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Original Article

Sinus floor augmentation using mineralized freeze-dried bone allograft combined with recombinant human bone morphogenetic protein-2 (rhBMP-2): A long-term retrospective study

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KEYWORDS
Allografts;
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Abstract *Background/purpose:* The combination of recombinant human bone morphogenetic protein-2 (rhBMP-2) with a carrier material has not been extensively studied. This study aimed to evaluate the clinical, radiological, and histomorphometric outcomes of sinus floor augmentation using a 3:7 mixture of cancellous and cortical freeze-dried bone allografts (mixed AG) combined with rhBMP-2.

Materials and methods: Mixed AG was used for sinus floor augmentation in a total of 21 patients with a residual alveolar bone height <5 mm. Among the total 47 sites, augmentation with and without rhBMP-2 was performed in 26 and 21 sites, respectively. Radiographic parameters were assessed using cone-beam computed tomography. After a six-month healing period, core biopsies were harvested for histomorphometric analysis.

Results: The bone gain after healing was 13.36 ± 3.9 mm and 12.07 ± 3.8 mm in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively. The survival rate of implants in both groups was 100% during the follow-up period. The proportion of newly formed bone was 24.6 ± 10.2% and 39.7 ± 18.3% in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively ($P < 0.05$). Moreover, the percentage of residual graft material was 21.0 ± 12.2% and 9.6 ± 10.0% in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively ($P < 0.05$).

Conclusion: Mixed AG combined with rhBMP-2 could be a suitable material for sinus floor augmentation. This combination may reduce the treatment time and improve the predictability of implant placement.

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Introduction

An adequate amount of alveolar bone around dental implants is necessary for long-term success.[1] In the maxillary posterior region, significant alveolar bone loss occurs owing to alveolar bone resorption starting from the buccal side after tooth extraction and pneumatization of the maxillary sinus.[2] Various techniques have been introduced to reconstruct the alveolar ridge and form adequate alveolar bone to support dental implants.[3] Among them, guided bone regeneration (GBR) is the most well-known and documented alveolar bone-augmentation technique.[4,5] However, in the maxillary posterior region, sufficient bone quantity and quality for implant placement may not be obtained with GBR alone. In such cases, elevation of the maxillary sinus membrane is required for bone augmentation. There are two main approaches for maxillary sinus floor augmentation: a two-step approach using a lateral approach and a one-step approach using a lateral or crestal approach.[6,7] The lateral approach should be performed if the available residual alveolar bone is $<$ 5 mm and it is difficult to secure the initial stability of the implant, the crestal approach is difficult to perform, or there are irregular anatomical structures in the maxillary sinus.[8]

Various bone graft materials are used for maxillary sinus floor augmentation, including autografts, allografts (AG), xenografts, and alloplasts.[3] Xenografts have been widely studied, whereas autografts have shown limited results in terms of space maintenance due to increased bone resorption over time.[9] However, studies on AG and alloplasts are relatively scarce compared with those on xenografts. In most studies on maxillary sinus floor augmentation using AG, AG was mixed with other bone graft materials, and it has rarely been used alone.[10] Additionally, the use of AG containing a mixture of cortical and cancellous bone particles (mixed AG) has not been extensively studied.[11] However, in our previous study, we confirmed that mixed AG demonstrated satisfactory outcomes in maxillary sinus floor augmentation.[12]

In recent years, growth factors have emerged as a promising strategy for improving bone regeneration outcomes while reducing the treatment time. The effectiveness of various growth factors, such as bone morphogenetic proteins (BMP), platelet-derived growth factor, insulin-like growth factor, and vascular endothelial growth factor, for bone regeneration has been extensively studied.[4] Recombinant human BMP-2 (rhBMP-2) is widely used in clinical practice and has been found to increase the number of cells required for bone formation through osteoblastogenesis.[13,14] However, the use of rhBMP-2 can lead to various complications, such as postoperative edema and the potential for carcinogenesis. Therefore, it is necessary to consider the appropriate concentration, dosage, and application method to minimize complications and maximize effectiveness.[15]

This long-term retrospective study aimed to evaluate the clinical, radiological, and histological outcomes of a 3:7 mixture of cancellous and cortical freeze-dried bone AGs (FDBA) combined with rhBMP-2 for maxillary sinus floor augmentation.

