CLINICAL ORAL IMPLANTS RESEARCH

*Tali Chackartchi**
*Giovana Iezzi**
*Moshe Goldstein**
Avigdor Klinger
Aubrey Soskolne
Adriano Piattelli
Lior Shapira

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

Authors' affiliations:
Tali Chackartchi, Moshe Goldstein, Avigdor Klinger, Aubrey Soskolne, Lior Shapira, Department of Periodontology, Hadassah and Hebrew University Medical Center, Faculty of Dental Medicine, Jerusalem, Israel
Giovana Iezzi, Adriano Piattelli, Faculty of Dental Medicine, University of Chieti-Pescara, Chiety, Italy

Corresponding author:
Prof. *Lior Shapira*
Department of Periodontology
Hadassah and Hebrew University Medical Center
Faculty of Dental Medicine
PO Box 12272
Jerusalem 91120
Israel
Tel.: +972 2 677 7826
Fax: +972 2 643 8705
e-mail: Shapiral@cc.huji.ac.il

Key words: bone augmentation, bovine bone mineral, human study, implant surgery, maxillary sinus, sinus floor augmentation

Abstract

Objectives: To compare the amount of newly formed bone after sinus floor augmentation with two different particle sizes of bovine bone mineral (BBM) using clinical, micro-computerized tomography (CT) and histological techniques.

Methods: Bilateral sinus floor augmentations were performed in 10 patients. Six to 9 months later, bone samples were retrieved and analyzed.

Results: Results: Both groups were not different in vertical bone height achieved after augmentation, post-operative complications and maximal torque for the insertion of implants. Micro-CT measurements could not detect a statistically significant difference in bone volume between the groups (with a tendency for new more bone in the small granules group). Histomorphometric analysis revealed that both granule sizes produced the same pattern of bone formation, surrounding the graft granules, and producing a shape of a network, "bridging" between the BBM particles. Multi-nucleated giant cells, probably osteoclasts, were observed directly on the BBM particle surface in both groups. The osteoclast-like cells preferred the small-size BBM particles and not the large particles both in the small-size and the large-size granules group.

Conclusion: Both sizes of BBM granules preformed equally and achieved the aim of the sinus floor augmentation procedure clinically and histologically.

Crestal bone resorption and pneumatization of the maxillary sinus are often evident after loss of maxillary posterior teeth (Tallgren 1972; Branemark et al. 1984; Blomqvist et al. 1996; Sharan & Madjar 2008). The edentulous posterior maxilla in these cases, often presents with an insufficient bone quantity for the restoration of posterior missing teeth with dental implants. One of the common methods for achieving sufficient bone volume is sinus floor augmentation. Clinical success has been obtained by grafting the maxillary sinus with different bone replacement materials (Hallman & Thor 2008; Nkenke & Stelzle 2009) before or simultaneously with implant placement.

Various bone grafting materials were described for sinus floor augmentation, such as autogenous bone (Boyne & James 1980; Tatum 1986; Moy et al. 1993), human demineralized bone matrix (Groenveld et al. 1999), bovine deproteinized bone matrix (Artzi et al. 2005), resorbable hydroxyapatite (Wagner 1991), porous hydroxyapatite (Smiler & Holmes 1987), tricalcium phosphate (Artzi et al. 2005) or bioactive glass particles (Turunen et al. 2004).

Deproteinized bovine bone mineral (BBM) is a well-documented grafting material and very popular for the augmentation of the maxillary sinus floor. It was proven as a scaffold for new bone formation in both animal models (Klinge et al. 1992; Spector 1994; Wetzel et al. 1995; Hurzeler et al. 1997) and human clinical trials (Valentini & Abensur 1997; Froum et al. 1998; Valentini et al. 1998; Piattelli et al. 1999; Tadjoedin et al. 2003).

BBM is a calcium-deficient carbon apatite with a crystal size of approximately 10 nm (Smiler et al. 1992; Hurzeler et al. 1997). This material was found to be osteoconductive and the bovine bone granules provided a scaffold for new bone formation (Klinge et al. 1992; Spector 1994; Wetzel et al. 1995; Hammerle et al. 1998). When the material was used for the augmentation of the

*These authors had equal contribution to the study. T. C. for the micro-CT, G. I. for the histology and M. G. for the clinical part.

Date:
Accepted 24 June 2010

To cite this article:
Chackartchi T, Iezzi G, Goldstein M, Klinger A, Soskolne A, Piattelli A, Shapira L. 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 Impl. Res **22**, 2011; 473–480
doi: 10.1111/j.1600-0501.2010.02032.x

sinus floor, it had led to the formation of lamellar bone (Wetzel et al. 1995; Hurzeler et al. 1997) and to an increase in bone density (McAllister et al. 1998). In humans, deproteinized bovine bone granules alone or mixed with other materials (autogenous bone or demineralized freeze dried bone allograft) was found to be highly osteoconductive and allowed the creation of bone bridges between and around the graft granules (Wallace et al. 1996; Valentini & Abensur 1997; Froum et al. 1998; Piattelli et al. 1999).

Geistlich BioOss® is a commercially popular BBM preparation, and it is available in two particle sizes, 0.25–1 or 1–2 mm. Despite the wide-scale research using this grafting material, there are no available data comparing these two particle sizes. Therefore, there is no scientific basis to the clinician's choice between these two commercially available products.

The aim of this prospective human study was to compare the amount of newly formed bone after bilateral sinus floor augmentation with BBM 0.25–1 or 1–2-mm-size particles using histomorphometry (primary outcome measures), micro-computerized tomography (CT) analysis, radiographic and clinical measurements (secondary outcome measures). Our working hypothesis was that due to the expected spaces between the granules, using large granules will induce the formation of more bone between the BBM particles.

Material and methods

Patient selection

Ten patients, four females and six males, all nonsmokers, age range from 46 to 65 years with an average age of 54.25, were included in this study. All patients were candidates for bilateral maxillary sinus augmentation, in a two-stage approach. The patients were included in the study if they were able to comply with the study-related procedures such as exercising good oral hygiene and attending all follow-up procedures. The exclusion criteria included pregnant and nursing women, people who smoke more than 10 cigarettes a day, alcohol and drug abusers, people suffering from uncontrolled diabetes (HbA1c>7.5), severe osteoporosis, rheumatic arthritis, precancerous or neoplastic lesions of oral cavity and people suffering from any diagnosed pathology in the maxillary sinus. All patients were fully informed of the study protocol and implications, and consent forms were signed before treatment. The study and the consent forms were approved by the Ethics Committee of the Hadassah Medical Center, and the guidelines for Good Clinical Practice were respected.

