# Low-Dose Recombinant Human Bone Morphogenetic Protein-2 to Enhance the Osteogenic Potential of the Schneiderian Membrane in the Early Healing Phase: In Vitro and In Vivo Studies

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**Purpose:** The objectives of this study were to confirm the osteogenic potential of the Schneiderian membrane and to elucidate the early healing pattern of low-dose recombinant human bone morphogenetic protein-2 (rhBMP-2)–coated biphasic calcium phosphate (BCP).

**Materials and Methods:** The osteogenic potential of the Schneiderian membrane and enhancement by rhBMP-2 were evaluated by in vitro analysis. RhBMP-2–coated BCP (experimental group) and BCP soaked with saline (control group) were applied to the maxillary sinus in rabbits. After 2 weeks, micro-computed tomographic and histometric analyses were performed.

**Results:** Enhanced osteogenic potential was found when cells from the Schneiderian membrane were treated with rhBMP-2. Micro-computed tomographic analysis showed that the total augmented volume was significantly larger in the experimental group. Different healing patterns were observed in 3 regions, although the area of new bone did not differ significantly. Although more newly formed bone appeared, particularly along the Schneiderian membrane in the experimental group, the difference was not statistically significant.

**Conclusions:** RhBMP-2 enhanced the osteogenic potential of the Schneiderian membrane in vitro. However, low-dose rhBMP-2–coated BCP failed to exert a statistically significant effect in vivo, although it appeared to be effective in sinus augmentation specifically for the volumetric increase in the early phase.

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The maxillary sinus floor augmentation procedure is usually performed successfully, in association with high survival rates of implants placed in severely atrophied posterior maxillae, irrespective of the graft materials used.[1] Kaneko et al[2] investigated sinus lifting using a titanium bone-fixation device without any grafting materials and found that new bone was generally observed around the implant on radiographs, and postoperative bone height was significantly higher than the residual alveolar crest height. It can be assumed that the formation of new bone was possible within the space made by the surrounding structures, such as the sinus walls and the membrane. However, the effect of the periosteum of the Schneiderian membrane on new bone formation remains a matter of controversy. Sharawy[3] suggested that the periosteal portion of this membrane differs from the periosteum that covers the cortical plates of the maxillary or mandibular residual ridges and jaws. The minimal presence of osteoblasts may account for the enlargement of the antrum after tooth loss, and Lundgren et al[4] suggested that the periosteum might be a source of bone-forming cells in the lifted sinus membrane.[5] Gruber et al[6] showed that cells derived from the porcine sinus-associated mucosa express STRO-1, which is a marker of osteoprogenitors, and respond to bone morphogenetic protein (BMP)-6 and BMP-7.

Despite the proven clinical usefulness of these bone substitutes, there is room for improvement. Because the bone substitutes act merely as osteoconductive scaffolds, an extended healing period is required for the osseointegration of dental implants at the grafted site.[7] In addition, the amount and distribution of regenerated vital bone vary widely, even after a long healing period, and bone substitutes in the vicinity of the Schneiderian membrane away from the basal bone generally tend to become embedded in connective tissue, without any actual bone regeneration occurring.[8] Therefore, although the grafted area exhibits marked radiopacity compared with other areas on a radiograph, this does not always mean that true bone regeneration has occurred.

To accelerate bone formation and induce homogeneous vital bone in the grafted sinus, recombinant human BMP-2 (rhBMP-2) carried in an absorbable collagen sponge (ACS) has been approved by the US Food and Drug Administration (FDA) for clinical use in sinus augmentation and ridge augmentation.[9] Lee et al[10] suggested that an ACS soaked in rhBMP-2 induced bone whose quality was significantly better than that of autogenous bone grafts obtained from the iliac crest. However, the authors' previous study that applied rhBMP-2–soaked ACSs to the rabbit maxillary sinus model found that, despite the enhanced osteogenic potential, it was unable to maintain the augmented volume in the sinus.[11] Volume stability under continuous pressure from respiration is an essential requirement for rhBMP-2 carrier systems when used for maxillary sinus augmentation.[12]

Biphasic calcium phosphate (BCP) is composed of hydroxyapatite (HA) and tricalcium phosphate (TCP) at a specific ratio and is not only chemically and structurally similar to human bone tissue, but also biocompatible.[13] Because BCP has osteoconductive and space-providing properties,[14-16] it may be a good candidate as a carrier system for rhBMP-2. However, the loading onto BCP of a diluted solution of rhBMP-2 can be associated with inaccurate dosing and uncontrolled flow. Uludag et al[17] suggested that up to 75% of the rhBMP-2 could be lost from the site within 1 hour from rhBMP-2–soaked BCP. A combination of ACS and BCP tended to retain a larger fraction of rhBMP-2 in the immediate postimplantation period than when using BCP alone, but the retention of rhBMP-2 decreased thereafter (between days 7 and 36).[17] Overall, Uludag et al[17] found that the retention kinetics of rhBMP-2 at the implant site were similar in the BCP group and the ACS-and-BCP combination group. To improve the retention of rhBMP-2, an alternative coating method, lyophilization, was used for loading rhBMP-2 onto BCP. Kim et al[18] used calcium phosphate particles and calcium phosphate blocks coated with rhBMP-2, using the same technique for the rabbit calvaria onlay model, and concluded that these carriers could be used successfully to enhance the formation of new bone, and found they were easy to handle for application regardless of their form.

Contrary to the findings of Kim et al,[18] Choi et al[19] observed adverse effects in the early healing phase, such as swelling and retarded bone formation, when using high doses of rhBMP-2 in the rabbit maxillary sinus. It also has been reported that high doses of rhBMP-2 might induce adverse clinical effects, such as cystlike bone formation, persistent soft tissue swelling, and pain.[20]

The minimum dose of BMP necessary to induce consistent bone formation is higher in nonhuman primates than in rodents.[21] The FDA approved the 1.5-mg/mL concentration used for initial human clinical trials based on these nonhuman primate data, and it remains the approved concentration for human use.[22,23] Higher concentrations have been approved when BMP is used in combination with ACS because it is thought to diffuse early from this carrier, but lower concentrations of rhBMP-2 are applied with other carriers with more optimal release kinetics. In addition, for the present study, it was assumed that a 0.15-mg dose of rhBMP-2 might be too high and had been the cause of detrimental outcomes in the previous studies using a rabbit sinus model.[19] The objective of this study was to evaluate the osteogenic potential of BCP coated with a lower dose of rhBMP-2 at 0.015 mg in continuation from the previous experiment[19]

in which the dosage was 10-fold and 0.0025 mg for the minimal dose in the early stage of healing (2 weeks); however, the same experimental protocol was used.

## Materials and Methods

### ANIMALS

Eight male New Zealand white rabbits weighing 2.5 to 3.0 kg were included in the study. The animals were maintained in separate cages under standard laboratory conditions with ad libitum access to water and a standard laboratory pellet diet. Animal selection and care, the surgical protocol, and the preparation procedures were certified by the institutional animal care and use committee of Yonsei Medical Center (Seoul, Korea; approval number 09-114).

### CELL CULTURE

Rabbit sinus membrane tissues were collected during the sinus floor augmentation procedure. Fibroblastic cells were obtained from those tissues using an enzymatic method. In brief, the rabbit sinus membrane tissues were treated with collagenase type I (3 mg/mL; Invitrogen, Carlsbad, CA) and dispase (4 mg/mL; Invitrogen) for 1 hour at 37°C and were filtered through a 70-$\mu$m cell strainer (BD Falcon, Lincoln Park, NJ). The sinus membrane cells were cultured in Dulbecco's Modified Eagle's Medium and F12 medium supplemented with 10% fetal bovine serum, penicillin 100 U/mL, and streptomycin 100 $\mu$g/mL (all from Invitrogen) at 37°C in 5% $CO_2$. Cells cultured at passages 3 to 5 were used for all experiments.