Materials and methods

Study population

This retrospective study enrolled 136 patients diagnosed with chronic periodontitis between 2012 and 2017 at the Department of Periodontology, Chosun University Dental Hospital, who underwent extraction of posterior teeth, including maxillary molars, followed by lateral-approach maxillary sinus floor augmentation and delayed implant placement because of limited residual bone height (RBH). This study was approved by the Ethics Committee of Chosun University Dental Hospital (CUDHIRB-2106-003).

Participants were selected based on the following inclusion criteria: 1) Patients with RBH $<$5 mm from the maxillary sinus floor who required delayed implant placement; 2) Patients with no systemic diseases that contraindicated implant placement; 3) Patients who underwent cone-beam computed tomography (CBCT) preoperatively and after bone healing to measure the amount of augmented bone after maxillary sinus floor elevation; 4) Patients who received 3:7 ratio allografts (cancellous FDBA 30%; cortical FDBA 70%) alone or combined with rhBMP-2 for maxillary sinus floor elevation; 5) Patients who underwent core biopsy at the time of implant placement for histomorphometric analysis.

The following patients were excluded from the study. 1) Patients with RBH $\geq$5 mm from the maxillary sinus floor; 2) Patients who underwent implant placement concurrently with maxillary sinus floor augmentation; 3) Patients with uncontrolled diabetes or cardiovascular disease; 4) Patients who received a composite graft containing different types of bone graft materials along with AG for maxillary sinus floor augmentation. Finally, a total of 21 patients were included based on the aforementioned criteria (Fig. 1).

Surgical procedures

Surgical procedures were performed under local anesthesia using 2% lidocaine (1:100,000 epinephrine; Yuhan Corporation, Seoul, Korea) by an experienced periodontal surgeon (SJY). After an alveolar incision and a vertical incision adjacent to the tooth mesial to the edentulous space, a full-thickness flap was elevated to expose the lateral wall

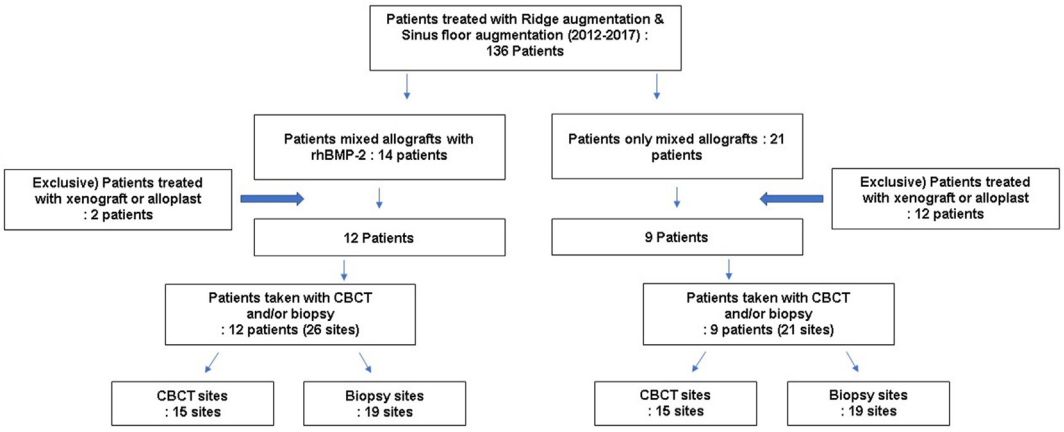

Figure 1 Flow chart depicting the selection process. rhBMP-2, recombinant human bone morphogenetic protein-2; CBCT, cone-beam computed tomography.

of the maxillary sinus. A square bony window was created at the level of the maxillary sinus floor at the implant placement site using a piezoelectric device (Piezotome; Mectron, Carasco, Italy) with a saw-shaped tip (OT-7TM; Mectron, Carasco, Italy) (Fig. 2a).

The maxillary sinus membrane was elevated using a membrane elevation instrument (DASK®; Dentium, Seoul, Korea). After membrane elevation, mixed AG (Do Bone®; Renew Medical, Bucheon, Korea) was mixed with platelet-rich fibrin formed using the patient's venous blood. Bone grafting was performed below the raised maxillary sinus floor till resistance was felt. In the experimental group, 0.25 mg/mL of rhBMP-2 (Novosis-Dent®; CGBIO, Seoul, Korea) was added to the mixture before bone grafting. The bony window was then repositioned (Fig. 2b—d), and suturing was performed using non-absorbable sutures (Happylon®; Purgo Biologics, Seongnam, Korea). To prevent postoperative infection, oral antibiotics (Augmentin 625 mg; Ilsung, Seoul, Korea) were administered three times a day for five days, and a mouth rinse with 0.12% chlorhexidine (Daewoong Pharmaceutical, Seoul, Korea) was administered twice daily for two weeks.

