



Original Article

Sinus floor augmentation using mixture of mineralized cortical bone and cancellous bone allografts: Radiographic and histomorphometric evaluation



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Abstract *Background/purpose:* Due to the pneumatization of the maxillary sinus, the sinus floor augmentation is often performed to implant placement in the maxillary posterior region. The aim was to perform radiographic and histomorphometric evaluation after placement of mixed allografts (cortical freeze-dried bone allograft [FDBA] 50%:cancellous FDBA 50%) during sinus floor augmentation.

Materials and methods: In 37 patients, anorganic bovine bone (ABB, sites = 16), mineralized cancellous bone allograft (MCBA, sites = 15), and mixed allografts (Mixed AG, sites = 20) were placed during sinus floor elevation via the lateral approach (LSFE), at total 51 sites with residual alveolar bone height (RBH) < 5 mm. Cone-beam computed tomography images were obtained before LSFE (T0), after surgery (T1), and 6 months after surgery (T2) for radiographic analysis. After a 6-month healing period, core biopsies were harvested and histomorphometric analysis was performed.

Results: The mean augmented bone height (ABH) of ABB, MCBA, and mixed AG groups after surgery was similar (13.86 ± 4.19 mm, 13.99 ± 4.07 mm, and 14.20 ± 3.12 mm, respectively; $P > 0.05$). The mean ABH of ABB, MCBA, and mixed AG groups after 6 months was similar

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(13.72 ± 4.55 mm, 11.83 ± 3.31 mm, and 12.53 ± 2.97 mm, respectively; $P > 0.05$). In the ABB, MCBA, and mixed AG groups, the proportion of newly formed bone (NB) was similar (36.13 ± 10.01%, 39.26 ± 10.72%, and 31.27 ± 18.31%, respectively; $P > 0.05$).

Conclusion: This result demonstrated that mixed AG led to sufficient bone augmentation and histologically comparable NB formation as compared to ABB and MCBA for sinus floor augmentation.

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Introduction

After loss of teeth in the maxillary posterior region, implant placement can be difficult due to the lack of residual alveolar bone height (RBH) resulting from pneumatization of the maxillary sinus.¹ To overcome this problem, sinus floor augmentation has been performed. The Schneiderian membrane of the maxillary sinus floor is lifted and graft materials are placed below the lifted Schneiderian membrane for sinus floor augmentation.

LSFE was first introduced by Tatum in the 1970s. In the 1980s, Boyne & James introduced bone grafting using autogenous bone (AB) grafts.² The technique has been adopted as a standard procedure, as AB grafting leads to formation of new bone (NB).³ However, recent studies have recommended the use of various bone substitute materials (BSM).⁴ The graft materials that are currently used after LSFE include AB, and BSMs that can replace AB, such as xenografts (XG), allografts (AG), and alloplasts (AP). AB has osteogenic, osteoinductive, and osteoconductive properties, and can be harvested from the mandibular ramus and the maxillary tuberosity. However, the amount that can be harvested is limited, and is difficult to estimate the amount of resorption that will occur after bone graft placement. Moreover, since additional surgical sites are required, the patient's morbidity increases.^{5,6} Among XG material, anorganic bovine bone (ABB) is used in various alveolar bone augmentation procedures as a single graft or as a mixed graft along with AB.^{7,8} AG can be divided into mineralized freeze-dried bone allograft (FDBA) and demineralized freeze-dried bone allograft (DFDBA), according to the method of processing the donor material. FDBA has osteoconductive properties and behaves as a scaffold. FDBA is divided into cortical FDBA and cancellous FDBA according to the components of the donor's bone during the manufacturing process. While both have osteoconductive potential, their healing patterns are different. In cancellous AG, NB formation occurs through creeping substitution, while in cortical AG, it occurs through reverse-creeping substitution.^{9,10}

The aim of this study was to assess radiographically the amount of augmented bone following anorganic bovine bone (ABB), mineralized cancellous bone allograft (MCBA), and mixed allograft (Mixed AG; cortical FDBA 50%: cancellous FDBA 50%) placement during LSFE and compare them histologically and histomorphometrically.

