Collagenated biphasic calcium phosphate loaded with bone morphogenetic protein 2 in rabbit calvarial defect model

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감사의 글

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이렇게 결실을 맺기까지, 길을 열어주시고 방패막이 되어주시고 한 없이 베풀어주셨던 최성호 지도교수님께 진심으로 감사드립니다. 학자로서의 소양뿐 아니라, 삶에 대한 태도, 스승으로서의 역할에 대한 많은 가르침 주셨습니다. 또한, 학과 첫 졸업생으로 나아갈 방향을 제시해주시고 애정 주시는 정원균 교수님께 진심으로 감사 드립니다. 두 분 교수님을 뵐 수 있었고 가르침 받을 수 있었던 것 모두 사랑과 보살핌 주시는 주님의 크신 뜻과 은혜라고 생각합니다. 모두 감사드립니다. 존경합니다.

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ABSTRACT

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Backgrounds and aim of this study

Being free from any consideration of donor, biphasic calcium phosphate (BCP) blended with hydroxyapatite and beta-tricalcium phosphate has been used as a bone substitutes. In recent years, a cylinder-form collagenated BCP that was incorporated with a type-I collagen matrix have been developed. To overcome the small augmentation by collagen-incorporated scaffolds, recombinant human bone morphogenetic protein 2 (rhBMP2) was treated with the collagenated BCP. The aim of this study was to determine the superiority of the recent collagenated BCP as a BMP2 carrier in healing of calvarial bone defect model in rabbit.

Materials and methods

Four circular defects were made in each rabbit calvarial (total 10 rabbits), and materials were delivered as follows: no scaffold (Control), collagen sponge + rhBMP2 (CS), BCP + rhBMP2 (BCP), collagenated BCP + rhBMP2

(CBCP). After 2 weeks and 8 weeks later, samples (n=5) were isolated and stained with hematoxylin and eosin solutions for histological and histomorphometrical analysis.

Results

BCP and CBCP groups exhibited the significantly larger areas of new bone formation, residual scaffold, total augmentation, and total minerals than Control and CS in 2 weeks, and showed similar results also in 8 week even thought there was no significant differences in amount of new bone formation and total augmentation ($p<0.05$). More importantly, the rates of parameters in CBCP were reached to 85.756±16.85 % in defect closure, to 36.94±8.1% in new bone formation, and to 60.73±6.1% in total mineral until 8 weeks, and these were the highest in all groups even though it was partially significant ($p<0.05$).

Conclusion

We have demonstrated that CBCP group created effective and reliable defect reconstructions in rabbit calvarial model. These results implicate that the moldable cylinder-form collagenated BCP loaded with rhBMP2 might be the practical and convenient biomaterial for bony defect reconstructions.

Key words : bone regeneration, biphasic calcium phosphate, collagen, bone morphogenic protein 2, rabbit calvarial defect

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I. INTRODUCTION

In oral-maxillofacial and orthopedic surgery, reconstruction of bone defects caused by injuries, infections, tumors, or congenital deformities is still challenging. Although autologous bone grafting is known as a gold standard, it has limitations such as insufficient supply, morbidity, secondary surgical site and postoperative pains, conflict with its excellent bone regenerative potential[1]. Therefore, approaches have been tried to develop a promising bone substitute which is biocompatible, biodegradable, and biofunctional[2, 3].

Being free from any consideration of donor, a calcium phosphate such as hydroxyapatite (HA) was used as the bone substitute. HA is chemically and structurally similar to bone and is biocompatible and osteoconductive.

However, HA has an inappropriate mechanical property that is prone to fragile and resorbed slowly, so does not match to the tissue repair rates, making it difficult for new bone to invade into[4]. Another calcium phosphate akin to bone minerals, beta-tricalcium phosphate (β-TCP) was examined as bone alternative. β-TCP is extremely porous, interconnected, and so resorbs at a quicker rate than HA[5]. The degraded portions of β-TCP provide bone integrations onto and the invasions of fibrovascular tissues, resulting in bony replacement. Although b-TCP can also provide initial structural strength with remodeling and replacement of bone, however, the structural stability is not persisted until the deposition and maturation of bony tissues[6]. The use of single β-TCP has limitation as the bone alternative.

To overcome these shortcomings, the concept of biphasic calcium phosphate (BCP), in which two or more ceramic phases were blended, was introduced. Previous studies showed BCP ceramics are biocompatible and effective in bone defect reconstructions, and can provide the benefits combining the biodegradability of β-TCP and the stability of HA[6]. So, various ratios of HA/β-TCP have been tried for predictable bone regeneration[7-10], and more in depth for convenient and practical use of BCP[11].