After a bone healing period of six months, CBCT was performed to assess the alveolar bone condition after healing, and the appropriate implant size for the area was determined. Implant placement was performed under local anesthesia using 2% lidocaine (Yuhan Corporation). A full-thickness flap was elevated after an alveolar incision and a vertical incision adjacent to the mesial tooth were made. A core biopsy was performed through the alveolar crest at the implant placement site using a trephine bur with an inner diameter of 2 mm, and the specimen was taken from the

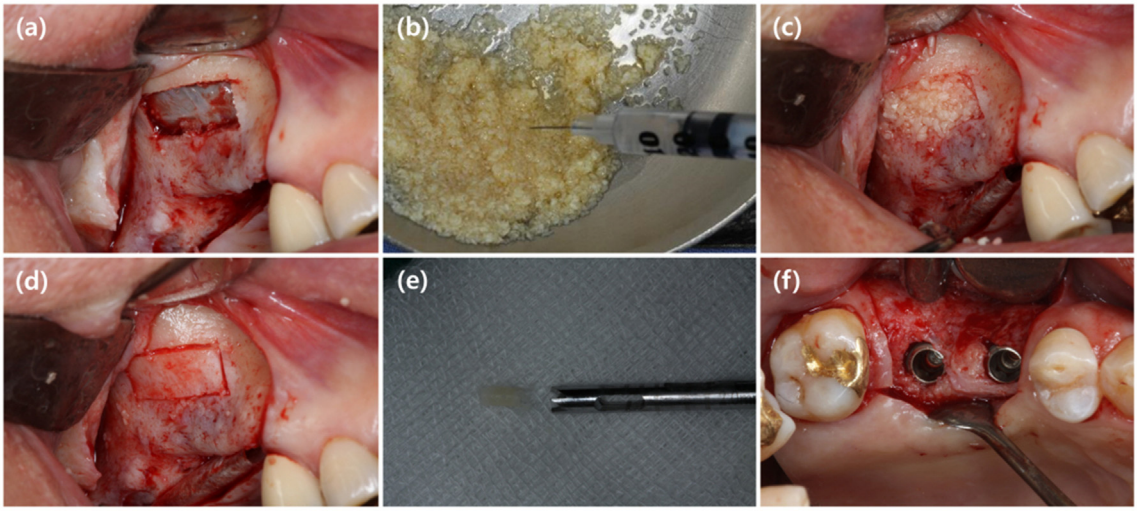

Figure 2 Surgical procedure of maxillary sinus floor augmentation and delayed implant placement. (a) after full-thickness flap elevation, a lateral window access has been created using a piezoelectric device. (b) The rhBMP-2 is combined with the mixed allograft. (c) The graft material is placed after elevation of the Schneiderian membrane. (d) The bone lid is repositioned. (e) Core biopsy harvested using a trephine bur (inner diameter 2 mm) after full-thickness flap elevation of the same site after a six-month healing period. (f) Implant fixtures have been placed.

central part of the maxillary sinus (Fig. 2e). After placement of an implant with an appropriate length and height, the wound was sutured using non-absorbable sutures (Purgo Biologics) (Fig. 2f).

Clinical and radiographic measurements

CBCT (CB MercuRayTM; Hitachi, Tokyo, Japan) was performed before and after bone healing in patients who underwent lateral-approach maxillary sinus floor augmentation. The RBH before surgery and augmented bone height (ABH) after bone healing were measured using a measurement software (PiViewStar 5.0.9.2, Infinitt, Seoul, Korea). The values were standardized after calculating the average of the measurements on tomographic images 2 mm anterior and posterior to the central position where a standard-implant was placed (Fig. 3).

To observe the marginal bone loss (MBL) around the implant, standardized digital panoramic radiographs (Planmeca OY, Helsinki, Finland) were obtained after final prosthesis delivery (baseline) and 12 months after functional loading and analyzed using a measurement program. MBL was measured linearly from the most mesial and distal positions of the implant platform to the alveolar bone by one examiner on each panoramic radiograph and was expressed as the average value. Magnification of the radiographs was corrected using clinical data (length and diameter) for each implant. Each linear measure corresponding to the MBL was calibrated and recalculated based on the radiographic image size using simple mathematical calculations.

