Vertical bone augmentation using collagenated or non-collagenated bone substitute materials with or without recombinant human bone morphogenetic protein-2 in a rabbit calvarial model

Lim, Hyun-Chang ; Paeng, Kyeong-Won ; Jung, Ui-Won ; Benic, Goran I

DOI: https://doi.org/10.5051/jpis.2204240212

J Periodontal Implant Sci. 2023 Dec;53(6):429-443
https://doi.org/10.5051/jpis.2204240212
pISSN 2093-2278·eISSN 2093-2286

JPIS
Journal of Periodontal & Implant Science

Research Article
Implant Science

Check for updates

OPEN ACCESS

Received: Oct 25, 2022
Revised: Jan 1, 2023
Accepted: Jan 17, 2023
Published online: Mar 7, 2023

***Correspondence:**
Ui-Won Jung
Department of Periodontology, Research Institute for Periodontal Regeneration, Yonsei University College of Dentistry, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea.
Email: drjew@yuhs.ac
Tel: +82-2-2228-3185
Fax: +82-2-392-0398

†Hyun-Chang Lim and Goran I. Benic contributed equally to the study.

ORCID iDs
Hyun-Chang Lim
https://orcid.org/0000-0001-7695-1708
Kyeong-Won Paeng
https://orcid.org/0000-0001-7262-7345
Ui-Won Jung
https://orcid.org/0000-0001-6371-4172
Goran I. Benic
https://orcid.org/0000-0001-7551-7934

Funding
This work was supported by Korea Medical Device Development Fund grant funded by the Korea government (the Ministry of Science and ICT, the Ministry of Trade, Industry and Energy, the Ministry of Health & Welfare, the Ministry of Food and Drug Safety) (Project Number: KMDF_PR_20200901_0240).

Vertical bone augmentation using collagenated or non-collagenated bone substitute materials with or without recombinant human bone morphogenetic protein-2 in a rabbit calvarial model

Hyun-Chang Lim [1,†], Kyeong-Won Paeng [2], Ui-Won Jung [2,*], Goran I. Benic [3,4,†]

[1]Department of Periodontology, Kyung Hee University, College of Dentistry, Kyung Hee University Medical Center, Seoul, Korea
[2]Department of Periodontology, Research Institute for Periodontal Regeneration, Yonsei University College of Dentistry, Seoul, Korea
[3]Clinic of Reconstructive Dentistry, Center of Dental Medicine, University of Zurich, Zurich, Switzerland
[4]Institute for Research and Education in Dental Medicine, Lugano, Switzerland

ABSTRACT

Purpose: The aim of this study was to determine 1) the bone-regenerative effect of porcine bone block materials with or without collagen matrix incorporation, 2) the effect of a collagen barrier, and 3) the effect of adding recombinant human bone morphogenetic protein-2 (rhBMP-2) to the experimental groups.
Methods: Four treatment modalities were applied to rabbit calvaria: 1) deproteinized bovine bone mineral blocks (DBBM), 2) porcine bone blocks with collagen matrix incorporation (PBC), 3) porcine bone blocks alone without collagen matrix incorporation (PB), and 4) PBC blocks covered by a collagen membrane (PBC+M). The experiments were repeated with the addition of rhBMP-2. The animals were sacrificed after either 2 or 12 weeks of healing. Micro-computed tomography (micro-CT), histologic, and histomorphometric analyses were performed.
Results: Micro-CT indicated adequate volume stability in all block materials. Histologically, the addition of rhBMP-2 increased the amount of newly formed bone (NB) in all the blocks. At 2 weeks, minimal differences were noted among the NB of groups with or without rhBMP-2. At 12 weeks, the PBC+M group with rhBMP-2 presented the greatest NB ($P<0.05$ vs. the DBBM group with rhBMP-2), and the PBC and PB groups had greater NB than the DBBM group ($P>0.05$ without rhBMP-2, $P<0.05$ with rhBMP-2).
Conclusions: The addition of rhBMP-2 enhanced NB formation in vertical augmentation using bone blocks, and a collagen barrier may augment the effect of rhBMP-2.

Keywords: Animal model; Bone morphogenetic protein 2; Bone substitutes; Histology

Author Contributions

Conceptualization: Ui-Won Jung, Goran I. Benic. Formal analysis: Hyun-Chang Lim. Data curation: Kyeong-Won Paeng. Investigation: Kyeong-Won Paeng, Ui-Won Jung, Goran I. Benic. Methodology: Ui-Won Jung. Project administration: Kyeong-Won Paeng. Writing - original draft: Hyun-Chang Lim. Writing - review & editing: Ui-Won Jung, Goran I. Benic.

Conflict of Interest

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

INTRODUCTION

The increasing number of dental implant treatments is resulting in the utilization of various bone augmentation modalities to support them. The severity and location of bone deficiency at implant sites affect the timing of bone augmentation. A severe bone deficit may lead to an unfavorable implant position, such as the implant surface being significantly exposed outside of the adjacent bone contour and in proximity to vital anatomic structures. Such situations require bone augmentation prior to implant placement—that is, primary bone augmentation [1,2].

Primary bone augmentation is generally a demanding treatment modality. One of its challenges lies in the material properties. Conventional particulated bone substitute materials are prone to displacement and scattering during surgery and the healing period, which leads to a suboptimal ridge shape [3]. Block-type bone substitute materials are beneficial in maintaining the augmented shape compared with particulated bone materials [4,5], but inadequate bone formation inside the block and brittleness can be problematic [6-8].

A novel bone substitute block material, collagenated bone substitute block, was recently introduced in dentistry. Its processing methods are proprietary, and the characteristics of the resulting blocks appear to vary according to the method used. The presence of collagen in the block material is known to provide a semisolid characteristic that allows adaptability to the defect [9,10] and passages for newly formed bone (NB) ingrowth into the augmentation [11]. These traits may compensate for the drawbacks of particulated and block bone materials.

Despite the development of these materials, extensive ridge defects may still require healing enhancers for predictable NB formation, a favorable ridge shape, and reduced healing time. Tissue engineering technology has introduced various growth factors for such purposes [12], with recombinant human bone morphogenetic protein-2 (rhBMP-2) being primarily investigated [13]. The effect of rhBMP-2 on primary bone augmentation was tested in our previous preclinical study [14], which found improved NB formation in bone block materials and compensation of the different bone-forming capabilities of the materials. Preclinical studies have been performed on collagenated bone substitute materials as carriers for rhBMP-2 [11,15,16], but they have not been compared with other types of bone substitute materials and other augmentation modalities that involve a barrier membrane, especially for primary bone augmentation.

The present study developed collagenated porcine bone blocks (PBs) by applying collagen slurry to bone blocks and freeze-drying them. This block material maintains solidity in a similar way to blocks without collagen, while the presence of collagen may facilitate blood clot stability and enhance growth factor absorption.

The aims of the present study were to determine 1) the bone-regenerative effect of PB materials with or without collagen matrix incorporation, 2) the effect of a collagen barrier, and 3) the effect of adding rhBMP-2 to the experimental groups.

MATERIALS AND METHODS

This study used 32 male New Zealand white rabbits that weighed 2.5–3.0 kg. Each animal was housed in an individual cage with *ad libitum* water access and a standard laboratory pellet diet. The entire study protocol, including animal provision, care, and surgical procedures,

https://doi.org/10.5051/jpis.2204240212

was approved and monitored by the Institutional Research Committee of Yonsei University College of Medicine (approval No.: 2014-0363). This study was a serial study investigating the effect of block bone substitute materials and rhBMP-2, following the study by Lim and colleagues [14]. The ARRIVE guidelines were followed in the present study.

