss in the CA group (24.44% ± 6.52% vs 14.60% ± 4.58%).*

Conclusion: *Both graft substitutes were integrated in bone, confirming their biocompatibility and effectiveness for MSFA. The CB group showed less bone height loss than the CA group. (Implant Dent 2016;25:1–9)*
Key Words: *biphasic calcium sulfate, graft height stability, osteoconduction, cone beam computed tomography*

*Research Assistant, Department of Oral Implantology, Istanbul University Faculty of Dentistry, Istanbul, Turkey.
†Professor, Department of Oral Implantology, Istanbul University Faculty of Dentistry, Istanbul, Turkey.
‡Associate Professor, Institute of Oncology, Medical Faculty, Istanbul University, Istanbul, Turkey.

Reprint requests and correspondence to: B. Alper Gultekin, DDS, PhD, Department of Oral Implantology, Istanbul University Faculty of Dentistry, Istanbul, 34390, Turkey, Phone: +90 5326757236, Fax: +90 212 5323254, E-mail: alpergultekin@hotmail.com

osteogenic potential, but its disadvantages are the limited amount of available bone and donor-site morbidity.[7,8] Its rapid resorption has also been reported in both animal and human studies.[9,10] Bone graft substitutes are attractive alternatives and are popular because of the unlimited amount of available biomaterial, no therapeutic approach needed under general anesthesia, no donor-site morbidity, and the possibility of being used as

matrix carriers for drugs, hormones, and growth factors.[10,11]

Deproteinized bovine bone (DBB) is a well-documented xenograft material consisting of 100% anorganic bovine bone.[12] Its interconnecting pore system simplifies the migration of osteoblasts and provides a scaffold for bone formation.[13,14] In humans, DBB used alone or mixed with other graft materials (ie, autogenous bone or allografts) was shown to be highly

Fig. 1. Midcrestal and vertical buccal incisions were made along the residual alveolar bone. A diamond bur or piezosurgery device was used to open a window into the buccal sinus wall.

Fig. 2. Patients requiring MSFA underwent grafting procedures using a 2:1 mixture of biphasic CS and DBB (group CB) or biphasic CS and alloplast (group CA).
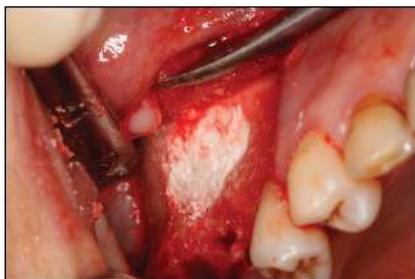

Fig. 3. Care was taken to place the composite graft in contact with as many bony walls as possible to facilitate the healing process.

Fig. 4. The resorbable membrane was placed such that migration of the composite graft and subsequent removal procedures would be avoided.

Fig. 5. The preoperative and postoperative images were used to evaluate the change in height after MSFA using a software tool.

osteoconductive and allowed the development of bone bridges between and around the grafts.[7,15,16]

The possibility of disease transmission from xenografts and allografts to humans has drawn attention to other bone graft substitutes that can be accepted by patients.[17] Alloplasts, which are synthetic and biocompatible, are also osteoconductive and have an interconnecting pore system that serves as a scaffold for the migration of osteogenic cells.[18] Various synthetic graft materials have been developed for use in MSFA, such as synthetic hydroxyapatite (HA), beta–tricalcium phosphate (β-TCP), calcium sulfate (CS), and bioglass. Synthetic HA shows a similar chemical composition to the mineral component of bone. It is highly biocompatible and osteoconductive, has a space-maintenance capacity, and easily connects chemically to local growth factors. Similarly, β-TCP is biocompatible and osteoconductive but is highly biodegradable and has less compressive strength than synthetic HA. By mixing adequate ratios of synthetic HA and β-TCP, the resorption rate can be controlled without losing their properties.[6,19,20] CS has also been used as a synthetic graft material and barrier membrane for over a century.[21] It is biocompatible,

osteoconductive, and completely resorbable. It can be used as a bone graft substitute, graft extender, and matrix carrier for growth factors and drugs.[22] However, it has poor mechanical properties and a rapid resorption rate. It is completely resorbed within 4 to 7 weeks, allowing grafted areas to be replaced by new bone.[23,24]

The ideal bone graft substitute should have the following characteristics: biocompatibility, bioresorbability, osteoconductivity or osteoinductivity, and replaceability by new bone.[25] DBB and alloplast (60% synthetic HA and 40% β-TCP) used alone or mixed with other materials (ie, autogenous bone or demineralized freeze-dried bone allograft [DFDBA]) for MSFA are highly osteoconductive and allow the creation of bone bridges between and around residual graft granules.[26–28] Slow and fast resorbable graft materials may be used as a composite to increase the new bone formation rates at planned implant sites.[2,25,27]

Over time, radiographic evaluation of the stability of grafted materials and changes in graft height are important to predict the success of MSFA. Panoramic radiographs allow only 2-dimensional evaluation, and distances may be affected by magnification and distortion.[29] Cone beam computed tomography (CBCT) is well suited for imaging of the craniofacial area, provides clear images of highly contrasted structures, and is extremely useful for evaluating hard tissue.[29] The development of high-quality detector systems and improvements in software have resulted in a reduction in metal artifacts, shortened scanning time, dose reduction, and submillimetric resolution of scans.[29] The absolute vertical stability of the augmented sinus floor during healing can also be evaluated.[30]

