A histomorphometric and micro−computed tomography study of bone regeneration in the maxillary sinus comparing biphasic calcium phosphate and deproteinized cancellous bovine bone in a human split-mouth model

Gert L. de Lange, DDS, PhD,[a] Janice R. Overman, MD,[b] Elisabet Farré-Guasch, DDS, PhD,[b] Clara M. Korstjens, DDS, PhD,[b] Bastiaan Hartman, DDS,[b] Geerling E.J. Langenbach, PhD,[c] Marion A. Van Duin, BA,[b] and Jenneke Klein-Nulend, PhD[b]
Academic Center Oral Implantology Amstelveen, Amstelveen; Academic Centre for Dentistry Amsterdam, University of Amsterdam, VU University Amsterdam, and MOVE Research Institute Amsterdam, Amsterdam, The Netherlands

Objective. The gain of mineralized bone was compared between deproteinized bovine bone allograft (DBA) and biphasic calcium phosphate (BCP) for dental implant placement.
Study Design. Five patients with atrophic maxillae underwent bilateral sinus elevation with DBA (Bio-Oss) and BCP (Straumann BoneCeramic). After 3 to 8 months, 32 Camlog implants were placed, and biopsies were retrieved. Bone and graft volume, degree of bone mineralization, and graft degradation gradient were determined using micro−computed tomography, and bone formation and resorption parameters were measured using histomorphometry. Implant functioning and peri-implant mucosa were evaluated up to 4 years.
Results. Patients were prosthetically successfully restored. All but one of the implants survived, and peri-implant mucosa showed healthy appearance and stability. Bone volume, graft volume, degree of bone mineralization, and osteoclast and osteocyte numbers were similar, but BCP-grafted biopsies had relatively more osteoid than DBA-grafted biopsies.
Conclusions. The BCP and DBA materials showed similar osteoconductive patterns and mineralized bone, although signs of more active bone formation and remodeling were observed in BCP- than in DBA-grafted biopsies. (Oral Surg Oral Med Oral Pathol Oral Radiol 2014;117:8-22)

Augmentation of the maxillary sinus floor with a grafting material is a well-established procedure to restore the bone height required for placing dental implants in the posterior edentulous maxillary region.[1-3] Autogenous bone grafts are often used for sinus augmentation and are considered the gold standard owing to their maintenance of cellular viability and presumptive osteogenic capacity. Nevertheless, drawbacks such as the requirement for an additional surgical site, graft resorption, and increased risk of morbidity[2,5] make bone substitutes an interesting alternative to autogenous grafts, with similar results when using some of these materials.[4,6,7]

Deproteinized bovine bone allograft (DBA) (Bio-Oss; Geistlich AG, Wolhusen, Switzerland) is a well-documented and well-established bone graft material that has been used frequently in sinus floor elevation procedures for nearly 2 decades.[7-9] DBA is a calcium-deficient carbonate derived from deproteinized bovine bone and is identical to human bone from a chemical and physical point of view. It performs well as a grafting material for sinus floor augmentation.[10] DBA material acts as an osteoconductive scaffold, leading to the formation of lamellar bone and increased bone density.[11] Osteoblasts are recruited from the adjacent preexisting bone and adhere directly to the surface of the graft particles using cell-matrix binding proteins.[12] However, in a few human cases, DBA led to a foreign body reaction,[13] which might have been due to residual protein.[14] Both immunologic and ethical considerations have created the need for a purely synthetic material.[15] The advantage of using synthetic materials is the predictable quality of production and the elimination of the risk to retain known and unknown proteins from an animal source.

Biphasic calcium phosphate (BCP) (Straumann Bone-Ceramic, Institut Straumann AG, Basel, Switzerland) is

Statement of Clinical Relevance

Both deproteinized bovine bone allograft (Bio-Oss) and biphasic calcium phosphate (Straumann Bone-Ceramic) graft materials were effective for regaining adequate maxillary bone height for implant placement and prosthetic rehabilitation after sinus floor elevation in patients with severe maxillary atrophy.

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[a]Academic Center Oral Implantology Amstelveen.
[b]Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam, and VU University Amsterdam; MOVE Research Institute Amsterdam.
[c]Department of Functional Anatomy, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam, and VU University Amsterdam; MOVE Research Institute Amsterdam.
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a new purely synthetic bone graft material consisting of a mixture of 60% hydroxyapatite (HA) and 40% β−tribasic calcium phosphate (β-TCP). HA has been found to be highly biocompatible with bone.[16-18] β-TCP has also been used successfully for sinus floor elevation.[19,20] However, β-TCP degrades rather fast and has a different resorption pattern than HA has.[21] BCP combines the bioactive properties of HA with the good bioresorbability of β-TCP and has been successfully used for maxillary sinus floor elevation and treatment of mandibular bone defects.[22-27] It has good biocompatibility and osteoconductivity, with an implant survival >90% and similar bone formation compared with allografts and xenografts such as DBA.[25,28-31]

Bone volumes measured with conventional 2-dimensional (2D) histologic techniques have been described to vary between 22% and 39% after grafting the sinus with BCP, with an increase in bone volume over time.[22,23,25-29,32] Micro−computed tomography (micro-CT) analysis is a nondestructive radiographic procedure providing high-resolution 3-dimensional (3D) images. This technique allows the distinction between graft material and (native) mineralized bone.[33] Micro-CT is useful for the investigation of hard tissue volume and bone structure after bone regeneration, which was first and independently reported in the same year by Ito (2005)[34] and Chappard et al. (2005).[35] Comparative studies of DBA and BCP performance in sinus floor elevation concluded that DBA and BCP produced similar amounts of newly formed bone, indicating that both materials are suitable for sinus floor augmentation to allow the placement of dental implants.[23,28] However, in these studies, most biopsies were obtained from different patients, and a comparison within one patient was not made.

In the current study, a split-mouth model was used to compare BCP and DBA for their capacity to augment maxillary bone when grafted in the maxillary sinus floor. We hypothesized that BCP, which combines the bioactive properties of HA with the good bioresorbability of β-TCP, may perform better in conjunction with dental implants placed in the augmented sinus floor for prosthetic rehabilitation. Five edentulous patients with thin residual sinus floors were selected for bilateral sinus floor elevation using BCP on one side and DBA on the other side in a split-mouth study design. Biopsies were retrieved at implant positions from previously augmented bony sites for histology, histomorphometry, and micro-CT analysis. The survival of implants in the augmented sites was evaluated during a 4-year follow-up period, including evaluation of the peri-implant mucosa and surrounding bone.

MATERIALS AND METHODS

Patients

Five healthy nonsmoking patients (4 women, 1 man; age, 64-71 years; mean age, 66 years; Table I), who had been without their maxillary dentition for many years and complained about the retention of their upper denture, were randomly selected to undergo sinus floor elevation and implant placement at 3 to 8 months after bone augmentation. All patients included had severe maxillary atrophy and were examined thoroughly. Radiographic examination included a dental panoramic tomography view and a lateral view, which revealed that the maxillary anatomy and residual sinus floor on the left and right sides were comparable. The maxillary bone height varied from 0.5 to 2 mm in the center and up to 4 mm mesially or distally, with a mean height of 2.2 mm on the left side and 2.3 mm on the right side (see Table I). All patients were informed about the necessity of sinus floor augmentation to achieve sufficient bone volume. A staged approach was used; implants were placed 3 to 8 months after bone augmentation (mean, 6 months) and were loaded after osseointegration. Early implant placement, that is, after 3 months, was considered in one patient owing to the patient's schedule. A total treatment time of 12 months was scheduled.

The protocol was reviewed by the appropriate institutional review board in compliance with the Helsinki Declaration, and ethical approval was obtained according to subcommittee CEN/TC 258 (clinical investigation of medical devices) of the European Committee for Standardization, Central Secretariat, Brussels, Belgium. Each subject was informed of the procedures and signed a detailed informed consent form.

