CLINICAL ORAL IMPLANTS RESEARCH

*Min-Soo Kim**
*Jung-Seok Lee**
Hyun-Ki Shin
Jae-Shin Kim
Jeong-Ho Yun
Kyoo-Sung Cho

Prospective randomized, controlled trial of sinus grafting using *Escherichia-coli*-produced rhBMP-2 with a biphasic calcium phosphate carrier compared to deproteinized bovine bone

Authors' affiliations:
Min-Soo Kim, Jung-Seok Lee, Hyun-Ki Shin, Jae-Shin Kim, Kyoo-Sung Cho, Department of Periodontology, Research Institute for Periodontal Regeneration, College of Dentistry, Yonsei University, Seoul, Korea
Jeong-Ho Yun, Division of Periodontology, Department of Dentistry, School of Medicine, Inha University, Incheon, Korea

Corresponding author:
Kyoo-Sung Cho
Department of Periodontology, Research Institute for Periodontal Regeneration, College of Dentistry, Yonsei University, 50 Yonsei-ro, Seodaemoon-gu, Seoul 120-752, Korea
Tel.: +82 22 228 3188
Fax: +82 23 92 0398
e-mail: kscho@yuhs.ac

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Abstract

Aim: This study compared the effects of *Escherichia-coli*-produced recombinant human bone morphogenetic protein 2 (ErhBMP-2) with a biphasic calcium phosphate (BCP) carrier to those of deproteinized bovine bone in human maxillary sinus floor augmentation.

Material and methods: Screening for this clinical trial selected 56 sites that provided informed consent to participate, of which 46 were ultimately enrolled and 41 were finally included in the study. The sites were divided into two groups using a random-number table, and the material was applied. A trephine biopsy was performed after 24 weeks, and implants wider than the biopsy site were inserted. Computed tomography and plain panoramic images were obtained immediately and then again at 24 weeks after the surgery. Radiographic images were reconstructed to allow measurement of the linear and volumetric changes. The biopsy samples were processed for histologic and histometric analyzes.

Results: All sites healed uneventfully with no complications. Radiographic analysis revealed a tendency for the volume to increase, but the difference was not statistically significant in either group. Comparison of volumetric changes between the two groups also revealed no significant difference. Moreover, none of the histometric parameters differed significantly between the groups, although different healing patterns were observed on histologic analysis.

Conclusions and clinical implications: It can be concluded that sinus augmentation with ErhBMP-2 carrying BCP carrier did not enhance bone regeneration compared to the conventional treatment using deproteinized bovine bone at 24 weeks after the surgery.

Since introduction of maxillary sinus augmentation by Boyne & James (1980), it has been the treatment of choice for restoration using dental implants in a maxillary posterior area showing a severely resorbed alveolar ridge or a pneumatized sinus (Wallace & Froum 2003). Many previous clinical studies have demonstrated successful bone regeneration in the augmented sinuses and comparable implant survival rates to those in the existing alveolar bone (Browaeys et al. 2007; Pjetursson et al. 2008; Esposito et al. 2009; Nkenke & Stelzle 2009). Mordenfeld et al. (2010) evaluated biopsy samples histologically at 11 years after maxillary sinus augmentation using deproteinized bovine bone and demonstrated the presence of mature regenerated bone tissue with unresorbed graft biomaterials.

Various graft biomaterials have been used for sinus augmentation for dental implant placement, such as autogenous, allogenic, and xenogenic bone, synthetic biomaterials, and a mixture of these biomaterials. The use of autogenous and allogenic bone is subject to limitations such as the morbidity at the donor site, insufficient quantity, and excessive resorption at the recipient site after a long period of healing (Clavero & Lundgren 2003; Andersson 2008). Therefore, many clinicians prefer using space-maintainable biomaterials for sinus augmentation, such as hydroxyapatite (HA), biphasic calcium phosphate (BCP), or deproteinized bovine bone (Lindgren et al. 2012; Oliveira et al. 2012; Schmitt et al. 2013). Because maxillary sinus is a contained defect surrounding osteogenic sources, the bony sinus

*These authors contributed equally to this work.

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floor and walls, and the Schneiderian membrane, successful bone regeneration and favorable clinical results can occur even in sinus augmentation using an osteoconductive biomaterial rather than an osteoinductive one. However, in cases of an extensive defect of the maxillary sinus, long healing periods will be needed for the production of bone that would be of clinically acceptable quality for implant placement (Nkenke & Stelzle 2009).

To reduce the healing time for bone regeneration, recombinant human bone morphogenetic protein-2 (rhBMP-2) has been introduced and evaluated for used in various bone augmentation techniques in both the research and clinical fields. rhBMP-2 is a protein obtained from mammalian Chinese hamster ovarian cells and was approved by the US FDA based on numerous clinical studies (Boyne et al. 1997, 2005; Triplett et al. 2009). However, several recent *in vitro* and *in vivo* pre-clinical studies have demonstrated that *Escherichia-coli*-derived rhBMP-2 (ErhBMP-2) is comparable to rhBMPs derived from mammalian cells. Lee et al. (Lee et al. 2010) found that when applied with an absorbable collagen sponge (ACS) carrier, ErhBMP-2 induced significantly superior bone regeneration in a rat calvarial defect model compared with the control condition, over a wide range of doses. Jung et al. (2011) used an absorbable collagen block as a carrier for ErhBMP-2 to observe bone formation in the rat calvarial defect model and confirmed the osteoinductivity of ErhBMP-2.