Materials and methods

This study was conducted after patients received an explanation about the study and gave written informed consent to participate in this study. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the Dental hospital of Chosun University, Gwangju, South Korea (Protocol No. CUDHIRB-1702-003). A total of 37 patients (20 men, 17 women, mean age: 52.88 ± 8.58 years [range: 37–72 years]) were selected. Sinus floor augmentation was performed between October 2011 and October 2014. All participants met the following inclusion criteria (Table 1):

- i) Patients who had RBH < 5 mm from the maxillary sinus floor, and thus required delayed implant placement (Del Fabbro et al., 2012; Schmitt et al., 2014)
- ii) Patients who did not have any systemic diseases that contraindicated implant placement
- iii) Patients who received ABB (ABB; Bio-Oss®; Geistlich Pharma AG, Wolhusen, Switzerland), mineralized cancellous bone allograft (MCBA; Puros®, Zimmer Biomet, Warsaw, USA), or mixed allografts (Mixed AG; cortical FDBA 50%: cancellous FDBA 50%; Allo-Oss®, CG-bio, Seoul, South Korea) during LSFE.
- iv) Patients who underwent biopsy planning for implant placement

Table 1 Characteristics of subjects.

	ABB	MCBA	Mixed AG
N of subjects	11	10	16
N of sites	16	15	20
Mean age (range)	53.00 ± 8.17 (46–72)	51.07 ± 9.67 (37–64)	54.15 ± 8.24 (38–67)
Gender			
Men, N (%)	7 (63.6)	5 (50.0)	8 (50.0)
Women, N (%)	4 (36.4)	5 (50.0)	8 (50.0)

N, number; ABB, anorganic bovine bone; MCBA, mineralized cancellous bone allograft; Mixed AG, mixed allografts (cancellous FDBA 50%: cortical FDBA 50%).

Clinical and radiographic measurements

The anatomical structure, pathological condition, and RBH of the maxillary sinus were assessed using cone-beam computed tomography (CBCT; CB Mercuray™; Hitachi, Tokyo, Japan) scans that were obtained before LSFE (T0), after surgery (T1), and at 6 months after surgery (T2), according to a surgical guide (Fig. 1). The cross-sectional images were reconstructed and sent to Picture Archiving and Communication System (PACS) of Chosun University Dental Hospital, using imaging analysis software (OnDemand3DTM; Cybermed, Seoul, Korea). The RBH and augmented bone height (ABH) was measured using software (PiViewStar™; Infinitt STARPACS, Seoul, South Korea).

Surgical procedures

Local anesthesia was induced at the surgical site using 2% lidocaine with 1:100,000 epinephrine (Yuhan, Seoul, South Korea). A crestal incision was made using a #15 scalpel, and a full-thickness flap was elevated to expose the lateral wall of the maxillary sinus. The window was created using a piezoelectric device (Piezosurgery®; Mectron, Carasco, Italy) and a 0.55 mm principal micro-saw (OT-7™; Mectron, Carasco, Italy) (Fig. 2a). The Schneiderian membrane was carefully elevated using sinus elevation instruments (DASK kit®; Dentium, Seoul, South Korea). Next, ABB, MCBA, and mixed AG were inserted until resistance to additional material placement occurs in the maxillary sinus (Fig. 2b). The