In all 10 patients participating in the study, a CT scan of the maxilla was taken. From the tomography of each patient, three sagittal cuts were selected from the mid portion of each sinus and the residual bone height was recorded. These three measurements were then used to obtain the average residual alveolar height.

Surgical procedures

In each patient, one side was randomly assigned (by a random table generated before the study by T.C.) to be grafted with BBM 0.25–1 mm and the contra-lateral side with BBM 1–2 mm (Geistlich Biomaterials Inc., Wolhusen, Switzerland). Thirty to 60 min before surgery, patients were given 875 mg of amoxicillin/clavulanate potassium (Augmentin, GSK, Worthing, West Sussex, UK) plus 1.5 g amoxicillin, and analgesics, according to the decision and choice of the treating surgeon. The maxillary sinus floor augmentation was then preformed according to the lateral window technique described by Boyne & James (1980) and Tatum (1986) (Fig. 1). Under local anasthesia, a muco-periosteal flap was elevated and the lateral wall of the maxillary sinus was exposed. An oval window was cut in the lateral wall to enable the gentle elevation of the Schneiderian membrane. BBM (0.25–1 or 1–2 mm) and blood clot were mixed and applied into the sinus cavity by avoiding extreme packing. Before soft-tissue closure, the entire obturated lateral window was covered with a resorbable collagen membrane (Bio-Gide, Geistlich, Switzerland). After surgery, the patients were prescribed Augmentin 875 mg twice a day for a week, and advised to rinse their mouth daily with chlorhexidine (0.2%) for 2 weeks. The patients were examined 1 week post-surgery when the sutures were removed. All patients were checked regularly to verify healing. Any adverse reactions, signs of infection, hamatomas or swelling were recorded.

Six to 9 months after the augmentation procedure, a second CT scan was performed (Fig. 2)

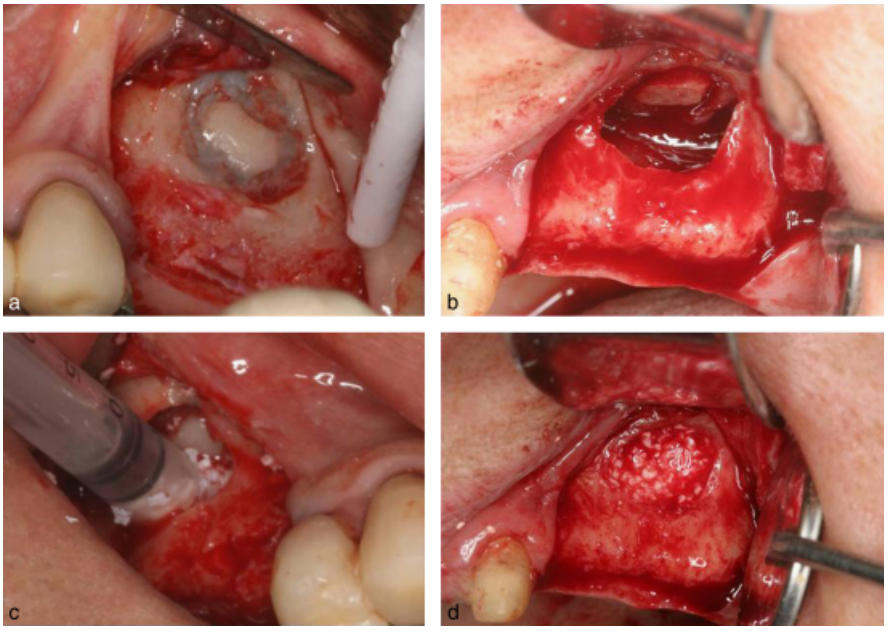

Fig. 1. Sinus floor augmentation technique used in this study. (a) Window preparation. (b) Membrane elevation. (c) Packing using a syringe. (d) The packed granules.

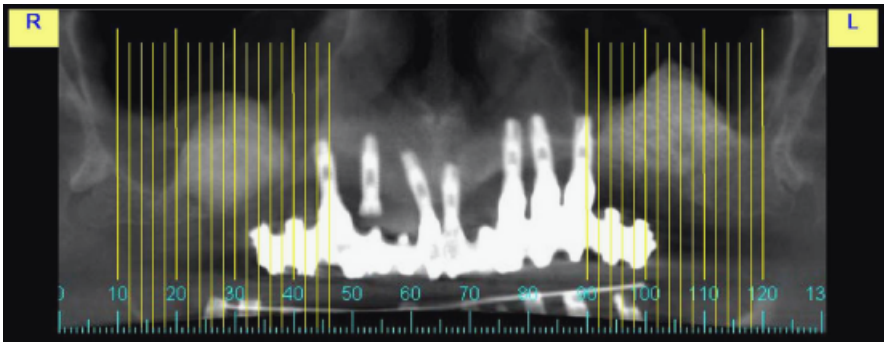

Fig. 2. Example of a second-stage computerized tomography scan (6–9 months after augmentation procedure).

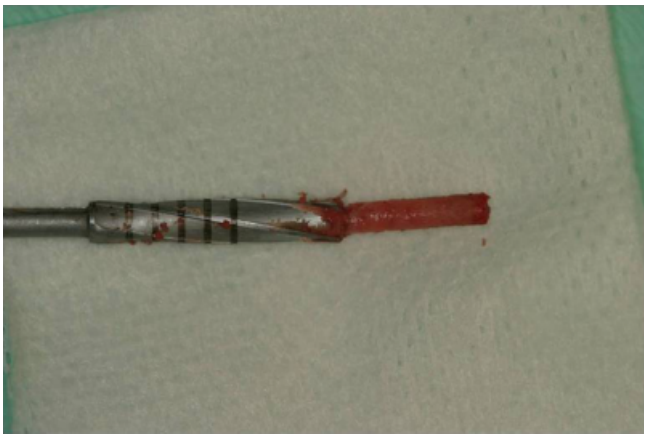

Fig. 3. Harvesting bone sample during second-stage surgery.