The aim of this study was to histologically, histomorphometrically, and radiographically compare the clinical performance of 2 composite bone graft materials for MSFA. To the best of our knowledge, this is the first clinical study evaluating biphasic CS radiographically, histologically, and histomorphometrically in composite bone graft substitutes for MSFA.
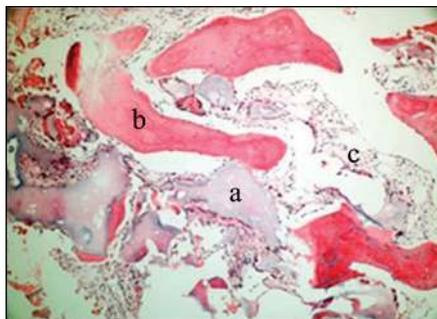
Fig. 6. Light micrograph of a ground section of a specimen collected 5 months after MSFA in the CA group. The grafted alloplastic particles (a) are well integrated in seemingly new bone (b). A smaller area of the specimen consisted of bone marrow tissue (c). Scale bar = 200 μm (H&E staining, ×100 magnification).
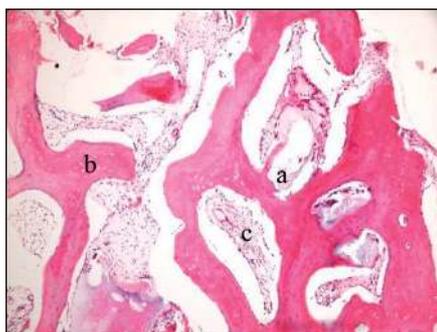

Fig. 7. Light micrograph of a ground section of a specimen collected 5 months after MSFA in the CB group. The grafted DBB particles are well integrated (a) and surrounded by immature woven bone (b). A smaller area of the specimen consisted of bone marrow tissue (c). The marrow cavity is rich in cells and blood vessels. Scale bar = 200 μm (H&E staining, ×100 magnification).

Materials and Methods

Patient Selection

This study was conducted at the Department of Oral Implantology, Istanbul University Faculty of Dentistry, and all participants were recruited from this department between April 2010 and November 2012; 20 patients (n = 8 women, 12 men; mean age = 53.8 years; age range = 47–65 years), with a residual bone height <5 mm and requiring unilateral or bilateral MSFA for posterior implant placement, received 2 graft options: (a) group CB (n = 14 maxillary sinuses), 2:1 mixture of biphasic CS (BondBone; Medical Implant System, Shlomi, Israel) and DBB (Bio-Oss; Geistlich Pharma AG, Wolhusen, Switzerland) or (b) group CA (n = 14 maxillary sinuses), 2:1 mixture of biphasic CS (BondBone) and alloplast (60% synthetic HA and 40% β-TCP) (4Bone; Medical Implant System). The allocation of patients to either group was randomized. In cases of bilateral MSFA, the sinuses were also independently randomized to the test or control group. Randomization was performed before the start of the study using a predetermined randomization table. Because of the therapeutic protocol, the surgeon could not be blinded to the procedure.

The study protocol was explained to each patient, and signed informed consent was obtained. Patients had the right to withdraw from the study at any time without explanation. This study was approved by the Ethical Committee of Istanbul University (Protocol no: 1429-685) and was conducted in accordance with the Declaration of Helsinki.

Exclusion criteria included smoking (≥10 cigarettes/day), maxillary sinus pathology and chronic sinusitis, systemic disease that would contraindicate oral surgery, chronic periodontitis in the remaining teeth, and large sinus membrane perforation that could not be repaired during MSFA.

Surgical Procedure

The partially edentulous patients initially underwent a careful periodontal examination including the assessment of plaque, gingivitis, and probing depth. If indicated, periodontal treatments were completed preoperatively. Before MSFA, patients were instructed to rinse with 0.2% chlorhexidine mouthwash (Klorhex; Drogsan, Istanbul, Turkey) for 1 minute. A 2-stage approach was used in all patients. All surgical procedures were performed under local anesthesia (Ultracaine DS Forte; Sanofi Aventis, Istanbul, Turkey). In brief, crestal and vertical incisions were made along the residual alveolar. The access window was designed according to the planned locations of the implants and anatomy of the maxillary sinus. A mucoperiosteal flap was elevated, and the sinus membrane was accessed by drilling a window into the buccal sinus wall using a dental carbide bur followed by a wide-diameter diamond bur in a high-speed handpiece. In thin walls, piezosurgery was also used for window preparation. The bone at the center of the access window was gently fractured, and the intact sinus membrane was elevated. The composite bone graft substitute was hydrated with saline. Sterile gauze sponges were gently applied to the mixture to reduce moisture and facilitate manipulation of the material. According to the experimental group, the graft was gently packed until it filled the entire cavity between the sinus floor and membrane. A resorbable collagen barrier membrane (Bio-Gide; Geistlich Pharma AG) was placed on the buccal wall of the sinus to avoid migration of the graft and its invasion by soft tissue (Figs. 1–4). The mucosal flap was sutured with 4-0 silk (Dogsan Surgical Sutures, Trabzon, Turkey) for primary closure.

Postoperative care included antibiotic prophylaxis on the day of the surgery and the following 7 days (1000 mg amoxicillin and clavulanic acid, twice daily), pain medication (600 mg ibuprofen to be taken as needed every 6 hours), and 0.2% chlorhexidine mouthwash twice daily for 2 weeks starting on the day after the operation. Dexamethasone (4 mg daily) was administered for 2 days to minimize edema. The sutures were removed 10 days after surgery. Grafts were allowed to heal for 5 months before implant placement. The implants were allowed to heal for 3 months before prosthodontic rehabilitation.

