Atrophic maxillary floor augmentation by mineralized human bone allograft in sinuses of different size: an histologic and histomorphometric analysis

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Abstract
Objective: The aims of this work were to histologically examine the healing of mineralized human bone allograft (MHBA) in sinus augmentation for elevating a severe maxillary atrophy ridge (≤2 mm residual ridge height) and to correlate the results to the sinus cavity size.

Material and methods: A two-stage protocol was conducted in 23 patients, all having crestal bone ≤2 mm. A mixture of 80/20 cortical/cancellous of MHBA particles was used to augment sinus using the lateral window approach in narrow (NS; < 15 mm bucco-palatal distance) and wide (WS; ≥ 15 mm bucco-palatal distance) sinuses, based upon computerized tomography (CT) assessment. A bone core biopsy was taken at implant placement, 6 and 9 months after surgery. Microradiography, histology and histochemistry of methacrylate-embedded sections were performed to analyze and to evaluate the bone and graft amount.

Results: Newly formed bone around MBHA particles was found in all 28 biopsies. Bone showed a woven structure at 6 months after surgery and a lamellar structure 9 months after surgery. At 6 months after surgery, the 13 NS and 15 WS had 30.5 ± 8.8% and 20.7 ± 4.9% mean ± SD bone formation, respectively. At 9 months after surgery, it was 38.8 ± 7% (NS) and 30.7 ± 3% (WS). Residual graft was about 16% (6 months) and 6% (9 months), in both NS and WS. The Mann–Whitney test showed a greater bone formation in NS than in WS (P < 0.005).

Conclusions: The used 80/20 MHBA mixture appears to promote, in the severe atrophic maxilla, a satisfactory bone formation. Our results prove that the larger the sinus, the longer the maturation time needed to achieve a suitable amount of new bone formation.
2007; Chiapasco et al. 2009) show that the survival rate of implants placed in augmented sinuses is the same as that of implants inserted into native bone of maxillary posterior areas. These reviews highlight that the exclusive use of autogenous bone does not improve the survival rate of the implant. On the contrary, 96% was the survival rate of 12,000 implants (6913 – Del Fabbro et al. 2004; 5128 – Aghaloo & Moy 2007) in sinus augmentations when a xenograft, without autogenous bone, was used. When only autogenous bone is used, the survival rate falls to 92% (Del Fabbro et al. 2004; Aghaloo & Moy 2007). A further decrease of the rate (88%) was recorded when autogenous bone from the iliac crest was the graft used (Aghaloo & Moy 2007). The survival rate falls to 82% when autogenous block graft is used (Wallace & Froum 2003; Del Fabbro et al. 2004).

Several materials have been used as graft for sinus floor augmentation [Wallace 2006]. Both xenograft and allograft materials have shown predictable and successful results [Aghaloo & Moy 2007; Browaeys et al. 2007; Galindo-Mor eno et al. 2008]. Mineralized human bone allograft (MHBA) was recently been introduced and has shown promising results in periodontal and bone regeneration [Block & Degen 2004; Wang et al. 2004; Noumbissi et al. 2005; Froum et al. 2006; Gapski et al. 2006; Tsao et al. 2006, Wang & Tsao 2007, 2008; Browning et al. 2009; Morelli et al. 2009]. This grafting material is a bone-derived product harvested from cadavers, solvent dehydration and processed and then sterilized. This graft type has been used in several clinical applications such as the treatment of periodontal infrabony defects (Tsao et al. 2006; Browning et al. 2009), in socket augmentation (Wang et al. 2004; Wang & Tsao 2007, 2008), in horizontal ridge regeneration (Block & Degen 2004; Morelli et al. 2009) and in sinus augmentation procedures [Noumbissi et al. 2005; Froum et al. 2006, Gapski et al. 2006].