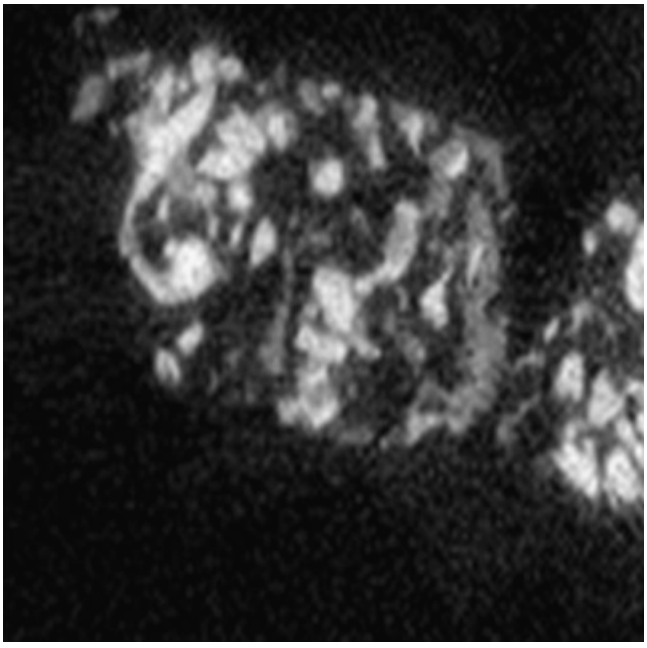

Fig. 4. An example of a micro-computerized tomography scan showing three gray-scale areas to be examined: white, gray and black.

and the bone height was measured as described above. The patient was scheduled for implant placement surgery. Before placing an implant, a biopsy of the augmented tissue was retrieved using a 3.5 mm internal diameter trephine bur (Fig. 3). The insertion torque of each implant was also recorded.

Initially, the samples were analyzed by three-dimensional (3D) micro-CT. Subsequently, histological sections were prepared for standard histomorphometric measurements.

Micro-CT processing

The samples were fixed in 10% buffered formalin, rinsed in saline and alcohol, and then scanned using a high-resolution micro-CT system (Scanco Medical, Bassersdorf, Switzerland). The resolution of the scanning was about 16 μm in a multi-slice mode. Each 3D image data set consisted of approximately 500 micro-CT slice images. Scanning time for each specimen was approximately 3 h. Micro-CT measurements of the bone/graft were obtained by working on the thresholds of the gray levels (gray for bone, white for graft and black for marrow spaces, Fig. 4). The micro-CT scan produces serial two-dimensional (2D) sagittal slides that could be reproduced to a 3D image (Fig. 5) using the scanner software. With the arbitrary threshold, it was possible to visualize and measure separately the graft particles alone and the bone segments alone. It was not possible to distinguish the residual and new bone segments. Fig. 6 was presenting the CT scan taken before second-stage surgery and the corresponding micro-CT 3D reproduction of the core biopsy. Using the micro-CT software, the 3D image reproduction was measured for the following: TV, total volume of the whole core (mm^3); BV, bone volume (mm^3); BBM-BV, graft volume (mm^3); BV/TV, % bone volume from the whole core; BS, linear calculation of the surface of the bone/graft trabecules (mm^2); BS/TV, total surface of bone/graft trabecules out of total core volume (mm^2/mm^3); Th, trabecular thickness (mm); Sp, spaces between bone/graft trabecules (mm); and TbN, trabecular number ($1\ mm^{-1}$).

Histological processing

Following micro-CT scanning, the specimens were processed to obtain thin ground sections with the Precise 1 Automated System (Assing, Rome, Italy). The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200 VLC; Kulzer, Wehrheim, Germany). After polymerization, the specimens were sectioned along their longitudinal axis with a high-precision diamond disk at about 150 μm and ground down to about 30 μm with a specially designed grinding machine. The slides were stained with acid fuchsin and toluidine blue. The slides were observed in normal transmitted light under a Leitz-Laborlux microscope (Laborlux S, Leitz, Wetzlar, Germany). The histomorphometry was performed using a light microscope (Laborlux S, Leitz) connected to a high-resolution video camera (3CCD JVC KYF55B), and interfaced to a monitor and personal computer. This optical system was associated with a digitizing pad (Matrix Vision GmbH, Brescia, Italy) and a histometry software package with image-capturing capabilities (Image-Pro Plus 4.5; Media Cybernetics Inc., Immagin, Milano, Italy).

Statistical analysis

The results of the CT analysis and clinical measurements were calculated as the means ± SD and ranges (95% confidence interval) for each variable. The Wilcoxon signed-rank test was used to assess the statistical significance of the micro-CT and histological data, and the differences between the large and small BBM granules. *P*-values ≤ 0.05 were considered significant.

Results

Patient demographic and clinical data

Ten patients participated in the study (four females and six males). The pre-operative alveolar ridge residual height was 2.45 ± 1.46 mm (90% confidence interval: 1–3.49 mm) in the side augmented with large BBM particles, and 1.95 ± 1.06 mm (95% confidence interval: 1.49–2.5 mm) in the side augmented with small BBM particles (Table 1). No statistically significant difference was measured between both sides

using the Wilcoxon signed-rank test. Six to 9 months after sinus floor augmentation, the increase in ridge height was measured from the second CT scan. The post-operative alveolar ridge residual height was 18 ± 2.915 mm (95% confidence interval: 14.99–20.5 mm) in the side augmented with the large BBM particles, and 18.3 ± 1.12 mm (95% confidence interval: 14.99–20.49 mm) in the side augmented with the small BBM particles (Table 1). Again, no statistically significant difference was measured between both sides using the Wilcoxon signed-rank test. At the second surgery, after taking the biopsy, implants were inserted into the reconstructed bone of the augmented sinus floor. Maximal implant insertion torque was >50 N for all the inserted implants (Table 1), with no statistically significant difference between both sides.

The post-operative complications included hematoma and swelling, regardless of the operated side or the granule size that was in use. In one of the patients, there was a post-operative infection, after the first surgery, on both sides operated, but he recovered using antibiotic therapy with no need for surgical intervention. In another patient, during the second-stage surgery, the augmented bone was found to be with a very low density and was very porous in both sides of the maxilla. No biopsies could be recovered from this subject, and the patient was excluded from the study.

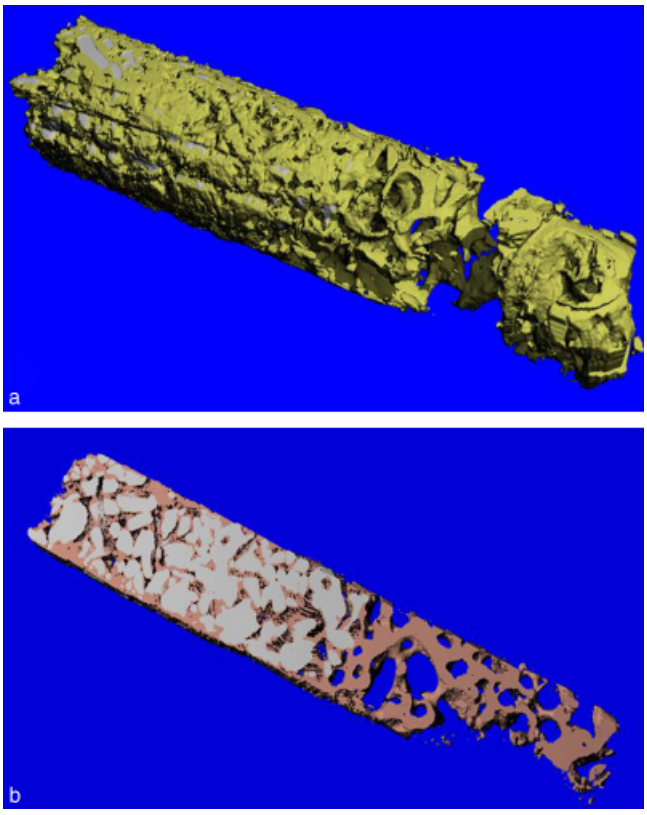

Fig. 5. (a) Example of a micro-computerized tomography (CT) three-dimensional (3D) reconstruction. (b) An example of micro-CT 3D slice reproduction from the same sample as in (a). The slice is made from 40 original CT slides, with different staining for thresholds – white for BioOss® and pink for bone.