Surgical procedures and postoperative care

Patients received one preoperative antibiotic oral dose of 3 g amoxicillin. Bilateral sinus augmentation was performed during one surgical procedure. The graft material was randomly assigned to one of the sides, with DBA (Bio-Oss; Geistlich AG) at one side and BCP (Straumann BoneCeramic; Institut Straumann AG) at the other side (see Table I). Graft material was infused with blood. A full-thickness buccal mucosa flap was elevated, and an opening was made in the lateral sinus wall. The bony window was pushed medially to detach the schneiderian membrane from the bone. The subantral cavity created was filled with granular DBA or BCP. The window at both sides was covered with a resorbable collagenous membrane (Bio-Gide; Geistlich AG). Complete wound closure was performed with resorbable sutures. Perforation of the schneiderian membrane did not occur. Postoperative examination was performed at the outpatient clinic. Patients were seen on a 3-week basis to check on healing. Chlorhexidine 0.2% rinse was used as an antiseptic therapy twice daily for 2 weeks. The differences in the healing period were partly due to the availability of the patients.

Table I. Patient and biopsy characteristics

Patient No./male or female/age (y)	Graft left	Mean residual sinus floor left (mm)	Mean gain bone height left (mm)	Graft right	Mean residual sinus floor right (mm)	Mean gain bone height right (mm)	No. of implants placed [No. of biopsies analyzed] Left	Right	Bone healing time (mo)	Implant healing time (mo)	Total healing time (mo)
1 M 63	DBA	1.3	15	BCP	1.6	16	3 [2]	3 [1]	8	9	17
2 F 65	BCP	2.0	22	DBA	2.3	21	4 [2]	4 [3]	5	12	17
3 F 64	BCP	1.6	24	DBA	2.0	23	3 [1]	3 [2]	3	5	8
4 F 65	DBA	4.0	20	BCP	3.6	25	2 [1]	2 [2]	6	3	8
5 F 71	DBA	2.0	25	BCP	2.2	24	4 [0]	4 [0]	6	2	8
Mean		2.2	21.2		2.3	21.8			5.6	6.2	11.6

DBA, deproteinized bovine bone allograft (Bio-Oss); *BCP*, biphasic calcium phosphate (Straumann BoneCeramic); *F*, female; *M*, male.

Implant surgery

Implant surgery was performed under appropriate local anesthesia. A total of 32 screw-type titanium implants (Camlog Screw Line, Camlog Biotechnologies AG, Wimsheim, Germany) were manufactured from commercially pure titanium. The core diameter of the implants was 3.8 mm, and the total length was 11 mm. Implants were sandblasted and acid-etched according to a standardized procedure (Promote; Altatec, Wimsheim, Germany) and inserted (see Table I) with an undersized drilling technique. The implant design, with a conical shape and a self-tapping screw thread, contributed to a good initial stabilization in the relatively soft regenerated bone (type D4 according to Lekholm and Zarb[36]). The implants were covered with mucosa, and the flap was sutured with 4-0 polyglactin 910 (Vicryl) resorbable sutures. The sutures were removed after 7 to 10 days, and the existing prosthesis was adapted with a soft material to the new situation. Patients were seen every 3 weeks to check on healing and ensure prevention of premature loading.

Abutment surgery was performed after implant healing, and the soft tissues were optimized for a sufficient amount and quality of peri-implant keratinized tissue. After 1 month of soft tissue maturation, prosthetic procedures were started, either for an overdenture with bar retention (2 patients) or for fixed bridges cemented on customized titanium abutments (3 patients).

Follow-up procedures

Patients were seen every 6 months for 4 years. The peri-implant mucosa and surrounding bone were examined at 4 positions (buccal, palatal, mesial, and distal) with probe angulation as the method for pathology detection.[37] Scoring was performed according to the peri-implant score of Mombelli et al.[38] for (1) healthy appearance, no bleeding on gentle probing, and pocket depth <5 mm; (2) bleeding, also when the probe was angulated, with pocket depth <5 mm and radiographic bone loss <2 mm; and (3) bleeding and pus, with pocket depth >5 mm and radiographic bone loss >2 mm. The highest score dominated. A score of 1 was considered successful, whereas a score of 3 was considered unsuccessful. A score of 2 indicated the need for treatment, and when it turned out to be reversible to a healthy situation (score 1), the implant was considered successful.

Laboratory biopsy procedure and histology

At 3 to 8 months after the sinus floor elevation, vertical bone biopsies of 4 patients were retrieved from the augmented sinus floor, at implant positions, during implant placement with a hollow trephine burr (3.5 mm outer diameter and 2 mm inner diameter) at approximately 12 mm depth. In total, 6 biopsies with DBA and 8 biopsies with BCP could be analyzed. One patient (No. 5) refused to have the biopsies taken. All biopsies ($n = 14$) were immediately fixed in 4% formaldehyde solution in 0.1M phosphate buffer, pH 7.3, at 4°C for 24 hours.[39] They were then rinsed 3 times in 0.1M phosphate buffer and stored in 70% ethanol at 4°C, until ready to be embedded in low-temperature polymerizing methyl methacrylate (Merck Schuchardt OHG, Hohenbrunn, Germany) without decalcification. Three series of 6 consecutive sections with thicknesses of 5 μm were cut using a Jung K microtome (R. Jung, Heidelberg, Germany). The distance between the 3 series was 100 μm. Two sections of each set of consecutive sections were stained with the Goldner trichrome method to highlight distinct mineralized bone tissue (green) and osteoid (red). A third section of the set of consecutive sections was stained for tartrate-resistant acid phosphatase (TRAP) to detect osteoclast-like cells. Three other sections served as backup.

Bone histomorphometry

Histomorphometric measurements were performed using a Leica DMR microscope (Leica Microsystems, Wetzlar, Germany) connected to a computer using an electronic stage table and a Leica DC 200 digital camera. The computer software used was Leica QWin (Leica