Regardless of graft type, it is not yet well defined how three-dimensional anatomical sinus cavity may influence the mineralization processes and healing. It has been reported that the greater the residual bone, the higher the success rate regardless of whether one-stage or two-stage approaches were used [Jensen & Greer 1992; Geurs et al. 2001; Peleg et al. 2006; Mardinger et al. 2007; Rios et al. 2009]. However, no studies has reported results in relation to the three-dimensional conformation of the vestibular and the palatal walls, or to the bone regeneration by the graft positioned between these walls. For example, if the graft placed in a narrow maxillary sinus might quickly and successfully heal and mineralize, when compared with a larger and wider sinus.

The aims of this study were to histologically examine the healing of MHBA in sinus augmentation for elevating a severe maxillary atrophy ridge (≤ 2 mm residual ridge height) and to correlate the results to the sinus cavity size.

Material and methods

Surgery

The patients of this study, examined and treated in private dental office, were selected from a pool of subjects requiring maxillary sinus augmentation for the placement of delayed posterior implants. All patients were partially or totally edentulous and needing either unilateral or bilateral maxillary sinus augmentation. Additional inclusion criteria were < 2 mm of crestal bone height of the sinus floor, as measured on the serial section of the computerized tomography (CT), general health, not a smoker, absence of diseases that affects bone metabolism or wound healing, absence of specific disease or problems within the maxillary sinus and no regular medications consumption for more than 3 months. All patients signed their informed consent in which all procedures of the study were detailed, according to the Helsinki protocols (World Medical Association Declaration of Helinski, 2002).

A pre-operative panoramic radiograph and a CT scan (at increasing depth of 1 mm intervals) of the maxilla were taken for each patient. The width of the sinus was evaluated in the CT section corresponding to the exact place where the implant had to be inserted based upon the prosthetic plan. The sagittal CT section was identified using a diagnostic template (radio-opaque coated material). Using a radiographic software [3 Diagnosis 3.0, 3DIEMME S.r.l., Cantù CO, Italy], the distance between the palatal and buccal wall (PBD) was measured 10 mm cranially to the residual bone crest (as a reference), in order to place at least a 10 mm implant (Winkler et al. 2000; Friberg et al. 2002; Petrie & Williams 2005), 6 or 9 months later.

Surgical sites were infiltrated by a local anesthetic (articain hydrochloride, Ultracain, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany). By CT scan pre-planning of window location and boundaries, a full-thickness flap was reflected to expose the lateral wall of the sinus. A bony window osteotomy (Vercellotti et al. 2001) was performed using a piezorsurgical device (Mectron S.p.A., Carasco GE, Italy). The bony window was lifted, without removal, at first wall movement, then the Schneiderian membrane was gently lifted using the piezorsurgical device and subsequently a broad curette.

A mixture of cortical and cancellous (80/20) MHBA (Puros, Zimmer Dental Inc., Carlsbad, CA, USA), each consisting of a 50 : 50 mixture of 0.25–1 and 1–2 mm particles, moistened by saline solution, was used as the grafting material. Depending on the sinus anatomy and number of implants to place, 2–4 g of graft was gently packed on each sinus floor, beneath the membrane. Before soft tissue closure, an absorbable collagen membrane (BioMend®, Zimmer Dental Inc.) was placed over the window and the vestibular flap repositioned using surgical Gore-Tex 5/0 (W. L. Gore & Associates, Flagstaff, AZ, USA). Patients were then treated with amoxicillin 1 g (Ratiopharm GmbH, Ulm, Germany) twice a day for 6–7 days, and Synflex forte 550 mg (Recordati SpA, Milano, Italy) as an analgesic, if needed, after surgery. Patients were directed to use a chlorhexidine mouthwash [0.12%], twice a day, and not to brush the surgical sites for 2 weeks. Sutures were removed 12–14 days after the surgery. Monthly follow-up was scheduled to check for wound dehiscence up to implant insertion.

A 3-mm-diameter trephine, under a saline jet at 600 rpm, was utilized [up to 10 mm] to collect bone core specimens before implant insertion (Tapered Screw Vent, Zimmer Dental Inc.). The bone core specimens were collected with the assistance of surgical guides that was based upon the individual prosthetic requirements, 6 or 9 months after sinus augmentation procedures. Implant sites were accurately planned to place implants exactly in purely augmented skeletal segments, including possible residual crest. The retrieved bone core samples were immediately fixed for the histological study.