Histological and Histomorphometric Analyses

Five months after MSFA, bone biopsy specimens were collected from the planned implant sites using a trephine bur with an internal diameter of 2.3 mm (Helmut Zepf Medizintechnik GmbH, Seitingen-Oberflacht, Germany). The specimens were stored in formaldehyde solution and sent to the Pathology Institute at the University of Istanbul for processing and histomorphometric analysis. The specialist (VO) who performed this analysis was not provided with any information
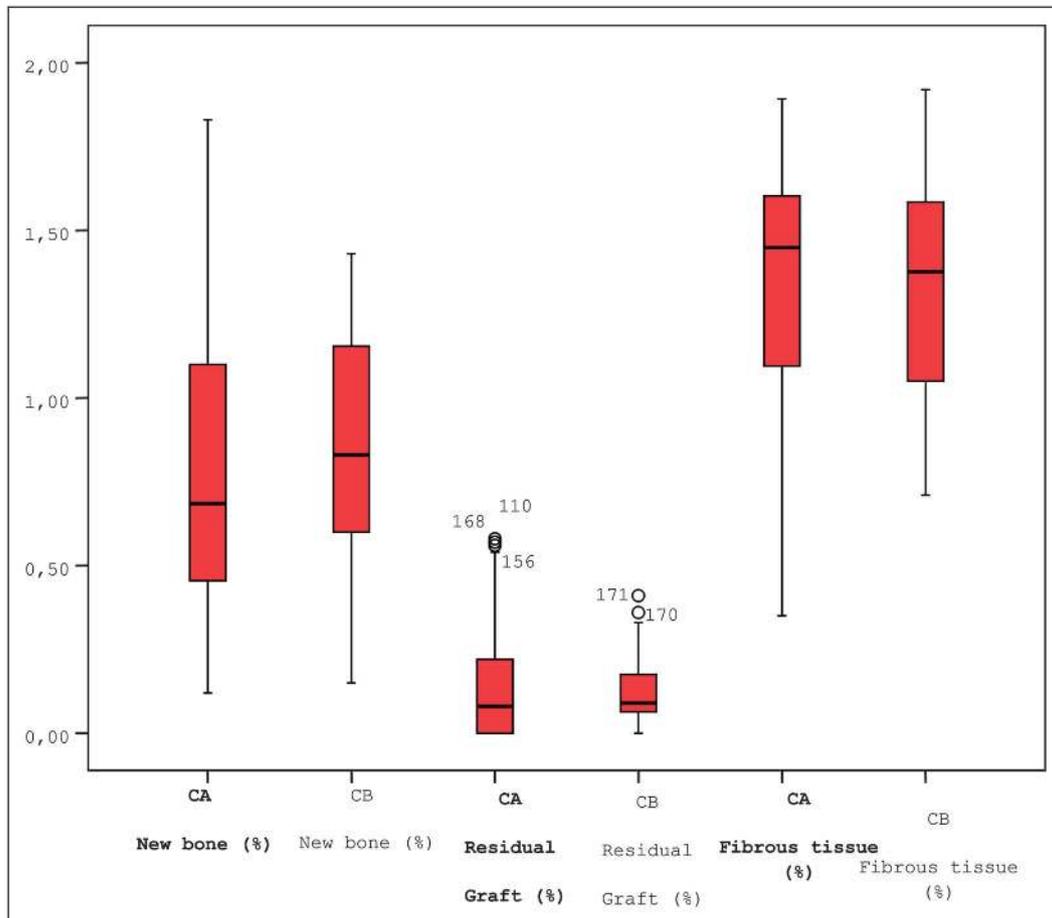
Fig. 8. The mean percentages of new bone in the CA and CB groups were 34.40% ± 18.91% and 36.71% ± 15.32%, respectively ($P >$ 0.05). The percentage of residual graft particles was 6.98% ± 5.09% in the CA group and 5.52% ± 4.12% in the CB group ($P >$ 0.05). In addition, the percentage of fibrous or bone marrow tissue was 58.61% ± 17.67% in the CA group and 57.76% ± 14.72% in the CB group ($P >$ 0.05).

regarding the experimental materials. In brief, cylindrical bone biopsy specimens were fixed in 10% neutral buffered formalin for 48 hours, decalcified in a mixture of 50% formic acid and 20% sodium citrate solution for 3 days, and embedded in paraffin according to standard protocols. Blocks were cut on a microtome obtaining 3-μm thick sections that were stained with hematoxylin and eosin (H&E). Qualitative and quantitative analyses were performed using a light microscope (Olympus BX60; Olympus Corp., Lake Success, NY) connected to a high-resolution video camera interfaced to a computer running Olympus Analysis 5 histomorphometric software package. Percentages of new bone, residual graft particles, and fibrous or bone marrow tissue in the regions of interest were calculated. Each region of interest was

chosen within the grafted area, but the initial crestal ridge was excluded because it was not representative of the entire specimen and could affect the histomorphometric measurements.

Radiographic Analysis

CBCT imaging was performed before MSFA, within 2 weeks after MSFA, and after a healing period of 5 months (Fig. 5). Scans were evaluated using an i-CAT 3D Imaging System (Imaging Sciences International Inc., Hatfield, PA). Alveolar bone height was evaluated according to the method of Pramstraller et al.[31] In this method, the mean distances from the incisive foramen to the computed tomography cross sections of the first premolar, second premolar, first molar, and second molar were 21.2, 28.2, 36.1, and 44.0 mm, respectively, in women and 22.0,

29.0, 37.1, and 45.0, respectively, in men. In our study, CBCT cross sections of the premolar and molar regions were regarded as the sections of interest for measuring the graft height. The preoperative and postoperative images were used to evaluate the change in graft height after MSFA.