The previous study also demonstrated enhancement osteoinductivity from Schneiderian membrane *in vitro* and *in vivo* experiments by ErhBMP-2, in which BCP was used as a scaffold due to its osteoconductivity and its ability to carry growth factors (Choi et al. 2013). BCP with ErhBMP-2 has also exhibited favorable results in the rat calvarial model (Jang et al. 2012). In addition, block-type BCP lyophilized with ErhBMP-2 was shown to enhance new bone formation (Kim et al. 2011). Various pre-clinical studies involving animal experiments have confirmed the osteoinductivity of ErhBMP-2 with a BCP carrier (ErhBMP-2/BCP), whereas few clinical studies have applied ErhBMP-2 to defect models that include the sinus graft. Therefore, this study used computed tomography (CT) and histologic analyzes to compare the effects of ErhBMP-2/BCP with those of deproteinized bovine bone as a conventional standard of graft biomaterial in human maxillary sinus augmentation.

Materials and methods

This study was designed as a single-blinded, randomized, controlled clinical trial performed at two single centers (Dental Hospital of Yonsei University and Inha University Hospital). The protocols used in this study were approved by the Institutional Review Board for Clinical Research at Dental Hospital of Yonsei University (approval no. 2-2011-0006) and observed the Helsinki Declaration (as revised in Tokyo in 2004) and the Good Clinical Practice Guidelines. All of the included patients provided written informed consent to take part in the clinical experiments, and this manuscript was prepared according to CONSORT guidelines (Appendix S1).

Sample size

The sample size was calculated using G Power software (Version 3; Faul et al. 2009). The null hypothesis was that ErhBMP-2/BCP has superior bone regeneration capacity relative to deproteinized bovine bone for sinus augmentation. The histometric difference in new bone area between two groups was assumed to be 10% with 90% power and an alpha level of 0.1. The standard deviation of the outcome was set to 10%, in accordance with (Cordaro et al. 2008). The required sample size per group was 18 subjects, and 20 subjects were scheduled to be enrolled in each group in this study based on an assumed dropout rate of 10%. Among the 52 patients who volunteered to participate in this study, 46 who conformed to the inclusion criteria were enrolled. The number of sites per institute was determined as followed based on the number of participating surgeons: 44 at Institute A and 12 at Institute B (Fig. 1).

Randomization

Random group assignment was performed using block randomization (SAS, SAS Institute, Cary, NC, USA). Computer-generated random numbers were sealed in opaque envelopes by an independent person. After elevating the Schneiderian membrane, the envelope was opened, and the corresponding assigned material was brought to the surgeon by an assistant. The enrolled patients were not informed of the assigned materials.

Preparation of ErhBMP-2/BCP particles

An *E. coli* expression system was used to produce ErhBMP-2 at the research institute of Cowellmedi (Busan, Korea). Microporous BCP (particle type, 0.5–1.0 mm diameter, 70% porosity; Bio-C, Cowellmedi) consisting of HA and beta-tricalcium phosphate at a ratio of 30:70 was used as the carrier for ErhBMP-2. ErhBMP-2 was coated onto BCP particles using a previously described lyophilization method (Kim et al. 2011). Briefly, ErhBMP-2 solution (0.67 ml in 1.5 mg/ml buffer) was added to 1 g of BCP particles and lyophilized in a freeze-dryer. The solution was frozen on shelves that were pre-cooled to −43°C. The formulations were dried in a condenser at −40°C (primary drying) and maintained at that temperature for 3 h, after which they were transferred to a pressure chamber at 5 millitorr for 2 h. Secondary drying was performed on a shelf using the following temperature sequence: −20°C for 4 h, −10°C for 4 h, 0°C for 2 h, and 20°C for 20 h. The pressure in the chamber was maintained constant throughout the procedure.

Timetable and surgical procedures

Before the start of this trial, all researchers attended a calibration meeting at which an experienced senior surgeon (K.S.C.) described all steps of the surgical procedures to the participating surgeons. Antibiotics (500-mg amoxicillin or 150-mg roxithromycin) and analgesics (200-mg ibuprofen) were administered to all patients 1 h before and then for 7 days after surgery. Sinus floor augmentation was performed by a lateral approach technique using a specially designed opening kit (LAS-KIT, Osstem, Seoul, Korea) and a membrane-elevation kit (DASK, Dentium, Seoul, Korea). A crestal incision and mesial vertical incision were made at the operation site with the patient under local anesthesia. A full-thickness mucoperiosteal flap was elevated to expose the alveolar crest and lateral wall of the maxillary sinus. A standard osteotomy was performed using a specially designed drill to make a round window in the bone (7 mm in diameter), and Schneiderian membrane was carefully elevated. According to random group allocation, the graft material—deproteinized bovine bone (Bio-Oss, Geistlich Biomaterials, Wolhuser, Switzerland) for control group and pre-fabricated ErhBMP-2/BCP (Cowell BMP, Cowellmedi, Busan, Korea)—was packed into the space once continuity of the Schneiderian membrane had been confirmed. A Valsalva maneuver was applied to patients after grafting of the materials to again check that the Schneiderian membrane had not been perforated. After the material was grafted, the mucoperiosteal flap was sutured closed with 4-0 glyconate absorbable monofilament (Mono-