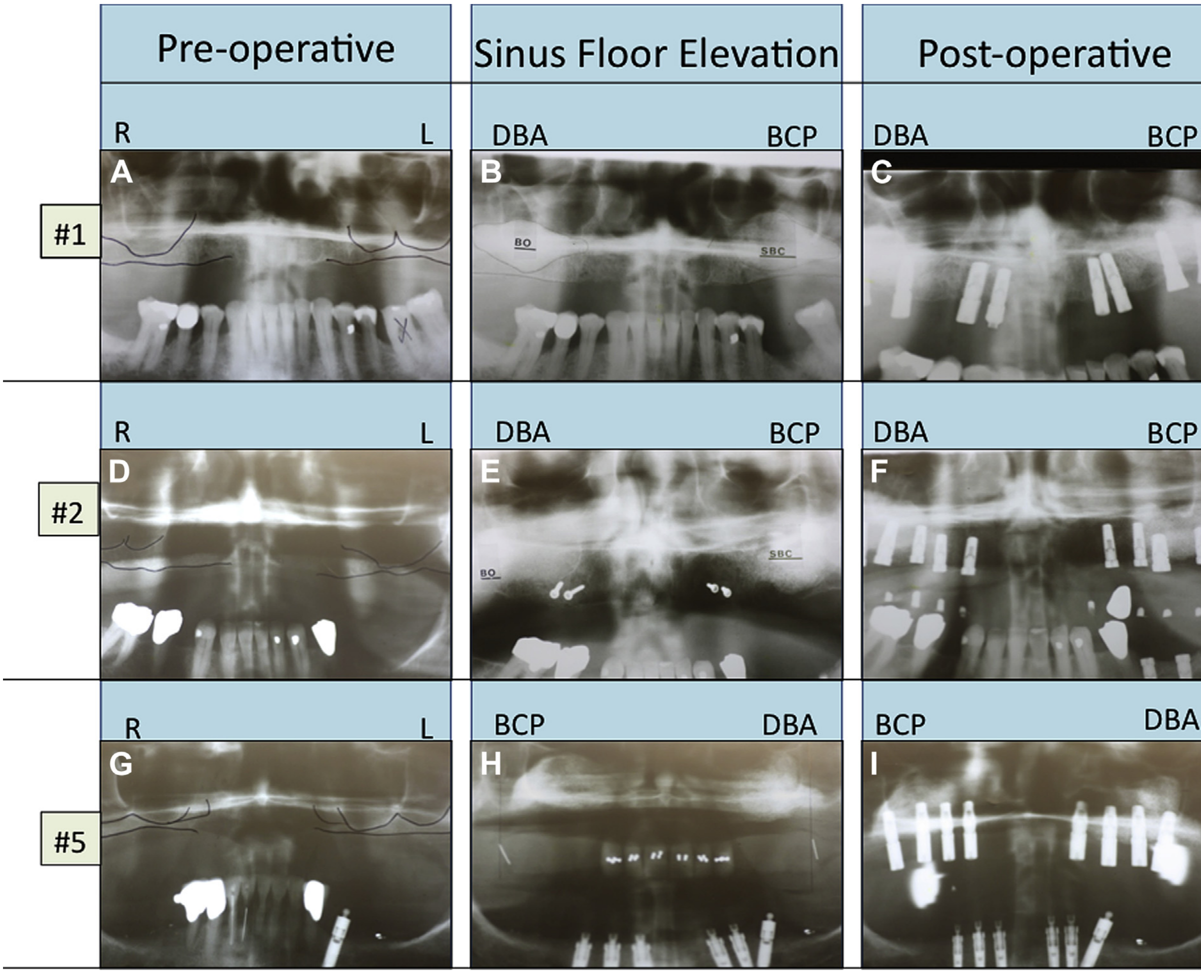

Fig. 1. Pre- and postoperative radiographs of 3 patients after bilateral sinus grafting with deproteinized bovine bone allograft (DBA, Bio-Oss) at one side and biphasic calcium phosphate (BCP, Straumann BoneCeramic) at the other side. **A, D,** and **G,** Preoperative radiographs (of patients No. 1, No. 2, and No. 5) showing severe maxillary resorption and thin residual sinus floors. **B, E,** and **H,** Radiographs of the same patients after sinus floor elevation with DBA and BCP. **C, F,** and **I,** Radiographs of the same patients after implant placement. Note that both DBA and BCP graft materials are more radiopaque than bone and that the augmented sites become less radiopaque over time.

Microsystems Image Solutions, Rijswijk, The Netherlands). The sections were digitized at ×125, ×250, and ×400 magnification. For every biopsy, one Goldner trichrome−stained section per series was analyzed, that is, 3 sections per biopsy. A demarcation line was indicated between the native (that is, original or background) alveolar bone of the residual sinus floor and the regenerated and grafted bone (Figure 1). Three consecutive areas of interest, each 625 μm^2 and at a 500-μm distance, were defined in the grafted bone from caudally, at a 500-μm distance from the sinus floor, up to the sinus bone end at the cranial side (see Figure 1).

Nomenclature, symbols, and units were used as recommended by the Nomenclature Committee of the American Society for Bone and Mineral Research.[40] In each area of interest, bone volume (BV) was calculated as the amount of mineralized tissue (mineralized volume, Md.V) plus the amount of osteoid tissue (osteoid volume, OV) as a percentage of the total tissue volume (TV) (thus BV/TV × 100). The relative osteoid volume was calculated as the amount of osteoid tissue as a percentage of the total bone volume (OV/BV × 100). The absolute graft particle volume (GV) was calculated as the amount of graft material as a percentage of the total tissue volume (GV/TV × 100). The number of osteocytes (N.Ot) and the number of osteocyte lacunae (N.lac) were counted and expressed per mineralized tissue area (mm^2). The number of TRAP-positive cells (osteoclasts, N.Oc) was expressed per total tissue area (mm^2).

Micro-CT

All biopsies (n = 14) were fixed in buffered formaldehyde and embedded in plastic resin before micro-CT analysis. Scanning was performed with the micro-CT equipment of Scanco Medical AG (model μCT40; Bassersdorf, Switzerland). This scanner has a tube voltage of 55 kV and a tube current of 145 mA. Scanning resolution was 15 μm. The micro-CT scanner measures the radiopacity of the material. The scanner is calibrated every week, and the calibration constants are used to convert the opacity values to mineralization degrees. The distinction between newly formed bone and graft material was made by using the highest value of the degree of mineralization in preexisting sinus floor bone as a cutoff point. The degree of mineralization, expressed in milligrams of hydroxyapatite per cubic centimeter (mgHA/cm^3), was found to be 550 to 1300 mgHA/cm^3. A threshold of 550 mgHA/cm^3 was used to differentiate between graft bone, newly formed bone, and background bone. Values above 1300 mgHA/cm^3 were assumed to be graft material. This way we were able to distinguish the graft material from the original nongrafted native bone of the residual sinus floor and newly formed bone. Volumes of interest (3 mm^3) of the scanned biopsies were analyzed, starting with the sinus floor bone (caudal), and continuing in the cranial direction, every subsequent 1 mm. The 3 areas selected represented different areas of interest in the grafted sinus, whereby area of interest 1 was close to the sinus floor, in a way similar to that in the study by Cordaro.[28] The ratios of bone and graft volume over total volume were calculated, as well as the average degree of mineralization of bone and graft contained in each volume of interest.

Data pooling and statistical analysis

Implant survival was calculated by the Kaplan-Meier method as described by Hu and Lagakos.[41] Bone and graft volumes, as well as the degree of mineralization of bone and graft in the volumes of interest retrieved from biopsies containing the same graft material, were pooled. The preexisting native bone from the sinus floor was used as a reference. This enabled overlay of data from biopsy sections with different lengths and eliminated possible differences in results due to different thickness of preexisting sinus floor bone, resulting in comparable areas with regard to their distance to the sinus floor to allow pooling of the data. The last millimeter of the sinus floor bone adjacent to the graft material was used as the starting point for the first volume of interest (number 1). The following volumes of interest contained the graft material in increasing distances, namely, in steps of 1 mm from the residual sinus floor. Data were expressed as mean ± standard error of the mean (SEM). Statistical testing was performed using paired *t* tests, Student independent *t* test, and analysis of variance using SPSS (version 16.1; SPSS Inc, Chicago, IL, USA), KyPlot 4.0 (KyensLab Inc, Tokyo, Japan), and GraphPad Prism 5.01 (GraphPad Software Inc, La Jolla, CA, USA). These methods allowed us to compare the mean bone volume, graft volume, degree of bone mineralization, and graft mineralization for the 2 graft materials, gradient of graft-degradation per millimeter in biopsy, osteoid volume, number of osteocytes, and number of TRAP-positive osteoclasts. Statistical analysis was performed on pooled data from corresponding volumes of interest obtained from at least 3 biopsies. Values of $P < .05$ were considered significant.

RESULTS

Clinical results

All patients had good postoperative healing (see Figure 1). The postoperative radiographs showed the presence of the bone substitute material in the augmented sinuses with an increase in height of 16 to 25 mm (see Figure 1). Two patients exceeded the time schedule; one (patient No. 1) owing to traveling and the other (patient No. 2) owing to a series of soft tissue augmentations in the anterior zone with connective tissue obtained from hyperplastic tuberosities, which was necessary to achieve maximal aesthetics for a fixed bridge. In one other patient (patient No. 5), the healing abutments were placed immediately after implant surgery. Unfortunately, one implant failed 6 months earlier, owing to premature loading at the BCP-grafted side. The height of the original sinus floor at the position of the failed implant (P2 ss) was only 1 mm (see Figure 1, *G*). The implant was removed, and a fixed bridge was made on the remaining 7 implants. All other 31 implants survived during the 4 years of follow-up. These implants resisted occlusal load and were successfully used for the prosthetic follow-up, which was 2 overdentures with bar retention (patients No. 1 and No. 3) or 3 fixed bridges (patients No. 2, No. 4, and No. 5).