Histology

The cylindrical bone samples were fixed in 4% paraformaldehyde [all reagents came from Fluka, Sigma-Aldrich Schweiz, Buchs SG, Switzerland] in 0.1 M phosphate buffer pH 7.2 for 4 h at room temperature. Specimens were dehydrated through ethanol series at 4°C, then embedded in methyl metacrylate (PMMA) using a water bath at 4°C as described elsewhere [Bertoldi et al. 2008].

The PMMA blocks were serially sectioned to obtain two sets of sections: thick (100 μm) and thin (5-μm-thick) sections.

First of all, each PMMA block was serially sectioned along the longitudinal axis of the cylindrical bone sample to its center using a diamond saw microtome (SP1600, Leica Microsystems, Wetzlar, Germany).

A thick section (200 μm) was obtained from the center of the cylindrical sample using the diamond saw microtome. The section was reduced to 100 μm by grinding, perfectly polished
with emery paper and alumina, and then X-ray microradiographed (KI, Italstructures, Riva del Garda TN, Italy) at 15 kV and 10 mA on high-resolution film (SO 343, Eastman Kodak Co., Rochester, NY, USA). This section was then glued to a slide and stained with 0.1% fast green FCF in 1% acetic acid.

A set of 5-μm-thick sections was obtained starting from the level at which the thick section was performed, using a tungsten carbide knife (Profile D) on a bone microtome (Autocut 1150, Reichert-Jung GmbH, Nussloch, Germany). Thin sections were stained with toluidine blue or trichrome Gomori stain. Some slides were stained with total alkaline phosphatase (TAP) histochemical method, a marker of osteogenesis (azo dye = fast blue BB) and tartrate-resistant acid phosphatase (TRAP) histochemical method, a marker of osteoclasts (azo dye = fast red RC) (Zaffe & D’Avenia 2007).

The microradiographs and the sections were analyzed and photographed using a microscope (Axiophot, Carl Zeiss AG, Oberkochen, Germany) under ordinary light. The amount of bone tissue [bone volume (BV)], graft [graft volume (GV)] per tissue volume (BV/TV and GV/TV, respectively), were evaluated on the microradiographs using a suitable program for image analyzer and software (AnalySIS®, Soft Imaging System GmbH, Münster, Germany).

Statistical analysis
Comparisons were performed by means of the non-parametric Mann–Whitney rank-sum test (Glantz 2003). The null hypothesis was rejected for a critical significance level of \( P < 0.05 \).

Results

Surgery
Twenty-three selected patients, 10 males and 13 females, aged between 35 and 70, mean = 54.5 years, underwent unilateral (\( n = 18 \)) or bilateral (\( n = 5 \)) maxillary sinus augmentation with a total of 28 treated sinuses (Fig. 1). Primary wound closure was obtained in all surgeries and no complaint or adverse effects were observed during the follow-up.

The distance between the PBD of the 28 sinuses, measured using 3Diagnosis software 3.0, had a mean ± SD value of 14.7 ± 4.47 mm and a median of 15 mm. Therefore, performing arbitrary cut-off, maxillary sinuses have been split into two categories: (A) narrow sinuses (NS), with PBD < 15 mm; (B) and wide sinuses (WS), with PBD ≥ 15 mm (Fig. 2).

A total of 28 core biopsies, and correspondingly a total of 28 implants was placed in those sites according to individual implant–prosthetic–treatment plan, were obtained from the 23 patients.

Sixteen core biopsies were performed at 6 months, and 12 were performed at 9 months. Seven core biopsies pertained to NS and nine biopsies to WS, 6 months after surgery. Six core biopsies pertained to NS and six biopsies to WS, 9 months after surgery.