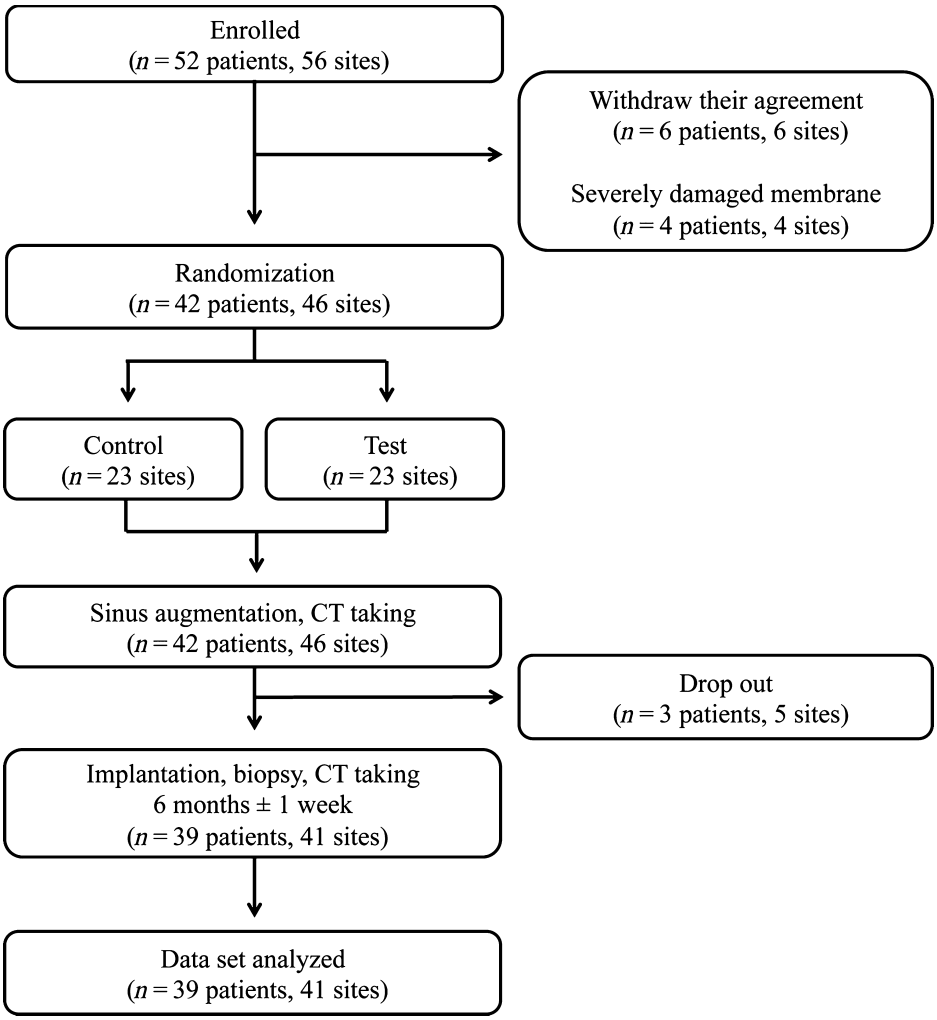

Fig. 1. Randomization and treatment allocation chart.

syn, B-Braun, Aesculap, Center Valley, PA, USA), which was subsequently removed after 10–18 days. To acquire the initial parameters, postoperative radiographs were obtained immediately after completion of the sinus floor augmentation.

Preoperative radiographs were obtained immediately before the implant surgery – which took place 24 weeks after the sinus floor augmentation – to evaluate the grafted site. Following elevation of the full-thickness flap, a biopsy sample of the grafted area was obtained carefully along the long axis of the planned implant site using a trephine bur with an internal diameter of 3 mm. One biopsy site per one grafted sinus was selected. Implants with a larger diameter than the biopsy preparation were inserted following a common surgical protocol. In cases of reduced bone quality, specific surgical methods such as undersized final drilling and osteotome sinus floor augmentation were performed to achieve primary stability.

Radiographic analysis

Various radiographs were obtained to evaluate the pathology, density, height, and width of the bone anatomy in the maxillary sinus. All acquired CT images were processed in DICOM format, and complete images that included the maxillary sinus area were analyzed using three-dimensional (3D) reconstruction software (OnDemand3D, CyberMed, Seoul, Korea). Sectional images acquired from the CT and plain panoramic radiographs were used to obtain the following linear measurement: the highest and lowest points of the grafted area (CT and plain images); the distance between the two points was calculated using a computer program. Volumetric changes were measured using polygonal cutting and color coding of the grafted area in reconstructed 3D images in each of three axes: coronal, sagittal, and axial. After processing, the volume of the isolated graft materials was measured automatically using a computer program (Fig. 2). The radiographic measurements were performed independently by two examiners (M.S.K. and H.K.S.).

Histologic analysis

After the surgery, the trephined bony core that was removed at biopsy was carefully separated from the drills and decalcified in 5% formic acid over 14 days and then embedded in paraffin. Serial coronal sections were cut along the center of each trephined specimen. The two central-most sections were chosen and stained using hematoxylin and eosin and Masson's trichrome. The histologic slides were observed under a light microscope (BX50, Olympus, Tokyo, Japan) and analyzed qualitatively.