Study design

Two experiments were designed in the present study based on the use of rhBMP-2. In the first experiment (experiment 1; n=16), the following 4 treatment modalities were applied to the rabbit calvaria: 1) deproteinized bovine bone mineral block (DBBM group, the control), 2) PB with collagen matrix incorporation (PBC group), 3) PB without collagen matrix incorporation (PB group), and 4) PBC block covered by a crosslinked collagen membrane (OssGuide, Hyundai Bioland, Cheongju-si, Korea) (PBC+M group). Screw holes (1.4 mm in diameter) were made on all blocks, which were then fixed using fixation screws, except for the PBC+M group. A crosslinked collagen membrane covered the block in the PBC+M group. In the second experiment (experiment 2; n=16), experiment 1 was repeated by using the same bone block material loaded with *Escherichia coli*-derived rhBMP-2 (Cowellmedi, Busan, Korea). All blocks had dimensions of 6 mm × 6 mm × 4 mm (width × length × height). For both experiments, healing periods of 2 weeks (n=8) and 12 weeks (n=8) were applied.

Spongious porcine bone block with or without collagen matrix

Bone block preparation

To produce the PBs, 100 mm × 100 mm × 20 mm bone blocks were harvested from the legs of pigs, which were then decellularized, sterilized, and sintered. The blocks were then cut into blocks of size 6 mm × 6 mm × 4 mm for the PB group. For the PBC group, the blocks were cut into blocks of size 20 mm × 20 mm × 10 mm, precipitated in a 2% atelocollagen solution (extracted from pig skin), treated with negative pressure for collagen incorporation into the blocks, and freeze-dried. Finally, the PBCs were cut to the same size as the PB blocks. The blocks then received gamma radiation at 25 kGy.

Scanning electron microscopy

After coating using an ion sputter coater (Q150V Plus; Quorum Technologies, Lewes, UK) on the bone blocks, field-emission scanning electron microscopy was performed at 3 kV (Ultra Plus, ZEISS, Oberkochen, Germany). Porous structures and uniform atelocollagen distribution were observed in the structures of the blocks (**Figure 1**).

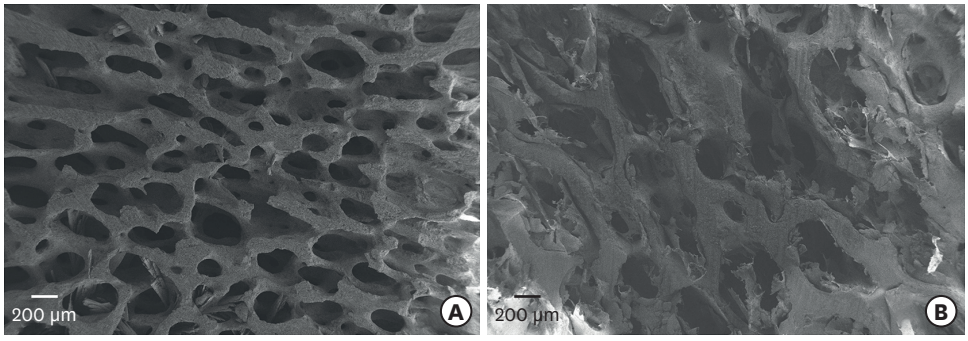

Figure 1. Scanning electron microscopy of non-collagenated/collagenated porcine bone blocks (PB and PBC, respectively). (A) A porous bone structure similar to that of human bone was observed in the PB blocks. (B) Atelocollagen was uniformly distributed in the porous structure of the PBC bone blocks.
PB: porcine bone block without collagen matrix incorporation, PBC: porcine bone block with collagen matrix incorporation.

https://doi.org/10.5051/jpis.2204240212

Surgical procedure

Surgery was performed under general anesthesia induced through an intramuscular injection of a mixture of xylazine hydrochloride (Rompun; Bayer, Seoul, Korea) and Zoletil 50 (Virbac; Virbac Laboratories, Carros, France). Calvarial areas were shaved and disinfected using an iodine solution. After local anesthesia with 2% lidocaine HCl containing 1:100,000 epinephrine (Huons, Seoul, Korea), a longitudinal skin incision was made along the center of the calvaria, followed by reflection of a full-thickness flap. Multiple cortical perforations were made around the predetermined sites using a round bur with bone block materials. Bone blocks were then passively stabilized on the calvaria using fixation screws, except for the PBC+M group (**Figure 2**). In the PBC+M group, the bone blocks were positioned on the designated area using the flat calvarial bone surface [17]. Bone blocks were allocated to the sites using a computer-generated random sequence. In experiment 2, a 0.1 mg/mL rhBMP-2 solution was prepared. All bone blocks were soaked in 1 mL of the rhBMP-2 solution for 5 minutes prior to application to the calvarium. The dose and soaking time of rhBMP-2 were determined according to our previous study, which showed an increase in NB formation in 4 block bone materials [14]. The experimental animals were sacrificed via anesthetic overdose after 2 and 12 weeks of healing. The skull specimens including the augmented sites were harvested and placed in a 10% buffered formalin solution.

Micro-computed tomography (micro-CT) analysis

Micro-CT scans were performed on skull specimens (SkyScan 1173; Bruker, Kontich, Belgium) under the following conditions: 130 kV, 60 μA, 13.85-μm pixel size, 500-ms exposure, and averaging of 4 frames. The obtained data were reconstructed using NRecon software (version 1.7.0.4; Bruker). The dimensions of the blocks (from the outline of the block to the outer surface of the skull) were measured as the total volume (TV).

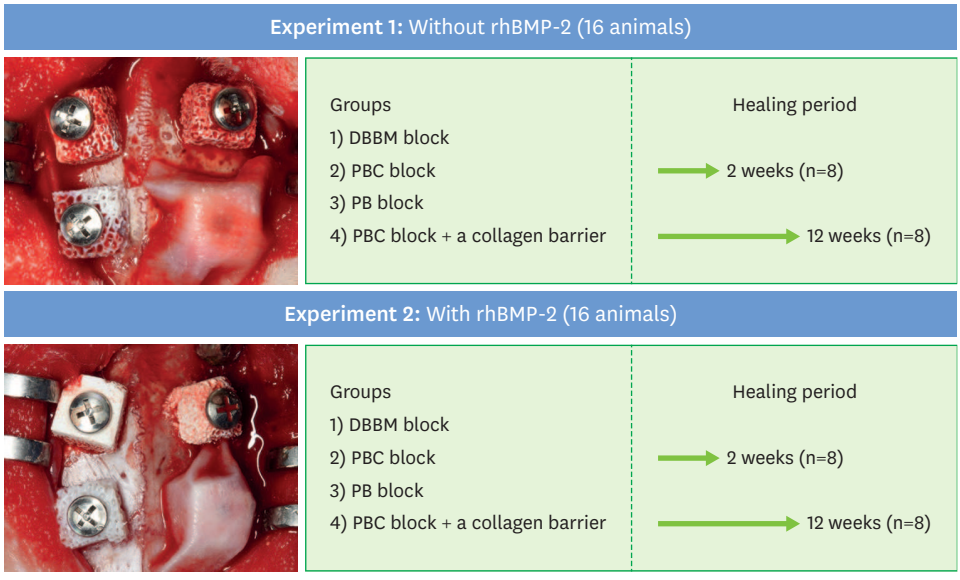

Figure 2. Flow chart and clinical photograph of the surgical procedure applied to the experimental rabbits. DBBM: deproteinized bovine bone mineral block, PBC: porcine bone block with collagen matrix incorporation, PB: porcine bone block without collagen matrix incorporation, PBC+M: PBC block covered by a collagen membrane.

https://doi.org/10.5051/jpis.2204240212

Histologic processing

After micro-CT scans, the specimens were decalcified in a 5% formic acid solution for 14 days and then embedded in paraffin blocks. The paraffin blocks were serially sectioned into 5-μm-thick sections. The 2 centermost sections of the augmentation were selected, which were then stained using hematoxylin-eosin and Masson's trichrome.