The mean peri-implant score for all implants was 1.2 and did not change over time. Patients with fixed prosthetics had excellent plaque control, and the mean peri-implant score of their 21 implants was 1.1. Patients with overdentures had slightly more plaque, and some of their implants had a peri-implant score of 2 as a result of bleeding on probing. With additional plaque control instructions and an intensive cleaning protocol, these patients were able to keep the peri-implant mucosa of their 10 implants supporting the overdentures healthy during the 4 years of follow-up. The mean peri-implant score did not exceed 1.4. Scores of 3, indicating severe bone loss, bleeding, or pus, were not seen. No differences were observed between the sites grafted with DBA or BCP. The bone height in the augmented sites, as observed in panoramic radiographs

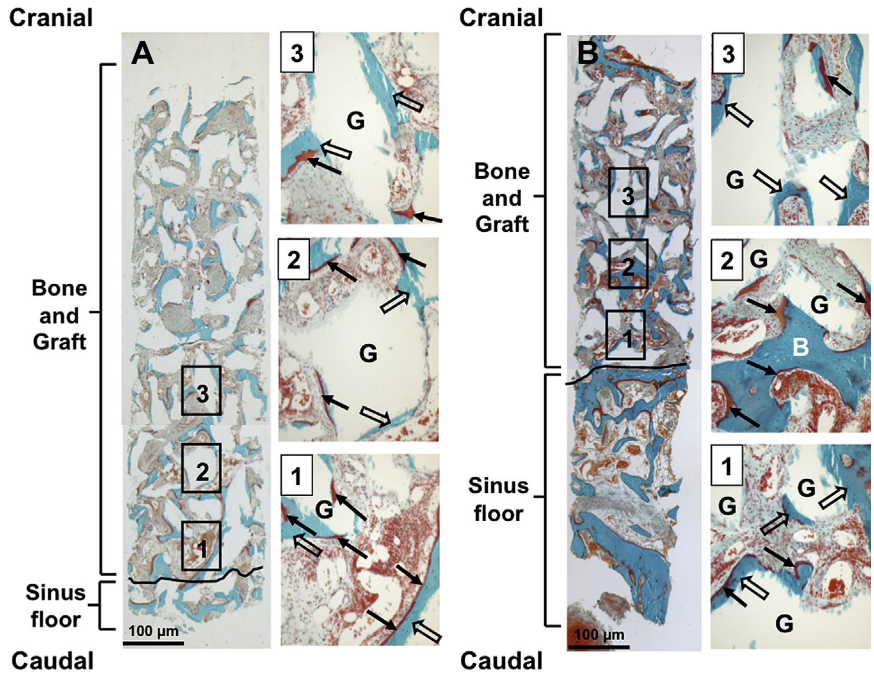

Fig. 2. Light microscopy of nondemineralized sections of whole human biopsies after sinus floor elevation with biphasic calcium phosphate (BCP, Straumann BoneCeramic) and deproteinized bovine bone allograft (DBA, Bio-Oss). **A** and **B,** Overviews of the whole biopsies of the augmented sinuses with BCP (**A**) and with DBA (**B**). The alveolar side (caudal) includes native mineralized bone (*green*) of the original sinus floor with coarse bone trabeculae ending in a dense layer of lamellar bone. The amount of mineralized bone in the augmented sinus decreases from caudal to cranial. Three areas of interest are located in the grafted bone, which are aligned at a 500 µm distance from each other and used for histomorphometric analysis. Both BCP and DBA graft particles are unstained (*white*) and surrounded by loose cell-rich connective tissue. No adverse tissue reactions are observed. The *black horizontal line* demarcates the native alveolar bone (*bottom*) and the grafted bone (*top*). The 3 areas of interest in the augmented sinus floor are seen with BCP (**A1** to **A3**) and with DBA (**B1** to **B3**). The BCP-grafted sinus shows particles with sharper angles, and their size is somewhat smaller compared with DBA. Mineralized bone (*green*) is deposited directly (*open arrows*) on the BCP graft particles (*unstained*, **G**). Osteoid tissue (*red*) borders mineralized bone, indicating new bone formation, and is located mainly at the marrow side (*black arrows*). Direct deposition of mineralized bone on the majority of the DBA particles is visible (**B1** to **B3**; *hollow arrows*). The bone trabeculae are thicker in area 1 than in the other areas, and some contain woven bone (**B1**). Most osteoid is located at the marrow side (*black arrows*). Goldner trichrome stained nondemineralized sections. Scale bar, 100 µm. Mineralized bone (*green*), osteoid tissue (red), cell nuclei (*black*).

(see Figure 1), was maintained during the 4 years of follow-up.

Histology

All biopsies obtained from patients after sinus floor elevation with BCP or DBA contained mineralized bone, osteoid, and remaining graft particles of BCP or DBA (Figure 2). The native bone showed the characteristic structure of lamellar bone, with coarse bone trabeculae and marrow spaces in between. Some trabeculae were covered by an osteoid layer, but osteoclasts were also present, lining the bone surface, indicating an ongoing normal bone remodeling process of living bone. The bone trabeculae were connected to each other and ended on a thick layer of mineralized lamellar bone with a flat surface representing the (old) residual sinus floor (see Figure 2). The regenerated bone, on the other hand, had thin bone trabeculae surrounding the remaining graft material up to the sinus end. The border between the residual sinus floor and the regenerated bone was clearly visible, which enabled us to draw a demarcation line between the residual sinus floor and the regenerated (grafted) bone (see Figure 2).

Histology showed direct bone deposition on graft particles, thin bone trabeculae, and woven bone with osteoid, characteristic for new bone formation, in all biopsies. Some graft material was still present in all biopsies, and both BCP and DBA grafted biopsies showed that remaining graft particles were embedded in a loose cell-rich connective tissue (see Figure 2, *A* and *B*). Most BCP and DBA particles were covered with a layer of mineralized bone that hardly contained cells (see Figure 2, *A* and *B*). This mineralized bone seemed to be deposited directly on the graft material without an intermediate osteoid layer. Direct apposition/deposition of osteoid on the BCP or DBA bone graft material was occasionally observed (see Figure 2, *A1* and *B2*). Most

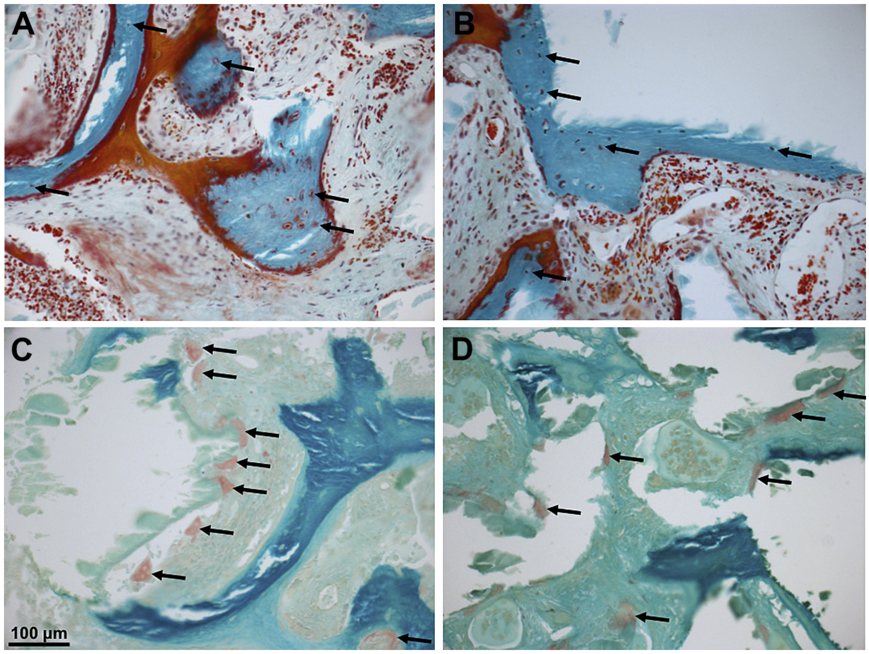

Fig. 3. Light microscopy of nondemineralized sections of whole human biopsies after sinus floor elevation with biphasic calcium phosphate (BCP, Straumann BoneCeramic) (**A** and **C**) and deproteinized bovine bone allograft (DBA, Bio-Oss) (**B** and **D**). In **A** and **B,** typical structures of a bone forming process are seen, with many blood vessels, thin bone trabeculae of woven bone containing numerous bone cells (*black arrows*), a mineralization front with osteoid, and osteoblasts embedded in a loose and cell-rich connective tissue. In **C** and **D,** tartrate-resistant acid phosphatase (TRAP)−positive cells (*red*) indicate osteoclast-like cells. TRAP-positive cells, bordering the BCP granules, seem to dissolve the material at the surface (*black arrows*). The DBA granules are also surrounded by TRAP-positive cells (*black arrows*), of which some invade the granule pores. Goldner trichrome stained sections. Scale bar, 100 µm.

osteoid was located on the bone trabeculae at the marrow side (see Figure 2, *A1* to *A3* and *B1* to *B3*). A gradient in bone maturation was observed from the cranial end (sinus end) down to the sinus floor; there was a decrease in BCP and DBA graft material and an increase in mineralized bone. In addition, the bone trabeculae were thicker close to the sinus floor than at the cranial end (see Figure 2, *A* and *B*).