For more practical and convenient approach, structured scaffold has been required. The success of particulate bone substitute implantation depends on the defect morphology and the user’s technical skills, since bone substitute particles can be spread out and cannot withstand the tissue pressures within uncontained defects. In comparison to granulated scaffolds, the rigid structure of solid-type materials can make it more possible to resist pressures of tissues, to set a frame of reconstruction, to enable three-dimensional tissue regenerations without material loss, and to simplify application approaches.

Furthermore, if a solid-form bone substitute contains a shape, it would provide improved structure of defect reconstruction in an appropriate three-dimensional shape for functional and aesthetic reasons. Indeed, studies showed that block-type of calcium phosphates provided the sufficient integrity, endured against tissue pressures, so created three-dimensional tissue augmentation[12-14]. However, the bone regeneration in the studies were limited or restricted to the outer side of block. Moreover, the brittle property of calcium phosphates can make it difficult to modify in a specific form since bending or torsional force might break the bone alternative blocks.

In an effort to improve the biofunctionality, investigators have focused on collagens. Collagen is the major organic component of bone that has excellent tensile and shear strength to mold in a desired shape of defects. Moreover, it was proven for collagen to regulate gene expressions and differentiations of osteoblasts[15]. Studies, therefore, tried collagen coating on ceramics, and showed increases in the proliferation rate and survival of osteoblasts[16, 17], and improvements in the infiltration of osteoblasts in vitro[18]. Unexpectedly, however, preclinical animal studies reported that the collagenated block-type scaffolds showed decreased augment volumes of tissues than uncollagenated ones due to imbalanced mechanical strength of fabricated collagen-coating bone substitutes[11, 19-23]. Thus, for more efficient bone augmentation, additional therapeutic approach such as growth factor treatment has been regard valuable to try.

As a one of the most potent osteogenic inducers, recombinant human bone morphogenetic protein-2 (rhBMP2) is extensively reviewed by numerous reports; rhBMP2 enhances the functions of the differentiated osteoblasts, stimulates the differentiation of cells to osteogenic lineage, and does a role in bone development and repairs[24]. Numerous preclinical and clinical studies reported the advanced bone regenerations produced by rhBMP2-treated bone substitutes[25, 26]. Thus, rhBMP2 has been expected to balance between scaffold degradation and new bone formation, so to create great reconstruction with the collagen-coating bone substitutes[27].

In recent years, solid-type BCP (HA/β-TCP, 30/70) incorporated with type-I collagen matrix have been developed. Several experiments showed previously intact bone formations bridging scaffolds[7] and successful osseointegrations with dental implant[28] with the collagenated BCP, although there was no significant difference. Thus, the present study treated rhBMP2 into the collagenated BCP (HA/β-TCP, 30/70) and assessed the reconstructive potential of calvarial bone defect in rabbit. The aim of this study was to determine the superiority of the current collagenated BCP as an rhBMP2 carrier.

II. MATERIALS AND METHODS

1. Study design

Groups of present study were designed as the followings;

1) No scaffold (Control),
2) Collagen sponge + rhBMP2 (0.05 mg/ml) (CS),
3) BCP (HA/β-TCP, 30/70) + rhBMP2 (0.05 mg/ml) (BCP),
4) Collagenated BCP (HA/β-TCP, 30/70) + rhBMP2 (0.05 mg/ml) (CBCP).

Four 8-mm circular defects were made in each rabbit calvarial (*n*=10), group assignment was randomly performed and each material was delivered to the assignment defect; and Control group was filled with blood coagulum. Then, the animals were divided into two groups and healed for either 2 or 8 weeks (n=5, each) (Figure 1).

2. Animals

In this study, ten male New Zealand white rabbits (14–16 weeks old; mean body weight, 3.0–3.5 kg) were used. The animals were cared under a standard laboratory condition in separate cages and fed standard diets. All the procedures involving animal selection, management, preparation and surgical protocols were approved by and followed the rules of the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea (approval no. 2011-0031).

3. Material preparations

For the CS group, cylinder-form of bovine type I collagen sponge (Genoss, Suwon, South Korea) was used, and it was manufactured in 8 x 2 mm to fit in the calvarial bone defect. For the BCP and CBCP groups, particlulated BCP (OSTEON II; Genoss, Suwon, South Korea) and cylinder-form of collagenated BCP (OSTEON II Collagen; Genoss, Suwon, South Korea) were used, respectively. The BCP particles and collagenated BCP cylinder each contained 0.1 cc of BCP components, had 250 μm of macropores, and consisted with 0.5–1 mm of BCP particles at a weight ratio of 30/70 (HA/β-TCP). Especially for the collagenated BCP, bovine type I collagen used as the scaffold to produce a cylinder form of BCP and was 8 x 2 mm in size. The Escherichia coli-derived rhBMP2 (Genoss, Suwon, South Korea) was thankfully given by Genoss, and 0.1ml of rhBMP2 solution (0.05 mg/ml) was loaded onto each material of CS, BCP and CBCP groups.