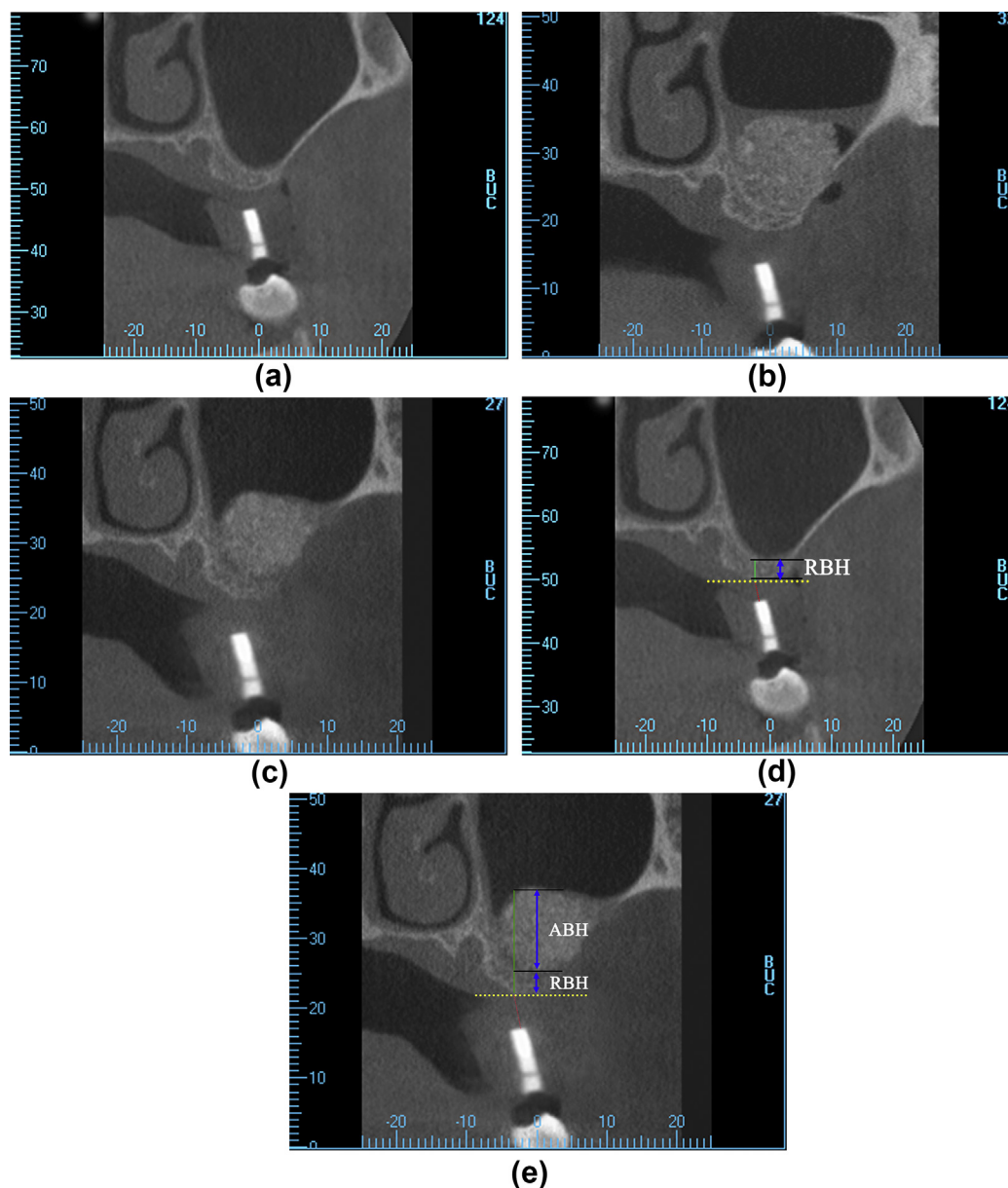


Figure 1 Cone-beam CT images obtained after the standardization of each location using a surgical guide (a) before sinus floor elevation via the lateral approach (LSFE) (T0), (b) after surgery (T1), (c) 6 months after surgery (T2), (d,e) standardized calculation of RBH(residual bone height) and ABH(alveolar bone height).