Histomorphometric analysis

Harvested specimens were fixed in 10% buffered formalin for one week and then demineralized using 10% formic acid for more than two weeks. The specimens embedded in paraffin blocks and cut into 7 μm-thick sections in the mesiodistal direction using a microsaw. Hematoxylin and eosin staining was performed and histomorphometric images were obtained using a digital camera connected to an optical microscope for evaluation. The i-SolutionTM software (IMT i-solution Inc., Burnaby, BC, Canada) was used for all measurements, and the proportions of newly formed bone (NB), residual bone graft material (RG), and connective tissue (CT) were calculated (Fig. 4).

Statistical analysis

Statistical analyses were performed using SPSS ver. 12.0 (SPSS Inc., Chicago, IL, USA), and measurements are expressed as mean ± standard deviation. Data were checked for normal distribution using the Kolmogorov–Smirnov and Shapiro–Wilk tests for each group. Fisher's exact test was used to analyze age, sex, distribution of implant diameter and length, healing period, and distribution of MBL. An independent *t*-test, a parametric test, was used to analyze the implant stability quotient (ISQ); bone height; average MBL; and proportions of NB, RG, and CT. The Wilcoxon test was used for the follow-up period analysis. Statistical significance was set as $P < 0.05$.

Results

Demographic information of patients and implants

This retrospective study included 21 patients who received a total of 47 implants. The detailed demographic information of the patients is summarized in Table 1. Nine patients (three men and six women) with a mean age of 53.4 ± 8.7 years (range, 43–70 years) received a total of 21 implants with mixed AGs containing a 3:7 mixture of cancellous and cortical freeze-dried bone. The remaining 12 patients (7 men and 5 women) with a mean age of 55.4 ± 8.0 years (range, 38–68 years) received mixed AG combined with rhBMP-2, and a total of 26 implants were placed. No statistically significant differences were observed between the two groups.

Table 2 summarizes the information regarding the implants used in this study. Six different systems of internal-connection implants were used: 3i (Implant Innovation, West Palm Beach, FL, USA), Astra (Astra Tech., MÖlndal, Sweden), ITI (Straumann AG, Waldenburg, Switzerland), Luna (Shinheung, Seoul, Korea), Superline (Dentium, Seoul, Korea), and TSIII (Osstem Implant Co., Busan, Korea). Most implants had a diameter ≥4 mm, with 5-mm diameter implants being the most common in both groups. The implant diameter was not significantly different between the two groups. All implants had length ≥10 mm, with length ≥11 mm accounting for 95.2% and 80.8% of implants in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively. The implant length was not significantly different between the two groups.

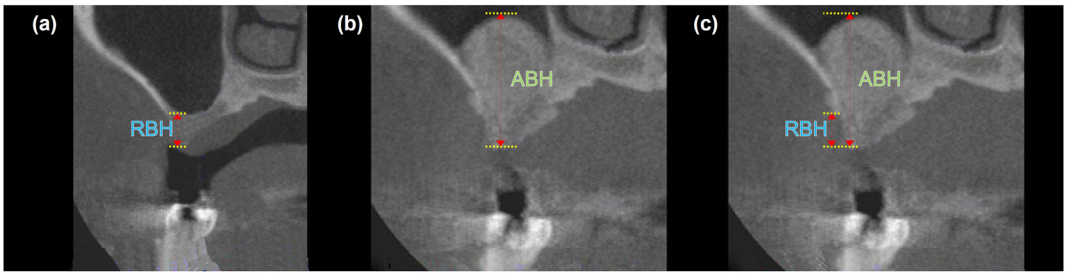

Figure 3 Cone-bean computed tomography images. (a) Preoperative image. (b) Image taken six months after bone grafting. (c) Standardized calculation of RBH and ABH. RBH, residual bone height; ABH, augmented bone height.