Statistical Analyses

A power analysis for the comparison of bone formation between the CB and CA groups yielded the following results: power = 0.80, β = 0.20, and α = 0.05. According to this calculation, the necessary sample size was at least 6 subjects per group. SPSS version 15.0 for Windows (IBM, Inc., Armonk, NY) was used for all statistical analyses. Data were obtained as mean and SD values. Because the measures were normally distributed, Student

Table 1. Changes in Mean Height in the Grafted Areas in Relation to the Time of CBCT Scans

Time of Scan	CA Mean ± SD	CB Mean ± SD	*P
Preoperative	3.60 ± 1.50	3.07 ± 1.55	**0.427†**
Postoperative	17.54 ± 2.92	17.58 ± 2.58	**0.976†**
5-mo postoperative	13.40 ± 3.15	15.06 ± 2.62	**0.211†**
‡P	0.001§	0.001§	
Preoperative to postoperative (¶P)	0.001§	0.001§	
Preoperative to 5-mo postoperative (¶P)	0.001§	0.001§	
Postoperative to 5-mo postoperative (¶P)	0.001§	0.001§	

*Student's *t*-test.
†*P* > 0.05.
‡Multiple comparisons by analysis of variance.
§*P* < 0.01.
¶Paired sample *t* test.

t-test was used to compare mean values between groups. Multiple comparisons were performed by analysis of variance; the variation within each group was analyzed using paired sample *t*-tests. The threshold of significance was set as $P < 0.05$.

RESULTS

Clinical Observations

Sixteen patients (n = 6 women and 10 men; age range = 47–65 years; mean age = 53.87 years) who underwent 23 grafting procedures (n = 13 CA and 10 CB) completed the study. Four patients were excluded because of noncompliance with the study protocol. Twenty-seven implants were placed. All patients experienced uneventful healing without complications associated with the grafts. Only 1 implant in the CA group was clinically mobile at the second-stage surgery and was removed, whereas the remaining implants were stable.

Histological and Histomorphometric Findings

Twenty-seven bone biopsy specimens were collected from the 16 patients. Four sections were obtained from each specimen, and 108 sections (n = 48 CB group and 60 CA group) were included in the histological and histomorphometric assessments.

In the CA group, trabecular bone and Haversian systems were observed around the graft particles. At low magnification, most of the residual graft particles could be seen lined by new bone (Fig. 6). Although, in some areas, an inflammatory cell infiltrate was present around the particles or at the bone-biomaterial interface, no signs of pathological inflammation were found. The residual graft particles seemed to be highly osteoconductive. In some specimens, a rim of osteoblasts lined the new bone (Fig. 6). Furthermore, the Haversian canals appeared to be colonized by capillaries and cells. The residual graft particles did not seem to undergo resorption. The soft tissue resembled bone marrow tissue and consisted of fat cells.

In the CB group, most of the graft particles appeared to be surrounded by new bone, with well-organized osteons (Fig. 7). The residual graft particles were easily identified because of their staining and morphology. Osteoblasts were observed in the process of opposing bone directly on the particle surface in some areas. Fibrotic bone marrow spaces were present at the bone-biomaterial interface, and the bone was in close contact with the particles. No inflammatory infiltrate was present around the particles or at the interface with bone.

The mean percentages of new bone in the CA and CB groups were 34.40% ± 18.91% and 36.71% ± 15.32%, respectively ($P > 0.05$; Fig. 8). The percentage of residual graft particles was 6.98% ± 5.09% in the CA group and 5.52% ± 4.12% in the CB group ($P > 0.05$; Fig. 8). In addition, the percentage of fibrous or bone marrow tissue was 58.61% ± 17.67% in the CA group and 57.76% ± 14.72% in the CB group ($P > 0.05$; Fig. 8).

Radiographic Findings

The intraexaminer accuracy was 0.971 on repeated measurements of 20% of measurements (data not shown). The mean baseline distance between the edentulous ridge crest and the sinus floor was 3.60 ± 1.50 mm in the CA group and 3.07 ± 1.55 mm in the CB group ($P > 0.05$; Table 1). Two weeks after MSFA, the distance was 17.54 ± 2.92 mm in the CA group and 17.58 ± 2.58 mm in the CB group ($P > 0.05$; Table 1). After 5 months of healing, the values were not significantly different between the 2 groups ($P < 0.05$; Table 1): 13.40 ± 3.15 mm in the CA group, and 15.06 ± 2.62 mm in the CB group. However, graft height loss values were significantly different between the two groups after 5 months of healing ($P < 0.01$). These results demonstrated an overall graft height loss of 4.14 ± 0.58 mm (24.44 ± 6.52%) and 2.52 ± 0.67 mm (14.60 ± 4.58%) in the CA and CB groups, respectively, after 5 months of healing.

DISCUSSION

In this study, the clinical performance of 2 composite bone graft materials for MSFA was evaluated. Our data showed sufficient augmentation of the maxillary sinus floor for stable implant placement in both groups. Progressive graft height loss at the augmented sites in both groups was observed from the immediate postoperative period to 5 months thereafter, with significantly less resorption in the CB group. The surgical technique, collapse of the graft material in the early phase of wound healing, and repneumatization of the sinus may be the major factors determining the amount of bone graft remodeling in MSFA.[27] The difference in graft composition between groups may have also affected the differential response.