4. Surgical protocol

The experimental rabbits were injected intramuscularly and anesthetized with a mixture of ketamine hydrochloride (Ketalar, Yuhan, Seoul, Korea) and xylazine (Rompun, Bayer Korea, Seoul, Korea). After shaving, the surgical sites were disinfected with alcohol and povidone iodine, and then locally anesthetized with 2 % lidocaine HCl (Huons, Seoul, Korea). Surgical procedures were performed by a skillful practitioner (E.W.L.) to remove the interexaminer errors. Incisions were made along the sagittal midline ranging from the frontal- to the occipital-bone. After dissections of skins, four circular holes in each rabbit were then created using 8-mm trephines bur (3i Implant

Innovation, Palm Beach Gardens, FL, USA) under a cool condition by saline irrigation, and then each defect was grafted according to the group assignment that was randomly performed. Soft tissue was repositioned and the surgical sites were sutured using absorbable synthetic monofilament suture (4-0 Monosyn; B-Braun, Melsungen, Germany). After 2 weeks and 8 weeks, five rabbits were sacrificed at each time point.

5. Histologic processing

The histological samples including defects and surrounding tissues were isolated from the sacrificed animals, rinsed briefly with sterilized saline, fixed in 10 % buffered formalin for 7 days, and then decalcified in 5 % formic acid for 14 days. After sorting out and labeling, the samples were dehydrated with a gradual series of ethanol, embedded in paraffin, and then sectioned serially. The sections were cut through the center of each defect and stained with hematoxylin and eosin solutions.

6. Clinical, Histologic and histomorphometric analysis

A. Clinical and histologic observation.

During healing period, animals were observed carefully for any allergic reactions, inflammations, and other complications. Histologically, each specimen were examined for the conditions of defect healing, tissue augmentation, new bone formation, remained materials, cell components, and vascularizations under a light microscope (Leica DM LB, Leica Microsystems Ltd., Wetzlar, Germany) by a single, blinded examiner (I.H.J.).

B. Histomorphometric analysis.

For the histomorphometrical analysis, the following five parameters were measured using an automated image-analysis system (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA):

① Defect closure (DC): the ratio of bone ingrowth within the defect.

② Newly formed bone tissues (NB): the area sum of bony tissues within the defect.

③ Residual graft materials (RS): the area sum of remained scaffold (CS, BCP or CBCP).

④ Total augmentation (TA): all the areas of tissues between the defect margins, which included the DC, RS and fibrovascular tissues.

⑤ Total mineral (TM): the area sum of NB and mineralized RS.

In a previous study, it was proved that the values of calcium phosphate transplanted and bone tissues newly formed are highly and negatively correlated (-93% especially in TCP)[29]. Thus, highly portions of the degraded calcium phosphates can be replaced by newly presented bony minerals. In many cases, however, the discrepancy in the rate between production and deposition of bony mineral causes changes of the initial volume of augmentation. Therefore, to check the changes of mineral sum in grafts can detect for investigators whether the mineral exchange is proceed in balance or not. Moreover, if the exchange rate is balanced, the TM can regard as the future area which would work as a bony part of the body. For all the parameters, measurement and analysis were performed by a single, blinded examiner (I.H.J.) to minimize inter-examiner errors.

7. Statistical analysis

Statistical analysis was performed using a statistic software program (SPSS 15.0; SPSS, Chicago, IL, USA). The histomorphometric records of groups (i.e., Control, CS, BCP, CBCP) were shown as the mean and standard deviation (SD) values. The multiple comparisons and the post hoc tests among groups were analyzed using Kruskal-Wallis test and the post hoc Bonferroni test, respectively. The statistical significance level was set as 5 % (p<0.05).

III. RESULTS

1. Clinical observations

The experimental animals were healed normally. Any sign of clinical complication or inflammation was not observed. However, due to technical complications of specimen sectioning, a sample in each group of 8 weeks was excluded. Thus, five and four samples were analyzed statistically in each group at 2 and 8 weeks, respectively.