### IN VITRO OSTEOGENIC DIFFERENTIATION

The cells were seeded in 12-well culture plates (BD Falcon) at a density of $1 \times 10^4$ cells/well. When they had reached approximately 100% confluency, the cells were treated with osteogenic induction medium (growth medium as described earlier supplemented with dexamethasone 0.1 mol/L, $\beta$-glycerophosphate 2 mmol/L, and ascorbic acid 2-phosphate 50 $\mu$mol/L; all from Sigma, St. Louis, MO), and then BMP was added at various concentrations (0.5, 1, and 10 $\mu$g/mL) for 4 weeks. The control was produced by culturing cells only in osteogenic induction medium.

### ALKALINE PHOSPHATASE STAINING

After in vitro osteogenic differentiation for 10 days, the cells were fixed for 30 minutes with 10% neutral buffered formalin (Sigma) at 4°C, washed, and stained with an alkaline phosphatase (ALP) staining solution (Tris-HCl 100 mmol/L, pH 8.4 [WELGENE, Daegu, Korea], 0.01% naphthol AS-MX phosphate [Sigma], and 0.06% fast red violet LB salt [Sigma]), which was modified from that of Kamon et al,[24] for 30 minutes at room temperature. The cells were washed with distilled water twice and then examined for color changes.

### ALP ACTIVITY

After in vitro osteogenic differentiation for 10 days, the level of ALP activity was measured using the SensoLyte *p*-nitrophenylphosphate (pNPP) Alkaline Phosphatase Assay Kit (AnaSpec, Fremont, CA). The cells were rinsed twice with phosphate buffered saline (pH 7.4; Invitrogen) and then lysed with Triton X-100 provided in the kit according to the manufacturer's instructions. The supernatant of the cell lysate was used to detect ALP activity. Then, pNPP was added to the cell extract, and ALP activity was measured according to the colorimetric change caused by the dephosphorylation of pNPP. Absorbance at 405 nm was measured using a spectrophotometer (Benchmark Plus Microplate, Bio-Rad Laboratories, Hercules, CA). The level of ALP activity was normalized against the total amount of protein in the supernatant of the same cell lysate using a protein assay kit (Thermo Scientific Pierce BCA, Thermo Fisher Scientific, Rockford, IL).

### VON KOSSA STAINING

After in vitro osteogenic differentiation for 4 weeks, the cells were fixed with 10% neutral buffered formalin (Sigma) for 30 minutes at 4°C and incubated with 1% silver nitrate solution (Junsei Chemical, Tokyo, Japan) under ultraviolet light for 3 hours. The cells were rinsed in distilled water and then incubated with 5% sodium thiosulfate (Wako, Osaka, Japan) for 5 minutes. The cells were rinsed again in distilled water and counterstained with 0.1% nuclear fast red solution containing 5% aluminum sulfate (Sigma) for 5 minutes.

### PREPARATION OF RHBMP-2–LOADED BCP AND STUDY DESIGN

BCP (Bio-C, Cowellmedi, Busan, Korea) was used as the graft material for the control group. This material is composed of HA and $\beta$-TCP at a specific ratio of 3:7. For the experimental group, the BCP lyophilized with rhBMP-2 (CowellBMP, Cowellmedi) was used as described by Kim et al.[18] A solution of rhBMP-2 produced by *Escherichia coli* was mixed with 1 g of the BCP granules and lyophilized. The solution was frozen by placing the sample on precooled shelves at −43°C. The formulations were maintained at this temperature for 3 hours, dried in a condenser at −40°C (primary drying), and then kept in a pressure chamber at 5 mmHg for 2 hours. Secondary drying was performed on a shelf in the following sequence: −20°C for 4 hours, −10°C for 4 hours, 0°C for 2 hours, and 20°C for 20 hours. The chamber pressure was kept constant throughout the procedure.

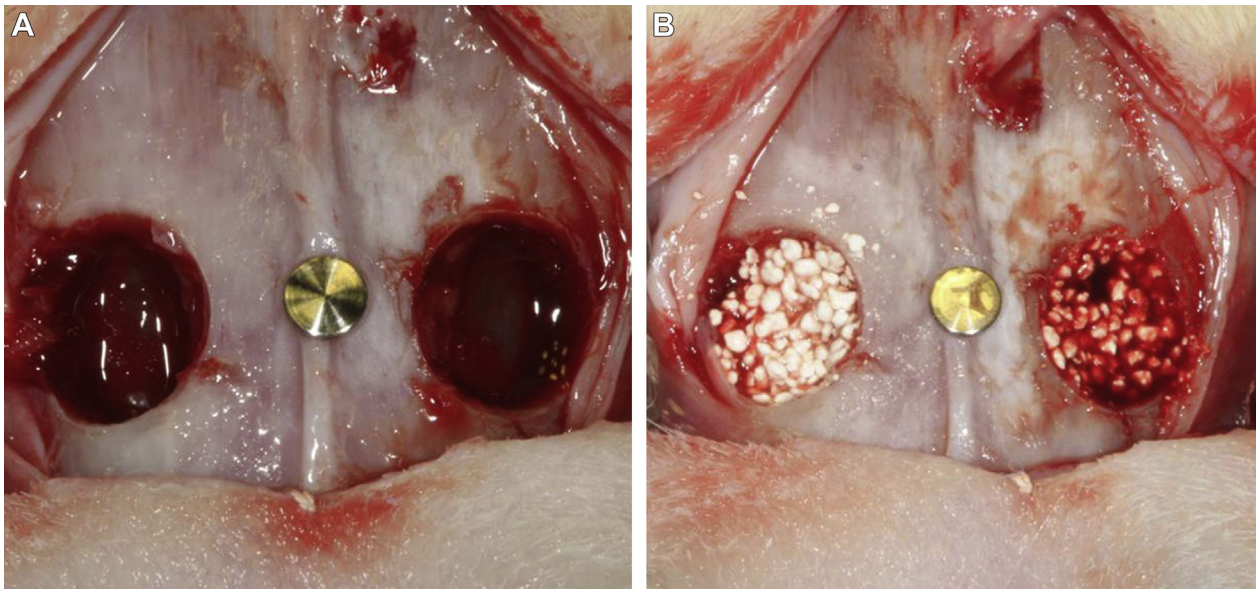


**FIGURE 1.** Clinical photograph of the window preparation. *A,* Windows were prepared bilaterally using a 5.5-mm trephine bur. *B,* Graft materials were inserted into each sinus.