Histomorphometric analysis

The histologic slides were digitally scanned and then observed using CaseViewer software (version 2.1; 3DHISTECH, Budapest, Hungary). All histologic slide images were captured and had their format transformed for histomorphometric analysis. The following parameters were measured (Photoshop CS6; Adobe, San Jose, CA, USA): 1) The area of total augmentation (TA, mm^2) bordering NB, bone blocks, and calvarial bone, 2) The area of NB (mm^2) within TA, 3) the area of NB in the inner part of the bone block (NB_in, mm^2), and 4) the area of NB in the outer part of the bone block (NB_out, mm^2) [14]. **Figure 3** shows the division of the inner and outer parts of the blocks. The percentages of NB, NB_in, and NB_out relative to TA were also calculated.

Statistical analysis

No sample size calculation was performed due to the pilot nature of this study. Instead, the required sample size was determined according to our previous study [14]. Data are presented as means, standard deviations, medians, and quartiles. Due to the small sample size, non-parametric tests were used. The Mann-Whitney *U* test was used to evaluate the statistical significance of differences between 2 and 12 weeks of healing, as well as between the groups with and without rhBMP-2 at the same time point. The Friedman test was used for to identify statistically significant differences among the groups without rhBMP-2 at the same time point, followed by the Wilcoxon signed-rank test for pairwise comparison (without the Bonferroni correction). The same analysis was performed for the groups with rhBMP-2. Statistical significance was set at $P<0.05$ (SPSS version 21.0; IBM Corp., Armonk, NY, USA).

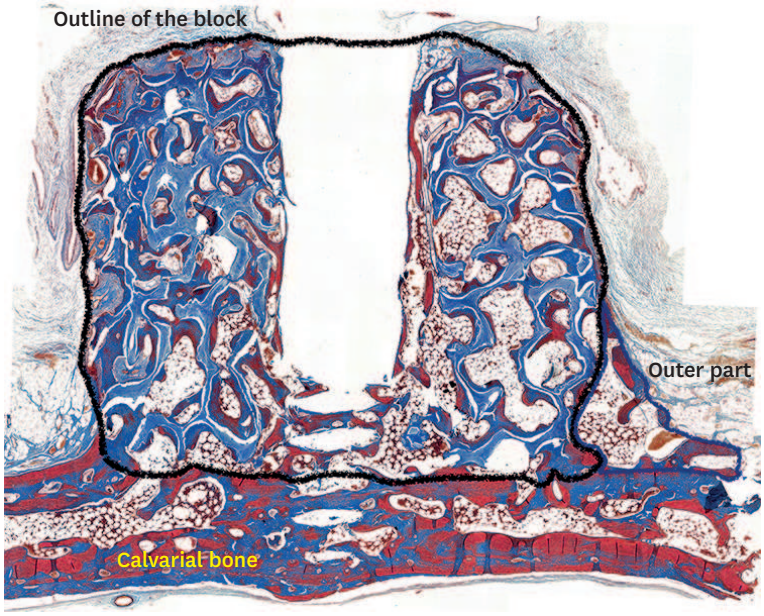

Figure 3. Diagram of the histomorphometric analysis.

https://doi.org/10.5051/jpis.2204240212

JPIS Journal of Periodontal & Implant Science

RESULTS

Clinical observations

No animal presented any signs of infection or adverse events during the study period.

Effects of treatment modalities in experiments 1 and 2

Micro-CT analysis

At 2 weeks, NB had generally formed at the interface of the block and the calvarial bone in all groups. At 12 weeks, the amount of NB appeared to be greater in the blocks than at 2 weeks. The blocks generally maintained a well-defined shape, but there was notable dimensional shrinkage in some specimens (**Figure 4**). One block in the PBC group without rhBMP-2 was lost due to screw exfoliation. Half of the blocks were lost in 1 specimen of the PB group without rhBMP-2. These 2 specimens were excluded from the analysis. TV differed significantly in the PBC group without rhBMP-2 and the PBC+M group with rhBMP-2 between 2 and 12 weeks ($P<0.05$; **Table 1**, **Figure 5**). Among the groups treated with rhBMP-2, statistically significant differences were found at 2 weeks between the DBBM and PBC groups, and between the PB and PBC+M groups ($P<0.05$ without the Bonferroni correction).

Histologic observations

At 2 weeks, NB formation occurred at the interface between the block and the calvarial bone in all groups with or without rhBMP-2. Irrespective of the group and rhBMP-2 application, NB was mostly observed around that interface. NB reached the middle of the blocks in some specimens (2 specimens in each group except the PBC+M group). At 12 weeks, NB formation in all groups increased compared with 2 weeks, but NB was still confined to the middle parts of the blocks in most specimens of the groups without rhBMP-2. The collagen membrane was totally resorbed at 12 weeks in PBC+M group with or without rhBMP-2 (**Figure 4**).

Histomorphometry

Some samples were not included in the histomorphometric analyses due to tissue handling and histologic processing errors. Histomorphometric data are presented in **Tables 2** and **3**, and **Figure 4**. Among the groups without rhBMP-2, NB was the greatest in the PBC group at 2 weeks (1.10±0.94 mm^2), followed by the PB, DBBM, and PBC+M groups ($P>0.05$). NB increased with time in all groups. The largest and smallest increases occurred in the PBC group (from 1.10±0.94 to 2.92±1.66 mm^2 at 12 weeks) and the PBC+M group (from 0.77±0.72 to 1.61±1.17 mm^2), respectively. However, there were no significant intergroup differences among NB, NB_in, and NB_out at 12 weeks ($P>0.05$).

Among the groups with rhBMP-2, the PBC+M group had the greatest NB (2.17±1.20 and 5.91±2.26 mm^2 at 2 and 12 weeks, respectively), NB_in (1.20±1.01 and 4.67±2.84 mm^2), and NB_out (0.97±0.52 and 1.24±1.57 mm^2). At 12 weeks, there was significantly less NB in the DBBM group (3.60±0.87 mm^2) than in the other groups ($p<0.05$ without Bonferroni correction). The differences in NB, NB_in, and NB_out values between the DBBM group and the other groups were greater at 12 weeks than at 2 weeks (**Tables 2** and **3**, **Figure 5**).