Histology
Six months after the surgery, the biopsies showed a composite formed by MHBA particles and newly formed bone trabeculae (Fig. 3). The majority of MHBA particles directly connected with the newly formed bone (Figs 4 and 5) with very few particles that were totally surrounded by the fibrous tissue. A recurrent event was the disappearance of the pre-existing “oral” bone forming the thin crestal wall before surgery. A network of newly formed bony trabeculae, with or without MHBA particles (Figs 3 and 4), was a common finding. Sometimes, a thickening of these trabeculae to form a thin and porous cortex could be observed (Fig. 4).

The common structure of newly formed bony trabeculae, all containing osteocytes, was the woven one (Fig. 5), in particular at the sinusal part or in the thickness of the newly formed maxillary floor. On the contrary, parallel fibered or lamellar bone with osteocytes, also enveloping
MHBA particles (Fig. 4), could be found at the oral part of the newly formed maxillary floor. Bone or particles surfaces covered by layered cuboidal osteoblasts (Fig. 5), i.e. active bone-forming cells, could form a recurring event. Only osteoblast sites and the fibrous tissue of the close neighbors expressed positive TAP (Fig. 5), whereas osteoclasts, TRAP positive (Fig. 5), preferably resorbed the newly formed bone, even if they do not disdain MHBA particles.

Nine months after surgery, the biopsies showed newly formed bone, all containing osteocytes with few MHBA particles remaining. The newly formed bone trabeculae turned from woven to lamellar structure in most of the maxillary floor thickness and woven bone could be found in the sinusal portion only. Very few were the bony surfaces covered by active osteoblasts, and the scanty TAP-positive surfaces corresponded to surfaces covered by flattened osteoblast, i.e. elements transforming into bone-lining cells. Very few TRAP-positive osteoclasts were observed at this time, but some mononucleated cells (probably pre-osteoclasts) were revealed by the TRAP in a few medullary spaces.

**Histology and statistics**

The histomorphometric evaluation of the amount of bone and MHBA per tissue volume (BV/TV and GV/TV, respectively) of all biopsies returned a mean ± SD value of 25.02 ± 6.34 (BV/TV) and 15.96 ± 7.07% (GV/TV), respectively, 6 months after surgery (n = 16). These indices turned to 34.75 ± 6.63 (BV/TV) and 5.85 ± 3.11% (GV/TV), respectively, 9 months after surgery (n = 12). Both the bone increase (+ 9.73%, P < 0.001) and the MHBA reduction (– 10.11%, P < 0.001) were statistically significant after the Mann–Whitney test.

Indices (mean ± SD) were 30.36 ± 8.77% (BV/TV) and 15.97 ± 8.86% (GV/TV) in NS (n = 7), and 20.71 ± 4.91% (BV/TV) and 15.95 ± 5.91% (GV/TV) in WS (n = 9), 6 months after surgery (Fig. 6). The greater bone value of NS was statistically significant (P = 0.03) after the Mann–Whitney test (Table 1). Those indices (mean ± SD) were 38.79 ± 6.97% (BV/TV) and 5.37 ± 2.15% (GV/TV) in NS (n = 6), and 30.71 ± 2.98% (BV/TV) and 6.33 ± 4.01% (GV/TV) in WS (n = 6), 9 months after surgery (Fig. 6). The greater bone value of NS was statistically significant (P = 0.04) after the Mann–Whitney test (Table 1).

The bone amount of NS and WS showed a similar behavior from 6 to 9 months after surgery (Fig. 7). The new bone formation had an almost parallel increasing trend in these sinuses, with a BV/TV differential of 8–9% for NS (Fig. 7). Longitudinal analyses on the increased bone content recorded 9 months after surgery highlighted a statistical significance for WS (P < 0.0015), but not for NS (P = 0.12), after the Mann–Whitney test.

**Discussion**

The MHBA used as particulate proved to be a safe and fully biocompatible material able to promote new bone formation in sinus augmentation procedures. This had been demonstrated previously when cancellous allograft was used alone [Noumbissi et al. 2005; Froum et al. 2006] or in combination with particulate autogenous bone [Gapski et al. 2006]. Nevertheless, this paper is...
Whitney rank-sum test results between 6 and 9 months after surgery. Table 1.

