Histomorphometric Analysis of Maxillary Sinus Grafts: A Pilot Study

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Purpose: *This pilot study evaluated and compared the degree of new bone formation following maxillary sinus graft using three different bone graft materials.* ***Materials and Methods:*** *Patients with an edentulous posterior maxilla (unilateral or bilateral) were included in this study and underwent a two-stage procedure. Each sinus was randomly assigned one of the three graft materials: anorganic bovine bone mineral (ABBM), anorganic equine bone mineral (AEBM), or mineralized cancellous bone allograft (MCBA). Bone core samples were obtained from the lateral wall of the grafted sites at least 8 months after maxillary sinus graft. Bone quality was evaluated during bone core retrieval. The samples were histomorphometrically analyzed using Kruskal-Wallis and Dunn-Bonferroni tests at the significance level of* $\alpha = .05$. ***Results:*** *A total of 28 sinuses (14 unilateral and 7 bilateral) from 21 subjects, with a mean age of 61.5 (range: 33 to 75) years, were included in the study. Twenty-eight bone cores (ABBM [n = 9], AEBM [n = 9], and MCBA [n = 10]) were obtained at a mean healing time of 9.1 (range: 8 to 12) months. Six maxillary sinus membrane perforations (≤ 5 mm) were noted and repaired during surgery (21.4%). Histomorphometric analysis of the harvested bone cores revealed statistically significant differences in the percentage of vital bone, residual bone materials, and connective tissue/marrow among the different graft materials (Kruskal-Wallis; P < .05). The percentage of vital bone in the MCBA group (32.0% ± 12.4%) was significantly greater than those in the ABBM (10.9% ± 8.9%) and AEBM (9.1% ± 5.9%) groups (P < .05). The percentage of residual bone materials in the MCBA group (5.5% ± 5.7%) was, however, significantly less than those in the ABBM (34.3% ± 12.1%) and AEBM (38.9% ± 5.3%) groups (P < .05). There were no significant differences in the percentage of vital bone and residual bone materials between ABBM and AEBM (P = 1.0). Newly formed bone and residual graft materials were integrated into the surrounding tissue with no sign of inflammation or foreign-body reaction.* ***Conclusion:*** *Within the confines of the study, MCBA has significantly greater new bone formation than ABBM and AEBM. AEBM showed comparable histomorphometric results in all parameters (percentage of vital bone, residual bone materials, and connective tissue/marrow) to ABBM.* Int J Oral Maxillofac Implants 2019;34:759–767. doi: 10.11607/jomi.6218

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Maxillary sinus graft is a common and predictable procedure used to increase bone height for implant placement in the posterior maxilla.[1–3] Since Boyne and James first described maxillary sinus graft using autogenous bone from the iliac crest,[4] autogenous bone has been considered as the "gold standard" due to its osteogenic potential.[5,6] Unfortunately, procuring autogenous bone (chin, ramus, iliac crest, calvaria) can increase risk of morbidity and discomfort.[7–10] Because of this, the use of nonautogenous bone graft materials (xenograft, allograft, and alloplast) has increased, and numerous studies have supported viability of bone substitute materials for maxillary sinus graft.[11–16]

Xenografts, especially anorganic bovine bone mineral (ABBM) (Bio-Oss, Geistlich) is one of the most studied nonautogenous bone graft materials for maxillary sinus graft, both histologically and histomorphometrically.[17–37] ABBM in the grafted sinus not only serves as an osteoconductive scaffold facilitating

revascularization,[17,18] the particles also integrate well with the newly formed bone.[19,20] Furthermore, implants placed in maxillary sinus graft with ABBM have shown high success rates.[38,39] Recently, another xenograft material, anorganic equine bone mineral (AEBM), has been introduced in the market and used for maxillary sinus graft, and studies demonstrated the percentage of vital bone after maxillary sinus graft in AEBM seems to be as effective as ABBM.[40–43] However, only limited studies have been published regarding its validity.[41,42]