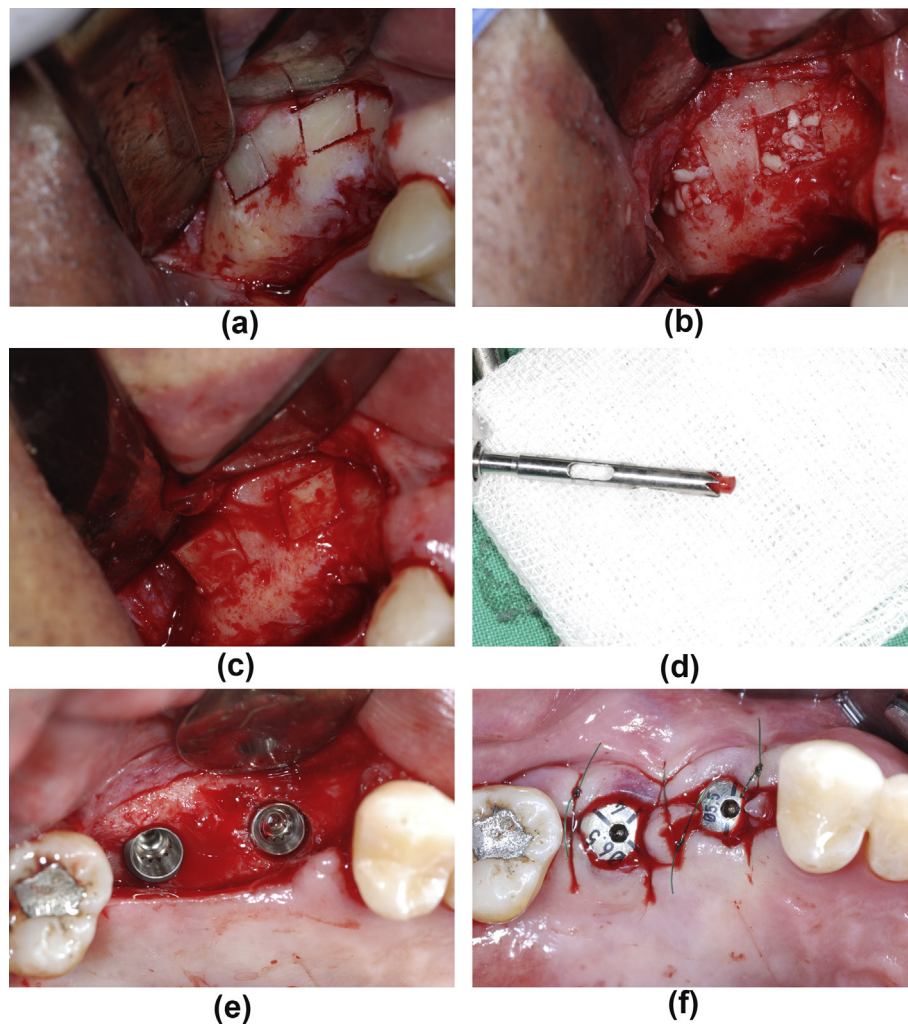


Figure 2 Surgical procedure of maxillary sinus floor augmentation and delayed implant placement. (a) After full-thickness flap was elevated, a lateral window access was formed using a piezoelectric device. (b) The graft material was placed after elevation of the Schneiderian membrane. (c) A barrier membrane was not applied after the bone lid was repositioned. (d) Core biopsies were harvested using a trephine bur (inner diameter 2 mm) after full-thickness flap elevation of the same site after a 6-month healing period. (e) After the alveolar bone was drilled, the implant fixtures were placed. (f) Primary closure performed using non-resorbable monofilament.

bone lid was repositioned, and primary closure was performed on the lateral wall using a non-resorbable monofilament (Happyton®; Purgo Biologics, Seongnam, South Korea) without covering with a barrier membrane (Fig. 2c). Patients were prescribed antibiotic medication (Augmentin® 625 mg; Il-Sung Drug Company, Seoul, South Korea) 3 times per day for 5 days.

Histomorphometric analysis

After a 6-month healing period following LSFE, core biopsies were taken before drilling was performed at the site of implant placement. Biopsies were harvested through the alveolar at a depth of 10 mm, using a trephine bur with an inner diameter of 2.0 mm (Fig. 2d). The internal type implants with an appropriate diameter and height for the site

were placed (Fig. 2e), and primary closure was performed using non-resorbable monofilament (Fig. 2f).

The harvested specimens were fixed for at least 2 days using 4% buffered paraformaldehyde (HT501128; Sigma–Aldrich, St. Louis, MO, USA), followed by demineralization for at least 2 weeks. The specimens were then processed into paraffin blocks and a microtome (RM2255; Leica, Nussloch, Germany) was used to produce 5-mm-thick microsections. Next, hematoxylin and eosin staining was performed, and the specimens were observed under a light microscope (Leica DM750; Leica Microsystems Ltd., Heerbrugg, Switzerland).

For histomorphometric analysis, a camera (Leica ICC50 HD; Leica Microsystems Ltd., Heerbrugg, Switzerland) connected to the light microscope was used to obtain images of the specimens. In this analysis, the proportion of NB, residual graft material (RG), and connective tissue (CT)

were measured. Three measurements were obtained for each specimen, and the mean was calculated. All measurements were made by a single examiner who had no knowledge about the procedure, and images were measured using i-Solution™ software (IMT i-Solution Inc., Burnaby, BC, Canada).