Histometric analysis

Histometric analysis was performed using an automated image-analysis system (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA) by two independent examiners (M.S.K.

and H.K.S.) to qualitatively evaluate the bone regeneration. The following parameters were evaluated: the area of residual graft material (RG), the area of newly formed bone (NB), and the non-mineralized area (NM). NM was calculated as the remaining area that was not RG or NB.

Statistical analysis

The mean, standard deviation, and median values of all parameters were calculated for both the experimental and control groups in the radiographic and histometric analyzes. Shapiro–Wilk test was used to determine the normality of all data, and all measured data except one result from volumetric analysis in CT images showed normal distribution. The unpaired *t*-tests for the normally distributed data and Mann–Whitney *U*-test for nonparametric data were used to determine the significance of any differences between time points and between the two groups, respectively ($P = 0.05$). The interexaminer reliability for radiologic and histometric measurements was quantified using the interclass correlation coefficient (ICC). The ICCs were 0.869 and 0.966 with a 95% confidence level in radiographic and histometric analyzes, respectively, which indicates a high level of reliability in the measurements made by the two examiners.

Results

Patient information

Fifty-six volunteers (44 at Institute A and 12 at Institute B) applied to take part in this clinical experiment between July 2011 and October 2012. Ten volunteers were excluded between the screening and enrollment phases: Six withdrew their agreement to participate before the surgery, and four had a severely damaged Schneiderian membrane during the membrane elevation and were therefore unable to receive sinus floor augmentation. Thus, 46 sites were enrolled in this clinical study, and they were randomly divided into 2 groups: control ($n = 23$) and experimental ($n = 23$), with 34 and 12 sites treated at Institutes A and B, respectively. Of these 46 sites, 5 were ultimately excluded after enrollment due to perforation of the Schneiderian membrane during the condensation of biomaterials in the elevated sinus ($n = 1$), uncontrolled blood sugar levels ($n = 1$), violation of the experimental schedule ($n = 1$), and withdrawing their agreement to participate ($n = 2$). This clinical trial was therefore completed with 41 sites (22 males and 19 females aged 52.37 ± 12.03 years, mean ± SD) that underwent sinus floor augmentation and implant surgery. Thirty-one of the sites were treated at Institute A (16 and 15 in the control and experimental groups, respectively), and 10 were treated at Institute B (5 in both groups; Table 1 and Fig. 1).

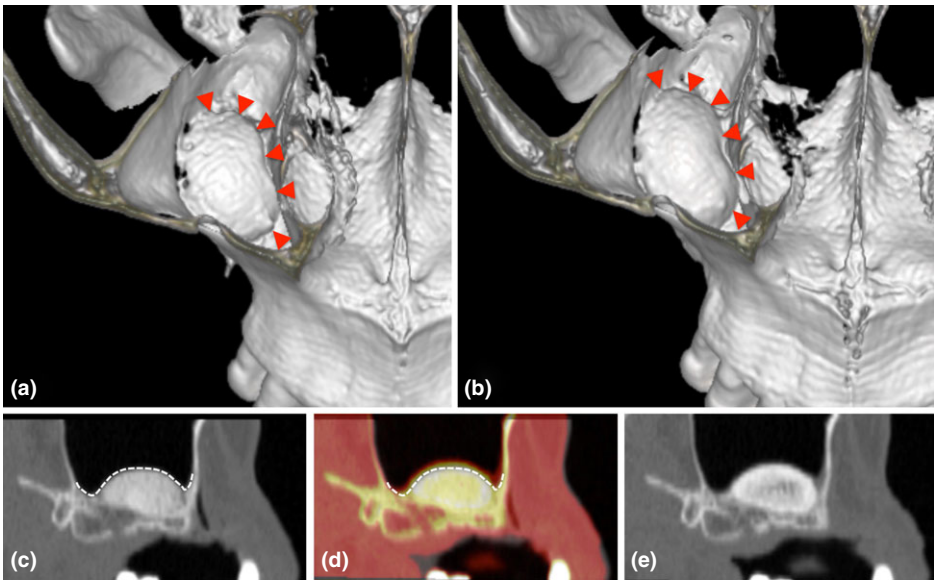

Fig. 2. Radiographic analysis based on superimposition of computed tomographies immediately and 24 weeks after surgery. The augmented area could be observed on three-dimensionally reconstructed images (red arrowheads) at immediately (a) and 24 weeks (b) after surgery, and these could be superimposed by unchangeable structures, like zygomatic arch, nasal sptum, etc. After superimposition of reconstructed images, superimposed sectional images (d) can provide the dimensional changes from the time point immediately after surgery (c) and 24 weeks after the augmentation (e). Colorfully accentuated image: samples from 24-week observational period, dotted line: roof of the augmented area immediately after surgery.