Using light microscopy, we observed in all biopsies the typical characteristics of an active bone forming process, namely, thin trabeculae of woven bone with many viable osteocytes (Figure 3, *A* and *B*). This woven bone was embedded in a loose and cell-rich connective tissue with many blood vessels, and it was surrounded with osteoid that was lined with active osteoblastic cells. Both BCP and DBA graft materials showed histologically a similar bone forming process. Signs of inflammation or foreign body reaction were not seen in BCP or DBA-grafted biopsies. TRAP-positive osteoclasts were observed at the tissue-graft interface of both materials. The surface of some BCP particles clearly showed resorptive activity in relation with TRAP-positive osteoclasts (see Figure 3, *C*) which was also the case for DBA particles (see Figure 3, *D*).

Histomorphometry

Bone volume (BV/TV $\times$ 100) in native bone was similar in all biopsies. Mineralized bone volume (Md.V/TV $\times$ 100) ranged between 12.6% and 11.7% close to the sinus floor (area 1) for both BCP and DBA-grafted biopsies, and it decreased cranially (area 3) to about 7% (Figure 4, *A*). There was no difference in mineralized bone volume between the 2 grafting materials (see Figure 4, *A*). However, the bone volume (BV/TV $\times$ 100) (including osteoid tissue and bone) in area 2 was 1.4-fold higher in BCP-grafted biopsies in comparison with DBA-grafted biopsies (see Figure 4, *B*). Whereas the bone volume (BV/TV $\times$ 100) increased with decreasing distance from the native bone, the graft volume (see Figure 4, *C*) increased significantly by 1.6-fold from area 2 to area 3 in the BCP-grafted biopsies ($P = .036$) and by 1.4-fold from area 1 to area 2 in the DBA-grafted biopsies ($P = .05$). The relative osteoid volume (OV/BV $\times$ 100) increased with increasing distance from the native sinus floor, especially in the BCP-grafted biopsies, indicating active bone formation (see Figure 4, *D*). The number of osteocytes per mineralized tissue area (mm^2) was slightly (but not significantly) higher in area 3 than in area 1 in BCP-grafted biopsies, but not in DBA-grafted

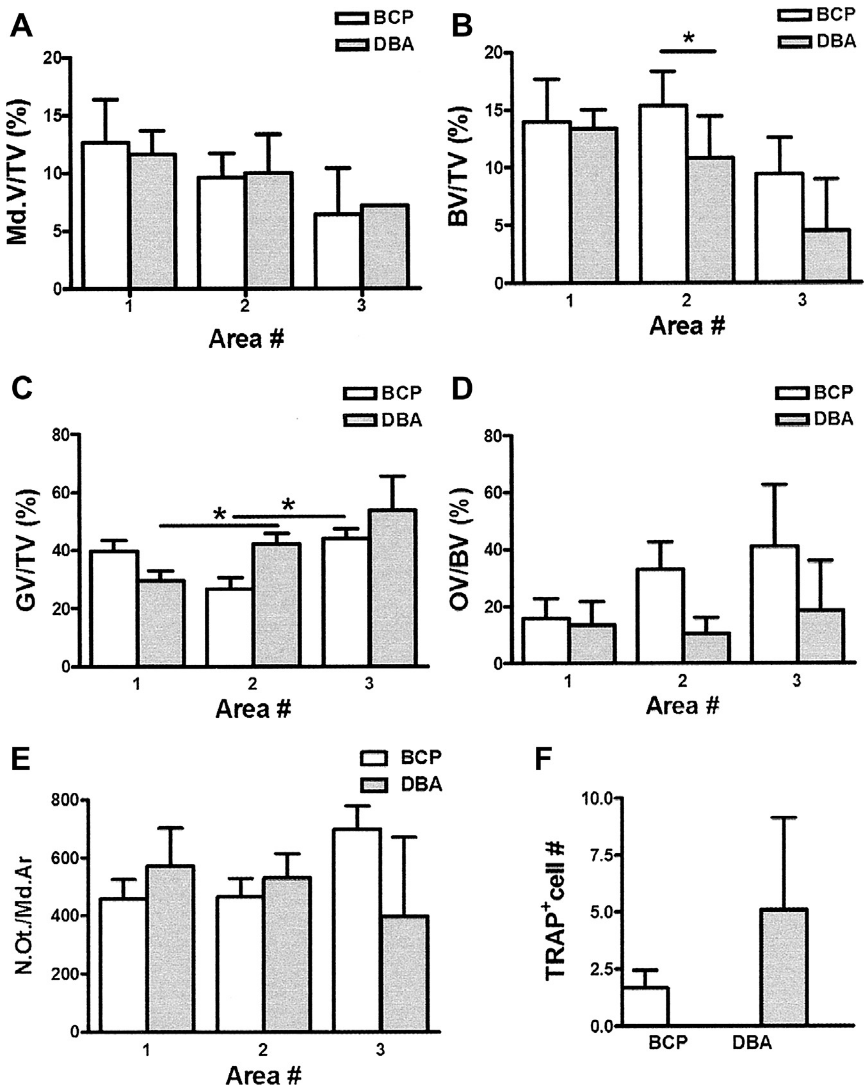

Fig. 4. Bone formation and bone resorption parameters measured in biopsies from biphasic calcium phosphate (BCP)−grafted and deproteinized bovine bone allograft (DBA)−grafted maxillary sinuses. **A,** Percentage of mineralized bone (Md.V) per total bone volume (TV) of the 3 areas of interest after sinus floor augmentation with BCP and DBA. The percentage of mineralized bone volume is highest in the area closest to the native sinus floor in both BCP- and DBA-grafted biopsies. **B,** Percentage of total bone including osteoid (BV) per TV of the 3 areas of interest after sinus floor augmentation with BCP or DBA. The amount of bone formation in area 2 was significantly higher in BCP-grafted biopsies than in DBA-grafted biopsies. **C,** Percentage of graft material (GV) per total tissue volume. The amount of graft material was decreasing from cranially to the sinus floor in BCP- and DBA-grafted biopsies ($P < .05$). **D,** Percentage of osteoid volume (OV) per BV in areas 1 to 3. **E,** Number of osteocytes per mineralized tissue area (mm^2) in the 3 consecutive areas, 1 to 3. **F,** Number of tartrate-resistant acid phosphatase (TRAP)−positive cells per mineralized tissue area (mm^2). Data from the 3 areas were pooled because the cell numbers were very low.

biopsies (see Figure 4, *E*). TRAP-positive cells were sparsely observed in the BCP and DBA-grafted biopsies. The number of TRAP-positive cells was slightly (but not significantly) higher in DBA-grafted biopsies than in BCP-grafted biopsies (see Figure 4, *F*).

Bone volume and remaining graft volume

Reconstruction by micro-CT showed mineralized bone and DBA graft material in the biopsies (Figure 5). The residual bone showed coarse trabeculae and abundant intermediate space, whereas the augmented part of the biopsy showed thin bone trabeculae with graft material, which filled up almost the entire remaining intermediate spaces, causing a dense appearance of the augmented bone (see Figure 5, *A*). The transition between residual bone and the augmented bone was clearly visible (see Figure 5, *B*). Graft material was seen throughout the entire length of the biopsies. The volume of graft material was slightly less than that of the newly formed mineralized bone (see Figure 5, *B* and *C*).