Statistical analysis

Shapiro–Wilk test was used to assess the normality of distribution of the data. Confirming that the normal distribution was not followed, all results were analyzed by Kruskal–Wallis test and Mann–Whitney test, which are nonparametric tests. The level of statistical significance was set at $P < 0.05$. All statistical analyses were performed using SPSS Version 22.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Results of clinical measurement

Tables 1 and 2 show the characteristics of subjects of three groups and Table 3 shows the results of the clinical measurements. The ABB group consisted of 11 patients (7 men, 4 women) with a mean age of 53.00 years (range: 46–72 years). The mean implant stability quotient (ISQ) value was 73.92 when implant fixture was placed. The implant survival rate was 100% (16/16). The MCBA group consisted of 10 patients (5 men, 5 women) with a mean age of 51.1 years (range: 37–64 years). The mean ISQ value was 78.63 when implant fixture was placed. The implant survival rate of 86.67% (13/15). The mixed AG group consisted of 16 patients (8 men, 8 women) with a mean age of 54.2 years (range: 38–67 years). The mean ISQ value was 74.61 when

implant fixture was placed. In this group, the implant survival rate of 95% (19/20). A significant difference was found for ISQ ($P = 0.005$). Significant differences were found between the MCBA and mixed ABB groups ($P = 0.006$) and between the MCBA and mixed AG groups ($P = 0.004$) (Table 3).

Radiographic evaluation

Table 4 shows the results of the radiographic analysis. ABB was measured in 11 patients, at 16 sites. MCBA was measured in 10 patients, at 15 sites, and mixed AG was measured in 16 patients, at 20 sites.

The mean ABH of ABB group at T1 and T2 were 13.86 ± 4.19 mm, 13.72 ± 4.55 mm, respectively. The mean ABH of MCBA group at T1 and T2 were 13.99 ± 4.07 mm, 11.83 ± 3.31 mm, respectively. The mean ABH of mixed AG group at T1 and T2 were 14.20 ± 3.12 mm, 12.90 ± 2.97 mm, respectively. Measurement of ABH on CBCT images showed expansion sites of increased ABH from T1 to T2 in a number of samples. The mean amount of increased ABH was 2.14 mm in 43.75% of ABB group (7/16 samples), 0.17 mm in 30% of MCBA group (3/15 samples) and 0.5 mm in 10% of the mixed AG group (2/20 samples). The mean amount of decreased ABH was 1.91 mm in 56.25% of ABB group (9/16 samples), 2.74 mm in 80% of MCBA group (12/15 samples) and 1.5 mm in 90% of the mixed AG group (18/20 samples). A significant difference was found between graft materials for the amount of increased ABH ($P = 0.021$). However, no significant differences in the amount of decreased ABH were found among the three groups ($P = 0.323$).

Histomorphometric evaluation

NB, RG, and CT were observed in all biopsy specimens harvested from the implant placement sites using a trephine bur (Fig. 3). Table 5 summarizes the results of the histomorphometric analysis. In the ABB group, the mean ratio of NB, RG, and CT was $36.13 \pm 10.01\%$, $13.38 \pm 11.03\%$, and $50.78 \pm 8.67\%$, respectively. In the MCBA group, the mean ratio of NB, RG, and CT was $39.26 \pm 10.72\%$, $3.60 \pm 4.42\%$, and $57.14 \pm 10.61\%$, respectively. In the mixed AG group, the mean ratio of NB, RG, and CT was $31.27 \pm 18.31\%$, $13.86 \pm 13.38\%$, and $54.89 \pm 12.96\%$, respectively (Table 5). No significant differences in the ratio of NB and CT were found among the three groups ($P = 0.361$, $P = 0.104$). A significant difference was found for RG ($P = 0.007$). Significant differences in the ratio of RG was found between the MCBA and mixed ABB groups ($P = 0.008$) and between the MCBA and mixed AG groups ($P = 0.004$).

Discussion

LSFE is a pre-prosthetic procedure with high predictability for the posterior maxilla with insufficient RBH due to pneumatization of the maxillary sinus.² Many factors should be considered for successful LSFE. The factors include the size of the lateral window, width of the maxillary sinus, and RBH.^{11,12} Kim et al. have reported that mean graft height

Table 2 Characteristics of implants.

Diameter (mm), N	Length (mm), N	ABB	MCBA	Mixed AG
4(4.1)	10	2	0	1
	11–12	2	0	1
	≥13	0	0	0
4.5	10	0	1	0
	11–12	1	0	4
	≥13	0	0	0
5.0(4.8)	10	0	5	4
	11–12	8	7	9
	≥13	3	2	0
6.0	10	0	0	1
Total		16	15	20

N, number; ABB, anorganic bovine bone; MCBA, mineralized cancellous bone allograft; Mixed AG, mixed allografts (cancellous FDBA 50%: cortical FDBA 50%).