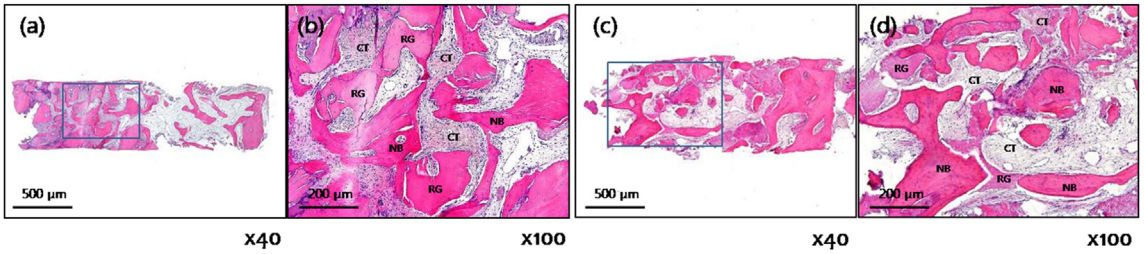

Figure 4 Histomorphometric analysis (hematoxylin and eosin stain). (a, b) 3:7 Mixed allografts (cancellous: cortical freeze-dried bone allograft, 30:70) group. The sample shows 24.6% NB, 21.0% RG, and 54.4% CT. (c, d) 3:7 Mixed allografts with rhBMP-2 group. The sample shows 39.7% NB, 9.6% RG, and 50.2% CT. NB, newly formed bone; RG, residual bone graft; CT, connective tissue.

Table 1 Characteristics of participants (patients = 21; sites = 47).

Variable	Mixed AG	Mixed AG with rhBMP-2	*P* -value
Participants	9	12	
Implants	21	26	
Mean age (yr)	53.4 ± 8.7 (43–70)	55.4 ± 8.0 (38–68)	0.568
Sex			0.387
Male	3 (33.3)	7 (58.3)	
Female	6 (66.7)	5 (41.7)	

Values are presented as mean ± standard deviation (range), number only, or number (%).
Mixed AG, mixed allograft (cancellous: cortical freeze-dried bone allograft, 30:70); rhBMP-2, recombinant human bone morphogenetic protein-2; yr, year.

Table 2 Characteristics of inserted implants.

Variable	Mixed AG (n = 21)	Mixed AG with rhBMP-2 (n = 26)	*P* -value
Implant diameter (mm)			0.535
3.5	0 (0)	1 (3.9)	
4.0 (4.1)	1 (4.8)	4 (11.5)	
4.5	8 (38.1)	9 (34.6)	
5.0 (4.8)	12 (57.1)	13 (50)	
Implant length (mm)			0.194
<11	1 (4.8)	5 (19.2)	
>11	20 (95.2)	21 (80.8)	

Values are presented as number (%).
Mixed AG, mixed allograft (cancellous: cortical freeze-dried bone allograft, 30: 70); rhBMP-2, recombinant human bone morphogenetic protein-2.

Table 3 Clinical assessment of augmented sites.

Variable	Mixed AG	Mixed AG with rhBMP-2	*P* -value
Sites	21	26	
Healing periods (mo)	6.11 ± 0.6	6.25 ± 0.6	0.910
ISQ	74.5 ± 6.2	75.5 ± 6.2	0.571
Failed implants (n)	0	0	
Implant survival rate (%)	100	100	
Follow-up period (mo)	71.0 ± 6.5	67.7 ± 7.2	0.694

Values are presented as number only or mean ± standard deviation.
Mixed AG, mixed allografts (cancellous: cortical freeze-dried bone allograft, 30:70); rhBMP-2, recombinant human bone morphogenetic protein-2; Healing period, period between ridge augmentation and implant insertion; mo, month; ISQ, implant stability quotient using Osstell ISQ (Osstell, Gothenburg, Sweden); n, number; Follow-up period, period from implant insertion to examination.

Clinical and radiological evaluation

The clinical evaluation results are summarized in Table 3. The bone healing periods after maxillary sinus floor augmentation were 6.11 ± 0.6 and 6.25 ± 0.6 months in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively. The bone healing period was not significantly different between the two groups. After bone healing, the ISQ for primary stability was 74.5 ± 6.2 and 75.5 ± 6.2 in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively. ISQ was not significantly different between the two groups. The follow-up period from implant placement to radiographic evaluation was 71 ± 6.5 and 67.7 ± 7.2 months in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively, and the difference was not statistically significant. The implant survival rate was 100% in both groups.