Many bone graft materials for MSFA tend to lose height over the healing period.[27] Air pressure from breathing may induce repneumatization after augmentation and could accelerate resorption of the graft material, particularly in 2-stage operations.[32] The stability of the graft material during the healing period and also the primary and secondary stabilities for implant placement are the major factors influencing the success of

MSFA.[27] Biomaterials used for this procedure should be resorbed and replaced with new bone over time.[26] DBB and the alloplast used in this study have low substitution rates[33,34]; however, biphasic CS resorbs more quickly and has a high turnover rate.[23,24] Materials with low turnover rates are good scaffolds for natural bone growth during healing and inhibit repneumatization of the sinus.[7] The use of both high-turnover and low-turnover rate materials as a composite offers several advantages. First, more space can be created for bone formation in the early phase of healing, and host bone can be formed quicker using high-turnover rate materials. Second, repneumatization of the sinus (ie, collapse of the graft material), which may result in a diminished overall height, can be inhibited during wound healing using low-turnover rate materials.

In this study, the baseline height of the residual host bone was similar between the 2 groups. However, a mean graft height loss of 24.44% ± 6.52% and 14.60% ± 4.58% from the postoperative period to 5 months postoperatively was noted in the CA and CB groups, respectively, with significantly greater height loss in the CA group. Postoperative reductions in bone height around 2-stage sinus lifting operations have been regarded as a normal consequence of healing.[27,32,34] The lesser graft height loss in the CB group could be explained as a result of DBB particles remaining intact, and therefore, not being resorbed after 5 months of healing, as previously reported.[35,36] Very slow or lack of resorption of DBB has been reported, particularly in early stages of healing.[36,37] In the study by Lindgren et al,[28] bone graft contact was significantly greater using DBB than alloplast (60% synthetic HA and 40% β-TCP), when used for MSFA. In another study, Lindgren et al[38] reported that TCP dissolved into phosphate and calcium ions, which reduced the calcium to phosphorus ratio in TCP during the healing period. Although theoretically the dissolution of β-TCP into phosphate and calcium ions should stimulate local bone formation, it could also decrease the mechanical resistance to repneumatization of the sinus according to the ratio with DBB in the composite graft. Even though we did not measure bone graft

contact, DBB may have offered better integration with new bone and higher-density properties because of lack of resorption, thus preserving the graft height in the CB group.

Wanschitz et al[39] reported a 10% to 13.9% resorption rate of a composite bone graft material (phycogenic HA and autologous bone chips) approximately 6 months after MSFA. In addition, Cho and Kim[40] reported a significant decrease in graft height when either autogenous bone or alloplastic material was used. Furthermore, Jensen et al noted a resorption tendency regardless of the graft material used, and they reported that the amount of resorption of an alloplast was 0.9 mm.[27] Hieu et al compared 2 xenogeneic materials solely for the sinus lift procedure (ie, the CB materials of this study) to evaluate changes in the height of the graft materials and found that both materials resorbed less than 1.5 mm during the 6 to 8 months of healing, without a significant difference in height change.[12] Moreover, Maiorana et al[41] reported that the resorption of a graft material containing DBB ranged from 0 to 1.5 mm, with an average value of 0.6 mm after 8 months of healing. In this study, CA and CB groups showed a mean graft height loss of 4.14 ± 0.58 and 2.52 ± 0.67 mm, respectively, 5 months after MSFA. Both groups presented a high level of graft shrinkage when compared to other MSFA-related studies.[27,39–42] This pronounced height loss has several possible causes. Although biphasic CS seems to be promising because of its subsequent replacement by new bone, its characteristics of fast and complete resorption during healing may hinder its ability to resist forces created by air pressure from breathing.[43,44] In this study, CS was mixed in a 2:1 ratio with either DBB or alloplast; therefore, both composite bone graft substitutes contained mainly biphasic CS. Another explanation may be the application procedure; although the preparation of biphasic CS with other bone graft materials as a composite is user friendly, its physical properties may be affected by the presence of blood during setting, which might not have been well controlled at the graft site. If implants were placed at

the time of grafting in less-resorbed ridges, advanced shrinkage may not have been observed because of the tenting effect of the implants.[23]

In the histological evaluation, we did not observe typical resorption lacunas at the remaining particle surfaces or the absence of a significant decrease in DBB and alloplastic (probably HA) particle size after 5 months. The bone-integrated residual graft particles seemed to be resistant to resorption.[45] In a previous study, biopsy specimens retrieved from patients with DBB-based grafts after 6 months did not reveal resorption in radiological assessments, and the histological evaluation showed no change in shape over that time. Our findings are in accordance with this previous study. On the contrary, Piatelli et al reported bone reactions to DBB after 4 years, and in their study, osteoclasts resorbing DBB particles were easily recognizable.[16] However, we could not find any significant differences in the amount of residual graft particles after a healing period of 5 months in both groups. Bone resorption by osteoclasts might be observable in histological specimens collected after several years of healing. No multinuclear cells were observed at the surface of the residual graft particles in both groups, and most of the particles were in direct contact with woven bone.[46] Multinuclear cells might have completed their function and disappeared.[46]