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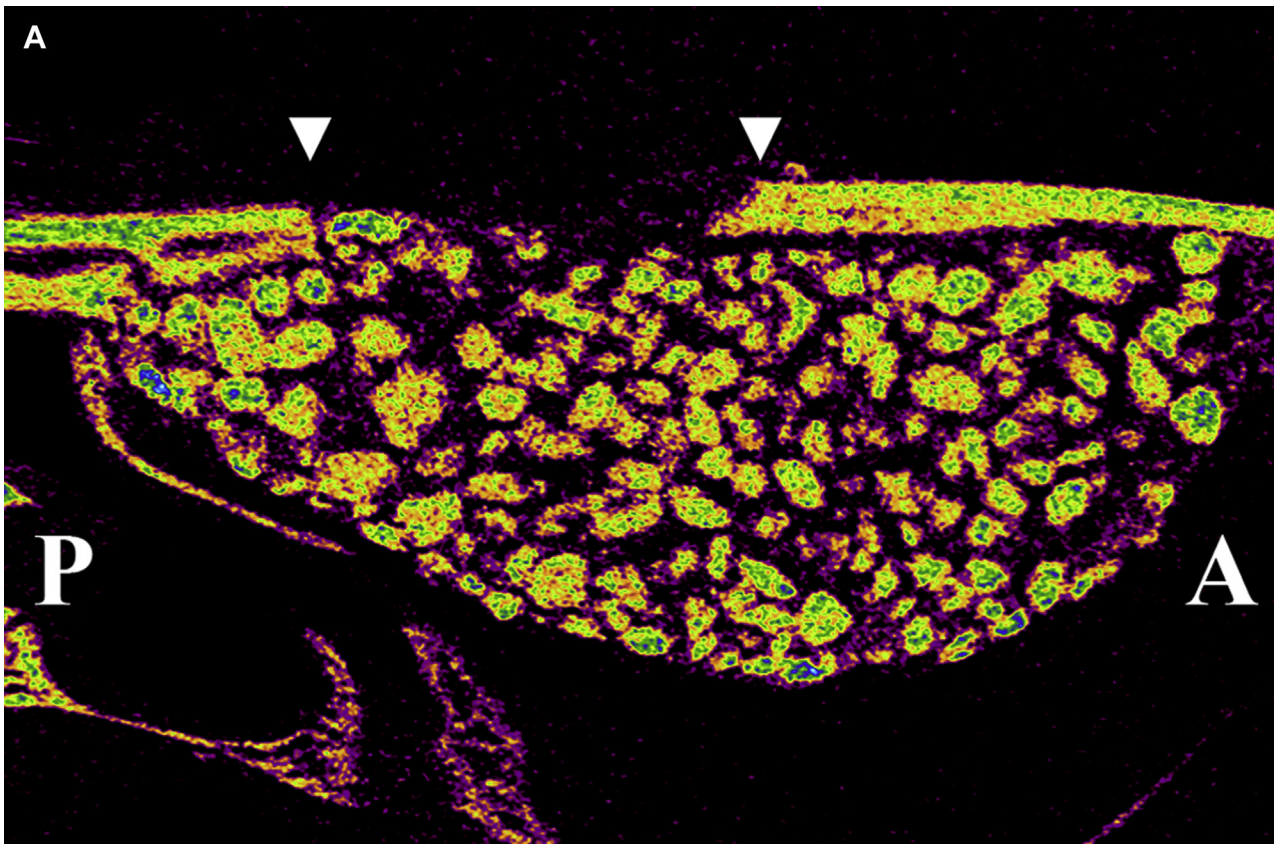


**FIGURE 2.** Radiographic findings. *A,* False-color representation of radiographic findings in a cross-sectional view. *Arrowheads* indicate sites of surgically created windows. Note that the grafted materials (*yellow and blue*) are well maintained within the maxillary sinus and have not escaped through the trephined window. **(Fig 2 continued on next page.)**

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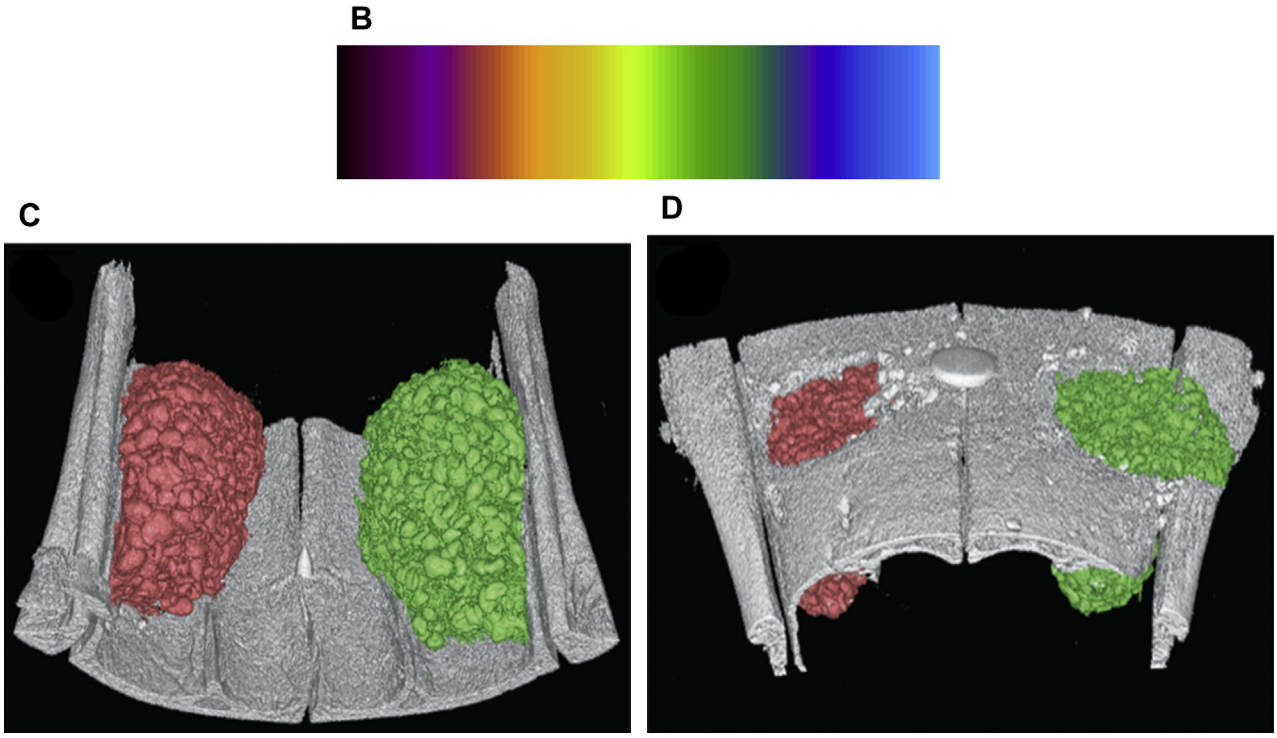


**FIGURE 2 (cont'd).** *B,* False-color radiographic images derived from threshold grayscale values. The color scale shows radiopacity increasing from left to right. *C,* Three-dimensional reconstructed image of the augmented site representing volumetric augmentation. The control site is on the left (*red*) and the experimental site is on the right (*green*). The total augmented volume was significantly larger in the experimental group than in the control group at 2 weeks after surgery. *D,* Note that grafted materials are relatively well maintained and not scattered in the control group, but have a slightly convex appearance at the window opening area in the experimental group, which was the result of swelling. A, anterior; P, posterior.

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For the experimental group, BCP 150 mg lyophilized with rhBMP-2 (0.15 mg/mL) was grafted into the maxillary sinus, and the equivalent BCP soaked with saline was grafted into the opposite maxillary sinus for the control group. For randomization, grafting in the experimental group was assigned to the right or left side alternately.

## EXPERIMENTAL PROCEDURES

The animals were anesthetized by an intramuscular injection of a mixture of ketamine hydrochloride (Ketalar, Yuhan, Seoul, Korea) and xylazine (Rompun, Bayer Korea, Seoul, Korea). The surgical sites were shaved, swabbed with povidone iodine and ethanol, and then anesthetized locally with 2% lidocaine HCl (Huons, Seoul, Korea). An incision was made from the frontal bone to the occipital bone along the sagittal midline, and a full-thickness flap, including the skin, subcutaneous tissues, and periosteum, was elevated laterally. Standardized circular windows were prepared bilaterally at the sites determined previously using normal skulls. To avoid damaging the soft tissues under the cranium, a 5.5-mm-diameter trephine bur (Neobiotech, Seoul, Korea) was used first in a counterclockwise direction. Then, clockwise drilling was applied until the grayish membrane appeared through the bone. The trephined bony disk was carefully removed from the nasal bone and the sinus membrane, which was elevated in pouch form to receive the grafting materials (Fig 1). A pin (Dentium, Seoul, Korea) was placed at the midline between the 2 windows as a reference point. After the graft material was applied, the periosteum was sutured over the window on each side. The flap consisting of subcutaneous tissues and skin was sutured layer by layer with 4-0 glyconate absorbable monofilament (Monosyn, B Braun, Aesculap, Center Valley, PA) and removed after 7 days. The animals were allowed to heal for 2 weeks postoperatively, after which euthanasia was performed by an overdose with anesthesia. Block sections, including the augmented sinus and the surrounding tissues, were cut and fixed in 10% formalin for 10 days.