Effects of rhBMP-2 (experiment 1 vs. experiment 2)

Micro-CT analysis

At both 2 and 12 weeks, there were generally no marked dimensional differences between the groups with and without rhBMP-2, except that TV differed significantly between the PB

https://doi.org/10.5051/jpis.2204240212

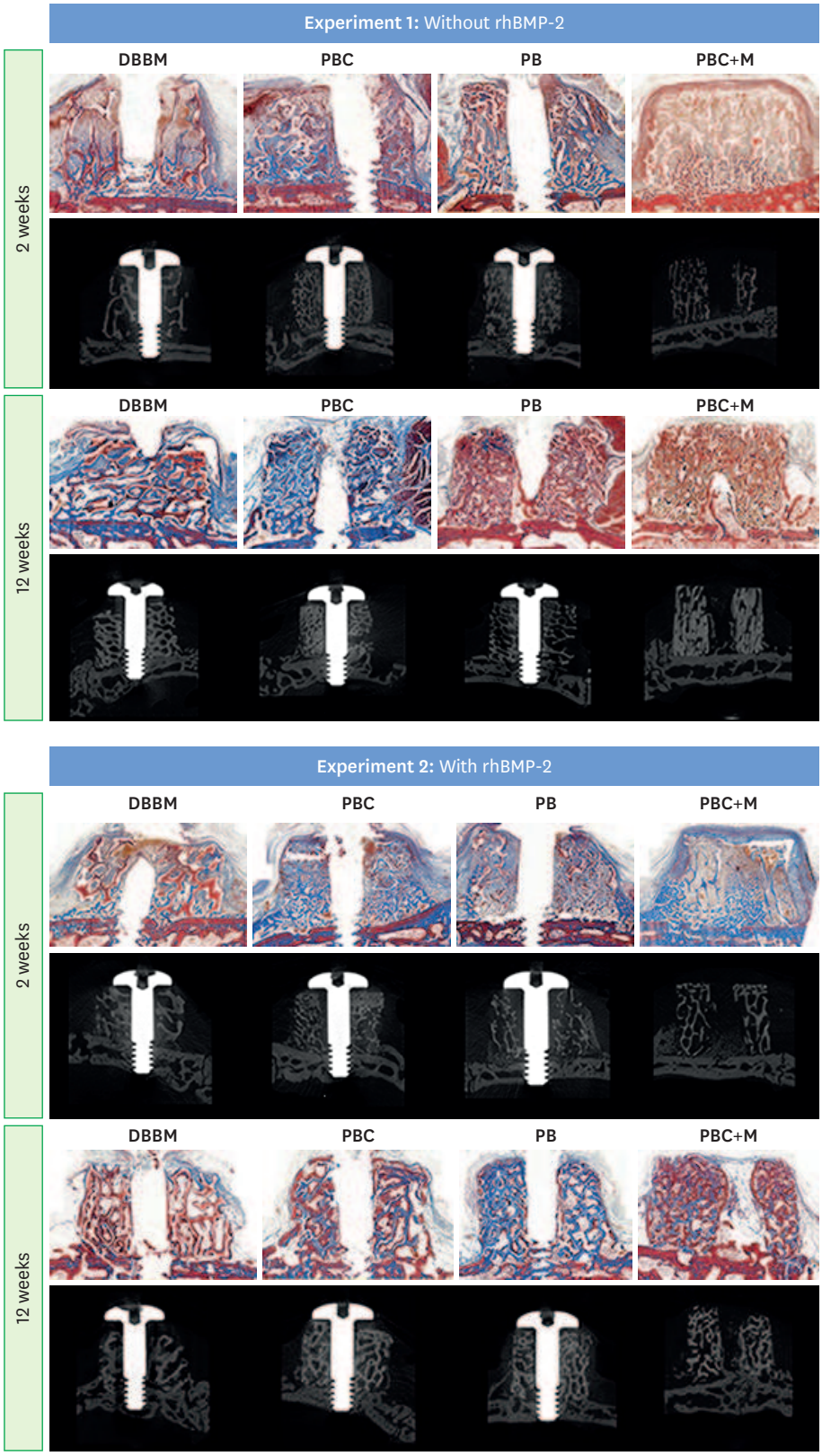

Figure 4. Representative histologic and radiographic views of the bone-block substitute materials with and without rhBMP-2.
rhBMP-2: recombinant human bone morphogenetic protein-2, DBBM: deproteinized bovine bone mineral block, PBC: porcine bone block with collagen matrix incorporation, PB: porcine bone block without collagen matrix incorporation, PBC+M: PBC block covered by a collagen membrane.

https://doi.org/10.5051/jpis.2204240212

JPIS Journal of Periodontal & Implant Science

Table 1. Total augmented volumes in all groups

Variables	DBBM	PBC	PB	PBC+M	Friedman test
Without rhBMP-2					
2 weeks	n=8	n=8	n=8	n=8	
	138.42±15.43, 139.11 (132.10–141.73)	150.61±21.94, 143.91 (133.92–172.81)	124.00±11.09, 122.30 (116.06–131.37)	133.10±29.99, 134.62 (114.62–156.53)	*P*=0.136
Intergroup comparison	N/A	N/A	N/A	N/A	
12 weeks	n=8	n=7	n=7	n=8	
	136.85±13.57, 137.45 (125.04–148.83)	124.21±16.76, 127.80 (117.45–132.71)	134.35±14.03, 128.52 (127.37–138.27)	142.84±19.57, 149.45 (127.40–157.20)	*P*=0.896
Intergroup comparison	N/A	N/A	N/A	N/A	
2 weeks vs. 12 weeks	*P*=1.000	*P*=0.037	*P*=0.189	*P*=0.600	-
With rhBMP-2					
2 weeks	n=8	n=8	n=8	n=8	
	138.05±15.98, 139.92 (127.76–146.67)	156.20±13.99, 153.32 (144.46–162.78)	149.69±13.82, 150.63 (136.95–161.53)	121.72±28.02, 127.02 (100.99–142.61)	*P*=0.024
Intergroup comparison	*P*=0.036 (vs. PBC)	*P*=0.036 (vs. DBBM)	*P*=0.161 (vs. DBBM)	*P*=0.263 (vs. DBBM)	
	P=0.161 (vs. PB)	*P*=0.401 (vs. PB)	*P*=0.401 (vs. PBC)	*P*=0.050 (vs. PBC)	
	P=0.263 (vs. PBC+M)	*P*=0.050 (vs. PBC+M)	*P*=0.012 (vs. PBC+M)	*P*=0.012 (vs. PB)	
12 weeks	n=8	n=8	n=8	n=8	
	148.51±11.82, 143.30 (141.51–153.18)	157.77±13.70, 153.31 (147.80–166.65)	146.75±18.41, 146.72 (135.37–157.21)	154.11±30.61, 153.50 (139.14–163.57)	*P*=0.415
Intergroup comparison	N/A	N/A	N/A	N/A	
2 weeks vs. 12 weeks	*P*=0.248	*P*=0.834	*P*=0.674	*P*=0.046	-

Data are mean±standard deviation, median (interquartile range) values in mm^3.
DBBM: deproteinized bovine bone mineral block, PBC: porcine bone block with collagen matrix incorporation, PB: porcine bone block without collagen matrix incorporation, PBC+M: PBC block covered by a collagen membrane, N/A: not applicable.

groups with and without rhBMP-2 at 2 weeks and between the PBC groups with and without rhBMP-2 at 12 weeks (P<0.05; **Figure 5**, **Table 1**).

Histologic observations

At both 2 and 12 weeks, NB formation appeared to be greater in the groups with rhBMP-2 than in their counterparts without rhBMP-2. This difference was noticeable at 2 weeks in the apical parts of the blocks. At 12 weeks, more blocks with rhBMP-2 exhibited NB formation up to the most-coronal parts of the augmentations compared with their counterparts without rhBMP-2. Some blocks with rhBMP-2 also presented distinct cortical bone lining along the block boundaries (1 out of 8 specimens in the DBBM group, 4 out of 8 in the PBC group, 3 out of 8 in the PB group, and 2 out of 8 in the PBC+M group), whereas this finding was not present in any block without rhBMP-2 (**Figure 4**).