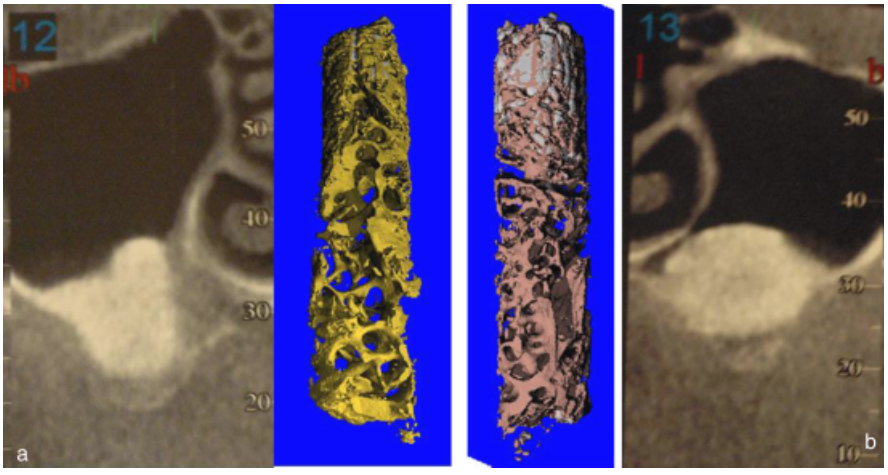

Fig. 6. Sections from of a computerized tomography (CT) scan taken before implant placement. With a reproduction of the micro-CT cores of the biopsies. (a) Right side, small particles, (b) left side, large particles.

Micro-CT analysis

The 2D slices produced from the CT scan allowed us to distinguish the BBM granules from the bone trabecules. Using the micro-CT software, we defined the radio-opacity threshold for bone and grafted material (white for grafted material and gray for bone). Then we were able to calculate from the 3D reconstruction the relative volume and percentage of these elements out of the whole core volume (TV) (Tables 2–5).

The morphometric results from the micro-CT quantitative analysis are summarized in Tables 2 and 3. The relative bone volume in the specimens retrieved from the large granules group was 7.99 ± 4.23% (95% confidence interval: 3.99–12.63%) while it was 14.64 ± 12.03% (95% confidence interval: 4.73–26.91%) for the small granules group. The differences between the two groups were not significant ($P = 0.15$). For large particles, the mean BV was 3.41 ± 2.38 mm^3 (95% confidence interval: 1.28–5.73 mm^3) and for the small particles, the mean BV was 5.39 ± 4.72 mm^3 (95% confidence interval: 1.79–10.34 mm^3). The difference between the volumes of the BBM between the two groups was also non-significant. For large particles, the mean BBM-BV was 9.41 ± 3.08 mm^3 (95%

Table 1. **Clinical information**

	Large particles			Small particles		
	Mean	SD	CI	Mean	SD	CI
Alveolar ridge height pre operative (mm)	2.45	1.46	1–3.49*	1.95	1.06	1.49–2.5**
Alveolar ridge height post operative (mm)	18	2.915	14.99–20.5**	18.3	1.12	14.99–20.49**
Max insertion torque			>50			>50

None of the differences between the groups were statistically significant (Wilcoxon's signed-rank test).
*CI 90%.
**CI 95%.
SD, standard deviation; CI, confidence interval.

Table 2. **Micro-CT-large vs. small particles**

	Large particles			Small particles			Large vs. small	
	Mean	SD	CI	Mean	SD	CI	CI	*P*-value
BBM (%)	23.12	6.02	15.82–28.22	22.89	8.32	13.86–9.87	− 3.18 to 2.96	0.68
Bone (%)	7.99	4.23	3.99–12.63	14.64	12.03	4.73–26.91	− 18.93 to 1.78	0.15
Soft tissue (%)	68.89	4.14	65.22–73.04	62.47	14.95	47.18–3.37	− 2.47 to 21.71	0.46

None of the differences were statistically significant (Wilcoxon's signed-rank test).
SD, standard deviation; CI, 95% confidence interval; CT, computerized tomography.

Table 3. **BBM, bone and soft-tissue volumes**

	Large particles			Small particles		
	Mean	SD	CI	Mean	SD	CI
Total volume (mm^3)	41.22	10.31	31.38–51.76	38.09	6.94	31.85–44.73
BBM volume (mm^3)	9.41	3.08	6.1–12.52	8.77	3.91	4.5–12.71
Bone volume (mm^3)	3.41	2.38	1.28–5.73	5.39	4.72	1.79–10.37
Soft tissue (mm^3)	28.4			23.9		

None of the difference were statistically significant (Wilcoxon's signed-rank test).
SD, standard deviation; CI, 95% confidence interval; BBM, bovine bone mineral.

Table 4. **Bone values**

	Large particles			Small particles		
	Mean	SD	CI	Mean	SD	CI
Bone/total volume (%)	7.99	4.23	3.99–12.63	14.64	12.03	4.73–26.91
Bone surface (mm^2)	71.58	41.56	29.83–114.99	112	103.13	36.91–219.12
BS/TV (mm^2/mm^3)	1.72	0.78	0.93–2.5	3.01	2.59	0.96–5.55
Th (mm)	0.15	0.03	0.11–0.17	0.15	0.02	0.13–0.15
Sp (mm)	0.97	0.19	0.77–1.1	0.8	0.43	0.43–1.19
TbN (1/mm)	1	0.22	0.87–1.21	1.45	0.66	1–2.16

None of the difference were statistically significant (Wilcoxon's signed-rank test).
SD, standard deviation; CI, 95% confidence interval; BS/TV, total surface of bone/graft trabecules out of total core volume; Th, trabecular thickness; Sp, spaces between bone/graft trabecules; TbN, trabecular number.