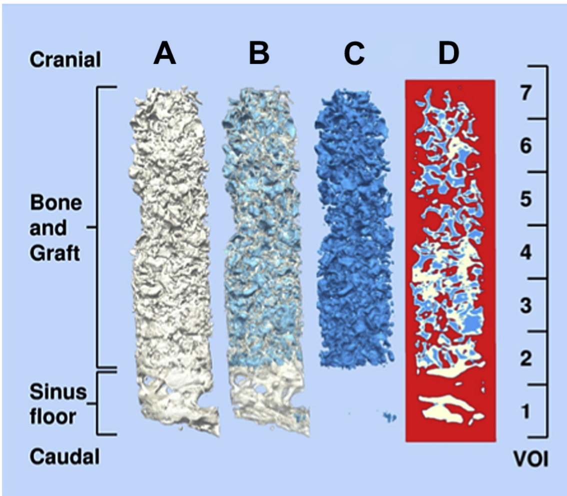

Fig. 5. Three-dimensional reconstruction by micro-CT of a vertical biopsy taken from previously augmented sites during implant surgery at implant locations after sinus floor elevation with deproteinized bovine bone allograft (DBA, Bio-Oss). **A,** Mineralized bone only. **B,** Mineralized bone and DBA graft material. Bone (*transparent gray*), DBA graft (*blue*). **C,** DBA graft material only. **D,** Longitudinal section through the center of the biopsy showing bone and DBA graft. Bone (*transparent gray*), DBA graft (*blue*). *VOI*, volume of interest; sample regions from caudal to cranial were separately analyzed for bone and graft volumes.

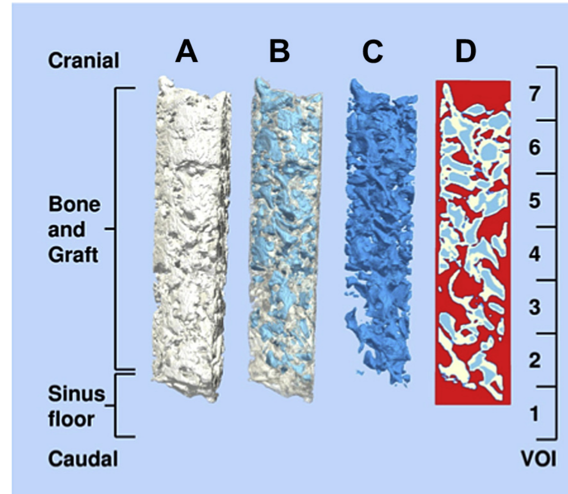

Fig. 6. Three-dimensional reconstruction by micro-CT of a vertical biopsy taken from previously augmented sites during implant surgery at implant locations after sinus floor elevation with biphasic calcium phosphate (BCP, Straumann BoneCeramic). **A,** Mineralized bone only. **B,** Bone and BCP graft material. Bone (*transparent gray*), BCP graft (*blue*). **C,** BCP graft only. **D,** Longitudinal section through the center of the biopsy showing bone and BCP graft. Bone (*transparent gray*), BCP graft (*blue*). *VOI*, volume of interest; sample regions from caudal to cranial were separately analyzed for bone and graft volumes.

The micro-CT reconstruction of the BCP-containing biopsies also showed a difference between the trabecular structure of the residual bone and the newly formed mineralized bone in the grafted area, similar to the DBA-containing biopsies (Figure 6). The newly formed bone and the remaining BCP graft (see Figure 6, *B* and *C*) displayed the same pattern in the cranial direction as was observed with DBA.

The volume of the newly formed bone in DBA-grafted biopsies was highest in the first 2 mm of the biopsy and decreased slowly in the cranial direction, whereas the graft material increased in volume more cranially (Figure 7, *A*). The volume of newly formed mineralized bone in BCP-grafted biopsies was highest in the first 2 mm of the biopsy, similar to DBA-grafted biopsies (see Figure 7, *B*), but this volume was 5% less in BCP-grafted biopsies than in DBA-grafted biopsies. BCP graft material was seen over the entire length of the biopsies, and the volume of the BCP graft material was similar to the volume of newly mineralized bone. More graft material than bone was found cranially. When comparing the total volume of mineralized tissue (the sum of bone and graft volumes) between DBA- and BCP-grafted sinuses, the DBA graft material was consistently more prevalent than the BCP material, except in the most cranial area of the biopsies. No significant differences between BCP- and DBA-grafted biopsies were found for either bone or graft volumes in volumes of interest 2 to 7 (see Figure 7).

Degree of mineralization

Micro-CT analysis of the degree of mineralization (expressed as mgHA/cm^3) of the newly formed bone showed the same mean degree of mineralization in the 7 consecutive volumes of interest for both DBA and BCP grafting materials (Figure 8). The degree of mineralization of the DBA graft material itself was significantly higher than that of BCP graft material. However, the degree of mineralization of the newly formed bone showed very little variation throughout the whole length of the biopsies for both DBA and BCP (see Figure 8) (DBA, 878 $\pm$ 48; BCP, 847 $\pm$ 51; mean $\pm$ SEM). A gradient in mineralization could not be detected.

Gradient

All biopsies showed a gradient of less bone volume and more graft material volume in the consecutive volumes of interest from residual sinus floor to cranial direction. Comparison of DBA and BCP graft materials found that the bone volume close to the residual sinus floor was 28% for DBA and 20% for BCP, and slowly decreased to 26% for DBA and 19% for BCP more

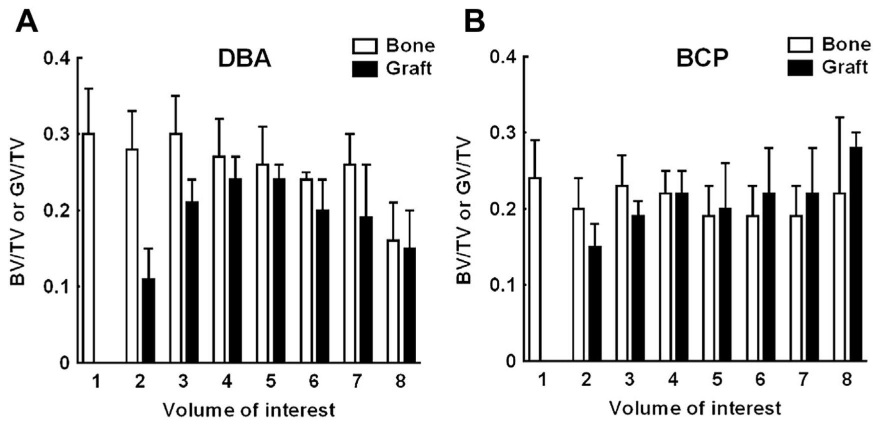

Fig. 7. Bone volume per total volume (BV/TV) and graft volume per total volume (GV/TV) assessed by micro-CT analysis in biopsies retrieved after bilateral sinus floor elevation with (**A**) deproteinized bovine bone allograft (DBA, Bio-Oss) and (**B**) biphasic calcium phosphate (BCP, Straumann BoneCeramic). Bone volume (*white*) and graft volume (*black*) were expressed per total tissue volume and assessed for each volume of interest containing DBA or BCP graft material. Volume of interest 1 represents the caudal-most millimeter of the biopsy (containing part of the original maxillary sinus floor and thus native bone), whereas volume of interest 8 represents the cranial-most millimeter of the biopsy.