Mineralized freeze-dried bone allograft is another type of bone graft material that has been used for maxillary sinus graft.[21,32,37,44–47] This material is osteoconductive in nature with porous microstructure giving the ability to incorporate with new bone. Some studies compared the efficacy of mineralized freeze-dried bone allograft to ABBM in new bone formation, and reported that newly formed bone was measured significantly greater when using mineralized freeze-dried bone allograft.[21,37] Contrarily, another study reported an insignificant difference in newly formed bone between ABBM and mineralized freeze-dried bone allograft.[32]

Comparing new bone formation through histomorphometric analysis using different bone graft materials may assist the clinician in determining graft materials to be used for sinus graft procedures. The purpose of this pilot study was to compare the degree of new bone formation through histomorphometric analysis using three bone graft materials: ABBM, AEBM, and mineralized cancellous bone allograft (MCBA) for maxillary sinus graft.

MATERIALS AND METHODS

Patient Selection

The study was approved by the Institutional Review Board of Loma Linda University (LLU) and was conducted at the Center for Implant Dentistry, LLU School of Dentistry. To be enrolled in this study, the subjects must *(1)* be older than 18 years of age; *(2)* have partially or completely edentulous posterior maxilla requiring two-stage maxillary sinus graft using a lateral window approach (unilateral or bilateral); and *(3)* be available for follow-up appointments, bone core biopsy, and implant placement. Study subjects were excluded if they presented with *(1)* medical or psychologic history that would complicate surgical procedures and/or the outcome of the study; *(2)* history of head and neck radiation treatment, uncontrolled diabetes, uncontrolled hypertension, blood disorder, alcohol or drug dependency, immunodeficiency diseases; *(3)* maxillary sinus disease; *(4)* smoking habits; *(5)* poor oral hygiene; and *(6)* pregnancy. Patient screening was performed by two of the investigators (J.K., M.N.). The study protocol was explained to each study subject, and consent was obtained before onset of the study.

The grafted site was excluded from the study if *(1)* a > 5 mm in diameter maxillary sinus membrane perforation was observed during the surgery,[48] or *(2)* maxillary sinus infection developed after the surgery. Subjects were dismissed from the study if they were uncooperative and failed to return at designated follow-up appointments.

The surgical sites (maxillary sinuses) were randomly allocated into three parallel groups (n = 10 per group) according to the graft material used: ABBM (Bio-Oss, Geistlich), AEBM (Equimatrix, Osteohealth), and MCBA (OSSIF-i sem, Osteohealth). A total sample size of 30, 10 per group, was estimated to provide an 80% chance of detecting a difference ≥ 1.25 standard deviations between any two population means with 95% confidence. In subjects that required bilateral maxillary sinus graft, each maxillary sinus was independently allocated. The subjects were blinded regarding group allocation. Randomization was performed by one investigator (M.N.) using Research Randomizer (Version 4.0) software (Urbaniak GC, Plous S [2013] from http://www.randomizer.org/). In the randomization process, sequential numbers were assigned to each graft material: 1 to 10 for ABBM, 11 to 20 for AEBM, and 21 to 30 for MCBA. Each surgical site was given a number from 1 to 30 through randomization process at the time of study enrollment. Histomorphometric examiners were also blinded. Each harvested bone core sample was stored in a numerically coded container and sent to an outside laboratory (Hard Tissue Research Laboratory, University of Minnesota School of Dentistry) for histologic and histomorphometric analysis.

Treatment Plan and Preoperative Procedures

Diagnostic wax patterns of the missing teeth were made for partially edentulous patients, and a vacuum-formed radiographic template (Polypropylene Coping Sheet, Ultradent Products) with auto-polymerizing acrylic resin (Jet, radiopaque teeth color, Lang Dental) was fabricated. For completely edentulous patients, conventional denture(s) were fabricated and a silicone index of the maxillary denture was made using condensation silicone lab putty (Polysiloxane Coltene Lab-Putty, Coltene Whaledent). A radiographic template was then fabricated using a clear auto-polymerizing acrylic resin (Splint Acrylic Resin, Great Lakes Orthodontics). A cone beam computed tomography (CBCT) scan (i-CAT Cone Beam 3-D Imaging System, Imaging Sciences International) was taken using the radiographic template for diagnosis. An additional template (biopsy template) with indexed groove(s) was fabricated to locate the area of bone core biopsy on the lateral window (Fig 1).