Histomorphometry

At both 2 and 12 weeks, NB, NB_in, and NB_out were greater in the groups with rhBMP-2 than in those without rhBMP-2. At 2 weeks, NB_out differed significantly between the PB groups with and without rhBMP-2, and between the PBC+M groups with and without rhBMP-2 (P<0.05). At 12 weeks, NB differed significantly between the counterpart groups in relation to rhBMP-2 (P<0.05), NB_in between the PBC+M groups with and without rhBMP-2 (P<0.05), and NB_out in all comparisons (P<0.05) except the comparison between the DBBM group with and without rhBMP-2 (P>0.05). Timewise, the increases in NB, NB_in, and NB_out were larger in the PBC+M group with rhBMP-2 than in its counterpart group without rhBMP-2 (**Tables 2** and **3**, **Figure 5**).

https://doi.org/10.5051/jpis.2204240212

JPIS
Journal of Periodontal & Implant Science

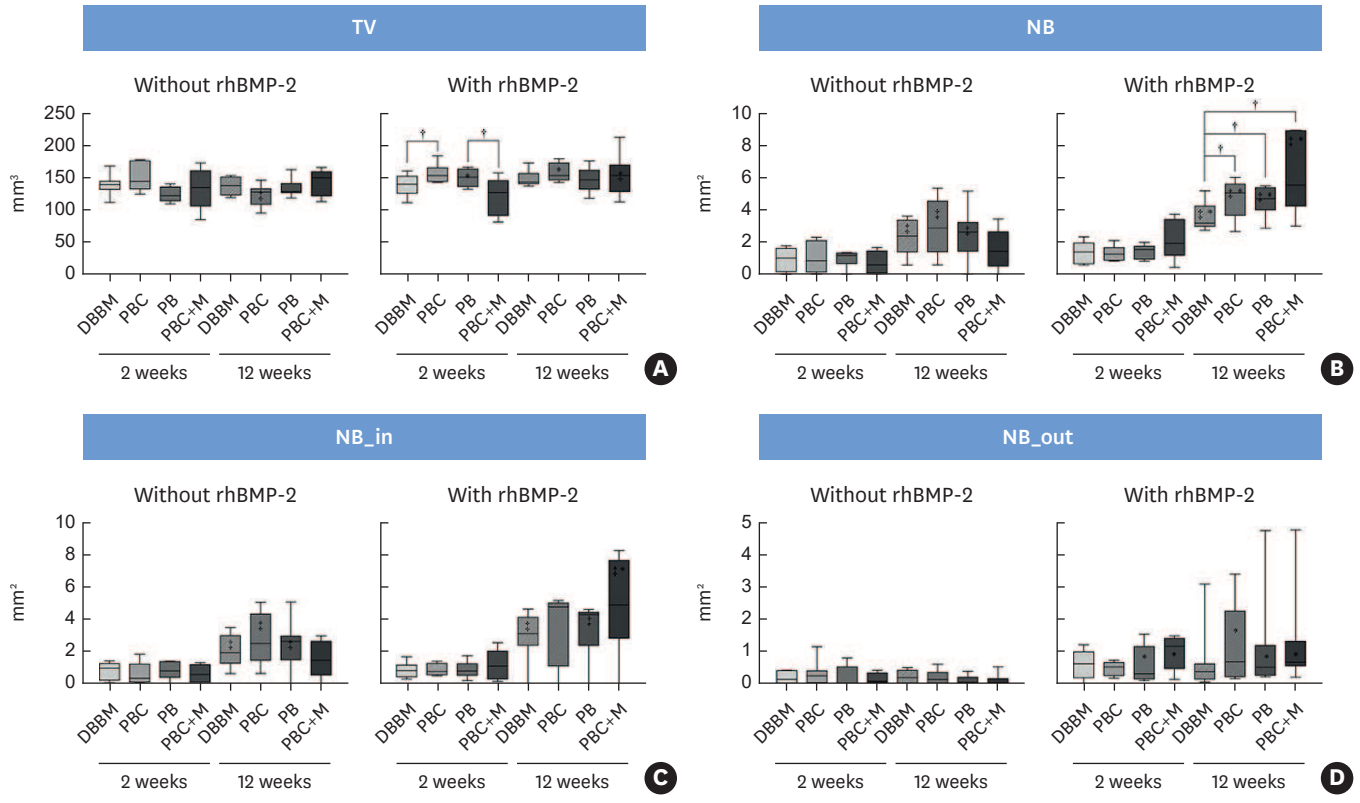

Figure 5. Box-and-whisker plots of the amount of NB in the groups with and without rhBMP-2. (A) TV, as measured using micro-computed tomography; (B) total NB; (C) NB_in; and (D) NB_out. The whiskers cover the entire data range.
NB: newly formed bone, rhBMP-2: recombinant human bone morphogenetic protein-2, TV: total volume, NB_in: area of NB in the inner part of the bone block, NB_out: area of NB in the outer part of the bone block, DBBM: deproteinized bovine bone mineral block, PBC: porcine bone block with collagen matrix incorporation, PB: porcine bone block without collagen matrix incorporation, PBC+M: PBC block covered by a collagen membrane.
*Significant difference between the blocks with and without rhBMP-2 at the same time point; †Significant difference between the blocks with the same treatment at the same time point; ‡Significant difference between the blocks at 2- and 12-week healing time points.

DISCUSSION

The present study investigated the bone-regenerative effect of PB materials with and without collagen matrix incorporation, as well as the effect of a barrier membrane and adding rhBMP-2 to bone blocks. The main findings were that 1) PBC blocks yielded the greatest NB among the blocks without rhBMP-2 at 12 weeks, followed by PB blocks (without significant differences), 2) rhBMP-2 increased NB in all groups, 3) the effect of rhBMP-2 was more prominent at 12 weeks than at 2 weeks, 4) PBC and PB blocks with rhBMP-2 led to greater NB than DBBM blocks with rhBMP-2, and 5) the presence of a collagen membrane further enhanced the effects of rhBMP-2.

Both particle and block types of bone substitute materials are utilized to overcome bone deficits, but advantages and disadvantages have been found for each type. Particle-type materials are prone to displacement and scattering during the healing period, whereas bone block materials exhibit high dimensional stability [3]. However, regarding NB ingrowth to the augmentation, bone blocks sometimes exhibit unfavorable histologic outcomes [8,18]. Using collagenated bone substitute materials can compensate for those limitations [11,19], because the collagen component may serve as a track for bone-forming cell infiltration and NB formation, while simultaneously preventing the collapse of augmented space.

https://doi.org/10.5051/jpis.2204240212

JPIS Journal of Periodontal & Implant Science

Table 2. Areas of NB in the groups without rhBMP-2 (in mm^2)