7 different types of internal type implant were placed in this study; 3i® (Implnat Innovations, West Palm Beach, FL), Astra® (Astra Tech., Mölndal, Sweden), DAS® (DAS Tech., Gwangju, South Korea), ITI® (Straumann AG, Waldenburg, Switzerland), Luna® (Shinheung, Seoul, South Korea), Superline® (Dentium, Seoul, South Korea), TSIII® (Osstem Implant Co., Busan, South Korea).

Table 3 Clinical examination of sites augmented with ABB, MCBA, and mixed AG.

	ABB	MCBA	Mixed AG	P-value
N of sites	16	15	20	
ISQ, Mean \pm SD	73.92 \pm 6.57	78.63 \pm 6.56	74.61 \pm 6.09	0.005*
Failure of implant, N	0	2	1	
Survival rate of implant (%)	100	86.67	95	

N, number; ISQ, implant stability quotient using Osstell ISQ® (Osstell, Gothenburg, Sweden); SD, standard deviation; ABB, anorganic bovine bone; MCBA, mineralized cancellous bone allograft; Mixed AG, mixed allografts.

* Statistically significant difference ($P < 0.05$; Kruskal–Wallis test).

Table 4 Radiological assessment of sites augmented with ABB, MCBA, and mixed AG.

	ABB	MCBA	Mixed AG	P-value
N of sites	16	15	20	
Mean amount of RBH				
Mean \pm SD (mm)	3.53 \pm 2.05	2.61 \pm 1.84	3.0 \pm 1.56	0.253
Mean amount of ABH(T1)				
Mean \pm SD (mm)	13.86 \pm 4.19	13.99 \pm 4.07	14.20 \pm 3.12	0.615
Mean amount of ABH(T2)				
Mean \pm SD (mm)	13.72 \pm 4.55	11.83 \pm 3.31	12.90 \pm 2.97	0.183
Sites of Expansion, N(%)	7 (43.75)	3 (20)	2 (10)	
Increased ABH				
Mean \pm SD (mm)	2.14 \pm 1.90	0.17 \pm 0.10	0.50 \pm 0.28	0.021*
Sites of Shrinkage, N(%)	9 (56.25)	12 (80)	18 (90)	
Decreased ABH				
Mean \pm SD (mm)	1.91 \pm 1.15	2.74 \pm 2.54	1.50 \pm 1.03	0.323

N, number; SD, standard deviation; ABB, anorganic bovine bone; MCBA, mineralized cancellous bone allograft; Mixed AG, mixed allografts; RBH, residual bone height; ABH, augmented bone height; Sites of expansion, sites of increased ABH from T1 to T2; Sites of Shrinkage, sites of decreased ABH from T1 to T2.

* Statistically significant difference ($P < 0.05$; Kruskal–Wallis test).

change was significantly different according to the residual bone height after 3 year healing period.¹³ In this study, there was not significant difference of RBH among three groups and the height of ABH was changed from T1 to T2. Most of cases showed the decrease of height after 6 months. But, the height of ABH was increased from T1 to T2 at some of cases. The increase in ABH can be associated with a change in thickness of schneiderian membrane postoperatively. After sinus floor elevation, the membrane is thickened. And sinus membrane thickness increased from 0.75 mm to 6.63 mm and the submucosal edema of sinus membrane was observed with 50% of graft exteriorization after 7 days.¹⁴ The space between the biomaterial particles was filled by new bone tissue after healing and allowed an increase in graft volume after sinus elevation.¹⁵

AG is divided into DFDBA and FDBA according to the processing method, and both are used in LSFE. Both grafts are used as osteoconductive scaffolds for NB formation.^{16,17} To obtain results with high predictability after bone graft placement, BSM must have excellent osteogenic, osteoinductive, and osteoconductive properties, as well as volumetric stability.^{18,19} However, DFDBA is reported to have high resorption rates and low volumetric stability.¹⁷ In this study, mixed AG, comprised of cortical FDBA and cancellous FDBA, was used. While most cancellous FDBA is resorbed during the healing period, cortical FDBA remains even after several years.^{9,10,20} In this study, 100% cancellous FDBA had