Our histomorphometric assessment indicated similar bone formation around the residual graft particles in the CA and CB groups. Trabecular bone with woven and lamellar architecture was observed bridging the graft particles. Remnants of alloplastic and DBB particles were detected in all areas. The CA and CB groups were associated with a mean bone formation rate of 34.40% ± 1 8.91% and 36.71% ± 15.32%, respectively, without significant differences. Furthermore, these groups showed a mean amount of residual graft particles of 6.98% ± 5.09% and 5.52% ± 4.12%, respectively, again without significant differences. Several authors reported bone formation rates with biomaterials in MSFA varying from 14% to 40% after a healing

period of 6 to 48 months.[27,35,47] These variations in bone-formation rate may depend on the surgical technique including pressure during the application of the graft material, particle size, biopsy technique, biological response, and healing time.[27,35] DBB is a well-documented graft material for MSFA.[12–14,26] Chackartchi et al[48] reported that mean percentage of new bone was 28% ± 6% using DBB alone 6 to 9 months after MSFA; they retrieved the biopsy specimens from the implant sites. Moreover, Froum et al[7] reported a mean bone-formation rate of 14.2% using bovine apatite (BA), 27.1% using BA and autogenous bone, and 27.8% using a mixture of DFDBA, BA, and autogenous bone, 6 to 9 months after MSFA. In another study, after 3 years of healing, the mean percentage of new bone formed using DBB was 32% ± 18.0%.[49] Hanisch et al[50] reported 20.7% ± 8.3% bone formation 12 months after healing using an allogeneic (DFDBA)-xenogeneic (bovine HA) bone graft for MSFA. Moreover, another study reported 24% and 33% bone formation 6 to 9 and 12 to 15 months after MSFA with a xenograft, respectively. In our study, the rate of bone formation was generally higher than previously reported.[7,27,49] However, Degidi et al observed an even higher bone-formation rate (ie, 38.7% ± 3.2%) than in this study, and residual graft particles constituted 14.4% ± 2.1% when a mixture of 50% autologous bone from an intraoral source and 50% DBB was used.[47] Many reports have shown that the use of graft materials with autologous bone may increase the bone-formation rate.[7,47] Our data demonstrate that the use of composite bone graft substitutes, without autologous bone, could be an alternative for MSFA with a higher bone-formation rate and less residual graft particles. Such treatment would decrease patient morbidity, surgical complications, and operating time.

The increased rate of bone formation may be the result of many factors. In this study, a greater amount of biphasic CS was used in both groups. Through 5 months of healing, biphasic CS undergoes complete resorption within the sinus, and the high calcium ion content may act as an ideal osteoconductive scaffold.[23] Another factor may be the host vascular supply, which is the source of cellular activity for wound healing in the maxillary sinus. During the fast resorption process of biphasic CS, a network of capillaries originating from the sinus walls may penetrate the innermost areas of the graft without any hindrance from solid obstacles. This is because there is less amount of nonresorbable or slowly resorbable bone graft particles in a composite form, and consequently angiogenesis may be increased in the residual space. The bone formation rate could be increased with a fast and sufficient blood supply because osteoblasts need high partial oxygen tension to produce bone matrix.[51] In addition, because of high shrinkage, the residual graft particles in both groups came closer to the crestal bone, and this process may increase the bone-formation rate, even in the most distant region of the graft. Finally, all specimens were harvested from the implant sites at the second-stage surgery and not from the lateral window as has been reported previously.[7] The institutional ethical committee did not permit specimen collection from sites other than the implant-placement sites. Therefore, the specimens, which were obtained from the superior aspect of the window (and thus distant from the bony walls), may have presented less vital bone-formation rates. The area adjacent to the crest would provide the most favorable environment for bone formation because it is narrow and close to multiple bony walls. Therefore, the bone-formation rate in this study may be higher than in studies in which specimens were collected from the lateral window.

The histomorphometric analysis easily discriminated between graft particles and surrounding new bone, based on staining and morphology. However, this analysis was a quantitative evaluation of 2-dimensional images, which were taken from the regions of interest of the specimens, and we can only assume that these images represented all the elements of the grafted sites. Therefore, 4 sections from each biopsy specimen (n = 108 sections) were used for the histomorphometric analysis, avoiding any bias.

A resorbable collagen barrier membrane was used on the buccal wall of the sinus to close the access window. This membrane prevented the mucosal tissue from collapsing into the window and thus maintained the volume of the augmented bone, in agreement with previous reports.[52] Although minor perforations in 2 of 23 sinuses were noted, no signs or symptoms of infection involving the maxillary sinuses or grafts were observed in the follow-up period. The perforations were covered with the same resorbable collagen barrier membrane, and none of the cases was abandoned.

Both CA and CB graft materials were easily recognizable by CBCT imaging after MSFA. CBCT yielded high-quality images for the exact assessment of the augmented bone in its true scale and without overlay or distortion.

CONCLUSION

The composite bone graft substitutes showed close integration with bone, confirming their biocompatibility and effectiveness for MSFA. A significant graft height reduction was found during healing in both groups. The CB group showed less bone height loss than the CA group. Further studies are needed to confirm the properties of this biomaterial with different mixture ratios and longer healing periods.

DISCLOSURE

The authors claim to have no financial interest, either directly or indirectly, in the products or information listed in the article.

APPROVAL

This study was approved by the Ethical Committee of Istanbul University (Protocol no: 1429-685) and was conducted in accordance with the Declaration of Helsinki.

REFERENCES

1. Chiapasco M, Zaniboni M, Rimondini L. Dental implants placed in grafted maxillary

sinuses: A retrospective analysis of clinical outcome according to the initial clinical situation and a proposal of defect classification. *Clin Oral Implants Res.* 2008;19: 416–428.

2. Hallman M, Sennerby L, Lundgren S. A clinical and histologic evaluation of implant integration in the posterior maxilla after sinus floor augmentation with autogenous bone, bovine hydroxyapatite, or a 20:80 mixture. *Int J Oral Maxillofac Implants.* 2002;17:635–643.