Variables	DBBM	PBC	PB	PBC+M	Friedman test
2 weeks	n=7	n=7	n=7	n=4	
NB	0.99±0.69, 1.07 (0.44–1.54)	1.10±0.94, 0.91 (0.34–1.86)	1.04±0.50, 1.24 (0.96–1.35)	0.77±0.72, 0.66 (0.35–1.08)	*P*=0.615
Intergroup comparison	N/A	N/A	N/A	N/A	
NB_in	0.81±0.53, 0.98 (0.38–1.20)	0.76±0.68, 0.60 (0.25–1.13)	0.86±0.52, 0.81 (0.57–1.31)	0.63±0.56, 0.60 (0.30–0.93)	*P*=0.308
Intergroup comparison	N/A	N/A	N/A	N/A	
NB_out	0.18±0.17, 0.12 (0.05–0.33)	0.29±0.39, 0.23 (0.04–0.31)	0.18±0.32, 0.00 (0.00–0.26)	0.14±0.18, 0.07 (0.05–0.15)	*P*=0.132
Intergroup comparison	N/A	N/A	N/A	N/A	
12 weeks	n=7	n=8	n=8	n=8	
NB	2.28±1.05, 2.41 (1.73–2.92)	2.92±1.66, 2.90 (1.75–3.82)	2.50±1.52, 2.65 (1.57–3.20)	1.61±1.17, 1.49 (1.01–2.14)	*P*=0.692
Intergroup comparison	N/A	N/A	N/A	N/A	
NB_in	2.08±0.97, 1.93 (1.55–2.72)	2.74±1.53, 2.49 (1.75–3.73)	2.40±1.45, 2.62 (1.57–2.92)	1.50±1.06, 1.47 (0.93–2.02)	*P*=0.934
Intergroup comparison	N/A	N/A	N/A	N/A	
NB_out	0.20±0.18, 0.17 (0.08–0.30)	0.18±0.22, 0.12 (0.00–0.33)	0.10±0.13, 0.04 (0.00–0.15)	0.10±0.17, 0.02 (0.00–0.12)	*P*=0.172
Intergroup comparison	N/A	N/A	N/A	N/A	
2 weeks vs. 12 weeks					
NB	*P*=0.018	*P*=0.028	*P*=0.015	*P*=0.308	
NB_in	*P*=0.013	*P*=0.010	*P*=0.015	*P*=0.173	
NB_out	*P*=0.698	*P*=0.267	*P*=1.000	*P*=0.387	

Data are mean±standard deviation, median (interquartile range) values in mm^2.
NB: newly formed bone, rhBMP-2: recombinant human bone morphogenetic protein-2, NB_in: area of NB in the inner part of the bone block, NB_out: area of NB in the outer part of the bone block, DBBM: deproteinized bovine bone mineral block, PBC: porcine bone block with collagen matrix incorporation, PB: porcine bone block without collagen matrix incorporation, PBC+M: PBC block covered by a collagen membrane, N/A: not applicable.

Table 3. Area of NB by bone block substitutes with rhBMP-2 (in mm^2)

Variables	DBBM	PBC	PB	PBC+M	Friedman test
2 weeks	n=8	n=6	n=8	n=6	
NB	1.44±0.64, 1.43 (0.91–1.95)	1.39±0.49, 1.30 (1.10–1.68)	1.46±0.43, 1.59 (1.06–1.78)	2.19±1.20, 1.97 (1.49–3.11)	*P*=0.165
Intergroup comparison	N/A	N/A	N/A	N/A	
NB_in	0.88±0.45, 0.84 (0.56–1.13)	0.91±0.38, 0.84 (0.60–1.19)	0.89±0.49, 0.82 (0.58–1.09)	1.20±1.01, 1.11 (0.37–1.86)	*P*=0.196
Intergroup comparison	N/A	N/A	N/A	N/A	
NB_out	0.56±0.43, 0.61 (0.18–0.77)	0.48±0.23, 0.51 (0.31–0.68)	0.57±0.55, 0.30 (0.22–0.91)	0.97±0.52, 1.15 (0.70–1.32)	*P*=0.075
Intergroup comparison	N/A	N/A	N/A	N/A	
12 weeks	n=7	n=8	n=7	n=7	
NB	3.60±0.87, 3.21 (3.01–3.99)	4.74±1.15, 5.08 (4.35–5.39)	4.56±0.89, 4.71 (4.29–5.11)	5.91±2.26, 5.55 (4.50–7.43)	*P*=0.029
Intergroup comparison	*P*=0.018 (vs. PBC) *P*=0.028 (vs. PB) *P*=0.043 (vs. PBC+M)	*P*=0.018 (vs. DBBM) *P*=0.735 (vs. PB) *P*=0.499 (vs. PBC+M)	*P*=0.028 (vs. DBBM) *P*=0.735 (vs. PBC) *P*=0.600 (vs. PBC+M)	*P*=0.043 (vs. DBBM) *P*=0.499 (vs. PBC) *P*=0.600 (vs. PB)	
NB_in	2.88±1.49, 3.12 (2.54–3.65)	3.64±2.25, 4.77 (3.36–4.92)	3.38±1.68, 4.30 (3.00–4.39)	4.67±2.84, 4.88 (3.28–6.48)	*P*=0.219
Intergroup comparison	N/A	N/A	N/A	N/A	
NB_out	0.73±1.05, 0.36 (0.23–0.58)	1.10±1.23, 0.67 (0.22–1.32)	1.17±1.60, 0.50 (0.34–1.07)	1.24±1.57, 0.65 (0.59–0.99)	*P*=0.706
Intergroup comparison	N/A	N/A	N/A	N/A	
2 weeks vs. 12 weeks					
NB	*P*=0.001	*P*=0.002	*P*=0.001	*P*=0.007	
NB-in	*P*=0.021	*P*=0.121	*P*=0.021	*P*=0.032	
NB_out	*P*=0.602	*P*=0.518	*P*=0.487	*P*=0.668	

Data are mean±standard deviation, median (interquartile range) values in mm^2.
NB: newly formed bone, rhBMP-2: recombinant human bone morphogenetic protein-2, NB_in: area of NB in the inner part of the bone block, NB_out: area of NB in the outer part of the bone block, DBBM: deproteinized bovine bone mineral block, PBC: porcine bone block with collagen matrix incorporation, PB: porcine bone block without collagen matrix incorporation, PBC+M: PBC block covered by a collagen membrane, N/A: not applicable.

https://doi.org/10.5051/jpis.2204240212

Bone substitute materials from different origins have previously been incorporated with collagen components. However, a few studies have compared bone blocks incorporated with and without collagen [20-22]. In a study by Kim and colleagues, biphasic calcium phosphate (BCP) was used to prepare collagenated bone blocks [21]. Using the same model as in the present study, collagenated BCP blocks presented significantly less NB formation than BCP blocks without collagen. Another study compared DBBM bone blocks with collagenated equine bone blocks, and found no difference in NB formation between them [20]. Another study found significantly greater amounts of NB at all healing time points in collagenated BCP blocks than in non-collagenated putty-type BCP blocks in a lateral augmentation model in dogs [9]. In the present study, the PBC group had greater NB formation than the DBBM and PB groups (albeit without significant differences at 2 and 12 weeks). The heterogeneity among these studies might be due to variations in the origins of the bone substitute materials, processing methods, and experimental models.

However, one may question whether the amount of NB (in experiment 1) was optimal or at least adequate. When calculating percentages of NB relative to the total augmented area, the PBC group yielded 6.46% at 2 weeks and 18.55% at 12 weeks (even though the 12-week value in PBC group was the greatest among the groups). Moreover, NB formation did not reach the most-coronal parts of the augmentations in the histologic sections. These observations suggest the need for additional material improvements to increase NB formation, such as by using rhBMP-2, as in experiment 2.

The application of rhBMP-2 requires carrier materials, and the most commonly used materials are absorbable collagen sponges and particle-type bone substitute materials [11,23-25]. Other carriers such as collagenated bone substitute materials have also been investigated, but data are still limited. These types of materials have previously been tested in sinus augmentation [26,27] and calvarial defect models in rabbits [11,21], and a lateral ridge augmentation model in dogs [15].