Fig 1 *(Above)* A biopsy template for partially edentulous patient.

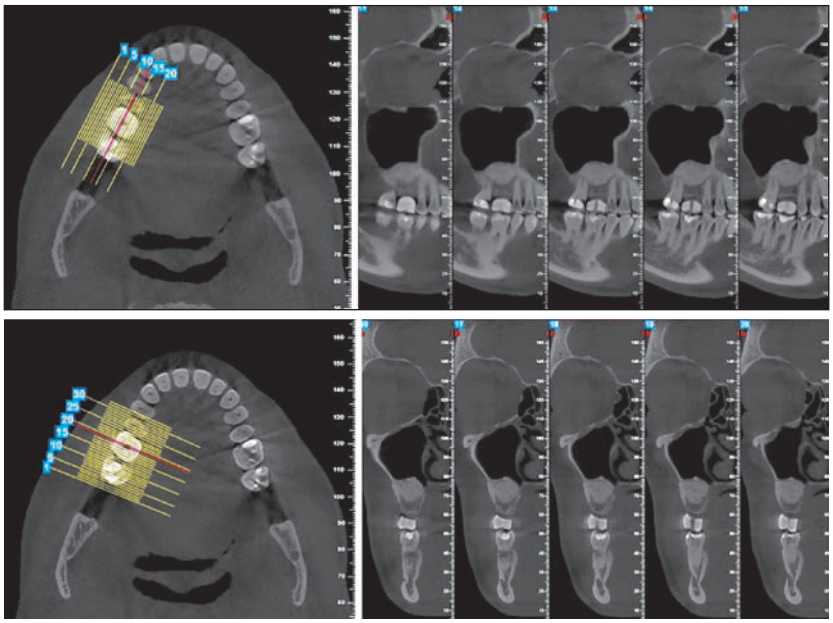

Fig 2 *(Right)* 9 months postoperative CBCT images in ABBM.

Surgical Procedures

Graduate students at the LLU Center for Implant Dentistry performed all surgeries after calibration was performed under the supervision of the principal investigator (J.K.). After administering local anesthesia (Xylocaine 2% 1:100,000, Astra Zeneca USA, Inc, Pharmaceuticals), midcrestal and vertical incisions were created to allow for adequate visualization and access the lateral wall of the maxillary sinus following full-thickness flap reflection. Each proposed lateral window was outlined using a sterile pencil approximately 3 mm apical to the maxillary sinus floor, 3 mm from the anterior wall of the maxillary sinus. The superior border was made approximately 15 mm from the alveolar crest to ensure adequate graft for bone core biopsy. The osteotomy was performed by completely removing the bone from the lateral wall using a dome shape bur (DASK Drill #5; Dentium Advanced Sinus Kit, Dentium) in all cases. The maxillary sinus membrane was then carefully elevated. The inferior and superior border of the window were measured with the biopsy template along the groove(s) using a periodontal probe (#12, South Dakota, Single End, Yellow, American Eagle Instruments, or Pearson Color-Coded Probe CP15UNC, Pearson Dental Supply Company) and served as a reference to locate the window for bone core biopsy. Bone graft materials (small particles), ABBM, AEBM, or MCBA, were randomly assigned and placed into the respective maxillary sinus. Prior to flap closure, resorbable collagen membrane (Bio-Gide, Geistlich) was placed over the lateral window extending approximately 3 mm in all directions.[1,25] In the event of a small perforation (≤ 5 mm in diameter), an attempt was made to isolate the perforation and a sheet of the same resorbable collagen membrane was placed over the perforation, and maxillary sinus graft was completed according to the protocol.

Primary closure was achieved using an expanded polytetrafluoroethylene (Gore-Tex, W.L. Gore and Associates) suture. Study subjects were placed on an appropriate antibiotic regimen (amoxicillin 500 mg, TEVA Pharmaceuticals USA), and analgesic (ibuprofen 800 mg, American Health Packaging), and were instructed to rinse twice daily with 0.12% chlorhexidine gluconate (Peridex, Zila Pharmaceuticals) for 2 weeks. The study subjects were to return for suture removal in 2 weeks and at 1, 3, and 6 months for follow-up examinations. Complications during and after the procedure as well as their management were also reported.