a significant low ratio of residual bone graft bone (3.6%) and a relatively low implant success rate of 87%. This indicates that 100% cancellous FDBA can have a high absorption rate, just as autogenous bone, resulting in sinus repneumatization and implant failure.²¹ Cortical allografts can thus be considered to be more effective as a scaffold than cancellous FDBA. Seebach et al. have reported that cancellous allografts are the best scaffold for NB formation, and that their macroporosity, microporosity, and collagen fiber structure make them useful for migrating mesenchymal stem cells in the early period after graft placement.²² Based on all these findings, mixed 2 AG (cancellous FDBA 50%: cortical FDBA 50%) produces morphologically excellent results. Radiological findings showed that mixed AG showed similar ABH after 6 months healing. And the difference of ABH among the ABB, MCBA and mixed AG was not statistically significant ($P = 0.183$).

To achieve higher implant survival rates after LSFE, sufficient bone volume and density, which contribute to excellent bone-implant contact (BIC), are necessary. For this reason, the amount of NB is an important criterion for assessing bone grafts.²³ Recent histomorphometric studies have analyzed specimens harvested during implant placement after alveolar ridge preservation, in which 100% cortical FDBA, 100% cancellous FDBA, and mixed AG (50% cortical FDBA and 50% cancellous FDBA) were placed. The ratio of NB was $24.54 \pm 8.65\%$, $28.81 \pm 14.09\%$, and