In the present study, the addition of rhBMP-2 increased NB formation at both 2 and 12 weeks. At 2 weeks, this increase was more than 2-fold, and the difference was larger at 12 weeks between the groups with and without rhBMP-2 (>2.5 times). Among the groups, the NB increase attributable to rhBMP-2 was the largest in the PBC+M group (>3 times at 2 weeks and >3.5 times at 12 weeks, compared with no rhBMP-2). Moreover, NB formation reached the coronal border only in the blocks with rhBMP-2. These findings indicate that the addition of rhBMP-2 can shorten the healing period and facilitate more favorable bone-forming patterns.

It was noted that the differences in NB between the DBBM with the rhBMP-2 group and the PBC/PB groups with rhBMP-2 were greater at 12 weeks than at 2 weeks, suggesting different capacities for rhBMP-2 delivery. It is conceivable that the inherent difference between the structures of bovine (DBBM) and porcine (PBC and PB) bones and resorption rates influenced this difference. An effect of different carrier characteristics was also observed in our previous study [14]. In that study, DBBM blocks with rhBMP-2 exhibited greater NB formation than other block materials with rhBMP-2 at 2 weeks, but the NB in BCP blocks and nano-hydroxyapatite blocks surpassed that in DBBM blocks at 12 weeks. When interpreting those 2 studies, it might not be proper to combine the outcomes (for example, the numerical values) from the studies due to different group settings in the experimental animals (except for DBBM, all materials from the above studies were not compared in the same animals).

https://doi.org/10.5051/jpis.2204240212

Incorporating the collagen component into the porcine blocks led to little difference in the action of rhBMP-2 (PB +rhBMP-2 group vs. PBC+rhBMP-2 group) in the present study, which does not corroborate the finding of another study that used the same model [21]. Kim et al. [21] investigated the effects of rhBMP-2 with BCP and collagenated BCP blocks, and found that the former combination resulted in significantly greater NB formation than the latter after 8 weeks of healing. This difference might be derived from the processing method and the origin of bone material.

One of the most interesting findings in the present study was enhanced NB formation in the PBC+M group with rhBMP-2 at 12 weeks. The PBC+M group with rhBMP-2 had the greatest NB, with a significant difference from the DBBM group with rhBMP-2. This can be explained as follows: First, a barrier membrane appeared to play a role in confining rhBMP-2 to the augmented site. After releasing rhBMP-2 from the PBC block, the nearby collagen membrane might have absorbed some of the rhBMP-2, and later provided rhBMP-2 again. Indeed, some studies have utilized collagen barriers as an rhBMP-2 carrier [28,29]. Second, the principle of guided bone regeneration requires a barrier membrane to populate bone-forming cells in the augmented area [30].

The type of barrier membrane may affect the action of rhBMP-2. One study found that nonresorbable expanded polytetrafluoroethylene (ePTFE) membranes did not impact rhBMP-2 treated defects [31]. Another study demonstrated that ePTFE membranes inhibited bone formation initially, but not later in the healing period [32]. The initial inhibition of bone formation observed in the latter study was also noted in the present study. At 2 weeks, the groups with rhBMP-2 had the smallest amount of NB. This delay might have been due to the characteristics of a cross-linked collagen barrier causing slow biodegradation and reduced vascularization [33], which might interfere with the influx of cells needed for bone regeneration during the initial healing, similar to the situation in nonresorbable membranes. This assumption should be further inspected by comparing non-cross-linked and cross-linked collagen barriers over the bone blocks.

A collagenated equine bone block has recently been clinically tested in lateral ridge augmentation for staged implant placement [34-37]. Those studies demonstrated sufficient ridge width gain for implants of appropriate diameters. One study demonstrated the histologic outcome of osseous organization in the bone blocks in 8 patients [36]. However, no clinical study has investigated vertical augmentation using collagenated bone substitute materials.

There were several limitations in the present study. First, some specimens were not included in the analysis due to errors in histologic processing. Second, there was no adjustment of the *P* values during multiple comparisons. Third, the structural characteristics of the bone blocks were not fully inspected, although these characteristics might be related to rhBMP-2 absorption and release kinetics. Fourth, the calvarial model in this study served as a screening model, indicating the necessity of further investigation in larger animal models that would be similar to the actual clinical situation.

In conclusion, this study found that NB formation was greater in PBC and PB blocks than in DBBM blocks. RhBMP-2 increased bone regeneration for all bone block types, and the additional collagen barrier further enhanced NB formation.

https://doi.org/10.5051/jpis.2204240212

REFERENCES

1. Naenni N, Lim HC, Papageorgiou SN, Hämmerle CH. Efficacy of lateral bone augmentation prior to implant placement: a systematic review and meta-analysis. J Clin Periodontol 2019;46 Suppl 21:287-306.
PUBMED | CROSSREF
2. Urban IA, Montero E, Monje A, Sanz-Sánchez I. Effectiveness of vertical ridge augmentation interventions: a systematic review and meta-analysis. J Clin Periodontol 2019;46 Suppl 21:319-39.
PUBMED | CROSSREF
3. Mir-Mari J, Wui H, Jung RE, Hämmerle CH, Benic GI. Influence of blinded wound closure on the volume stability of different GBR materials: an *in vitro* cone-beam computed tomographic examination. Clin Oral Implants Res 2016;27:258-65.
PUBMED | CROSSREF
4. Benic GI, Eisner BM, Jung RE, Basler T, Schneider D, Hämmerle CH. Hard tissue changes after guided bone regeneration of peri-implant defects comparing block versus particulate bone substitutes: 6-month results of a randomized controlled clinical trial. Clin Oral Implants Res 2019;30:1016-26.
PUBMED | CROSSREF
5. Benic GI, Thoma DS, Jung RE, Sanz-Martin I, Unger S, Cantalapiedra A, et al. Guided bone regeneration with particulate vs. block xenogenic bone substitutes: a pilot cone beam computed tomographic investigation. Clin Oral Implants Res 2017;28:e262-70.
PUBMED | CROSSREF
6. De Santis E, Lang NP, Favero G, Beolchini M, Morelli F, Botticelli D. Healing at mandibular block-grafted sites. An experimental study in dogs. Clin Oral Implants Res 2015;26:516-22.
PUBMED | CROSSREF
7. De Santis E, Lang NP, Scala A, Viganò P, Salata LA, Botticelli D. Healing outcomes at implants installed in grafted sites: an experimental study in dogs. Clin Oral Implants Res 2012;23:340-50.
PUBMED | CROSSREF
8. Schmitt C, Lutz R, Doering H, Lell M, Ratky J, Schlegel KA. Bio-Oss® blocks combined with BMP-2 and VEGF for the regeneration of bony defects and vertical augmentation. Clin Oral Implants Res 2013;24:450-60.
PUBMED | CROSSREF
9. Lee JT, Cha JK, Kim S, Jung UW, Thoma DS, Jung RE. Lateral onlay grafting using different combinations of soft-type synthetic block grafts and resorbable collagen membranes: an experimental in vivo study. Clin Oral Implants Res 2020;31:303-14.
PUBMED | CROSSREF
10. Mir-Mari J, Benic GI, Valmaseda-Castellón E, Hämmerle CH, Jung RE. Influence of wound closure on the volume stability of particulate and non-particulate GBR materials: an *in vitro* cone-beam computed tomographic examination. Part II. Clin Oral Implants Res 2017;28:631-9.
PUBMED | CROSSREF
11. Lee EU, Lim HC, Hong JY, Lee JS, Jung UW, Choi SH. Bone regenerative efficacy of biphasic calcium phosphate collagen composite as a carrier of rhBMP-2. Clin Oral Implants Res 2016;27:e91-9.
PUBMED | CROSSREF
12. Jung RE, Thoma DS, Hammerle CH. Assessment of the potential of growth factors for localized alveolar ridge augmentation: a systematic review. J Clin Periodontol 2008;35 Suppl:255-81.
PUBMED | CROSSREF
13. Gomes-Ferreira PH, Okamoto R, Ferreira S, De Oliveira D, Momesso GA, Faverani LP. Scientific evidence on the use of recombinant human bone morphogenetic protein-2 (rhBMP-2) in oral and maxillofacial surgery. Oral Maxillofac Surg 2016;20:223-32.
PUBMED | CROSSREF
14. Lim HC, Paeng KW, Jung UW, Benic GI. Effectiveness of xenogeneic and synthetic bone-block substitute materials with/without recombinant human bone morphogenetic protein-2: a preclinical study using a rabbit calvarium model. J Clin Periodontol 2021;48:1126-36.
PUBMED | CROSSREF
15. Benic GI, Joo MJ, Yoon SR, Cha JK, Jung UW. Primary ridge augmentation with collagenated xenogenic block bone substitute in combination with collagen membrane and rhBMP-2: a pilot histological investigation. Clin Oral Implants Res 2017;28:1543-52.
PUBMED | CROSSREF
16. Jung IH, Lim HC, Lee EU, Lee JS, Jung UW, Choi SH. Comparative analysis of carrier systems for delivering bone morphogenetic proteins. J Periodontal Implant Sci 2015;45:136-44.
PUBMED | CROSSREF