Table 5. **BBM values**

	Large particles			Small particles		
	Mean	SD	CI	Mean	SD	CI
BBM/total volume (%)	23.12	6.02	15.82–28.22	22.89	8.32	13.86–29.87
BBM surface (mm^2)	189.84	57.25	115.98–237.85	195.89	93.07	95.3–288.67
BBM BS/TV (mm^2/mm^3)	4.64	1.06	3.56–5.72	5.1	2.03	2.45–6.7
Th (mm)	0.18	0.02	0.16–0.19	0.17	0.01	0.15–0.18
Sp (mm)	0.51	0.23	0.3–0.72	0.67	0.37	0.39–1.11
TbN (1/mm)	1.98	0.55	1.51–2.45	1.69	0.59	0.92–2.18

None of the difference were statistically significant (Wilcoxon's signed-rank test).
SD, standard deviation; CI, 95% confidence interval; BS/TV, total surface of bone/graft trabecules out of total core volume; Th, trabecular thickness; Sp, spaces between bone/graft trabecules; TbN, trabecular number; BBM, bovine bone mineral.

confidence interval: 6.1–12.52 mm^3) and for the small particles, the mean BBM-BV was 8.77 ± 3.91 mm^3 (95% confidence interval: 4.5–12.71 mm^3). When subtracting the bone volume (BV) and the graft volume (BBM-BV) from the whole core (TV), we could calculate the volume of the soft-tissue in-between this calcified material (Tables 2 and 3). The percentage of soft-tissue volume in the cores containing large granules was 68.89 ± 4.14% (95% confidence interval: 65.22–73.04%) and the percentage of soft-tissue volume in the cores containing small granules was 62.47 ± 14.95% (95% confidence interval: 47.18–73.37%).

Table 4 presents the micro-CT data calculated for bone. The residual and new bone cannot be distinguished, and therefore were calculated together. The mean structural values of connectivity were as follows: TbN = 1 ($1\,mm^{-1}$), Sp = 0.97 mm, Th = 0.15 mm for large size granules and TbN = 1.45 ($1\,mm^{-1}$), Sp = 0.8 mm, Th = 0.15 mm for small size granules. For any of the tested parameters, there was no statistical difference between the groups using the Wilcoxon signed rank test. Table 5 presents the same parameters calculated for the BBM granules. There was no statistical difference between the groups in all measured parameters using the Wilcoxon signed rank test.

Histology and histomorphometric analysis

At low magnification, the differences in the histological sections between the large and the small particles were clear (Fig. 7). In both experimental groups, after 6–8 month of healing, most of the particles were surrounded by newly formed bone with well-organized osteons. The new bone produced a shape of a network, ''bridging'' between the BBM particles. The BBM particles presented marked staining differences from the host bone and had a lower affinity for the stains. The spaces between the mineralized tissues were occupied by small and large blood vessels, with the small blood vessels in close proximity to the new bone and the BBM particles. In many fields, multi-nucleated giant cells, probably osteoclasts, were observed directly on the BBM particles surface (Figs 8 and 9). It was interesting to see that in both groups (large vs. small particles), the osteoclast-like cells preferred the small-size BBM particles and not the large particles (Fig. 9). No gaps were present at the bone–particles interface, and the bone was always in close contact with the particles. No inflammatory cell infiltrate was present around the particles or at the interface with bone.

The morphometric results from the standard histological quantative analysis were summarized in Table 6. From the samples representing the large granules, the mean percentage of BioOss® granules out of the whole slide was 33.71 ± 8.28% (95% confidence interval: 26–40%). The percentage of bone volume was 27.14 + 3.89% (95% confidence interval: 23–31.99%) and the percentage of spaces was 39.14 + 8.47% (95% confidence interval: 32.5–48.5%). From the samples representing the small granules, the mean percentage of BBM granules out of the whole slide was 34.57 + 8.08% (95% confidence interval: 26.5–42%). The percentage of bone volume was 28 + 6% (22–34%), and the percentage of spaces was 37.42 + 4.15% (95% confidence interval: 34–42%). There was no statistical difference between the groups for all measurements (Table 6).

Discussion

In the present study, the clinical performances of the two different particle size of BBM were compared in sinus floor augmentation procedures. The results imply that there were no differences between these two BBM preparations

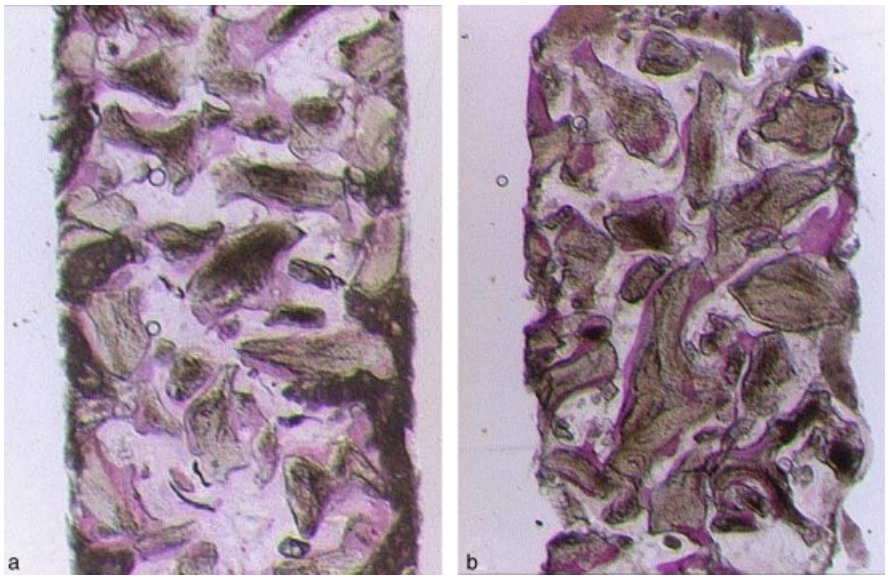

Fig. 7. A sample of the histological sections of the biopsies from one patient, × 30 magnification. (a) Small particles; (b) large particle. The difference in size of the BioOss® particles is evident.