Table 1. **Demographics of patient population and maxillary sinus (mean ± sd (median))**

	Experimental	Control
Distribution of Patients		
Age	52.70 ± 13.55 (50.50)	51.95 ± 10.80 (52.00)
Male/Female	9/11	13/8
Institute A/B	15/5	16/5
Right/Left	12/8	9/12
Distribution of Maxillary Sinuses		
Pre-existing bone height (mm)	1.74 ± 0.72 (1.54)	2.31 ± 1.43 (2.15)
Augmented height immediately after surgery (mm)	12.67 ± 2.47 (13.15)	13.22 ± 1.97 (13.08)
Augmented height 24 weeks after surgery (mm)	12.32 ± 3.16 (12.60)	14.51 ± 3.27 (14.00)
Mesio-distal dimension (mm)	34.70 ± 5.06 (34.26)	34.72 ± 6.28 (33.46)
Bucco-palatal angle (°)	67.23 ± 17.93 (62.54)	70.80 ± 14.99 (69.57)

Clinical findings

All sites healed without major complications associated with the failure of sinus augmentation, including postoperative infection. However, specific clinical findings including minor complications presented in some subjects, comprising postoperative swelling, pain, and bleeding ($n = 7$ and 1 for control and experimental group, respectively), perforation of the Schneiderian membrane (smaller than 3 mm in all cases) ($n = 4$ and 3), separated two chambers in one maxillary sinus area by septum, which led preparation of two window openings ($n = 1$ and 1), poor bone quality where the implant was installed ($n = 0$ and 2), and the presence of protruding grafted particles to oral cavity postoperatively near the window opening ($n = 0$ and 1). In cases with a small perforation of the Schneiderian membrane, grafting biomaterials were applied after application of a collagen membrane (Bio-Gide, Geistlich Biomaterials, Wolhuser, Switzerland) at the perforated area. After 24 weeks, the grafted sites remained stable prior to the implant surgery, and primary stability of all inserted implants was obtained in all subjects except for one in the experimental group, who experienced osseointegration failure. The mean follow-up period after delivery of the final restoration of

the inserted implants was 94.83 days at Institute A and 203.67 days at Institute B, and the cumulative survival rate of the total implants was 98.63%.

Radiographic findings

Among the 41 sites, those with unusual scattering of the grafted material in the radiographs and in whom the proportional changes after 24 weeks exceeded 200% were regarded as having loss of continuity or breakdown of the Schneiderian membrane and were excluded from the analysis (n = 4). The height of the grafted area immediately after sinus floor augmentation was 13.41 ± 4.27 mm in the experimental group and 13.86 ± 3.59 mm in the control group on plain panoramic radiographs; after 24 weeks these heights had changed to 13.66 ± 4.75 mm and 14.17 ± 4.19 mm, respectively. In sectional CT images, the initial height of the grafted area was 12.55 ± 3.27 mm in the experimental group and 13.11 ± 2.03 mm in the control group; at 24 weeks after the sinus floor augmentation these heights were 13.29 ± 3.44 mm and 13.84 ± 2.14 mm, respectively. Two different methods were used in the volumetric analysis to reduce measurement errors: (1) using computer software to demarcate the augmented area based on the presence of radiopacity, with the acquired volume was directly applied as data, and (2) using the air volume in the maxillary sinus to calculate the difference between two time points. As dental CT images that showed the whole maxillary sinus (especially the upper portion) were not available, two images from different time points of the same patient were superimposed to determine the reference points. The outlined maxillary sinus comprised radiolucent (air) and radiopaque (grafted materials) areas, and the changes in the augmented volume were calculated based on changes in the air volume (Fig. 2). Radiographic analysis revealed a slight tendency toward an increase in both linear and volumetric changes in both groups, but the difference was not statistically significant between the two time points in either group. Comparison of volumetric changes between the two groups also revealed no significant differences (Figs 3 and 4).

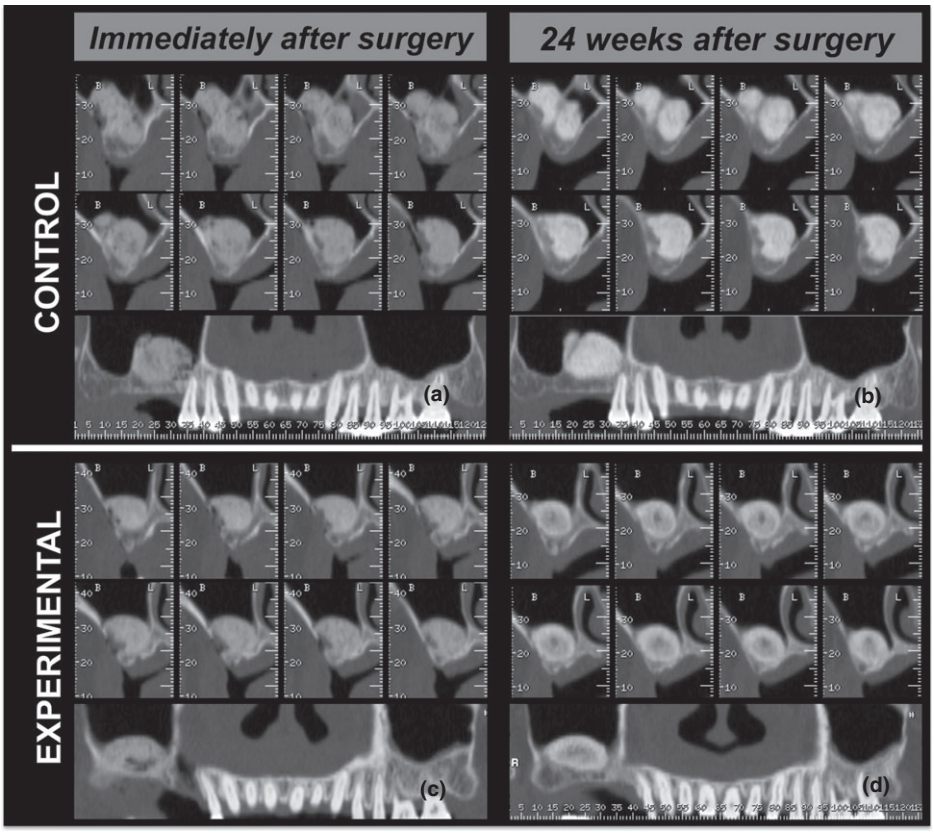

Fig. 3. Representive radiographic views of control and experimental sites immediately and 24 weeks after sinus augmentation. (a) Control sites immediately after the surgery, (b) control sites after 24 weeks from the surgery. (c) Experimental sites immediately after the surgery, (d) Experimental sites after 24 weeks from the surgery.