2. Histologic observations

At 2 week, the empty defects of Control were filled with connective or muscular tissues, so the central regions were depressed and flattened. A minimal bone ingrowth was seen at only the defect margins. In CS, pressed collagen material covering the dura mater and the vascularizations where a few large blood vessels were embedded inside were observed. However, there was limited bone regeneration only at the edge of margins. In BCP and CBCP, defects were packed with implanted graft materials engaged with fibrous tissues, so the volume integrities of implantation were almost maintained. And, prominent bone formations were presented throughout the implants in BCP and CBCP, where tissues creating bony bridges among scaffolds, and cellular components including osteoblasts and osteocytes were observed near grafts. Especially in the augmented tissues of CBCP, reversal lines of bony tissues and several large capillaries like harversian canals of bone were displayed.
At 8 weeks in Control and CS, the increases in defect volume were minimal

and partial bone ingrowths were displayed at the defect edges, containing several bony islands (Figure 4). And, the transplanted scaffold of CS was rarely remained. In groups of BCP and CBCP, both scaffolds still remained a lot, although they were degraded and so presented irregular surfaces containing osteoclasts. Thus, the defect volumes of BCP and CBCP seemed to be great and superior to those of Control and CS. Moreover, both BCP and CBCP showed great bone ingrowths and bony maturations filling the interspaces of scaffolds in defect, where a lot of developed reversal lines, blood vessels, and osteocytes embedded in bone tissues were detected.

3. Histomorphometric analysis

The histomorphometric parameters of each grafting modalities are summarized in Table 1, Table 2, and Figure 5. The ratios of DC were increased in all groups during the experimental period even though there was no defect fully closed (Table 1 and Figure 5A). Areas of NB were also increased in all groups until 8 weeks, and the densities of NB in TA were ranged from 29.11±11.6 to 36.94±8.1 % (Table 2). Conversely, areas of RS were decreased throughout the healing period in all the scaffold-transplanted groups (CS, BCP and CBCP), but the amount of remains were very similar between BCP and CBCP in all periods. On the other hand, TAs of BCP and CBCP which had increased at 2 weeks were a little decreased. The amounts of TA in BCP and CBCP were very similar at 2 weeks, but it became different at 8 weeks and CBCP showed the highest TA, although it was no significant ($p<0.05$; Table 2 and Figure 5B). For TM, the each recording of BCP and CBCP was almost retained until 8 weeks, despite a little difference where the TM of CBCP was a little increased at 8 weeks, whereas that of BCP was a little decreased. In the aspect of groups, Control revealed the lowest recordings in all parameters related, including DC, NB, TA, and TM. CS group showed only a partial DC, NB, TM, and TA during all the healing periods, and the parameters were significantly less than those of BCP and CBCP, although it was not significant in 8-week NB and TA ($p<0.05$; Figure 5). Yet, BCP and CBCP groups exhibited the significantly higher NB, RS, TA, and TM than Control and CS in 2 weeks, and showed similar results also in 8 week even thought there was no significant differences in NB and TA ($p<0.05$; Figure 5). More importantly, the rates of parameters in CBCP were reached to 85.756±16.85 % in DC, to 36.94±8.1% in NB, and to 60.73±6.1% in TM until 8 weeks, and these were the highest in all groups even though it was partially significant ($p<0.05$; Figure 5, Table 1 and Table 2).

IV. DISCUSSION

In recent years, a BCP ceramic (HA/β-TCP, 30/70) incorporated with type-I collagen matrix was developed, and the present study evaluated the bone regeneration by and the biodegradation pattern of the scaffolds under a condition of low-dose rhBMP2 treatment using a calvarial bone defect model in rabbit.

For rabbit calvarial defect model, different-sized defects ranging from 6 to 15 mm, in which 15 mm is the critical sized one[30], have been used, but a current report compared the healing capacity of defects in different sizes over time and showed 8-mm defect is useful to evaluate the earlier healing responses including late phases[31]. Therefore, although it was smaller than the critical-sized one, our study used the 8-mm-sized calvarial defect and observed that defects were filled with fibrous tissues and the Control was not healed spontaneously until 8 weeks.

The rhBMP2 is a great osteoinductive growth factor that had been reviewed in many reports[24]. Numerous bone substitutes including collagen sponge and BCP scaffold at various HA/b-TCP ratios (i.e. 20/80, 30/70, 60/40) produced significantly greater new bone formations with rhBMP2 than those rhBMP2-uncontained[13, 14, 24, 27, 32-34]. Therefore, it is no doubt that the bony reconstruction by a bone substitute can be improved by rhBMP2 treatment in an appropriate dose. The final goal of our study is to find the best and optimal modality for bone defect reconstruction. Toward this end, the present study used rhBMP2 in all groups except for Control, and compared the bone regenerations by the rhBMP2-loaded bone substitutes.