The radiological evaluation results are summarized in Table 4. The preoperative RBH was 3.06 ± 3.0 and 3.82 ± 2.4 mm in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively, and the difference was not statistically significant. ABH after bone healing was 16.42 ± 2.4 and 15.89 ± 2.8 mm in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively, and the difference was not statistically significant. Bone height increased by 13.36 ± 3.9 and 12.07 ± 3.8 mm in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively, and the difference was not statistically significant.

Table 4 Radiographical assessment of augmented sites.

Variable	Mixed AG	Mixed AG with rhBMP-2	*P* -value
Sites	21	26	
RBH (mm)	3.06 ± 3.0	3.82 ± 2.4	0.339
ABH (mm)	16.42 ± 2.4	15.89 ± 2.8	0.598
Increased ABH (mm)	13.36 ± 3.9	12.07 ± 3.8	0.311

Values are presented as mean ± standard deviation.
Mixed AG, mixed allografts (cancellous: cortical freeze-dried bone allograft, 30:70); rhBMP-2, recombinant human bone morphogenetic protein-2; RBH, residual bone height; ABH, augmented bone height; Increased ABH, difference between RBH and ABH.

Table 5 summarizes the MBL at one year after the installation of the final prosthesis and functional loading. The most frequently observed MBL was 1–2 mm (85.7% and 76.9% in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively), but the difference between the groups was not statistically significant. The mean MBL at 1 year after functional loading was 0.49 ± 0.91 and 0.3 ± 0.66 mm in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively, and the difference was not statistically significant.

Histomorphometric evaluation

The histomorphometric evaluation results are summarized in Table 6. The proportion of NB was 24.6 ± 10.2% and 39.7 ± 18.3% in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively, and the difference was statistically significant ($P < 0.05$). The proportion of RG was 21.0 ± 12.2% and 9.6 ± 10.0% in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively, and the difference was statistically significant ($P < 0.05$). The proportion of CT was 54.4 ± 10.3% and 50.2 ± 14.2% in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively. CT formation was not significantly different between the two groups.

Table 5 Marginal bone loss after one year of functional loading.

Variable	Mixed AG	Mixed AG with rhBMP-2	*P* -value
Sites	21	26	
Marginal bone loss (mm)			
≤1	2 (9.5)	4 (15.3)	0.887
1–2	18 (85.7)	20 (76.9)	0.446
>2	2 (7.7)	1 (4.8)	0.851
Average bone loss (mm)	0.49 ± 0.91	0.3 ± 0.66	0.425

Values are presented as mean ± standard deviation, number only, or number (%).
AG: mixed allograft (cancellous: cortical freeze-dried bone allograft, 30:70); rhBMP-2, recombinant human bone morphogenetic protein-2.

Table 6 Histomorphometric evaluation of augmented sites.

Variable	Mixed AG	Mixed AG with BMP	*P* -value
No of sites	21	26	
Newly formed bone (%)	24.6 ± 10.2	39.7 ± 18.3	0.003[a]
Residual bone graft (%)	21.0 ± 12.2	9.6 ± 10.0	0.003[a]
Connective tissue (%)	54.4 ± 10.3	50.2 ± 14.2	0.306

Values are presented as mean ± standard deviation.
Mixed AG, mixed allograft (cancellous: cortical freeze-dried bone allograft, 30:70); rhBMP-2: recombinant human bone morphogenetic protein-2.
[a] Statistically significant difference ($P < 0.05$, independent *t*-test).

Discussion

Maxillary sinus floor augmentation is a highly predictable procedure performed in the posterior maxilla in patients with insufficient residual bone. The bone graft material used during this procedure acts as a scaffold for the growth of blood vessels and cells, allowing the initiation of fibrous connective tissue and bone remodeling. Initially, osteoconduction is rapidly initiated by the cells whereas osteoinduction occurs slowly over many years.[16] Autografts are the only graft materials that not only exhibit osteoconduction but also osteoinduction and osteogenesis and are primarily used for maxillary sinus floor augmentation. In particular, if the maxillary sinus is extremely pneumatized in the posterior part of the maxilla, the use autografts alone or in combination with other graft materials is recommended to preserve BMP in the graft material and enable successful bone regeneration.[17] However, obtaining an autograft requires an intraoral or extraoral donor site and is associated with complications such as postoperative pain, edema, and nerve damage.[18] To overcome the disadvantages of autograft harvesting, alternative graft materials such as xenografts, AGs, and alloplasts have been used effectively to achieve bone regeneration.[19,20] AGs are theoretically believed to induce osteoinduction, but this has never been proven in human studies.[21] Therefore, rhBMP-2 is being studied as a substitute for autograft that can induce osteoinduction during maxillary sinus floor augmentation.