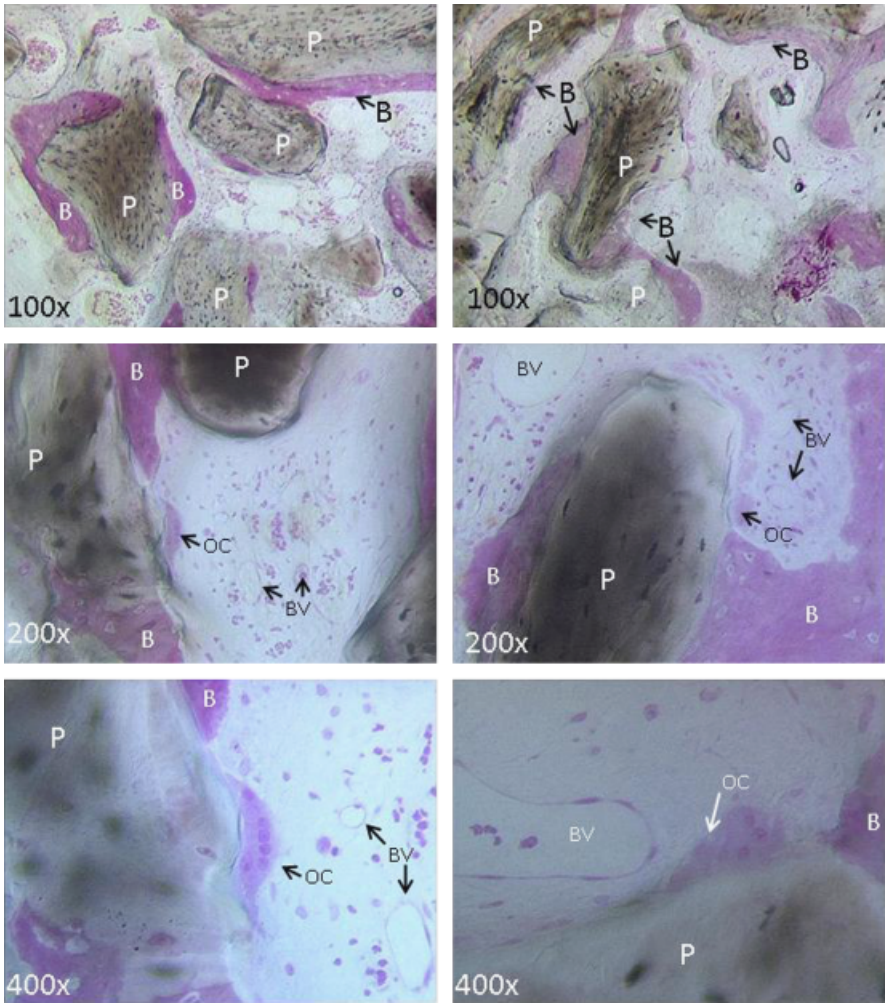

Fig. 8. Histological sections of the bone biopsies from the small particle group. P, BBM particle; B, new bone; OC, osteoclast; BV, blood vessel. Left and right panels are two different patients.

in sinus floor augmentation procedures in humans, using clinical, histological and micro-CT measurements. Both groups performed equally well, allowing stable implant placement and high insertion torque (>50 N). As expected, there was a large variability between the individual data from patient to patient, but the intra-individual design minimizes problems of variability between individuals.

BBM is a well-documented material for the augmentation of the maxillary sinus (Wallace et al. 1996; Valentini & Abensur 1997; Froum et al. 1998; Piattelli et al. 1999). The most popular and documented preparation of BBM, BioOss®, is commercially available for clinical use in two particle sizes – 0.25–1 and 1–2 mm, with no available data regarding the histological, clinical or other aspects related to the different particle sizes. There are studies investigating the effect of different particle sizes of harvested autogenous bone with no conclusive results (Zaner & Yukna 1984; Fucini et al. 1993; Xu et al. 2003; Springer et al. 2004; Murai et al. 2006; Coradazzi et al. 2007; Walsh et al. 2008; Kon et al. 2009). In another type of study, demineralized freeze-dried bone allografts with 850–1000 mm particles yielded more favorable results than the 250–500 mm particles in human periodontal defects (Fucini et al. 1993). However, no clinical or histological study was made so far relating the particle size of BBM.

In the present study, two quantitative methods for the evaluation of the mineralized material occupying the sinus orifice were used. The micro-CT system was first introduced by Feldkamp et al. (1989). This system was further validated as a 3D analysis method by Muller et al. (1998) when demonstrating the good correlation between the 3D analysis results with the well-accepted 2D histology. This method of analysis allows 3D measurements of bone quantity and a 3D assessment of the architecture and microstructure of the bone, which are important for the estimation of bone durability and strength. One of the great advantages of the micro-CT system is that this is a not destructive system. Therefore, all of these parameters can still be validated in the same biopsy sample with the 2D conventional histology. The histomorphometric analysis has the ability to more accurately evaluate the inter-phase between the graft particles and the newly formed bone, the cellular characterization, and will allow validation of the volumetric results. Using micro-CT volumetric analysis, there were no significant differences between the two groups for any of the tested parameters, as defined using radio-opacity thresholds to discriminate between bone, BBM and non-calcified tissue. There was a tendency toward the creation of more new bone when