3. Tatum H. Maxillary and sinus implant reconstructions. *Dent Clin North Am.* 1986;30:207–229.

4. Boyne PJ, James RA. Grafting the maxillary sinus floor with autogenous marrow and bone. *J Oral Surg.* 1980;38: 613–616.

5. Avila G, Neiva R, Misch CE, et al. Clinical and histological outcomes after the use of a novel allograft for maxillary sinus augmentation: A case series. *Implant Dent.* 2010;19:330–341.

6. Degidi M, Daprile G, Piattelli A. Primary stability determination of implants inserted in sinus augmented sites: 1-step versus 2-step procedure. *Implant Dent.* 2013;22:530–533.

7. Froum SJ, Tarnow DP, Wallace SS, et al. Sinus floor elevation using anorganic bovine bone matrix (OsteoGraf/N) with and without autogenous bone: A clinical, histologic, radiographic, and histomorphometric analysis—Part 2 of an ongoing prospective study. *Int J Periodontics Restor Dent.* 1998;18:528–543.

8. Blomqvist JE, Alberius P, Isaksson S, et al. Importance of bone graft quality for implant integration after maxillary sinus reconstruction. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1998;86:268–274.

9. Arasawa M, Oda Y, Kobayashi T, et al. Evaluation of bone volume changes after sinus floor augmentation with autogenous bone grafts. *Int J Oral Maxillofac Surg.* 2012;41:853–857.

10. Browaeys H, Bouvry P, De Bruyn H. A literature review on biomaterials in sinus augmentation procedures. *Clin Implant Dent Relat Res.* 2007;9:166–177.

11. Ozaki W, Buchman SR. Volume maintenance of onlay bone grafts in the craniofacial skeleton: Micro-architecture versus embryologic origin. *Plast Reconstr Surg.* 1998;102:291–299.

12. Hieu PD, Chung JH, Yim SB, et al. A radiographical study on the changes in height of grafting materials after sinus lift: A comparison between two types of xenogenic materials. *J Periodontal Implant Sci.* 2010;40:25–32.

13. Klinge B, Alberius P, Isaksson S, et al. Osseous response to implanted natural bone mineral and synthetic hydroxylapatite

ceramic in the repair of experimental skull bone defects. *J Oral Maxillofac Surg.* 1992; 50:241–249.

14. Spector M. Anorganic bovine bone and ceramic analogs of bone mineral as implants to facilitate bone regeneration. *Clin Plast Surg.* 1994;21:437–444.

15. Wallace SS, Froum SJ, Tarnow DP. Histologic evaluation of a sinus elevation procedure: A clinical report. *Int J Periodontics Restorative Dent.* 1996;16:46–51.

16. Piattelli M, Favero GA, Scarano A, et al. Bone reactions to anorganic bovine bone (Bio-Oss) used in sinus augmentation procedures: A histologic long-term report of 20 cases in humans. *Int J Oral Maxillofac Implants.* 1999;14:835–840.

17. Sogal A, Tofe AJ. Risk assessment of bovine spongiform encephalopathy transmission through bone graft material derived from bovine bone used for dental applications. *J Periodontol.* 1999;70: 1053–1063.

18. Barrack RL. Bone graft extenders, substitutes and osteogenic proteins. *J Arthroplasty.* 2005;20:94–97.

19. Giannoudis PV, Dinopoulos H, Tsiridis E. Bone substitutes: An update. *Injury.* 2005;36:S20–S27.

20. Thorwarth M, Wehrhan F, Srour S, et al. Evaluation of substitutes for bone: Comparison of microradiographic and histological assessments. *Br J Oral Maxillofac Surg.* 2007;45:41–47.

21. De Leonardis D, Pecora GE. Augmentation of the maxillary sinus with calcium sulfate: One-year clinical report from a prospective longitudinal study. *Int J Oral Maxillofac Implants.* 1999;14:869–878.

22. De Leonardis D, Pecora GE. Prospective study on the augmentation of the maxillary sinus with calcium sulfate: Histological results. *J Periodontol.* 2000;71: 940–947.

23. Guarnieri R, Grassi R, Ripari M, et al. Maxillary sinus augmentation using granular calcium sulfate (SurgiPlaster sinus): Radiographic and histologic study at 2 years. *Int J Periodontics Restorative Dent.* 2006;26:79–85.

24. Rauschmann MA, Wichelhaus TA, Stirnal V, et al. Nanocrystalline hydroxyapatite and calcium sulphate as biodegradable composite carrier material for local delivery of antibiotics in bone infections. *Biomaterials.* 2005;26:2677–2684.

25. Jensen SS, Aaboe M, Pinholt EM, et al. Tissue reaction and material characteristics of four bone substitutes. *Int J Oral Maxillofac Implants.* 1996;11:55–66.

26. McAllister BS, Margolin MD, Cogan AG, et al. Eighteen-month radiographic and histologic evaluation of sinus grafting with anorganic bovine bone in the chimpanzee. *Int J Oral Maxillofac Implants.* 1999;14:361–368.

27. Schulman LB, Jensen OT. Academy of osseointegration: Sinus graft consensus conference. *Int J Oral Maxillofac Implants.* 1998;13:5–46.

28. Lindgren C, Mordenfeld A, Hallman M. A prospective 1-year clinical and radiographic study of implants placed after maxillary sinus floor augmentation with synthetic biphasic calcium phosphate or deproteinized bovine bone. *Clin Implant Dent Relat Res.* 2012;14:41–50.