Harvesting of Bone Cores

A CBCT scan (i-CAT, or NewTom VGi, NewTom) was taken at least 8 months after maxillary sinus graft to ascertain sufficient bone quantity for the bone core biopsy (Fig 2). A full-thickness flap was reflected, and the previous surgical site was exposed. The location for biopsy was identified using the biopsy template within the previously prepared lateral window (Fig 3). One bone core biopsy specimen per sinus was harvested using a trephine (3.2 mm in internal diameter) with a minimum length of 8 mm (EasyRetrieve, ACE Surgical Supply Company) (Fig 4). The quality of bone[49] was evaluated during bone core biopsy using the trephine bur. The bone core remained in the trephine bur to avoid damage during removal, and was stored in 10% buffered formalin using a coded container (Fig 5). All samples were sent to a laboratory (Hard

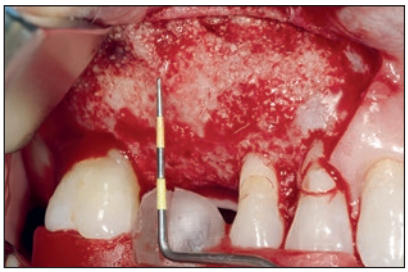

Fig 3 Locating the area for biopsy using the biopsy template.

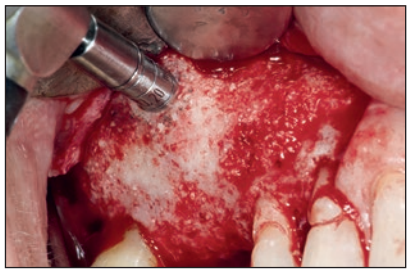

Fig 4 Harvesting a bone core biopsy specimen within the previously prepared lateral window using a trephine.

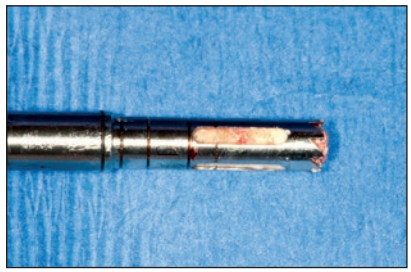

Fig 5 An obtained bone core biopsy specimen (ABBM).

Table 1 Patient Demographics

	ABBM	AEBM	MCBA	Total
Overall no. of enrolled participants	11	10	10	22
Overall no. of baseline participants	9	9	10	21
Overall no. of maxillary sinuses analyzed	9	9	10	28
Age Mean (± SD) (y)	60.9 (± 12.4)	65.6 (± 6.1)	57.9 (± 14.5)	59.5 (± 12.9)
Sex				
Female	5	3	5	11
Male	4	6	5	10

ABBM = anorganic bovine bone mineral; AEBM = anorganic equine bone mineral; MCBA = mineralized cancellous bone allograft.

Tissue Research Laboratory, University of Minnesota School of Dentistry) for histologic and histomorphometric analysis.

Histomorphometric Analysis

The specimens were dehydrated with a graded series of alcohols (Decon Labs) for a period of 3 to 4 weeks. The specimens were then embedded in light polymerizing resin for 10 hours (Technovit 7200 VLC, Kulzer), and were cut in a longitudinal section through the center of the core to a thickness of 150 µm (Exakt Apparatebau).[50,51] Subsequently, the samples were ground down and hand polished to a thickness of 45 to 50 µm, and were stained with Stevenel's blue and van Gieson picro fuchsin.[52] All specimens were digitized at the same magnification using a Nikon Eclipse 50i microscope (Nikon) and a SPOT Insight 2 megasample digital camera (Diagnostic instruments). Histomorphometric measurements were completed using a combination of SPOT 5.1 Advanced Software (Diagnostic instruments) and Adobe PhotoShop (Adobe Systems). The percentage of vital bone formation, residual graft material, and connective tissue/marrow were calculated, and the histomorphometric data for each specimen were recorded as the average of the two slides from the specimen.