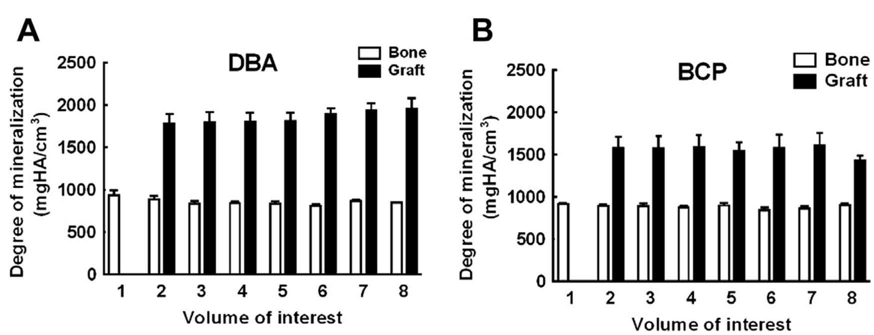

Fig. 8. Degree of mineralization of bone and graft material as assessed by micro-CT analysis in biopsies retrieved after bilateral sinus floor elevation with (**A**) deproteinized bovine bone allograft (DBA, Bio-Oss) and (**B**) biphasic calcium phosphate (BCP, Straumann BoneCeramic). Bone mineralization and graft mineralization were expressed as mgHA/cm^3 and calculated for each volume of interest containing DBA or BCP graft material. Volume of interest 1 represents the caudal-most millimeter at the end of the biopsy (containing part of the maxillary sinus floor and thus native bone), whereas volume of interest 8 represents the cranial-most millimeter of the biopsy.

cranially (Figure 9, *A*). The volume of DBA and BCP graft materials showed the opposite (see Figure 9, *B*); graft material increased from 12% for DBA and 15% for BCP close to the residual sinus floor to 19% for DBA and 22% for BCP more cranially.

DISCUSSION

This study found that both DBA and BCP facilitate the formation of new bone when placed in the maxillary sinus. Similar results were obtained for the volume of newly formed bone, the remaining graft volume, the gradient of graft consolidation, and the degree of mineralization of the newly formed bone in DBA- and BCP-grafted biopsies using histomorphometry and micro-CT. Traditionally, autologous bone is used for sinus augmentation and has proven its efficacy in the formation of new bone, as seen in histologic sections of biopsies taken from the grafted sites.[42,43] However, many patients as well as clinicians want to avoid the use of autologous bone, given that it is accompanied by donor site morbidity and that time is spent on the donor site surgery and hospitalization.[5]

As previously explained, we selected 2 promising bone substitute materials, DBA and BCP, the latter of which is a mixture of 60% HA and 40% β-TCP. HA is often used because of its high biocompatibility and low solubility[16,44,45] and because it can serve as a scaffold for osteoblasts.[18] β-TCP is also a biocompatible calcium phosphate and has been used successfully for sinus floor elevation.[19,20] However, β-TCP degrades rather fast and has a different resorption pattern than HA has.[21,46,47] β-TCP also has a relatively late-occurring remodeling phase.[20] Osteogenic cells infiltrate around and into the pores of the β-TCP particles, and degradation of these

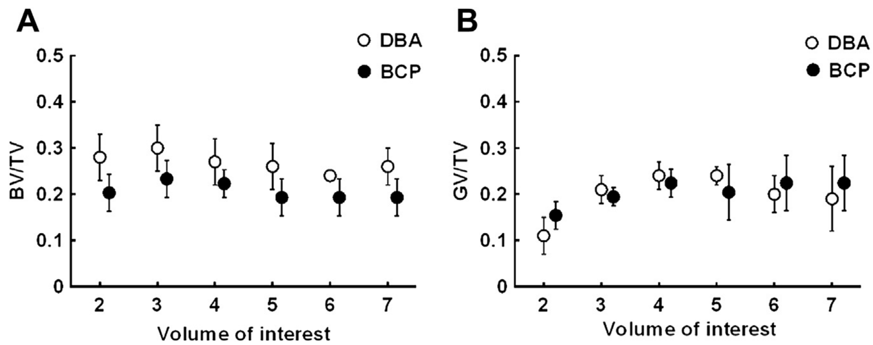

Fig. 9. Comparison of the gradient of (**A**) bone volume per total volume (BV/TV) and (**B**) graft volume per total volume (GV/TV) as assessed by micro-CT analysis in biopsies retrieved after bilateral sinus floor elevation with deproteinized bovine bone allograft (DBA, Bio-Oss; *white dots*) and biphasic calcium phosphate (BCP, Straumann BoneCeramic; *black dots*). Bone volume and graft volume were expressed per total tissue volume, and assessed for each volume of interest containing DBA or BCP graft material. Volume of interest 2 represents the caudal-most millimeter of the biopsy that contains graft material, whereas volume of interest 7 represents the last area where >3 measurements in different biopsies could be made. BV/TV, bone volume per total volume; GV/TV, graft volume per total volume.

particles mainly occurs through chemical dissolution of the material rather than by osteoclastic resorption.[48] Mixtures of HA/β-TCP in different ratios have been studied for their osteoconductive properties. These mixtures were osteoconductive in animal models, with 60:40 and 80:20 mixtures of HA/β-TCP having similar degradation and bone formation rates as DBA.[24] DBA is also widely used for sinus floor augmentation. Sometimes DBA is combined with autologous bone.[49] Both DBA and BCP are osteoconductive and therefore need more time for bone regeneration compared with autologous bone. Some patients fear protein transmission when using DBA, although this has never been detected so far[50] and is very unlikely to occur because the donor bone is subjected to heat preparation before transplantation.[51]

Most studies comparing bone substitutes in sinus floor elevation use biopsies obtained from unilateral sinus floor elevation and different patients. Because the sinus anatomy of these patients varies, reliable results are obtained only when the study includes a high number of patients. To reduce the number of patients while maintaining statistical reliability, we used a split-mouth design for graft comparison and obtained biopsies from 4 patients with similar anatomy and maxillary atrophy on the left and right sides. A split-mouth design is a strong design, allowing within-subject comparison of different bone grafts, thereby removing interindividual intrinsic variation in healing time, physiology, general health, and oral health[20] and reducing the number of patients needed to reach statistical significance. This design is an excellent way to compare the performance of BCP and DBA, especially when it concerns edentulous patients with an atrophic maxilla, comparable large sinus spaces, and thin sinus floors.[11,42] It would be interesting to use the split-mouth design in studies comparing different time points in bone healing as well as implant healing for bone regeneration in sinus floor elevation.

CLINICAL RESULTS

All treatments were considered successful, because all patients experienced uneventful treatment, and the peri-implant tissues around all implants were healthy during the 4 years of follow-up. Plaque control was only a minor issue and was easily solved with standard procedures. Variations in bone height, as measured on radiographs, were not observed, which is in agreement with a previous report.[52]

The 32 Camlog screw-type implants performed well in all patients, and a relatively high initial stability was experienced despite the softness of the grafted bone after 6 months of healing. In one patient, 4-mm-long healing abutments were placed immediately on the 8 implants. These implants were loaded by the removable denture, which was not part of the protocol for unloaded implant healing. Unfortunately, one implant failed in this patient at the site where the original bone height was only 0.5 mm. We consider this failure to be due to premature loading rather than to an insufficient performance of the BCP in conjunction with dental implants. Including this failure, the implant survival rates were 100% at the DBA-grafted site and 94.1% at the BCP-grafted site. The mean implant survival for all implants placed in the augmented sinus was 97.1%. One study reported an implant survival of 97.3% when the sinus floor is thin (≤4 mm) and an implant survival decreasing to 90.2% with increasing intermaxillary distance.[53] All patients in our study had a sinus floor height of <4 mm, and one patient had a large intermaxillary distance. Most clinicians still prefer to use autologous bone for sinus floor elevation and use DBA or BCP only when the residual sinus floor height is more than 4 or 5 mm. This provides sufficient implant stability also when there is limited newly formed bone.[2] Our results indicate that both DBA and BCP grafting materials performed clinically well in relation to the limited sinus floor height and implant survival. This suggests that it is not necessary to use autologous bone as long as the bone and implant are allowed sufficient time to heal.

Histology and histomorphometry

No differences in bone volume were observed between DBA and BCP materials. We measured a bone volume between 5% and 14%, which was less than was found in other studies, which reported bone volumes between 20% and 37% after 5 to 8 months of healing.[11,22,23,28] In our study, the healing time was only 3 to 8 months, and longer healing times may lead to increased bone volume.[11,24] Another factor that might have contributed to less bone volume in our biopsies is the maxillary bone anatomy. The patients included in this study were edentulous for many years. They were classified according to the Cawood classification with scores from VI to VII, because they had severe maxillary atrophy, with an initial sinus floor height ranging from 0.5 to 4 mm. These values were small but not a contraindication for inclusion in the present study. A thin original sinus floor (<5 mm) is related to low implant survival.[53] From a biologic view, atrophic maxillary bone and a thin sinus floor are also unfavorable, because the recruitment of bone cells from the surrounding bone will be easier if the patient has thick bone walls and a thick residual sinus floor with a high amount of vital bone. Thus the bone regeneration process in our patients is not the result of spontaneous bone repair but rather is dependent on the bone graft material properties.