## RADIOGRAPHIC ANALYSIS: MICRO-COMPUTED TOMOGRAPHY

The fixed block specimens were scanned in a micro-computed tomographic system (SkyScan 1072, SkyScan, Aartselaar, Belgium) at a resolution of 35 mm (achieved using 100 kV and 100 mA). The scanned dataset was processed in Digital

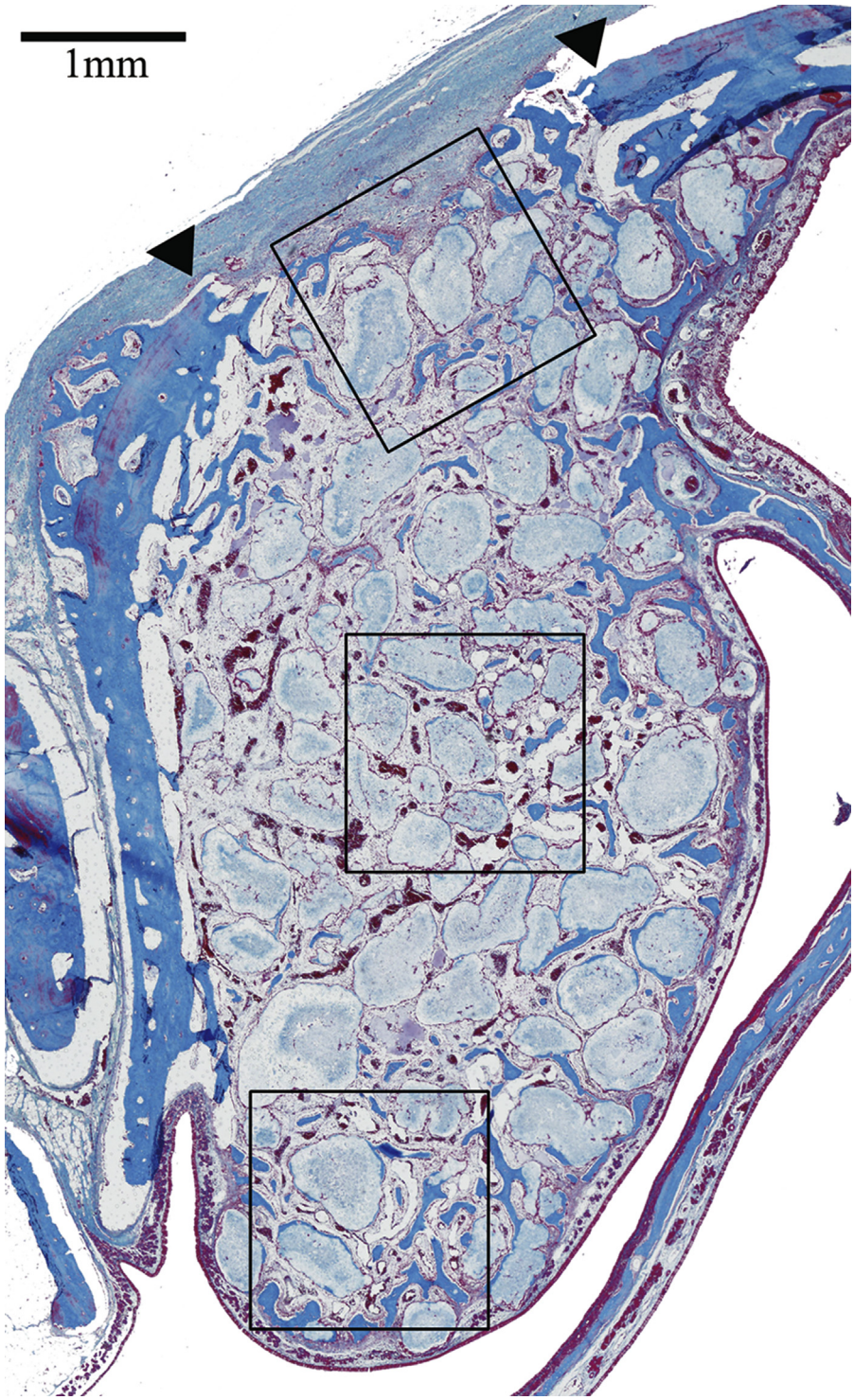


**FIGURE 3.** Histologic findings. *Arrowheads* indicate the site of the surgically created window. Each *square* represents an area of interest (window, center, and membrane; Masson trichrome staining).

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Imaging and Communications in Medicine format and the area of interest was reconstructed with On-Demand 3-dimensional (3D) software (Cybermed, Seoul, Korea). The overall dimensional topography of the maxillary sinus and supporting bone was visualized in a 3D reconstructed image. The augmented volume in the 2 groups was calculated on the 3D images. To perform more detailed analysis of augmented volume, all sectioned images were processed with NRecon 1.6.9 reconstruction software

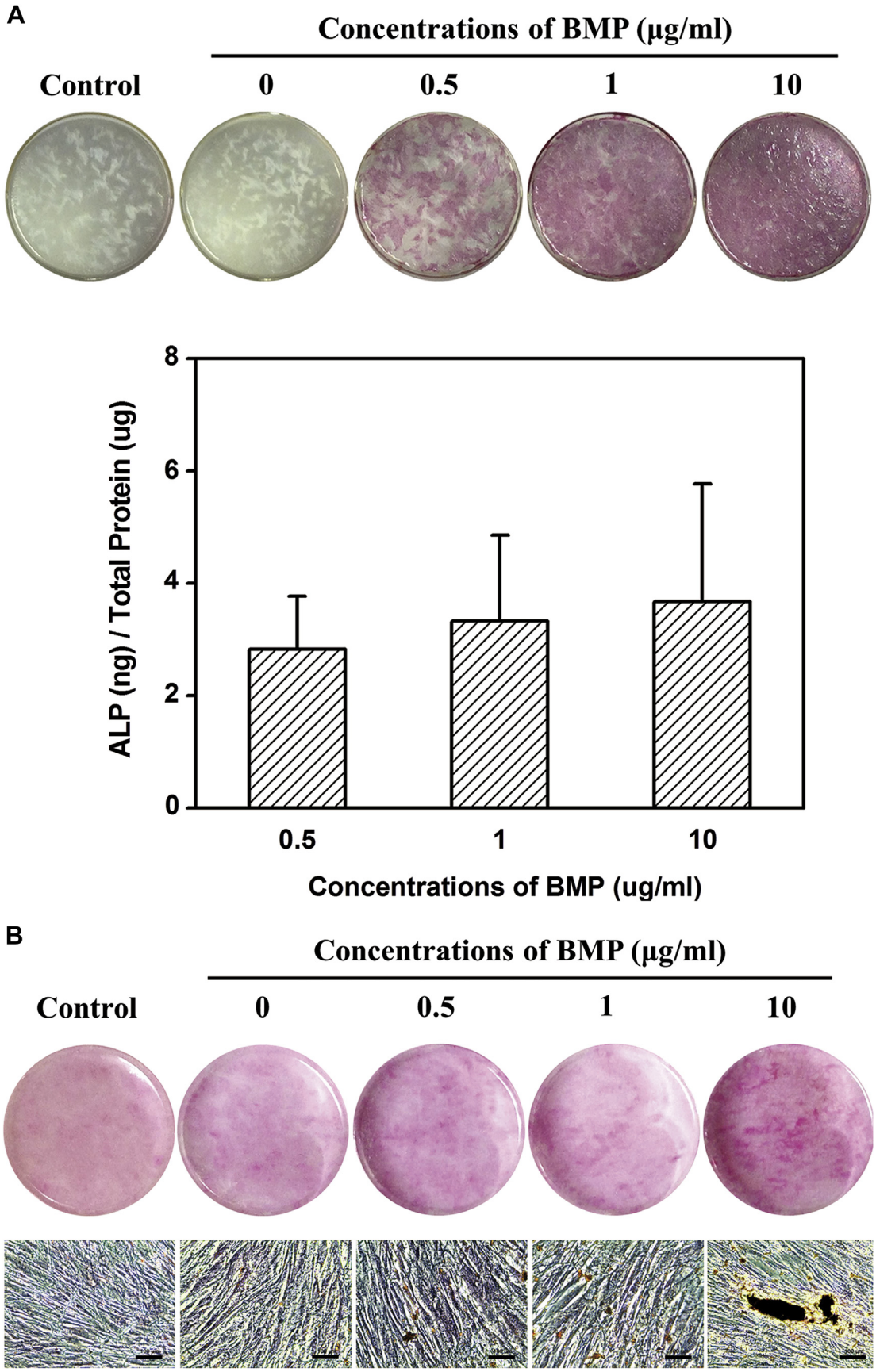


**FIGURE 4.** In vitro osteogenic differentiation of fibroblast cells from the rabbit sinus membrane. *A,* ALP staining and ALP activity in osteogenically differentiated cells. *B,* Von Kossa staining in osteogenically differentiated cells. *Black* and *brown and black areas* indicate calcium salts. Data were obtained from 3 independent experiments, with all samples run in duplicate. Data are presented as mean and standard deviation. ALP, alkaline phosphatase; BMP, bone morphogenetic protein.