29. Ziegler CM, Woertche R, Brief J, et al. Clinical indications for digital volume tomography in oral and maxillofacial surgery. *Dentomaxillofac Radiol.* 2002;3: 126–130.

30. Klijn RJ, van den Beucken JJ, Bronkhorst EM, et al. Predictive value of ridge dimensions on autologous bone graft resorption in staged maxillary sinus augmentation surgery using Cone-Beam CT. *Clin Oral Implants Res.* 2012;23:409–415.

31. Pramstaller M, Farina R, Franceschetti G, et al. Ridge dimensions of the edentulous posterior maxilla: A retrospective analysis of a cohort of 127 patients using computerized tomography data. *Clin Oral Implants Res.* 2011;22:54–61.

32. Chanavaz M. Maxillary sinus: Anatomy, physiology, surgery, and bone grafting related to implantology—Eleven years of surgical experience (1979-1990). *J Oral Implantol.* 1990;16:199–209.

33. Berglundh T, Lindhe J. Healing around implants placed in bone defects treated with Bio-Oss. *Clin Oral Implants Res.* 1997;8:117–124.

34. Wheeler SL. Sinus augmentation for dental implants: The use of alloplastic materials. *J Oral Maxillofac Surg.* 1997;55: 1287–1293.

35. Mordenfeld A, Hallman M, Johansson CB, et al. Histological and histomorphometrical analyses of biopsies harvested 11 years after maxillary sinus floor augmentation with deproteinized bovine and autogenous bone. *Clin Oral Implants Res.* 2010;21:961–970.

36. Hallman M, Lundgren S, Sennerby L. Histologic analysis of clinical biopsies taken 6 months and 3 years after maxillary sinus floor augmentation with 80% bovine hydroxyapatite and 20% autogenous bone mixed with fibrin glue. *Clin Implant Dent Relat Res.* 2001; 3:87–96.

37. Ramírez-Fernández MP, Calvo-Guirado JL, Delgado-Ruiz RA, et al. Ultrastructural study by backscattered electron imaging and elemental microanalysis of biomaterial-to-bone interface and mineral degradation of bovine xenografts in maxillary sinus floor elevation. *Clin Oral Implants Res.* 2013;24:645–651.

38. Lindgren C, Hallman M, Sennerby L, et al. Back-scattered electron imaging

and elemental analysis of retrieved bone tissue following sinus augmentation with deproteinized bovine bone or biphasic calcium phosphate. *Clin Oral Implants Res.* 2010;21:924–930.

39. Wanschitz F, Figl M, Wagner A, et al. Measurement of volume changes after sinus floor augmentation with a phycogenic hydroxyapatite. *Int J Oral Maxillofac Implants.* 2006;21:433–438.

40. Cho SH, Kim OS. Radiographic change of grafted sinus floor after maxillary sinus floor elevation and placement of dental implant. *J Korean Acad Periodontol.* 2006;36:345–359.

41. Maiorana C, Sigurtà D, Mirandola A, et al. Sinus elevation with alloplasts or xenogenic materials and implants: An up-to-4-year clinical and radiologic follow-up. *Int J Oral Maxillofac Implants.* 2006;21:426–432.

42. Mazzocco F, Lops D, Gobbato L, et al. Three-dimensional volume change of grafted bone in the maxillary sinus. *Int J Oral Maxillofac Implants.* 2014;29:178–184.

43. Peltier LF, Bickel EY, Lillo R, et al. The use of plaster of paris to fill defects in bone. *Ann Surg.* 1957;146:61–69.

44. Coetzee AS. Regeneration of bone in the presence of calcium sulfate. *Arch Otolaryngol.* 1980;106:405–409.

45. Hallman M, Cederlund A, Lindskog S, et al. A clinical histologic study of bovine hydroxyapatite in combination with autogenous bone and fibrin glue for maxillary sinus floor augmentation. Results after 6 to 8 months of healing. *Clin Oral Implants Res.* 2001;12:135–143.

46. Araujo M, Linder E, Wennstrom J, et al. The influence of Bio-Oss collagen on healing of an extraction socket: An experimental study in the dog. *Int J Periodontics Restorative Dent.* 2008;28:123–135.

47. Degidi M, Piattelli M, Scarano A, et al. Maxillary sinus augmentation with a synthetic cell-binding peptide: Histological and histomorphometrical results in humans. *J Oral Implantol.* 2004;30:376–383.

48. Chackartchi T, Iezzi G, Goldstein M, et al. Sinus floor augmentation using large (1-2 mm) or small (0.25-1 mm)

bovine bone mineral particles: A prospective, intra-individual controlled clinical, micro-computerized tomography and histomorphometric study. *Clin Oral Implants Res.* 2011;22:473–480.

49. Lindgren C, Mordenfeld A, Johansson CB, et al. A 3-year clinical follow-up of implants placed in two different biomaterials used for sinus augmentation. *Int J Oral Maxillofac Implants.* 2012;27:1151–1162.

50. Hanisch O, Lozada JL, Holmes RE, et al. Maxillary sinus augmentation prior to placement of endosseous implants: A histomorphometric analysis. *Int J Oral Maxillofac Implants.* 1999;14:329–336.

51. Schenk RK, Buser D, Hardwick WR, et al. Healing pattern of bone regeneration in membrane-protected defects: A histologic study in the canine mandible. *Int J Oral Maxillofac Implants.* 1994;9:13–29.

52. Mellonig JT, Triplett RG. Guided tissue regeneration and endosseous dental implants. *Int J Periodontics Restor Dent.* 1993;13:108–119.