The BCP-grafted sinuses had a higher amount of osteoid in the area between the native bone end and the sinus bone end of the biopsies. When osteoid volume was related to the bone volume, BCP-grafted biopsies showed relatively more osteoid in all 3 areas of interest in comparison with DBA-grafted biopsies, suggesting more active bone formation in BCP-grafted sinuses than in DBA-grafted sinuses. High amounts of osteoid in vertical biopsies 6 months after grafting with BCP for sinus floor elevation have been reported by others as well.[22] We conclude that the bone was vital in all areas of interest in both BCP- and DBA-grafted biopsies, and no foreign body reaction or signs of inflammation were noticed.

Micro-CT results: bone volume and remaining graft volume

Micro-CT analysis of biopsies has several advantages over traditional histology: it provides 3D information about the tissue sample without cutting the material; it delivers radiographic images of mineralized material that can be 3D-reconstructed; and if the biopsy is properly fixed and embedded in plastic, then histologic sections can be obtained afterward. There is, however, also a disadvantage. The micro-CT reconstruction algorithm does not produce sharp transitions between the graft material and the other materials but rather produces gradual transitions over several voxels. The consequence is that all graft material appears to be surrounded by a thin layer of material with a lower mineral density. During the evaluations, this layer was identified as mineralized bone. This is clearly visible in Figure 5, *D*, where the graft material is covered by a thin white layer at all transitions from graft material to unmineralized tissue. In this study, the volume of all these layers (which is not negligible) was erroneously added to the mineralized bone volume. Because we mainly looked at gradients in the cranial direction and because the volume of the graft material was almost constant in the cranial direction, we are convinced that this artifact did not influence our conclusions.

We found that both DBA and BCP are osteoconductive when used as a sinus augmentation material in patients with an estimated linear growth speed between 0.5 and 1.0 mm per month. BCP-containing biopsies showed a more evenly distributed bone growth, whereas bone formation in DBA-containing biopsies seemed to decline in areas with increasing distance from the preexisting sinus floor bone. This result could be due to a higher rate of bone formation in BCP-grafted sinuses and to a faster rate of degradation of the material compared with DBA. We did not find a significant difference in the degree of mineralization of the newly formed bone when using DBA and BCP. Interestingly, our micro-CT data indicate the existence of a certain volume relation between graft and regenerated bone. As in most patient studies, the newly formed maxillary bone plus remaining graft material composed about half of the total volume. When the graft material decreased, the bone volume increased and replaced the graft material over time. The total volume of graft plus bone was maintained (approximately 50%), and the other half was composed of soft tissue. Valentini et al. (2000)[54] found a similar shift in bone volume and graft volume between 6 and 12 months after grafting. Biopsies retrieved from native maxillary bone at the molar region also showed 45% to 50% bone volume.[42] Higher percentages are seldom seen.

In maxillary sinus grafting, a fast resorption of the bone substitute to be replaced by new bone would not be preferable. A long-lasting active osteoconductive guiding scaffold is needed to support osseointegrated implants without bone destabilization.[52] Therefore the stability of the graft material in the maxillary sinus and height changes of the graft material over time are important issues to consider for a successful bone regeneration in maxillary sinus floor elevation procedures. A bone substitute that gradually degrades may be desirable for these bone augmentation procedures. BCP is purely synthetic and is a mixture of HA and β-TCP in a 60:40 ratio. The resorption rate of BCP has been found to be dependent on the HA/β-TCP ratio and proportional to the amount of β-TCP present; HA/β-TCP 20:80 resorbs more rapidly than does HA/β-TCP 60:40, whereas DBA does not degrade within 52 weeks.[24] Another study found that resorption of BCP graft occurs faster than resorption of DBA after 1 year of functional loading (0.43 mm vs 0.29 mm mean graft resorption).[30] Cordaro[28] found less graft substitute in BCP-grafted sinuses than in DBA-grafted sinuses, a finding similar to ours.

Degree of mineralization

For micro-CT analysis, we have used the highest degree of mineralization value of residual bone (1300 mgHA/cm^3) as a maximum standard and 550 mgHA/cm^3 as a minimum standard for mineralized bone. It is possible that the amount of newly formed and less mineralized bone is slightly underestimated, because new bone can have a lower degree of mineralization. This also applies to uncalcified bone. Graft material and bone could be easily distinguished from each other, because both DBA and BCP graft materials contained more than 1300 mgHA/cm^3, and shape and contrast differed from mineralized bone. BCP particles had sharp edges, whereas DBA particles were more rounded, and both BCP and DBA particles differed in shape from bone trabeculae. When colors were used instead of grayscale, the images closely mimicked those obtained by traditional histology of undecalcified (ground) sections. Micro-CT analysis and histology of biopsies obtained after sinus grafting with β-TCP have been reported after pseudocoloring of the graft material.[55] However, the gray value for bone or graft material was not provided in this study, and a comparison between micro-CT and histologic analysis was not made.[55]

The main information of bone volume and bone growth after bone regeneration in the maxillary sinus stems from traditional 2D histology and histomorphometry. These reports show much variation between patients, which is caused by anatomic and biologic differences as well as different healing times. This makes a comparison between 2 materials difficult. The reported mean volumes of newly formed bone after sinus grafting using various materials are as follows: for β-TCP, 19%[20] and 27%[55]; for DBA, 20%,[28] 22%,[23] 23%,[11] 25%,[29] 28%,[23,56] and 33%[25]; and for BCP, 22%,[28] 27%,[22] 28%,[23] 30%,[29] 31%,[25] 34%,[27] and 39%.[26] A histologic comparison between DBA and BCP has been described in an elegant study reporting similar mean volumes of newly formed bone of 19.8% for DBA and 21.6% for BCP,[28] indicating that both materials produced similar amounts of newly formed bone. DBA was significantly more retained than BCP, and a higher amount of mineralized tissue in biopsies containing DBA was observed.[28] The latter was consistent with our observation of a higher mineralization of DBA graft material, but not the retaining of DBA.

Gradient

Using histology, we observed that with increasing bone volume, the graft material was decreasing, with a mean volume of 36.8% for BCP and 41.7% for DBA. A similar shift in the gradient between bone volume and graft material was reported when using DBA in maxillary sinus floor elevation.[11,28] Using micro-CT, we also observed a gradient of decreasing bone volume and increasing graft volume from residual sinus floor to cranial direction, suggesting osteoconduction. Such a gradient has also been observed in histologic sections of biopsies from mini pigs.[57]

In our study, we performed histomorphometry and micro-CT analysis separately. The correlation between micro-CT analysis and traditional histology is still unclear. Obviously, the 2D data from histomorphometry provide an incomplete picture of what is actually a 3D specimen. A discrepancy for bone volume of about 8% to 10% has been reported when histomorphometry and micro-CT data were compared.[58,59] There was still a strong correlation ($r = 0.93$) of bone volumes found in 2D slices of specimens analyzed by micro-CT and histomorphometry.[59]

CONCLUSION

Our findings suggest that both DBA and BCP graft materials were effective for regaining adequate maxillary bone height for implant placement and prosthetic rehabilitation after sinus floor elevation in patients with severe maxillary atrophy. Both materials had similar osteoconductive patterns and similar volumes of mineralized bone. Although our split-mouth study found more osteoid in BCP-grafted biopsies than in DBA-grafted biopsies, indicating more active new bone formation and remodeling, we may not conclude that BCP performed better in conjunction with dental implants.

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Reprint requests:

Jenneke Klein-Nulend, PhD
ACTA-University of Amsterdam and VU University Amsterdam
Department of Oral Cell Biology
Gustav Mahlerlaan 3004
1081 LA Amsterdam, The Netherlands
j.kleinnulend@acta.nl