**Table 1. VOLUME OF EACH COMPONENT IN THE AUGMENTED SINUS AS DETERMINED BY RADIOGRAPHIC ANALYSIS (N = 8)**

| | Experimental | Control |
|---|---|---|
| NB ($mm^3$) | 37.67 ± 2.86 | 32.32 ± 4.08 |
| RG ($mm^3$) | 71.60 ± 13.76 | 80.06 ± 13.12 |
| FV ($mm^3$) | 179.68 ± 15.21 | 117.45 ± 11.29 |
| TAV ($mm^3$) | 288.95 ± 17.09* | 229.83 ± 17.22* |

* Significant difference between experimental and control groups.

*Note:* Values are presented as mean ± standard deviation.

Abbreviations: FV, fibrovascular tissue; NB, newly formed bone; RG, residual graft; TAV, total augmented volume.

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(SkyScan). Radiopaque areas were distinguished from the total augmented area (TAA) according to specific 8-bit threshold grayscale values. The grayscale values were set from 110 to 225 for BCP and from 90 to 110 for newly formed bone (NB), and then a false-color scale was applied to aid visualization of the augmented area with an analysis program (CTAn 1.13, SkyScan). Areas with grayscale values lower than 90 were considered fibrovascular tissue (FV; Fig 2).

### HISTOLOGIC AND HISTOMETRIC ANALYSES

Sections were decalcified in 5% formic acid for 14 days and then embedded in paraffin. Serial 5-$\mu$m-thick sections were cut coronally along the center of the augmented sinus. The 2 central-most sections were chosen and stained with hematoxylin and eosin and Masson trichrome. The histologic slides were observed and digitally captured with the aid of a light microscope (BX50, Olympus, Tokyo, Japan). The TAA and the areas of NB, residual graft (RG) particles, and FV were measured (in square millimeters) by tracing the surface on the microscopic field along the outlines of the RG and NB, which were distinguishable histologically from the other tissues. FV was calculated by subtracting the areas of NB and RG from TAA. Then, each slide was divided into 3 areas of interest (window, center, and membrane; 1.3 × 1.3 mm each), and each component was analyzed separately in each of the 3 areas (Fig 3).

### STATISTICAL ANALYSIS

Statistical analysis was performed using a statistical software program (SPSS 12.0, SPSS, Inc, Chicago, IL). The Kruskal-Wallis test was used to compare differences among the window, center, and membrane groups, with the post hoc Bonferroni test used to analyze these differences ($P < .05$). The Mann-Whitney $U$ test was used to compare differences between the experimental and control groups. Data are presented as mean ± standard deviation.

## Results

### IN VITRO OSTEOGENIC DIFFERENTIATION

Fibroblast cells from the rabbit sinus membrane can differentiate into cells to produce mineralized nodules. ALP staining showed a BMP-concentration–dependent increase in ALP production after in vitro osteogenic differentiation for 10 days. This finding concurs with the results of the quantitative analysis of ALP in osteodifferentiated cells at 10 days, although the difference between the quantitative findings did not reach statistical significance (Fig 4). After 3 weeks of in vitro osteogenic differentiation, von Kossa staining showed an increase in the number of mineralized nodules in the BMP treatment groups. Although this increase in mineralized nodules appeared to occur in parallel with increases in BMP concentration, the increase was not statistically significant (Fig 4).

### CLINICAL OBSERVATIONS

All animals were healthy throughout the entire experimental period, with no major complications occurring. Healing after the sinus graft procedure was uneventful, although small tears (<1 mm) occurred in 1 sinus on the control side. None of the animals had influential complications, such as infection or maxillary sinusitis.

### RADIOGRAPHIC ANALYSIS

In the 3D reconstructed view, the augmented sinuses were anteroposteriorly oblong and the grafted materials were well localized, although some grafted particles overflowed through the window in the experimental group (Fig 2). The augmented volume was significantly larger in the experimental group (288.95 ± 17.09 $mm^3$) than in the control group (229.83 ± 17.22 $mm^3$; $P < .05$). More radiopaque RG was distinguishable from the surrounding tissues in the experimental group, including the original bone, the NB, and the soft tissues. The RG in the sinus cavity was denser in the control group than in the experimental group. Detailed analysis of the grayscale values of the radiographic images showed that RG occupied a larger volume in the control group, and that the experimental group appeared to have more NB and FV. However, none of these components differed significantly between the 2 groups (Table 1).