Statistical Analysis

The nonparametric Kruskal-Wallis test was used to determine the statistical difference in the degree of new bone formation, and percentage of vital bone, residual graft material, and connective tissue/marrow independently, among the three graft materials. Post hoc comparisons were conducted with the Dunn-Bonferroni pairwise test in cases of statistically significant differences in the percentage of vital bone, residual graft material, and connective tissue/marrow. The null hypothesis was that there was no statistically significant histomorphometric difference in new bone formation among graft materials. The Chi-square test was used to compare bone quality. All hypothesis tests were two-sided and tested at the significance level of $\alpha = .05$ using Statistical package for the social science (SPSS) version 22 (SPSS).

RESULTS

Clinical Results

After 27 patients were screened between January 2014 and May 2015, 21 patients with a total of 30 maxillary sinuses (12 unilateral and 9 bilateral) fulfilled the inclusion criteria. One sinus in the ABBM group developed an infection, and the infected graft material was removed 10 days after the grafting procedure. The site was irrigated with copious saline water and chlorhexidine gluconate solution. A drainage tube was inserted into the sinus cavity for 1 week, and the flap was closed using polytetrafluoroethylene suture. The site healed uneventfully, and implants were placed simultaneously with maxillary sinus graft 1 year later. This sinus was replaced with another subject (Table 1). One subject

with a bilateral sinus dropped out of the study due to relocation. Consequently, 21 study subjects, 10 men and 11 women, with a mean age of 61.5 (range = 33 to 75) years with 28 sinuses (14 unilateral and 7 bilateral) completed the study after the predetermined follow-up period (Table 1).

A total of 28 bone biopsy specimens were harvested and analyzed (ABBM [n = 9], AEBM [n = 9], and MCBA [n = 10]). The mean healing time at biopsy was 9.1 (range = 8 to 12) months (ABBM [8 to 11 months], AEBM [8 to 11 months], and MCBA [8 to 12 months]). The bone quality during biopsy is shown in Table 2. Though bone tended to be more dense in ABBM and AEBM than MCBA, the differences were not statistically significant (Chi-square test; P =.152).

Six small maxillary sinus membrane perforations (≤ 5 mm) were observed in ABBM (3), AEBM (1), and MCBA (2) groups. The perforation was repaired using resorbable collagen membrane, and the sinus graft procedure was completed as planned. These maxillary sinuses healed without further complications. Suture breakdowns, approximately 15 mm in length along the crestal incision line, were observed on both sides of bilateral surgical sites in one patient 2 weeks following the surgery. There were no signs of infection, and complete wound closure was noted at the 1-month follow-up.

Histomorphometric Analysis

Histologic findings are presented in Figs 6 to 13. Newly formed bone was in close contact to the residual graft materials and interconnected the bone particles in all graft materials. Osteoblasts were observed in contiguity with the newly formed bone, and vascular structures were evident in the connective tissue space. Similar histologic findings were observed in sites with the same graft materials regardless of the occurrence of membrane perforation (Figs 6, 8, 12, and 13).

The mean ± SD in each parameter from the histomorphometric analysis is shown in Table 3. Statistically significant differences were observed in percentage of vital bone, residual graft material, and connective tissue/marrow among three bone graft materials (Kruskal-Wallis, P = .001, P < .001, P = .028, respectively; Table 3). An insignificant difference in percentage of vital bone was noted between ABBM (10.9% ± 8.9%) and AEBM (9.1% ± 5.9%) (P = 1.0; Table 3), but percentage of vital bone in ABBM and AEBM was significantly less than MCBA (32.0% ± 12.4%) (ABBM [P = .004], AEBM [P = .001]; Table 3). Likewise, an insignificant difference in percentage of residual graft material was observed between ABBM (34.3% ± 12.1%) and AEBM (38.9% ± 5.3%) (P = 1.0; Table 3); however, the percentage of residual graft material in both xenograft materials was significantly higher than MCBA (5.5% ± 5.7%) (ABBM [P = .005], AEBM [P = .003]; Table 3). There was no significant difference observed in percentage of connective tissue/marrow between ABBM (54.8% ± 7.7%) and AEBM (52.0% ± 6.1%) (P = 1.0; Table 3), and ABBM and MCBA (62.5% ± 9.8%) (P = .179), but MCBA was significantly higher than AEBM (P = .031) (Table 3).