rhBMP-2 is a growth factor that acts locally and induces the differentiation of osteoprogenitor cells into osteoblasts through the chemotaxis of stem cells. However, when used alone, rhBMP-2 is soluble and immediately degrades, resulting in a limited osteoinductive effect.[22] To maintain the bone regeneration ability of rhBMP-2, a carrier material is required.[23] The carrier material should be easy to apply, maintain space for bone regeneration, and control BMP release.[24] Currently, the most widely used scaffold in

clinical practice is the absorbable collagen sponge (ACS). Several studies have reported promising results of rhBMP-2/ACS as an autograft substitute for maxillary sinus floor augmentation.[25,26] However, ridge augmentation using rhBMP-2/ACS is limited because of the inability of ACS to maintain space.[27] To overcome the lack of structural space maintenance, rhBMP-2/ACS has been used for ridge augmentation along with hydroxyapatite and xenografts, and several studies have demonstrated its efficacy as a scaffold.[28,29] Although studies on AGs are limited, a mixture of AGs and rhBMP-2 has been reported to be as safe and effective as an autograft for tibial fractures.[30] Therefore, we aimed to investigate whether AGs could serve as scaffolds to effectively transport rhBMP-2 in dentistry.

AGs can be divided into demineralized FDBA and FDBA based on the processing method. Both types are used as osteoconductive scaffolds for new bone formation.[31] Demineralized FDBA exhibits a high resorption rate and low volume stability. In this study, a mixture of cortical and cancellous FDBA was used. Most cancellous FDBA is absorbed during the healing period, whereas cortical FDBA persists for several years.[11] Therefore, cancellous FDBA acts as an optimal scaffold for new bone formation, and its macroporosity, microporosity, and collagen fiber structure are useful for the migration of mesenchymal stem cells during the initial healing process.[9] Kim et al. reported sufficient bone augmentation in maxillary sinus floor augmentation using a 5:5 mixed AG containing cancellous and cortical FDBA, similar to that in maxillary sinus floor augmentation using xenograft or cancellous FDBA (12.90 $\pm$ 2.97 mm).[12] However, the amount of increased ABH after 6 months was 0.50 $\pm$ 0.28 mm, which was less compared to the use of anorganic bovine bone (2.14 $\pm$ 1.90 mm). Furthermore, new bone formation was lower in the 5:5 mixed AG group (31.27 $\pm$ 18.31%) compared to the mineralized cancellous bone allograft (39.26 $\pm$ 10.72%). To overcome this, we employed a 3:7 mixed AG to enhance volume stability, and utilized rhBMP-2 to stimulate new bone formation. In this study, a similar increase in bone height was observed (13.36 $\pm$ 3.9 mm) when maxillary sinus floor augmentation was performed using mixed AG. Additionally, in the mixed AG with rhBMP-2 group, an increase of 12.07 $\pm$ 3.8 mm was observed, which was similar to that in the mixed AG alone group, but the difference was not statistically significant. Therefore, it can be concluded that mixed AG can be used as a scaffold for rhBMP-2 with a significant increase in bone formation.

Several systematic reviews have reported a high survival rate (95.5–100%) of implants placed after maxillary sinus floor augmentation.[20,32] Furthermore, the survival rate of implants placed after maxillary sinus floor augmentation was found to be similar to that of implants placed in natural bone.[19] A study with a long-term follow-up >10 years also reported a high survival rate of 96.9%.[33] In the present study, the implant survival rate after maxillary sinus floor augmentation using mixed AG combined with rhBMP-2 was 100% at a mean follow-up of 67.7 $\pm$ 7.2 months. Although the small sample size of 26 patients was a limitation of this study, the survival rate was higher than that in other studies. A peri-implant MBL of 2 mm at one year after functional loading is considered normal.[34] In a retrospective study, at one year after functional loading, MBL around implants placed after sinus floor augmentation was significantly greater than that around implants placed in natural bone.[35] Particularly, in internal-connection implants, MBL mesial and distal to implants with maxillary floor elevation was 0.59 $\pm$ 0.93 and 0.74 $\pm$ 0.89 mm, respectively. In most patients in this study, MBL was <2 mm at one year after functional loading, and 1–2 mm MBL was the most common (85.7% and 76.9% in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively). The average MBL in the mixed AG alone and mixed AG with rhBMP-2 groups was 0.49 $\pm$ 0.91 and 0.3 $\pm$ 0.66 mm, respectively. Thus, the bone loss was lesser than that in previous studies. Although the difference was not statistically significant, the incidence of bone loss >2 mm was lower in the mixed AG with rhBMP-2 group (7.7% and 4.8% in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively), and the average bone loss was lesser in the mixed AG with rhBMP-2 group. This shows that rhBMP-2 can better resist physiological bone resorption that occurs one year after functional loading.