17. Bae SY, Park JC, Shin HS, Lee YK, Choi SH, Jung UW. Tomographic and histometric analysis of autogenous bone block and synthetic hydroxyapatite block grafts without rigid fixation on rabbit calvaria. J Periodontal Implant Sci 2014;44:251-8.
PUBMED | CROSSREF

18. Laass A, Eisner BM, Hämmerle CH, Jung RE, Thoma DS, Benic GI. Histologic outcomes after guided bone regeneration of peri-implant defects comparing individually shaped block versus particulate bone substitutes. Int J Periodont Restor Dent 2020;40:519-27.
PUBMED | CROSSREF

19. Park JI, Yang C, Kim YT, Kim MS, Lee JS, Choi SH, et al. Space maintenance using crosslinked collagenated porcine bone grafted without a barrier membrane in one-wall intrabony defects. J Biomed Mater Res B Appl Biomater 2014;102:1454-61.
PUBMED | CROSSREF

20. Benic GI, Thoma DS, Muñoz F, Sanz Martin I, Jung RE, Hämmerle CH. Guided bone regeneration of peri-implant defects with particulated and block xenogenic bone substitutes. Clin Oral Implants Res 2016;27:567-76.
PUBMED | CROSSREF

21. Kim JW, Jung IH, Lee KI, Jung UW, Kim CS, Choi SH, et al. Volumetric bone regenerative efficacy of biphasic calcium phosphate-collagen composite block loaded with rhBMP-2 in vertical bone augmentation model of a rabbit calvarium. J Biomed Mater Res A 2012;100:3304-13.
PUBMED | CROSSREF

22. Moest T, Wehrhan F, Lutz R, Schmitt CM, Neukam FW, Schlegel KA. Extra-oral defect augmentation using autologous, bovine and equine bone blocks: a preclinical histomorphometrical comparative study. J Craniomaxillofac Surg 2015;43:559-66.
PUBMED | CROSSREF

23. de Freitas RM, Susin C, Tamashiro WM, Chaves de Souza JA, Marcantonio C, Wikesjö UM, et al. Histological analysis and gene expression profile following augmentation of the anterior maxilla using rhBMP-2/ACS versus autogenous bone graft. J Clin Periodontol 2016;43:1200-7.
PUBMED | CROSSREF

24. Susin C, Lee J, Fiorini T, de Freitas RM, Chiu HC, Prasad HS, et al. Sinus augmentation using rhBMP-2/ACS in a mini-pig model: Influence of an adjunctive ceramic bone biomaterial. J Clin Periodontol 2018;45:1005-13.
PUBMED | CROSSREF

25. Visser R, Arrabal PM, Becerra J, Rinas U, Cifuentes M. The effect of an rhBMP-2 absorbable collagen sponge-targeted system on bone formation in vivo. Biomaterials 2009;30:2032-7.
PUBMED | CROSSREF

26. Cha JK, Song YW, Kim S, Thoma DS, Jung UW, Jung RE. Core ossification of bone morphogenetic protein-2-loaded collagenated bone mineral in the sinus. Tissue Eng Part A 2021;27:905-13.
PUBMED | CROSSREF

27. Kim JS, Cha JK, Cho AR, Kim MS, Lee JS, Hong JY, et al. Acceleration of bone regeneration by BMP-2-loaded collagenated biphasic calcium phosphate in rabbit sinus. Clin Implant Dent Relat Res 2015;17:1103-13.
PUBMED | CROSSREF

28. Chang YY, Lee JS, Kim MS, Choi SH, Chai JK, Jung UW. Comparison of collagen membrane and bone substitute as a carrier for rhBMP-2 in lateral onlay graft. Clin Oral Implants Res 2015;26:e13-9.
PUBMED | CROSSREF

29. Sun YK, Cha JK, Thoma DS, Yoon SR, Lee JS, Choi SH, et al. Bone regeneration of peri-implant defects using a collagen membrane as a carrier for recombinant human bone morphogenetic protein-2. BioMed Res Int 2018;2018:5437361.
PUBMED | CROSSREF

30. Wang HL, Boyapati L. "PASS" principles for predictable bone regeneration. Implant Dent 2006;15:8-17.
PUBMED | CROSSREF

31. Linde A, Hedner E. Recombinant bone morphogenetic protein-2 enhances bone healing, guided by osteopromotive e-PTFE membranes: an experimental study in rats. Calcif Tissue Int 1995;56:549-53.
PUBMED | CROSSREF

32. Cochran DL, Schenk R, Buser D, Wozney JM, Jones AA. Recombinant human bone morphogenetic protein-2 stimulation of bone formation around endosseous dental implants. J Periodontol 1999;70:139-50.
PUBMED | CROSSREF

33. Rothamel D, Schwarz F, Sager M, Herten M, Sculean A, Becker J. Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat. Clin Oral Implants Res 2005;16:369-78.
PUBMED | CROSSREF

34. Ortiz-Vigón A, Suarez I, Martínez-Villa S, Sanz-Martín I, Bollain J, Sanz M. Safety and performance of a novel collagenated xenogeneic bone block for lateral alveolar crest augmentation for staged implant placement. Clin Oral Implants Res 2018;29:36-45.
PUBMED | CROSSREF

35. Parvini P, Mihatovic I, Sahin D, Becker J, Schwarz F. Lateral alveolar ridge augmentation using an equine-derived collagen-containing bone block: a prospective case series. Clin Oral Implants Res 2022;33:142-9.
PUBMED | CROSSREF

36. Schwarz F, Mihatovic I, Ghanaati S, Becker J. Performance and safety of collagenated xenogeneic bone block for lateral alveolar ridge augmentation and staged implant placement. A monocenter, prospective single-arm clinical study. Clin Oral Implants Res 2017;28:954-60.
PUBMED | CROSSREF

37. Schwarz F, Sahin D, Civale-Schweighöfer S, Becker J. Long-term outcomes following lateral alveolar ridge augmentation using a collagenated xenogeneic bone block: a monocenter, prospective single-arm clinical study. Int J Implant Dent 2021;7:9.
PUBMED | CROSSREF

https://doi.org/10.5051/jpis.2204240212