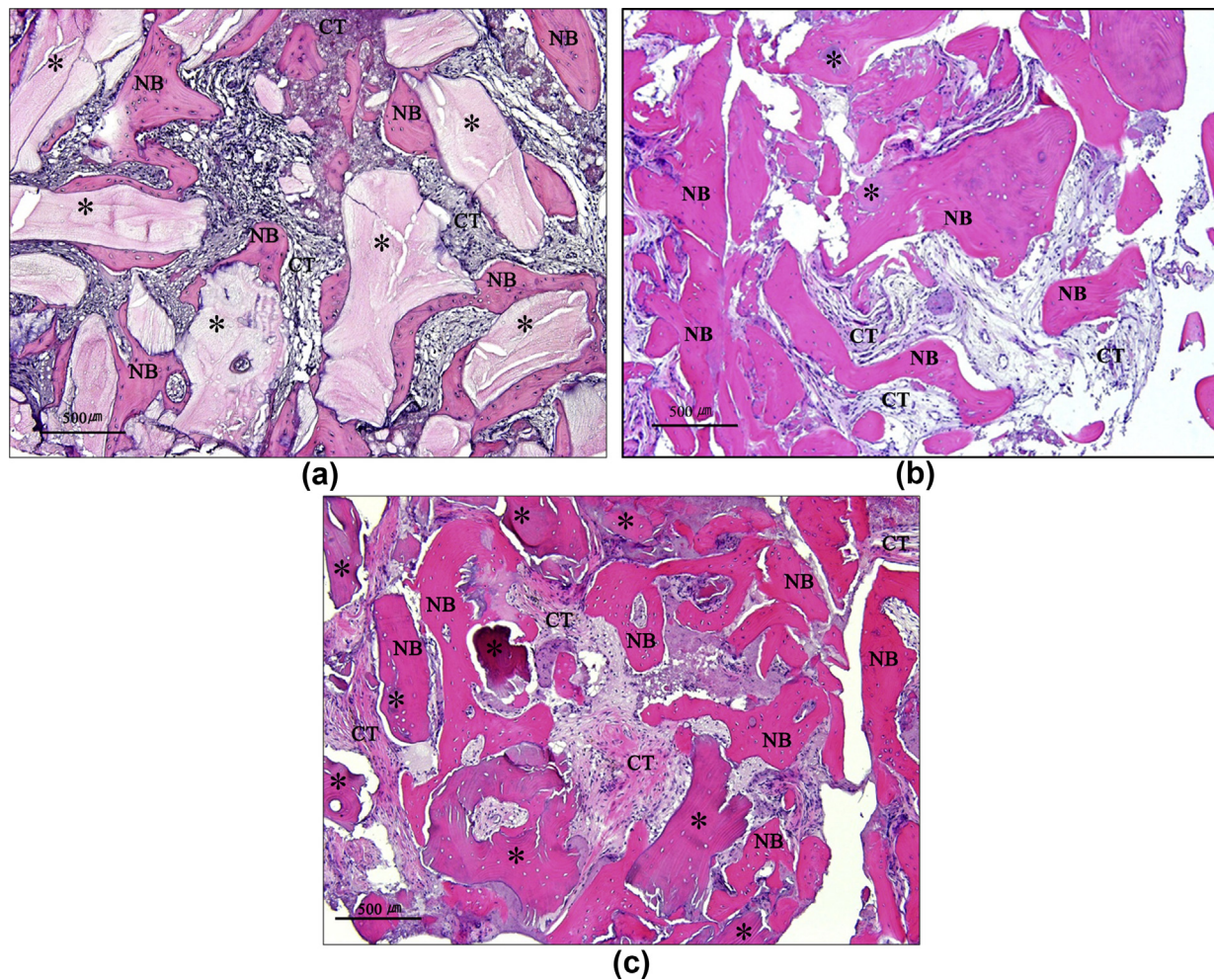


Figure 3 Histologic view and histomorphometric analysis (Hematoxylin and eosin (H & E) stained. Magnification $\times 100$). (a) An anorganic bovine bone (ABB) group: Deposition of newly formed bone (NB) around the residual bone graft (RG) was observed. Satisfactory incorporation between the NB and RG was observed. The sample showed 34.9% NB, 19.8% RG, and 45.3% connective tissue (CT). (b) A mineralized cancellous bone allograft (MCBA) group: A mature trabeculae of bone with a clear lamellar structure, and immature NB without a clear lamellar structure were observed. The gaps between the trabeculae of bone were filled with loose CT. The sample showed 40.3 NB, 2.7% RG, and 57.0% CT. (c) A mixed allograft (cortical FDBA 50%: cancellous FDBA 50%) group: Several RGs combined with the NB to form cancellous bone. The sample showed 32.3% NB, 8.4% RG, and 59.3% CT.

Table 5 Histomorphometric evaluation of sites augmented with ABB, MCBA, and Mixed AG.

	ABB	MCBA	Mixed AG	P-value
N of samples	16	15	20	
Newly formed bone (NB)				
Mean \pm SD (%)	36.13 \pm 10.01	39.26 \pm 10.72	31.27 \pm 18.31	0.361
Residual bone graft (RG)				
Mean \pm SD (%)	13.38 \pm 11.03	3.60 \pm 4.42	13.86 \pm 13.38	0.007*
Connective tissue (CT)				
Mean \pm SD (%)	50.78 \pm 8.67	57.14 \pm 10.61	54.89 \pm 12.96	0.104

N, number; SD, standard deviation; ABB, anorganic bovine bone; MCBA, mineralized cancellous bone allograft; Mixed AG, mixed allografts.

* Statistically significant difference ($P < 0.05$; Kruskal–Wallis test).

26.40 ± 13.18% in the respective bone grafts, and there was no significant difference in the ratio among the three groups. However, the ratio of RG was significantly higher in the 100% cortical FDBA group.²⁴ These results demonstrate that mixed AG has smaller resorption rates and higher volumetric stability than 100% cancellous FDBA. Furthermore, in this study, no significant difference in the ratio of RG was found between mixed AG and ABB, which is a highly non-resorbable material. This result indicates that mixed AG has excellent volumetric stability. Such a result can be attributed to the aforementioned role of cortical FDBA.

A systematic review was recently reported on the healing period after bone graft placement followed by LSFE. A histomorphometric analysis was performed on biopsies harvested after a healing period of 9–13.5 months (mean: 10.36 months) and of 4.5–9 months (mean: 6.22 months) after LSFE using AB, XG, AG and AP. The ratios of NB ranged from 19 to 44%. And the ratio of NB was higher among patients who used AG and had a healing period of 9.5 months.²⁵ In this study, biopsies were harvested after a mean healing period of 6.62 ± 1.30 months days (range: 4.40–10.23 months), and a histomorphometric analysis was performed. The ratio of NB was 36.13 ± 10.01%, 39.26 ± 10.72%, and 31.27 ± 18.31% in the ABB, MCBA, and mixed AG groups, respectively. The results were similar or slightly better than those reported in previous studies.

In conclusion, this study demonstrated that LSFE with mixed AG could increase sufficiently the alveolar bone height and show comparable NB formation. Our results suggest that LSFE with mixed AG would be helpful for sinus floor augmentation at the atrophic posterior maxilla area.

Declaration of Competing Interest

No potential conflict of interest relevant to this article was reported.

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