Table 2 Bone Quality at Grafted Sites During Biopsy

Bone quality	ABBM	AEBM	MCBA	Total
Type 1	0	0	0	0
Type 2	3	1	0	4
Type 3	5	6	5	16
Type 4	1	2	5	8

Classification of bone quality by Lekholm and Zarb (1985)[49] (Chi-square test; P =.152).
ABBM = anorganic bovine bone mineral;
AEBM = anorganic equine bone mineral;
MCBA = mineralized cancellous bone allograft.

DISCUSSION

Histomorphometry is commonly used to quantify bone architecture, as it provides information on the volume of bone and its cellular activity. Studies evaluating the consolidation of bone graft materials typically express the findings as percentage of vital bone, residual graft material, and connective tissue/marrow. Factors that can affect the histomorphometric outcome of the grafted bone in maxillary sinus graft are the type of graft materials used, time of biopsy, and incidence of complications.

In this study, one allograft (MCBA), and two xenograft (ABBM and AEBM) materials were evaluated. Although the sample size was small, statistically significant differences were observed in percentage of vital bone, residual graft material, and connective tissue/marrow between allograft and both xenograft materials (P < .05; Table 3). MCBA products are processed and sterilized by different protocols, such as low-dose gamma irradiation or chemical solvent disinfection.[53] These processes tend to maintain the inherent characteristics of allograft material.[54] Since new bone formation is associated with bone resorption by osteoclast activity during the bone remodeling process, graft material should be conductive to cellular functions. As MCBA contains retained collagen within the bone matrix, osteoclasts can attach to the surface of MCBA particles and adhere to Type I collagen in the same manner as native bone.[55] This feature allows the graft materials to be replaced by new bone. Histomorphometric studies using mineralized freeze-dried bone allograft for maxillary sinus graft has resulted in varying percentage of

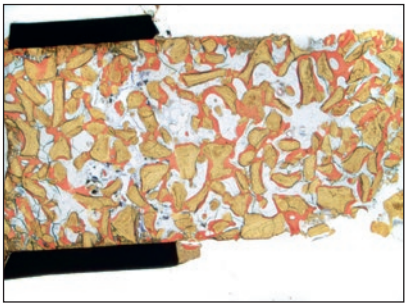

Fig 6 Newly formed bone interconnects ABBM bone particles throughout the field (Stevenel's blue and Van Gieson picro fuchsin stain × 25).

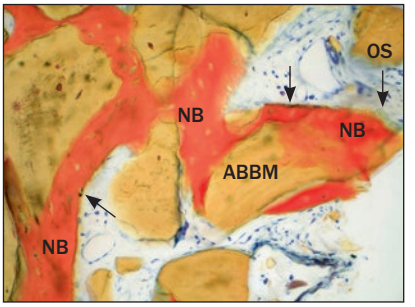

Fig 7 Newly formed bone (NB) shows close contact to bone particles (ABBM). Osteoblasts *(arrows)* and osteoid (OS) are observed in contiguity with the newly formed bone, and vascular structures are evident in the connective tissue space (Stevenel's blue and Van Gieson picro fuchsin stain ×200).

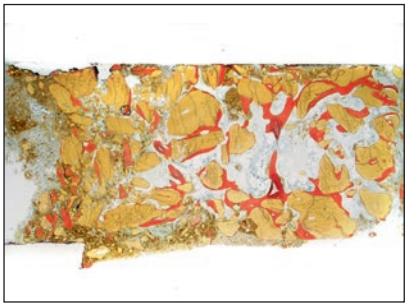

Fig 8 Newly formed bone interconnects with AEBM bone particles (Stevenel's blue and Van Gieson picro fuchsin stain ×25).

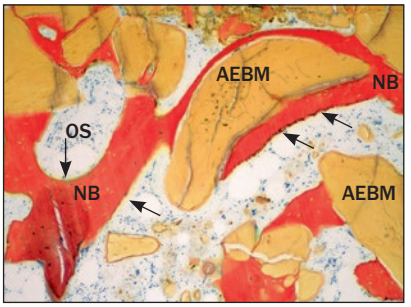

Fig 9 Newly formed bone (NB) shows direct contact to AEBM particles (AEBM). Osteoblast line *(arrows)*, osteoid (OS), and vascular structures are seen (Stevenel's blue and Van Gieson picro fuchsin stain × 100).