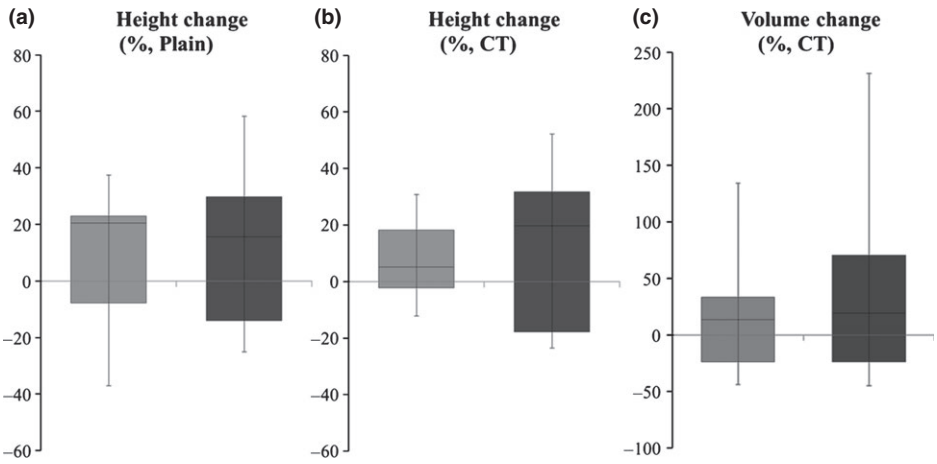

Fig. 4. Results of height and volume change between immediately and 24 weeks after sinus augmentation measured in radiographs. Height changes were measured in both plain panoramic radiographies (a) and computed tomographies (b) and volume change was calculated from the computed tomographic images (c). Bright gray bars indicate control group and dark gray bars for experimental group.

Histologic findings

Some differences in the healing pattern were observed on histologic analysis. In the experimental group, the surface of the BCP exhibited irregular morphology, possibly as a result of superficial resorption of the grafted material. Fatty marrow-like spaces were present between the RG and NB, especially in the experimental group. In contrast, the RG appeared to maintain its original morphology in the control group, even at 24 weeks after the surgery (Figs 5 and 6).

Histometric findings

A region of interest was selected at most central area from the sinus floor to the uppermost point of the biopsy samples in all histologic sections. A biopsy was considered to have failed in subjects without native bone on the histologic slides and was thus excluded from the histometric analysis. This resulted in three sites from the experimental group being excluded. The total areas were 10.27 ± 5.56 mm^2 and 7.69 ± 11.07 mm^2 in the control and experimental groups, respectively. RG was distinguished based on the

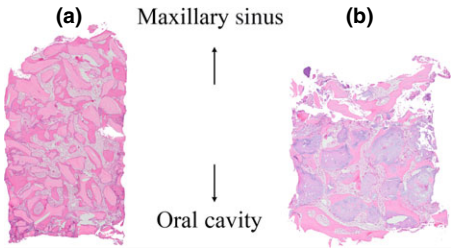

Fig. 5. Representative histologic view of low magnification used as an region of interest (ROI) used in histomorphometric analysis. The lower border of the ROI corresponded to the lowest portion of the graft material, and the upper border corresponded to the highest portion of the trephined biopsy. (a) Control group, (b) experimental group (H-E; ×40).

presence of specific morphology in both groups. In the control group, grafted particles had a similar morphology to the NB, but was characterized by sharp edges, trabecular patterns, and hollow lacunae without viable cells. The grafted material in both groups was surrounded by fibrous tissues and NB. NM mainly comprised fibrous tissue, fatty marrow-like adipose tissue, and blood vessels. The area of NB was 2.02 ± 1.11 mm^2 in the control group and 1.85 ± 4.08 mm^2 in the experimental group; the corresponding RG areas were 1.79 ± 1.24 mm^2 and 1.45 ± 1.21 mm^2, respectively, and those of NM were 6.45 ± 3.80 mm^2 and 4.39 ± 6.23 mm^2. None of these parameters differed significantly between the two groups (Table 2 and Fig. 7).

All samples of both control and experimental groups were subcategorized to two types of sites, by whether small perforation of sinus membrane was occurred, to evaluate its effects as a confounding factor. These results were presented in Table 3. Although there were no differences in radiographically dimensional change or the histologic proportion of newly formed bone, experimental sites with small perforation showed reduced area of newly formed bone (0.41 ± 0.19 mm^2) compared to other subcategorical groups (2.14 ± 4.44, 3.39 ± 1.17, and 2.05 ± 1.13 mm^2 for experimental site without perforation, and control sites with/without perforation, respectively).