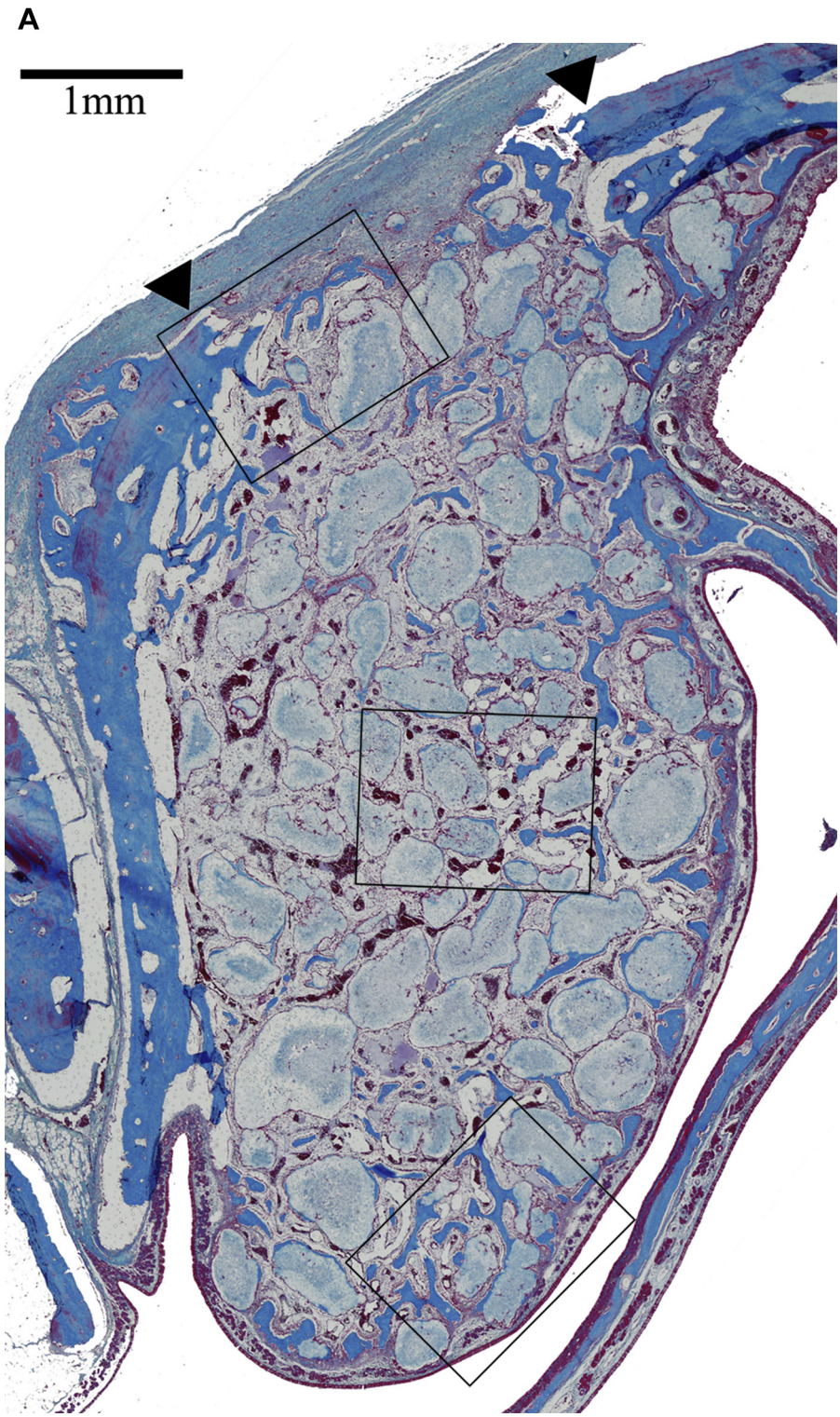


**FIGURE 5.** Histologic findings of experimental group. *A,* Total augmented area. Arrowheads indicates margin of surgically-induced window opening. **(Fig 5 continued on next page.)**

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## HISTOLOGIC AND HISTOMETRIC ANALYSES

Bone formation was initiated in the 2 groups within the 2-week experimental period. The augmented space was convex, and more newly formed trabeculae were found close to the parent bony wall and lifted membrane. In the experimental group, bony ingrowths were found between the BCP particles in the center area, and

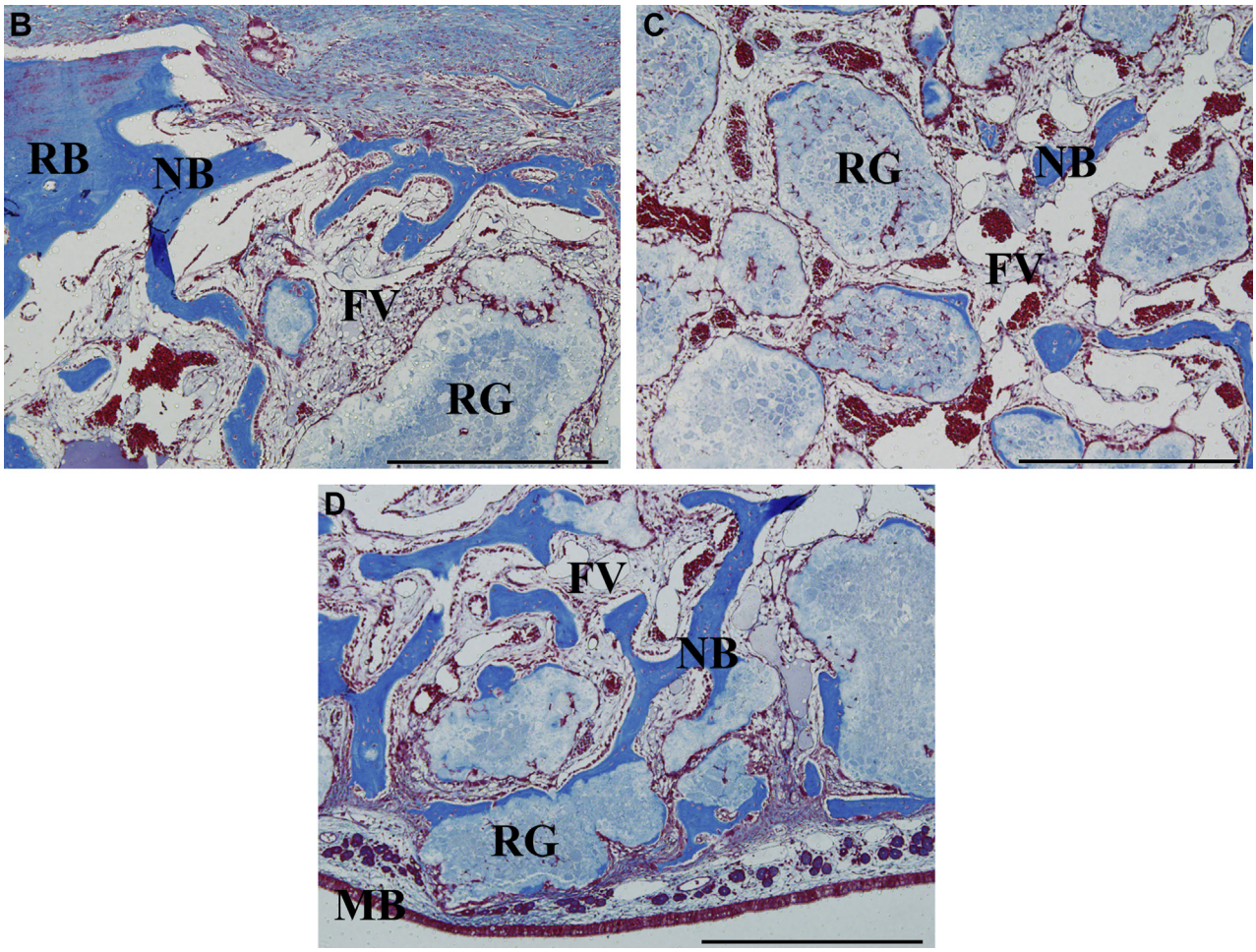


**FIGURE 5 (cont'd).** *B*, Window area. *C*, Center area. Note that the RG is surrounded by NB. *D*, Membrane area. Note the new bone formation along the membrane and between the RG particles. Pseudostratified ciliated columnar epithelium and glandular tissues indicate continuity of the Schneiderian membrane without tearing (Masson trichrome stain; scale bar, 500 $\mu$m). FV, fibrovascular tissue; NB, newly formed bone; RB, remaining bone; RG, residual graft.

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newly formed woven bone was deposited in close contact with the surfaces of the RG. Osteoclast-like multinucleated cells were occasionally seen along the remaining particles in some specimens. NB was observed along the membrane in the experimental group, and this was well integrated into FV and RG (Fig 5). Greater new bone formation was evident at the periphery of the defect margin of the windows in the control group (Fig 6). The center area exhibited minimal bone formation and irregular marginal morphology of the RG, representing biodegradation with osteoclasts. Many blood vessels were observed throughout the augmented area, which was composed mainly of connective tissue cells, blood vessels, and adipocytes. Furthermore, the membrane areas were composed mainly of FV and the structural characteristics of the respiratory mucosa indicated this to be a pseudostratified ciliated columnar epithelium (Fig 7).

Histometric analysis showed that although the area of NB appeared to be slightly smaller in the experimental group ($0.21 \pm 0.09$ mm$^2$) than in the control group ($0.24 \pm 0.12$ mm$^2$), the difference was not statistically significant (Fig 7). Comparison of the proportions of NB, FV, and RG in the experimental and control groups showed that although the proportion of FV appeared to be larger in the experimental group (42.6%) than in the control group (37.3%), this difference was not statistically significant. In the center area there was significantly proportionally more FV and less RG in the experimental group than in the control group. In the 2 groups the amount of NB detected in the center area was minimal and significantly less than in other areas, with connective tissue filling the space (Fig 7).