The initial stability of an implant is related to the quality of NB. A histomorphometric meta-analysis of maxillary sinus floor augmentation using various graft materials showed that autografts provided the highest total bone volume relatively early after grafting.[36] Interestingly, over time, this difference gradually decreased, regardless of the type of graft material, and difference in the total bone volume between graft materials was not statistically significant at nine months. Therefore, using an autograft is advantageous when a short graft healing period and early loading are required. In this study, we investigated whether the healing period with mixed AG with rhBMP-2 is shorter healing than that with autografts. In specimens obtained after a healing period of approximately six months, the proportion of NB was 39.7 $\pm$ 18.3% and 24.6 $\pm$ 10.2% in the mixed AG with rhBMP-2 and mixed AG alone groups, respectively. The proportion of NB was significantly greater in the mixed AG with rhBMP-2 group ($P < 0.05$), and the value was higher than that reported in a study in which ridge augmentation was performed using rhBMP-2/xenografts (37%).[29] This suggests that allografts are more effective scaffolds for rhBMP-2 than xenografts for new bone formation. Furthermore, the proportion of RG was 9.6 $\pm$ 10.0% and 21.0 $\pm$ 12.2% in the mixed AG with rhBMP-2, and mixed AG was alone groups, respectively, indicating significant absorption in the mixed AG with rhBMP-2 group ($P < 0.05$). This suggests that bone regeneration occurs faster in the early stages when rhBMP-2 is used with AGs. Therefore, the initial healing period after sinus floor augmentation can be shortened, and mixed AG with rhBMP-2 can be applied when early loading is required. In addition, the formation of hard tissue and soft tissue at a certain ratio enables the implant to function properly under masticatory forces after implant prosthesis insertion. The connective-tissue ratio was 54.4 $\pm$ 10.3% and 50.2 $\pm$ 14.2% in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively. It is believed that mixed AG with rhBMP-2 can function properly even when a load is applied after prosthesis insertion. This can be inferred by comparing the ISQ value representing the initial stability of the implant (74.5 $\pm$ 6.2 and 75.5 $\pm$ 6.2 in the mixed AG alone and mixed AG with rhBMP-2 groups, respectively).

Higher doses of rhBMP-2 have a more positive effect on bone formation, and a dose of 1.5 mg/mL of rhBMP-2 has the maximum positive effect on bone growth.[37,38] Furthermore, a systematic review suggested that the optimal dose of rhBMP-2 varies depending on the type and location of bone and the characteristics of the scaffold.[39] Clinically, the concentration of rhBMP-2 should be reduced to maximize the local therapeutic effects while minimizing the systemic effects. Moreover, there are concerns regarding the potential proto-oncogenicity of rhBMP-2. However, the risk of cancer appears to be dose dependent, and no threshold for increased risk has been defined to date.[15] Additionally, no evidence of rhBMP-2 proto-oncogenicity was found in the craniofacial region.[40] This may be because the average dose of rhBMP-2 used in the craniofacial region is usually much lower than that in other regions. In recent studies, bone regeneration was reported at a rhBMP-2 concentration of 0.2 mg/mL. Based on the results, the rhBMP-2 concentration used in this study was 0.25 mg/mL.[41,42] Nevertheless, the clinical, radiological, and histomorphometric results of this study were similar to those of previous studies. Therefore, we suggest that effective bone regeneration is possible using a lower concentration of rhBMP-2.

In this study mixed AG (cancellous: cortical FDBA, 30:70) combined with rhBMP-2 showed excellent clinical, radiological, and histomorphometric outcomes for maxillary sinus floor elevation. However, further studies are required to determine the optimal concentration of rhBMP-2 with mixed AG. Therefore, more comprehensive, large-scale, and long-term studies are warranted to further explore this subject.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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