Fig 10 Newly formed bone bridges together with MCBA particles (Stevenel's blue and Van Gieson picro fuchsin stain × 25).

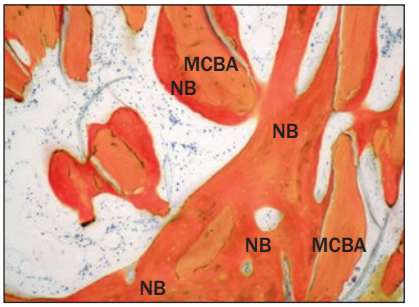

Fig 11 Newly formed bone (NB) is well incorporated into MCBA particles (MCBA) and difficult to distinguish between NB and MCBA particles in some areas. Abundant vascular structures are seen in the connective tissue (Stevenel's blue and Van Gieson picro fuchsin × 100).

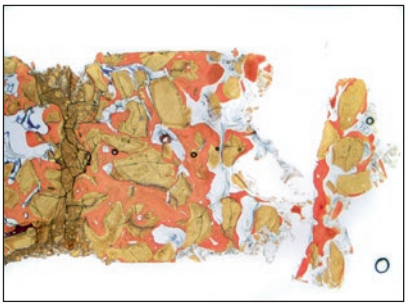

Fig 12 *(Left)* Microphotograph of a perforated site in ABBM shows bridging between newly formed bone and ABBM particles (Stevenel's blue and Van Gieson picro fuchsin stain × 25).

Fig 13 *(Right)* Microphotograph of a perforated site in AEBM. Newly formed bone interconnects with AEBM bone particles (Stevenel's blue and Van Gieson picro fuchsin stain × 25).

Table 3 Histomorphometric Comparison of Percentage of Vital Bone, Residual Bone Material, and Connective Tissue/Marrow Among Three Bone Graft Materials (Mean ± SD)

	ABBM (n = 9)	AEBM (n = 9)	MCBA (n = 10)	*P* value*
VB%	10.9 ± 8.9[a]	9.1 ± 5.9[a]	32.0 ± 12.4[b]	.001
RBM%	34.3 ± 12.1[a]	38.9 ± 5.3[a]	5.5 ± 5.7[b]	< .001
CT%	54.8 ± 7.7[a,b]	52.0 ± 6.1[a]	62.5 ± 9.8[b]	.028

*Kruskal-Wallis test.
[a,b]Different letters denote statistically significant difference using Dunn-Bonferroni pairwise tests at $\alpha = .05$.
VB% = the percentage of vital bone; RBM% = percentage of residual bone material; CT% = percentage of connective tissue/marrow; ABBM = anorganic bovine bone mineral; AEBM = anorganic equine bone mineral; MCBA = mineralized cancellous bone allograft.

Table 4 Histomorphometric Findings in Studies Using FDBA for Maxillary Sinus Grafts

Studies	No. of bone cores analyzed	Healing time (mo)	Type of FDBA	VB%	RBM%	CT%
Noumbissi et al[44]	3	6.5–8	Puros, Cancellous[a]	40.33	4.67	54.33
Froum et al[21]	10	10	Puros, Cancellous[a]	28.25	7.65	64.10
Kolerman et al[45]	23	9	Oragraft, Cortical[b]	29.09	19.00	51.91
Kolerman et al[32]	5	9	Oragraft, Cortical[b]	31.8	21.5	46.7
Avila et al[46]	39	6–7	MinerOss, Cortical/Cancellous[c]	23.02	22.25	54.73
Avil-Ortez et al[47]	23	6	MinerOss, Cortical/Cancellous[c]	21.69	23.51	55.08

[a]Puros (Zimmer Dental).
[b]OraGraft (LifeNet).
[c]MinerOss (BioHorizons).
VB% = the percentage of vital bone; RBM% = percentage of residual bone material; CT% = percentage of connective tissue/marrow.