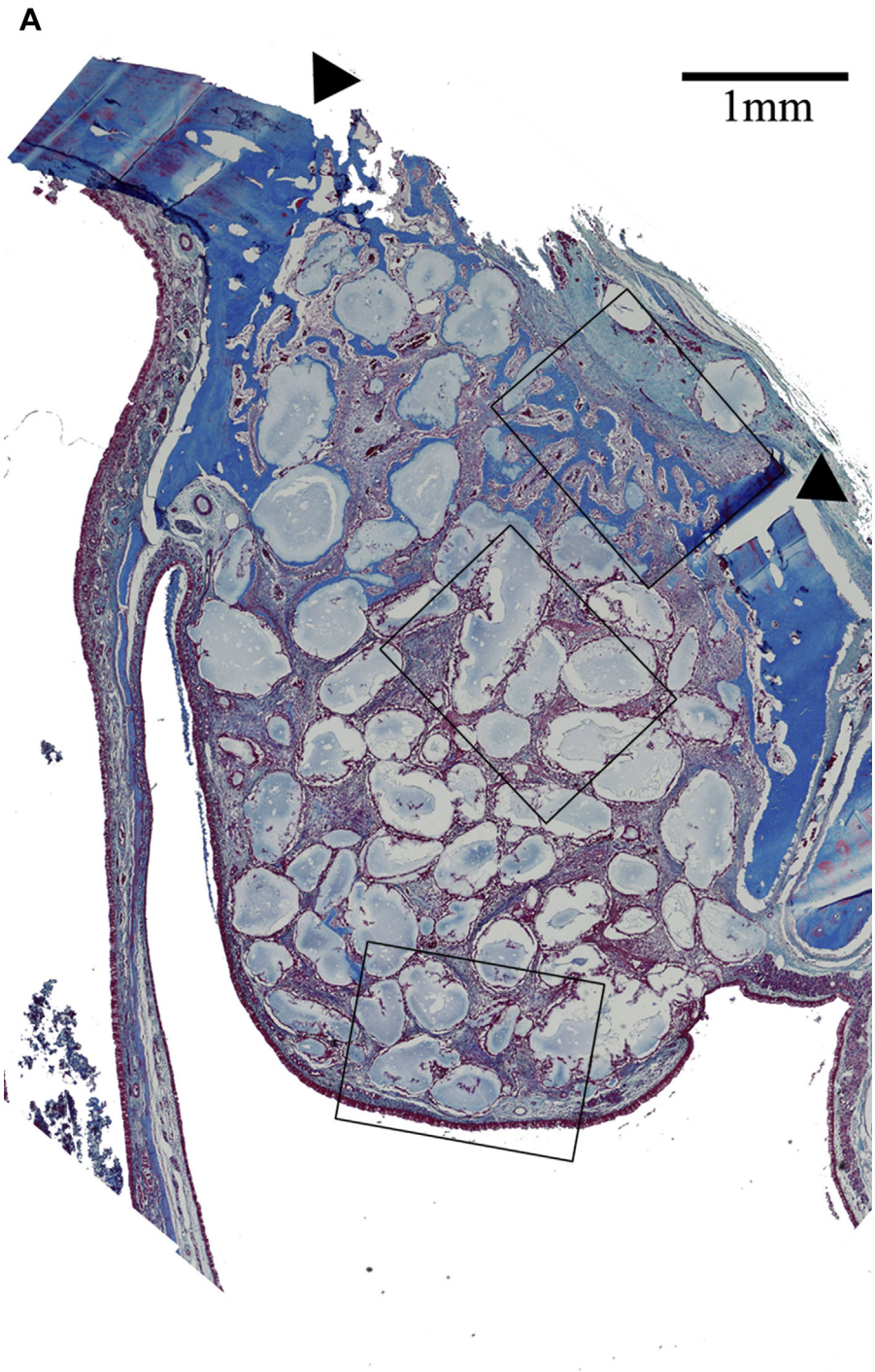


**FIGURE 6.** Histologic findings of control group. *A*, Total augmented area. Arrowheads indicates margin of surgically-induced window opening. **(Fig 6 continued on next page.)**

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## Discussion

Bone formation after maxillary sinus augmentation takes place by promoting osteoconduction from the surrounding adjacent bone,[25,26] which is a major source of revascularization and osteoblast recruitment. [26]

Srouji et al[27] tested the osteogenic potential of the Schneiderian membrane and found that ectopic bone formed in conjunction with an osteoconductive scaffold. Furthermore, when cultured with osteogenic supplements, cells in the Schneiderian membrane exhibited ALP activity and mRNA expression of

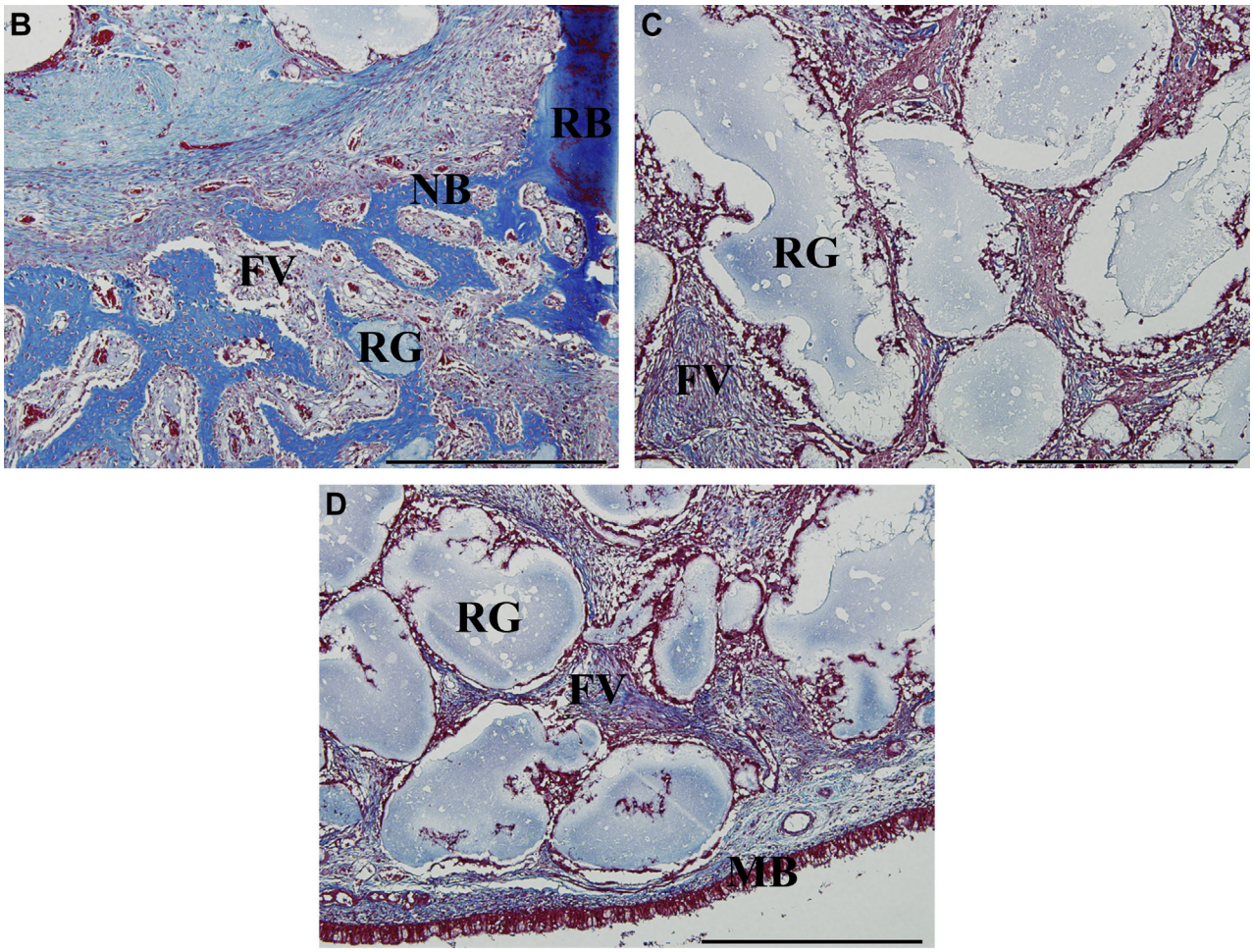


**FIGURE 6 (cont'd).** *B,* Window area. Note that NB contains viable cells and extends from the RB. *C,* Center area. Note that there is almost no bone formation, and that this area is rich in FV. *D,* Membrane area. Note the minimal new bone formation (Masson trichrome stain; scale bar, 500 $\mu$m). FV, fibrovascular tissue; MB, membrane; NB, newly formed bone; RB, remaining bone; RG, residual graft.