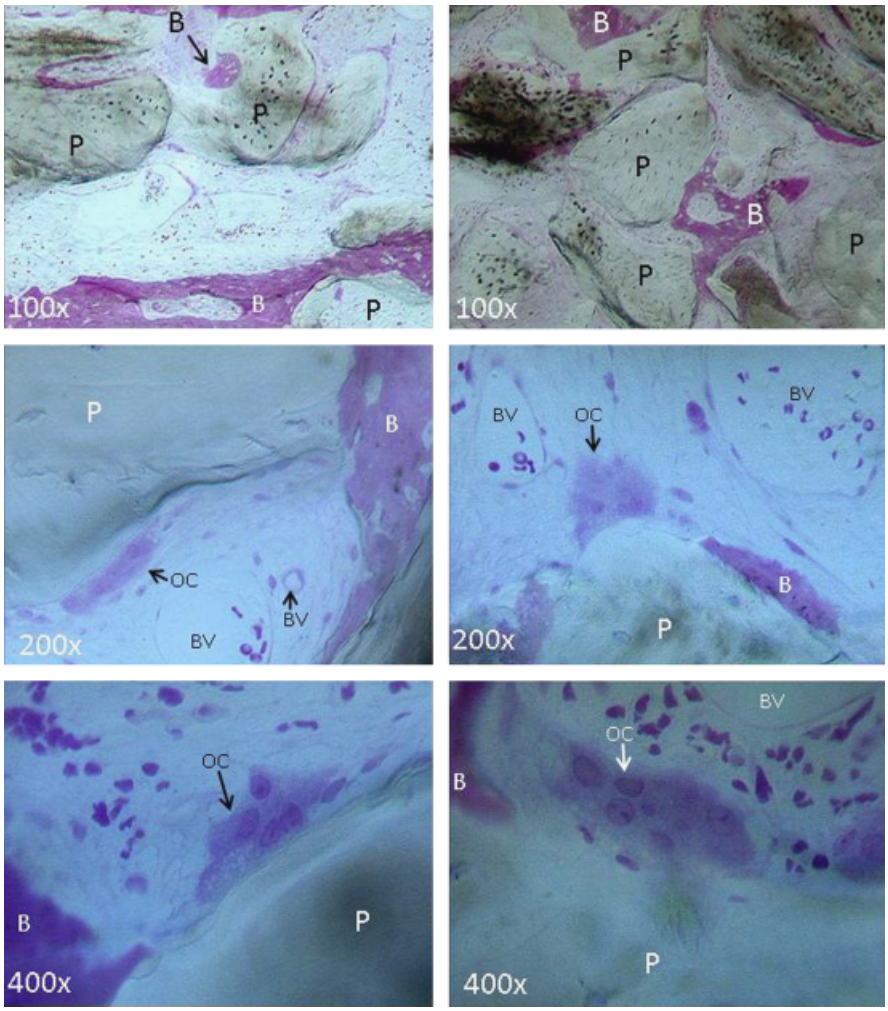

Fig. 9. Histological sections of the bone biopsies from the large particle group. P, BBM particle; B, new bone; OC, osteoclast; BV, blood vessel. Left and right panels are two different patients.

Table 6. **Histomorphometry – large vs. small**

	Large			Small			Large vs. small	
	Mean	SD	CI	Mean	SD	CI	CI	*P*-value
BBM (%)	33.71	8.28	26–40	34.57	8.08	26.5–42	−8 to 8.5	1
Bone (%)	27.14	3.89	23–31.99	28	6.53	22–34	−6.49 to 4	0.83
Spaces (%)	39.14	8.47	32.5–48.5	37.42	4.15	34–42	−4.5 to 9.99	0.67

None of the differences were statistically significant (Wilcoxon's signed-rank test).
SD, standard deviation; CI, 95% confidence interval; BBM, bovine bone mineral.

using the small granules. This tendency was not found in the histomorphometry analysis.

It seems that the micro-CT technique underestimated the amount of the histological bone and graft values. This is probably due to the differences in analysis methodology between these two techniques. Although the micro-CT was shown in the past to correlate to the 2D histological measurements (Muller et al. 1998), it was not true in the present study. The micro-CT analysis is based on our ability to define correct thresholds for separating the gray levels in the micro-CT image. It was difficult to distinguish in the micro-CT image the exact borders between the new bone and graft particles, which are in direct contact and show a similar pattern. Graft particles surrounded by new bone, as presented in Figs 7–9, will therefore be calculated in the micro-CT analysis as a large BBM particle, under-estimating the amount of new bone present. The histological analysis can easily discriminate between the graft particles and the surrounding new bone, based on color and morphology. On the other hand, the histological analysis is a quantitative calculation of a 2D image, taken from the central portion of the sample, and we can only assume that this represents the dispersing of all measured elements in the whole core. This is in contrast to the micro-CT analysis, which is made from the complete volume of the sample, and therefore, more complete in this aspect.

When the micro-CT scans were examined, it was hard for the naked eye to distinguish between small and large BBM granules. It was suspected that the surgical technique of applying the particles into the sinus space may changes the granule size, i.e. the condensation of large granules into a tube syringe (Fig. 1c) may crush them into smaller sized granules. The lack of significant difference between the values of BBM trabecular size (Table 5), which represented the thickness of graft particles, also supported this hypothesis. This may be one of the reasons for the fact that it was not possible to demonstrate a significant difference between the groups.

In conclusion, the present study suggested that there is no clinical or histological difference between the two BBM preparations for sinus floor augmentation procedures. Both size of the BBM granules preformed equally well and achieved the aim of the sinus floor augmentation procedure – insertion of implants of 10 mm or longer with high primary fixation. The present results suggested that the selection of small or large BBM particles should be depended on the personal preference of the surgeon, sinus anatomy or the presence of membrane rupture.

Acknowledgements: The study was partially supported by a research grant from Geistlich Biomaterials Inc., Wolhusen, Switzerland.

References

Artzi, Z., Koslovsky, A., Nemcovsky, C.E. & Weinreb, M. (2005) The amount of newly formed bone in sinus grafting procedures depends on tissue depth as well as the type and residual amount of the grafted material. *Journal of Clinical Periodontology* **32**: 193–199.

Blomqvist, J.E., Alberius, P. & Isaksson, S. (1996) Retrospective analysis of one-stage maxillary sinus augmentation with endosseous implants. *The Inter-*

national Journal of Oral & Maxillofacial Implants **11**: 512–521.

Boyne, P.J. & James, R.A. (1980) Grafting of the maxillary sinus floor with autogenous marrow and bone. *Journal of Oral Surgery* **38**: 613–616.

Branemark, P.I., Adell, R., Albrektsson, T., Lekholm, U., Lindstrom, J. & Rockler, B. (1984) An experimental and clinical study of osseointegrated implants penetrating the nasal cavity and maxillary sinus. *Journal of Oral and Maxillofacial Surgery* **42**: 497–505.

Coradazzi, L.F., Garcia, I.R Jr. & Manfrin, T.M. (2007) Evaluation of autogenous bone grafts, particulate or collected during osteotomy with implant burs: histologic and histomorphometric analysis in rabbits. *The International Journal of Oral & Maxillofacial Implants* **22**: 201–207.

Feldkamp, L.A., Goldstein, S.A., Parfitt, A.M., Jesion, G. & Kleerekoper, M. (1989) The direct examination of three-dimensional bone architecture in vitro by computed tomography. *Journal of Bone and Mineral Research* **4**: 3–11.

Froum, S.J., Tarnow, D.P., Wallace, S.S., Rohrer, M.D. & Cho, S.C. (1998) 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. *The International Journal of Periodontics and Restorative Dentistry* **18**: 528–543.

Fucini, S.E., Quintero, G., Gher, M.E., Black, B.S. & Richardson, A.C. (1993) Small versus large particles of demineralized freeze-dried bone allografts in human intrabony periodontal defects. *Journal of Periodontology* **64**: 844–847.

Groenveld, H.H., van den Bergh, J.P., Holzmann, P., ten Bruggenkate, C.M., Tuinzing, D.B. & Burger, E.H. (1999) Histological observations of a bilateral maxillary sinus floor elevation 6 and 12 months after grafting with osteogenic protein-1 device. *Journal of Clinical Periodontology* **26**: 841–846.

Hallman, M. & Thor, A. (2008) Bone substitutes and growth factors as an alternative/complement to autogenous bone for grafting in implant dentistry. *Periodontology 2000* **47**: 172–192.

Hammerle, C.H., Chiantella, G.C., Karring, T. & Lang, N.P. (1998) The effect of a deproteinized bovine bone mineral on bone regeneration around titanium dental implants. *Clinical Oral Implants Research* **9**: 151–162.