The amount of graft particles were reducing while narrow (N) and wide (W) sinuses, 6 and 9 months after surgery. The two lines have been plotted using the mean values (as the regression lines). Even if the bone content was statistically significant only for wide sinuses, the trend is almost parallel between narrow and wide sinuses, both increasing over time.

Histologic results highlight that bone, mostly woven structured, was formed in a moderate amount (about 35%) 6 months after surgery. Osteogenic and osteoclastic processes remain active at/after this time. Contrary to what was recorded in maxillary sinus augmentation by autologous bone (Browaeys et al. 2007; Consolo et al. 2007), the bone amount does not reduce but increased (reaching the 35%) 3 months later. The structure of the bone was more ordered and remodeling processes rather ineffective. MHBA most likely requires longer healing time when compared with the autologous bone.

Histomorphometric results highlight that the amount of newly formed bone recorded in this study in 16 core biopsies is almost similar to that reported by Froum et al. (2006), which used the same MHBA; however, the amount of residual graft recorded (about 16%) is almost twice that of Froum and colleagues, 6 months after surgery. This latter could be attributed to only cancellous allograft, resorbed quicker than the cortical/cancellous mixture, and was used in Froum et al.’s (2006) study. On the contrary, the amount of newly formed bone recorded in this study is greater than the studies using deproteinized bovine bone mineral (DBBM) as the graft material (Valentini et al. 2000; Yildirim et al. 2001; Froum et al. 2006 – control group) 6 months after surgery.

The amount of newly formed bone recorded in 12 core biopsies is still higher than that obtained in studies using DBBM (Valentini et al. 2000) or a mineralized freeze-dried bone allograft (Kolerman et al. 2008), 9 months after surgery. Contrary to what was observed with autologous bone (Consolo et al. 2007), this result seems to support the suggestion that a longer healing period allows for a more deposition of newly formed bone, as demonstrated previously (Landi et al. 2000; Artzi et al. 2008). In the opinion of Handschel et al. (2009), a healing period of 9 months would be sufficient to guarantee adequate outcomes for several grafting materials used in sinus augmentation procedures.

As reported in the case of fractures or distractive procedures (Zaffe et al. 2002), in sinus augmentation it is expected that bone processes expand skeletal formation from the peripheral wall towards the center of the grafted cavity. The bone expansion is more rapid and effective in proximity of medial and lateral sinus walls, but not only one of the few studies on sinus augmentation procedures focusing on severe atrophic maxilla ($\leq 2$ mm remaining ridge height) but also correlating the outcomes to the dimension of sinus cavity. Results obtained from this study showed that the 80/20 mixture of cortical/cancellous MHBA can be a predictable grafting material used to augment the sinus.

To favor better results, we used specific and restricted criteria for inclusions of patients in this research, undoubtedly limiting the number of patients enrolled. The selected group, equivalent enough against gender, ranged between 35 and 70 years, with a mean age of about 55 years, i.e. a not exactly elder population showing a severe maxillary atrophy. The decision to place implants in a two-stage procedure was because of the inability to ensure initial implant stability due to minimal amount of residual crestal bone ($\leq 2$ mm).

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it is slower and less effective at the center [Boyne & James 1980; Jensen et al. 1998]. As our results seem to indicate, WS may express a reduced osteogenic potential owing to a greater distance from the peripheral sources of osteogenic cells. Conversely, NS may have an increased osteogenic potential due to the relatively short distance from peripheral walls. In the 28 augmented sinuses, results obtained showed a statistically significant lower bone formation in WS ($n = 15$) than the NS ($n = 13$) at 6 and 9 months after surgery. The lacking of statistical significance recorded in the longitudinal analysis of NS is probably due to its numerosity, pointing out recorded in the longitudinal analysis of NS is probably due to its numerosity, pointing out

Conclusion

Within the limits of this study, the following conclusions can be drawn: (1) 80/20 mixture of cortical/cancellous MHBA is a biocompatible and effective material for augment sinus floor and (2) the larger the sinus cavity, the longer the maturation time required to achieve a suitable amount of new bone formation.

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