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osteogenic markers, verifying its osteogenic potential.[27,28] These findings are in agreement with those of the present in vitro study. Although the differences were not statistically significant, enhanced ALP production and an increase in the number of mineralized nodules were noted in sections stained with von Kossa stain. There also was a tendency for the in vitro osteogenic differentiation to increase with the rhBMP-2 concentration.

The use of rabbits as a model for maxillary sinus elevation has been well documented. It has been suggested that rabbits have the same ventilation system as humans, with air exchanges through the nasal cavity and a well-defined ostium opening to their nasal cavities.[29] Air pressure causes movement of the maxillary sinus membrane, which could affect the graft material and bone-healing process,[11,30] and therefore the graft material is an important determinant of success or failure.[31] It has been suggested that autogenous bone graft materials cannot withstand sinus pressures for long periods, and start to lose their density and height during the first several weeks of healing.[32] Johansson[33] suggested that the absorption rate of autogenous bone in sinus augmentation is 47% at 6 months after surgery. Xu et al[5] suggested that the positive air pressure within the sinus may play a role in osteoclast activation and cause absorption of NB in the rabbit sinus model. These processes resulted in a thin continuous layer of cortical bone forming under the elevated sinus membrane at 10 weeks after surgery.[31] The 2 groups in the present study exhibited a convex augmented space, which suggests that BCP can withstand the sinus air pressure and maintain the augmented space. In particular, newly formed trabeculae in the experimental group were found mainly close to the elevated membrane, thereby forming a partial shell adjacent to the membrane. It is possible that this shell can withstand the sinus pressure and retain its density

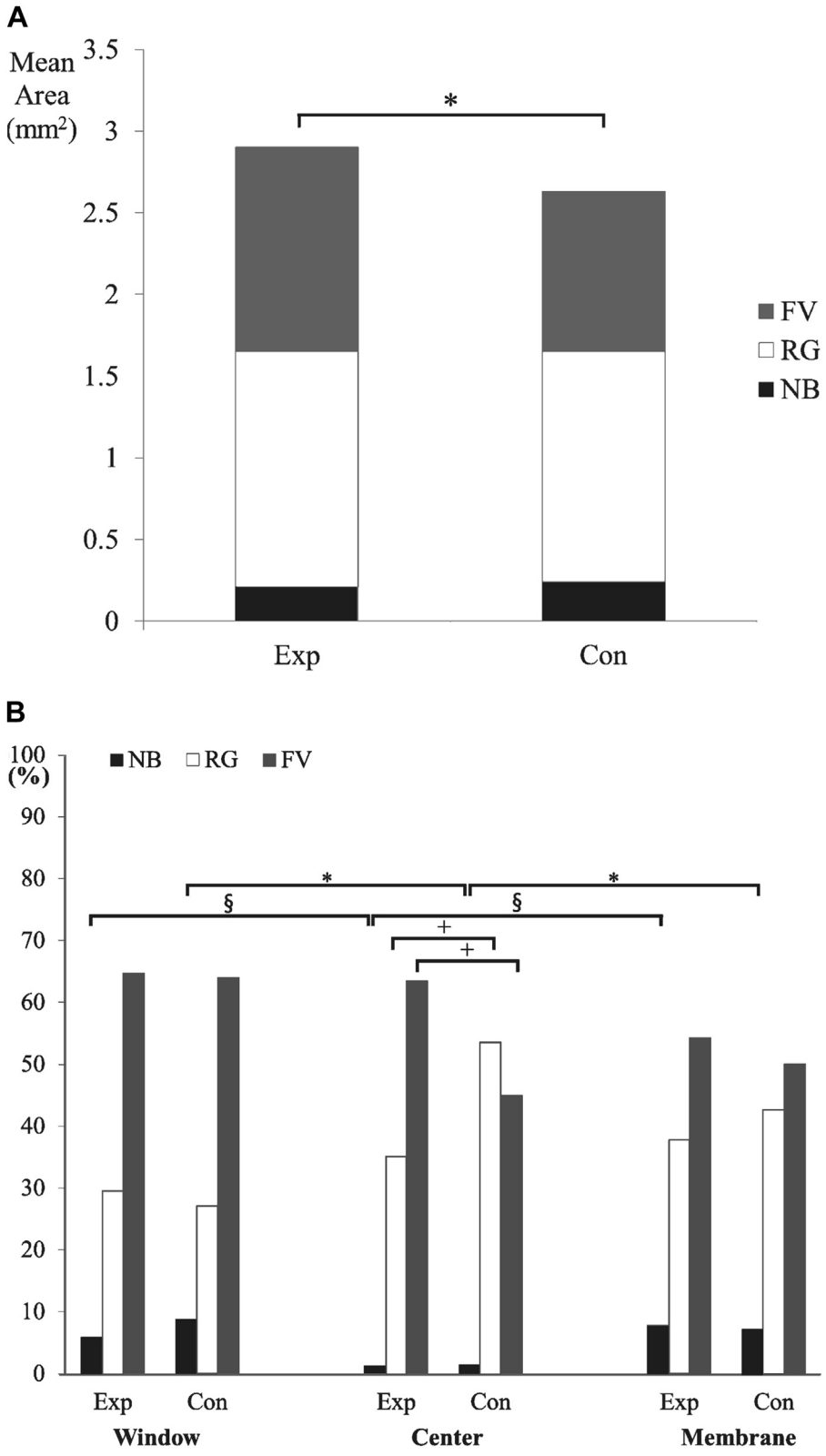


**FIGURE 7.** Histometric analyses. *A*, Mean area of each component in the augmented sinus. The compositions did not differ significantly between groups, although the total augmented area was significantly larger in the experimental group. *B*, Composition of the augmented area in each area of interest. +Statistically significant difference between the 2 groups; §*statistically significant difference compared with the other 2 areas. Con, control group; Exp, experimental group; FV, fibrovascular tissue; NB, newly formed bone; RG, residual graft.

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and height during the first several weeks of healing. In addition, there was 7.86% more NB in the membrane area in the experimental group compared with the control group, which might have been due to rhBMP-2–induced enhancement in the osteogenic potential of the sinus membrane.

The authors' previous studies showed the predictability of rhBMP-2 in an animal model, but adverse effects also were observed when the dose of rhBMP-2 was excessive.[18,19] To avoid such problems, in the present study a lower dose of rhBMP-2 was tested and BCP was used as a verified carrier. It is known that the physiologic concentration of BMP in the human bone is 1 $\mu$g/g,[34] which is much lower than the clinically applied concentration. In the present study, the mean dose of rhBMP-2 per animal was decreased to 0.015 mg given the expectations of the BCP carrier for its controlled releasing characteristics. However, 0.015 mg of rhBMP-2 also induced only volumetric augmentation in the grafted area at the early stage of healing, without significantly increased new bone formation. The histometric analysis showed no significant difference in the compositions of NB and RG between the experimental and control groups, although the FV area and TAA were slightly larger in the experimental group. From these results it can be assumed that the rhBMP-2 caused swelling, resulting in overflow of the grafted particles (as seen on the radiographs) through the window in the experimental group (Fig 2). In addition, the control group exhibited 50.1% more new bone formation in the window area than in the membrane area. This suggests that the bone formation started from the remaining bone, from the sinus walls and the septa, in the control group. This is in agreement with previous experimental studies performed in rabbit models.[5,11]

In conclusion, the present study found no apparent osteogenic potential or remarkable new bone formation in the early healing period when using a low dose of rhBMP-2, although in vitro analysis showed the enhanced osteogenic potential of the Schneiderian membrane with rhBMP-2. A 2-week healing period might be too short for properly evaluating bone formation in the rabbit sinus model, and a study design with the healing time increased to longer than 4 weeks is needed. Supplementary studies also are essential to clarify the role of rhBMP-2 in inducing new bone formation at the sinus membrane and the releasing characteristics of rhBMP-2 with appropriate carriers in the early healing stage. In addition, a precise characterization of the sinus-derived osteoprogenitors and an explanation of their role in new bone formation and maturation after sinus augmentation are required.

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