Discussion

This study aimed to quantify bone healing in a conventional sinus graft and in an alternative sinus graft that used ErhBMP-2, aided by radiographic and histologic analyzes in humans, and was performed at two single centers with a prospective, randomized, and single-blinded trial design. The authors hypothesized that ErhBMP-2 would enhance bone regeneration in augmented maxillary sinus, based on previous pre-clinical studies showing acceleration of bone healing processes and ectopic bone formation in various experimental models. However, the results obtained in the present study failed to show any superiority of ErhBMP-2 in sinus augmentation at 24 weeks after surgery relative to conventional sinus augmentation using the osteoconductive biomaterial.

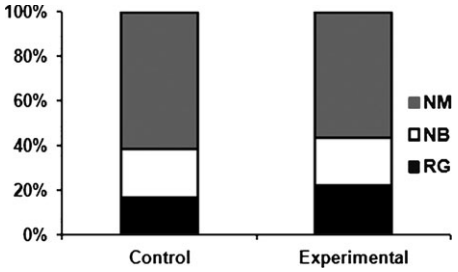

Fig. 7. Histometric analysis measured in region of interest (NB, new bone; RG, residual graft; NM, non-mineralized area).

In radiographic analysis, dimensional changes were evaluated by comparing between the time immediately and 24 weeks after surgery, and the experimental and control groups showed similar patterns of volumetric and linear changes between two time points; there was a slight increase in the volume and height of the augmented area, with no significant differences between the two groups. However, the experimental sites that received ErhBMP-2/BCP showed wider ranges of height and volume changes after 24 weeks, with a higher maximum value for both the height and volume. These results are consistent with a previous study finding a significantly increased augmentation volume at sites that received ErhBMP-2/BCP compared to BCP only in the early healing period in a rabbit sinus augmentation model (Choi et al. 2013). That study found that the augmented volume was decreased at a later time period but still showed a slightly increased volume. ErhBMP-2 was found to increase postoperative tissue swelling or seroma during the

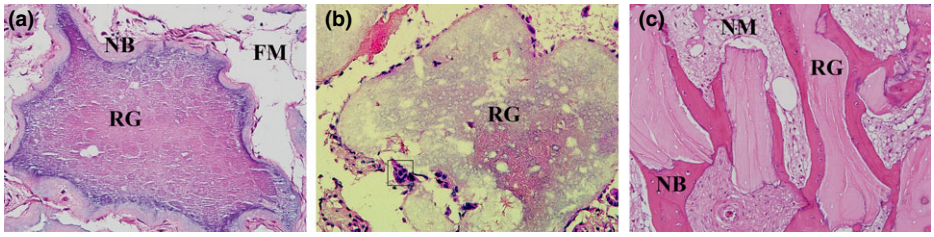

Fig. 6. Representative histologic view of high magnification. (a) Histologic findings in the experimental group. The grafted particles were surrounded by NB as immature bony tissue. Non-mineralized tissues were composed mainly of fatty marrow-like tissue (FM) and there appeared to be adipose-tissue-rich zones. (b) A multinucleated cell (square box), assumed to be an osteoclast, was in contact with the grafted material in the experimental group. (c) Histologic findings in the control group. NB with many viable cells was observed between the grafted particles. The original morphology of the material was a cell-poor bony tissue [hematoxylin and eosin stain (H-E); ×100].

Table 2. **Measurements of radiographic and histometric analyzes (mean ± SD [median])**

	Control		Experimental	
	Surgery	6 months	Surgery	6 months
Radiographic analyzes				
Augmented height (mm), 2D	12.89 ± 3.76 (12.13)	12.75 ± 2.97 (12.58)	13.70 ± 3.32 (12.89)	14.51 ± 4.11 (13.49)
Augmented height (mm), 3D	12.53 ± 2.49 (12.55)	12.39 ± 3.18 (12.66)	13.06 ± 1.96 (12.86)	13.41 ± 2.26 (13.57)
Augmented volume (mm^3)	267.96 ± 182.40 (246.28)	279.94 ± 186.76 (248.83)	197.59 ± 88.29 (196.72)	205.41 ± 81.20 (203.12)
Histometric analyzes				
Newly formed bone (mm^2)	2.02 ± 1.11 (1.67)		1.85 ± 4.08 (0.72)	
Residual biomaterials (mm^2)	1.79 ± 1.24 (1.44)		1.45 ± 1.21 (1.03)	
Non-mineralized tissue (mm^2)	6.45 ± 3.80 (6.20)		4.39 ± 6.23 (2.74)	

Augmented height (mm) was measured in 2D (plain panoramic view) and 3D (reconstructed CT view), but augmented volume (mm^3) was measured in 3D.