vital bone to residual graft material ratios (1:1 to 8:1), based on the amount of cortical or cancellous bone presence in the graft material (Table 4).[21,32,44–47] Based on the above studies, the percentage of vital bone to residual graft material ratios for mineralized freeze-dried bone allograft with cortical bone is 1.5:1,[32,45] for cancellous bone it ranges from 4:1 to 8:1, and for cortical/cancellous bone it is 1:1.[46,47] The presence of cortical bone in mineralized freeze-dried bone allograft serves as a scaffold, resulting in a slower resorption rate and lower percentage of vital bone to residual graft material ratio.[46] In this study, the high percentage of vital bone and residual graft material ratio (6:1) of the MCBA evaluated is comparable to the findings of other studies utilizing cancellous allograft material.[21,44]

The ability of ABBM to resorb and be replaced by bone has been controversial.[19,20,56–61] Several animal and human histomorphometric studies have reported signs of resorption with the presence of osteoclasts and osteoclastic activities on ABBM particle surfaces.[19,20,57,58] On the other hand, other studies claimed that there were no signs of resorption (eg, no changes in the area fraction or particle size) of ABBM particles.[30,59–61] ABBM and AEBM used in this study are unsintered heat-deproteinized mineral matrix (with 75% to 80% porosity in ABBM)[62,63] and possess similar physical structure to human bone.[64] The percentage of vital bone to residual graft material ratios for ABBM (10.9%: 34.3% = 1:3) and AEBM (9.1%: 38.9% = 1:4) in this study are similar and seem to support the notion that both are unlikely to resorb.

Despite the lack of resorption, ABBM is osteoconductive and facilitates revascularization.[17,18,22] Even with residual bone graft particles, the percentage of vital bone to residual graft material ratio is likely to increase over time as new bone ingrowths infiltrate during the healing process.[20,30,60,65] ABBM particles also have been shown to be well integrated with newly formed bone.[19,61,63] Therefore, even though histologic results showed osteoclasts or resorption lacunae on the surface of ABBM particles from human biopsy specimens, the slow resorbing property of ABBM may provide density to the graft and structural integrity for the long term. This is substantiated by the results of this study where, though not statistically significant, bone quality of the grafted sites tended to be higher in ABBM and AEBM than in MCBA (Chi-square test; $P = .152$) (Table 2). Furthermore, while high bone-to-implant contact has been observed in the maxillary sinus grafted with ABBM, no contact between ABBM particles and the implant was detected.[65] This suggests that lack of particle resorption did not prevent bone formation nor implant osseointegration.[16,66]

It had been suggested that percentage of vital bone may increase with time.[22,34] Hanisch et al reported significantly greater percentage of vital bone at 12 months compared with 6 or 8 months after maxillary sinus graft.[65] However, in this study, no significant correlations were noted between biopsy time and the percentage of vital bone in all graft materials (ABBM: $P = .330$, AEBM: $P = .175$, and MCBA: $P = .197$).

The rate of maxillary sinus membrane perforations, 6/28 (21.4%), in this study is similar to the rate (19.5%) reported by others.[2] Although maxillary sinus membrane perforations can be associated with an increase in postoperative complications such as sinusitis, graft infection, and/or graft failure,[67,68] Froum et al reported no adverse effect on vital bone formation if small to middle size membrane perforations (< 10 mm) were properly repaired.[69] In this study, the histomorphometric analysis of the six repaired sinuses showed the mean percentage of vital bone as 12.2% in ABBM, 18.6% in AEBM, and 27.8% in MCBA. Although the sample size is too small to be used to conclusively determine the effect of membrane perforation on the new bone formation, these results suggest that properly repaired membrane perforation during maxillary sinus graft does not seem to compromise the amount of vital bone formation.

The limitation of this study was the small sample size. A larger sample size would allow for subgroup analyses and adjustment for potential confounders.

CONCLUSIONS

Within the confines of this study, a statistically significant difference in new bone formation was noted among the three graft materials at a mean follow-up period of 9.1 months. The MCBA revealed significantly greater new bone formation than ABBM and AEBM. The AEBM showed comparable histomorphometric results in all parameters (percentage of vital bone, residual graft material, and connective tissue/marrow) compared with ABBM.

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