Hurzeler, M.B., Quinones, C.R., Kirsch, A., Gloker, C., Schupbach, P., Strub, J.R. & Caffesse, R.G. (1997) Maxillary sinus augmentation using different grafting materials and dental implants in monkeys. Part i. Evaluation of anorganic bovine-derived bone matrix. *Clinical Oral Implants Research* **8**: 476–486.

Klinge, B., Alberius, P., Isaksson, S. & Jonsson, J. (1992) Osseous response to implanted natural bone mineral and synthetic hydroxylapatite ceramic in the repair of experimental skull bone defects. *Journal of Oral and Maxillofacial Surgery* **50**: 241–249.

Kon, K., Shiota, M., Ozeki, M., Yamashita, Y. & Kasugai, S. (2009) Bone augmentation ability of autogenous bone graft particles with different sizes: a histological and micro-computed tomography study. *Clinical Oral Implants Research* **20**: 1240–1246.

McAllister, B.S., Margolin, M.D., Cogan, A.G., Taylor, M. & Wollins, J. (1998) Residual lateral wall defects following sinus grafting with recombinant human osteogenic protein-1 or bio-oss in the chimpanzee. *The International Journal of Periodontics and Restorative Dentistry* **18**: 227–239.

Moy, P.K., Lundgren, S. & Holmes, R.E. (1993) Maxillary sinus augmentation: histomorphometric analysis of graft materials for maxillary sinus floor augmentation. *Journal of Oral and Maxillofacial Surgery* **51**: 857–862.

Muller, R., Van Campenhout, H., Van Damme, B., Van Der Perre, G., Dequeker, J., Hildebrand, T. & Ruegsegger, P. (1998) Morphometric analysis of human bone biopsies: a quantitative structural comparison of histological sections and micro-computed tomography. *Bone* **23**: 59–66.

Murai, M., Sato, S., Fukase, Y., Yamada, Y., Komiyama, K. & Ito, K. (2006) Effects of different sizes of beta-tricalcium phosphate particles on bone augmentation within a titanium cap in rabbit calvarium. *Dental Materials Journal* **25**: 87–96.

Nkenke, E. & Stelzle, F. (2009) Clinical outcomes of sinus floor augmentation for implant placement using autogenous bone or bone substitutes: a systematic review. *Clinical Oral Implants Research* **20** (Suppl. 4): 124–133.

Piattelli, M., Favero, G.A., Scarano, A., Orsini, G. & Piattelli, A. (1999) Bone reactions to anorganic bovine bone (bio-oss) used in sinus augmentation procedures: a histologic long-term report of 20 cases in humans. *The International Journal of Oral & Maxillofacial Implants* **14**: 835–840.

Sharan, A. & Madjar, D. (2008) Maxillary sinus pneumatization following extractions: a radiographic study. *The International Journal of Oral & Maxillofacial Implants* **23**: 48–56.

Smiler, D.G. & Holmes, R.E. (1987) Sinus lift procedure using porous hydroxyapatite: a preliminary clinical report. *Journal of Oral Implantology* **13**: 239–253.

Smiler, D.G., Johnson, P.W., Lozada, J.L., Misch, C., Rosenlicht, J.L., Tatum, O.H Jr. & Wagner, J.R. (1992) Sinus lift grafts and endosseous implants. Treatment of the atrophic posterior maxilla. *Dental Clinics of North America* **36**: 151–187.

Spector, M. (1994) Anorganic bovine bone and ceramic analogs of bone mineral as implants to facilitate bone regeneration. *Clinical Plastic Surgery* **21**: 437–444.

Springer, I.N., Terheyden, H., Geiss, S., Harle, F., Hedderich, J. & Acil, Y. (2004) Particulated bone grafts – effectiveness of bone cell supply. *Clinical Oral Implants Research* **15**: 205–212.

Tadjoedin, E.S., de Lange, G.L., Bronckers, A.L., Lyaruu, D.M. & Burger, E.H. (2003) Deproteinized cancellous bovine bone (bio-oss) as bone substitute for sinus floor elevation. A retrospective, histomorphometrical study of five cases. *Journal of Clinical Periodontology* **30**: 261–270.

Tallgren, A. (1972) The continuing reduction of the residual alveolar ridges in complete denture wearers: a mixed-longitudinal study covering 25 years. *Journal of Prosthetic Dentistry* **27**: 120–132.

Tatum, H. Jr. (1986) Maxillary and sinus implant reconstructions. *Dental Clinics of North America* **30**: 207–229.

Turunen, T., Peltola, J., Yli-Urpo, A. & Happonen, R.P. (2004) Bioactive glass granules as a bone adjunctive material in maxillary sinus floor augmentation. *Clinical Oral Implant Research* **15**: 135–141.

Valentini, P. & Abensur, D. (1997) Maxillary sinus floor elevation for implant placement with demineralized freeze-dried bone and bovine bone (bio-oss): a clinical study of 20 patients. *The International Journal of Periodontics and Restorative Dentistry* **17**: 232–241.

Valentini, P., Abensur, D., Densari, D., Graziani, J.N. & Hammerle, C. (1998) Histological evaluation of bio-oss in a 2-stage sinus floor elevation and implantation procedure. A human case report. *Clinical Oral Implants Research* **9**: 59–64.

Wagner, J.R. (1991) A 3 1/2-year clinical evaluation of resorbable hydroxylapatite osteogen (ha resorb) used for sinus lift augmentations in conjunction with the insertion of endosseous implants. *Journal of Oral Implantology* **17**: 152–164.

Wallace, S.S., Froum, S.J. & Tarnow, D.P. (1996) Histologic evaluation of a sinus elevation procedure: a clinical report. *The International Journal of Periodontics and Restorative Dentistry* **16**: 46–51.

Walsh, W.R., Vizesi, F., Michael, D., Auld, J., Langdown, A., Oliver, R., Yu, Y., Irie, H. & Bruce, W. (2008) Beta-tcp bone graft substitutes in a bilateral rabbit tibial defect model. *Biomaterials* **29**: 266–271.

Wetzel, A.C., Stich, H. & Caffesse, R.G. (1995) Bone apposition onto oral implants in the sinus area filled with different grafting materials. A histological study in beagle dogs. *Clinical Oral Implants Research* **6**: 155–163.

Xu, H., Shimizu, Y., Asai, S. & Ooya, K. (2003) Experimental sinus grafting with the use of deproteinized bone particles of different sizes. *Clinical Oral Implants Research* **14**: 548–555.

Zaner, D.J. & Yukna, R.A. (1984) Particle size of periodontal bone grafting materials. *Journal of Periodontology* **55**: 406–409.