Table 3. **Measurements of radiographic and histometric analyzes in subcategorical groups according to the existence of membrane perforation during sinus augmentation. (mean ± SD [median])**

		Control		Experimental	
		Surgery	6 months	Surgery	6 months
Radiographic analyzes					
Augmented height (mm), 2D	Perfo.	14.72 ± 2.30 (14.18)	13.63 ± 3.60 (14.50)	14.82 ± 3.80 (14.82)	13.57 ± 3.68 (13.57)
	None	13.44 ± 3.56 (12.89)	14.74 ± 4.31 (13.35)	12.63 ± 3.81 (12.00)	12.64 ± 3.00 (12.58)
Augmented height (mm), 3D	Perfo.	13.65 ± 0.75 (13.72)	13.71 ± 1.20 (13.74)	13.70 ± 2.01 (12.80)	13.47 ± 1.95 (12.86)
	None	12.88 ± 2.19 (12.22)	13.31 ± 2.54 (13.57)	12.31 ± 2.56 (11.72)	12.20 ± 3.36 (12.60)
Augmented volume (mm^3)	Perfo.	202.50 ± 63.54 (205.37)	189.25 ± 53.86 (189.51)	235.67 ± 87.93 (199.18)	214.33 ± 49.97 (203.86)
	None	288.11 ± 203.67 (246.17)	307.85 ± 205.42 (254.09)	189.43 ± 89.43 (176.27)	203.50 ± 87.79 (205.78)
Histometric analyzes					
Newly formed bone (mm^2)	Perfo.	3.39 ± 1.17 (1.79)		0.41 ± 0.19 (0.42)	
	None	2.05 ± 1.13 (1.67)		2.14 ± 4.44 (0.81)	
Residual biomaterials (mm^2)	Perfo.	3.04 ± 1.30 (1.64)		0.47 ± 0.32 (0.59)	
	None	1.83 ± 1.26 (1.44)		1.65 ± 1.23 (1.48)	
Non-mineralized tissue (mm^2)	Perfo.	6.38 ± 6.25 (4.49)		1.94 ± 2.21 (0.95)	
	None	6.51 ± 3.31 (6.45)		4.88 ± 6.70 (2.81)	

Augmented height (mm) was measured in 2D (plain panoramic view) and 3D (reconstructed CT view), but augmented volume (mm^3) was measured in 3D.
Perfo.: subcategorical group with small sinus membrane perforation in both control and experimental group.
None: subcategorical group without small sinus membrane perforation in both control and experimental group.

early healing processes (Smucker et al. 2006; Leknes et al. 2008; Shah et al. 2008), and this could affect the volumetric change. However, all specimens in both of the groups showed a clinically acceptable volume for dental implant placement and that this volume did not change significantly.

All specimens analyzed histologically or histometrically in the present study showed evidence of new bone formation in the biopsy samples. As these samples were taken at the site of a dental implant, histologic analyzes were limited to within 10 mm from the alveolar ridge crest regardless of the pre-existing alveolar bone and augmentation height. The bone density and the proportion of residual biomaterials were comparable within these areas at the control and experimental sites. These results were in accordance with a previous pre-clinical study finding similar bone healing in the entire augmented sinus area in a rabbit sinus model at 8 weeks after surgery using the same biomaterials as those used in the present clinical study (Choi et al. 2013).

At the 2-week observational period, Choi et al. (2013) found a peculiar healing pattern at the sites treated with ErhBMP-2 but not at the control sites without ErhBMP-2. While newly formed bone was concentrated in the peripheral area around the Schneiderian membrane, limited bone tissue could be observed in the central area of the augmented sinus. Although the healing period was much longer in the present clinical study (24 weeks) than in that previous pre-clinical study, this healing pattern could have affected the bone density in the central area of the augmented sinus and hindered the acquisition of histologic biopsy samples. The use of a trephine drill resulted in smaller histologic specimens being taken despite using the same protocol at all sites (10.27 ± 5.56 and 7.69 ± 11.07 mm^2 at the control and experimental sites, respectively). Interestingly, three experimental sites with small membrane perforation showed limited new bone tissue in histologic samples, while control sites with perforation showed comparable results to the other sites. This might be caused by healing mechanism that was dependent on the increase of osteoinductivity from Schneiderian membrane by ErhBMP-2, which had demonstrated in the previous animal study (Choi et al. 2013). These results are consistent with a previous clinical study also finding that rhBMP-2 exerted negative effects on bone regeneration and density in sinus augmentation (Kao et al. 2012). However, all of the other clinical, radiographic, and histologic parameters were indicative of comparable results in the control and experimental groups in the present study. Only one implant (in the experimental group) failed to acquire initial stability, and successful bone regeneration contacting with the residual biomaterial particles was evident in all of the acquired histologic samples. Therefore, like deproteinized bovine bone, ErhBMP-2/BCP can be used as a grafting biomaterial in sinus augmentation.

The present clinical study was subject to some limitations in evaluating the separate effects of ErhBMP-2. To evaluate the effects of ErhBMP-2 on accelerating bone healing processes in sinus augmentation, biopsy and implantation should have been performed earlier than when the final conventional clinical outcome occurs to compare the early phase of bone healing processes. However, the present clinical study was designed conservatively due to ethical issues, and this could have affected comparisons between the control and experimental groups. In addition, the scaffolding biomaterials differed between the two groups, with deproteinized bovine bone used at control sites as a conventional standard of graft biomaterial and BCP used at experimental sites, because this is the only biomaterial approved for use in conjunction with ErhBMP-2 in Korea according to domestic law. Therefore, the present results would be more conservative than those obtained in previous pre-clinical studies using the same biomaterials, and so, further studies should be performed using various observational periods and scaffolding biomaterials.

Within the limitations of this study, it can be concluded that sinus augmentation with ErhBMP-2 carrying BCP carrier did not enhance bone regeneration compared to the conventional treatment using deproteinized bovine bone in terms of clinical, radiographic, and histologic parameters at 24 weeks after the surgery.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. CONSORT 2010 checklist of information to include when reporting a randomised trial.