Generally in animal study, rhBMP2 is used at supraphysiological dosages ranged from 0.01 mg/ml for small animals such as rats, to 0.4 mg/ml for rabbits, and to more than 1.5 mg/ml for non-human primates[26]. However, the highly overdoses of rhBMP2 may cause adverse complications: aggressive defect swelling, cyst-like bone void emergence, or ectopic bone formation[35, 36]. Thus, a minimal therapeutic dose of rhBMP2 is recommended to use. The appropriate dose of growth factors including rhBMP2 depends on the species and the anatomical sites which has different vascularization, size of defect, and the number of resident cells[26]. To the best of our knowledge, the dose of rhBMP2 for 8-mm calvarial defect models in rabbit was ranged from 0.1 to 0.2 mg/ml[11, 37], and a further report using even a lower concentration (0.05 mg/ml) of rhBMP2 showed fine bone formation in rabbit sinus model[27]. Therefore, the present study used the minimal concentration of rhBMP2 (0.05 mg/ml), which is the lowest dose that has shown osteoinductive effects in rabbit model.

Under the rhBMP2-treatment condition, the BCP ceramics in BCP and CBCP performed well as the osteoconductive scaffolds. In comparison to Control and CS, the both BCP contained groups showed greater new bone formations and more bone ingrowths within defects. Moreover, firm tissue augmentations were built and maintained better than CS, like the earlier studies of rhBMP2-loaded BCP (HA/β-TCP, 30/70) scaffold [7, 28, 29]. These results showed that under the rhBMP2 treatment, the BCP and CBCP containing only a 30 percent of HA could fully endure against tissue pressures and protect the spaces for osteoblasts to move into. β-TCP is known to be gradually resorbed at a higher dissolution rate and released faster calcium and phosphate than HA, and a number of studies reported that BCP ceramics containing greater β-TCP promoted larger and faster mineralization of bone tissues[20, 38, 39]. Hence, the present BCP (HA/β-TCP, 30/70) ceramic could serve as a good component of osteoconductive bone substitute.

Previous studies reported the difficulty in optimizing the mechanical properties of collagenated scaffolds[18, 22]. Although various collagenated bone alternatives consisting of porcine bone block, solid type of bovine HA, or various ratios of BCP blocks were investigated, studies revealed only uncontrolled resorption rate of scaffold, collapse of scaffold or/and minimal tissue augmentation[11, 19, 21, 40]. However, the present study revealed that CBCP group produced the largest new bone formation and tissue augmentation, despite the amount of remained scaffolds was very similar to that of BCP group. Furthermore, TM, the future functional bony mineral, of CBCP was more stable than that of BCP which was decreased by 8 weeks. Showing a sum of NB and mineralized RS, TM can help to estimate the replacement condition of minerals in scaffold and bone tissue, and the future amount of bony minerals resulted in by checking the changes in TM. Thus, the stable TM in CBCP was assumed that the scaffold dissolution and bony mineral deposition were conducted at a similar speed, so the replacement of minerals was better balanced than BCP. Collagen coating on bone substitutes might promote the innate osteoblasts' proliferation, differentiation, and invasion into the graft materials[16-18] and to make controlled releasing of growth factors be more possible[11, 41]. Additionally, the osteoinductive rhBMP2 might provide better circumstanced for the innate progenitor cells and the migrated osteoblasts to make more bony minerals[24, 27]. Therefore, such finer mineralization of CBCP was supposed to come from the incorporations of collagen and rhBMP2.

On the other hand, a report showed that rhBMP2-loaded collagen sponge healed and bridged the calvarial defects with even a lower concentration of rhBMP2 (0.025 mg/ml), although it was a rat model that was differ from ours[40]. However, the CS in our study displayed only bony islands within defect, and there was no defect closing until 8 weeks. Although it is not clear since there was no rhBMP2-uncontained group in our study, the activity of rhBMP2 in our study seems to be lower than that in the previous study. Given the differences in animal model, scaffold used, and the manufacturing protocols of rhBMP2 in each company, further study that uses more efficient rhBMP2 and larger sample size for more long-term period is essential to confirm our results.

In our study, CBCP group showed great vital bone formation and voluminous defect reconstructions containing balanced replacement condition of bony and scaffold minerals. The present modality of rhBMP2-loaded BCP (HA/β-TCP) incorporated with type I collagen might provide clinicians effective and more convenient reconstructions in lots of bony defects.

V. CONCLUSION

In conclusion, we have demonstrated that a recent BCP ceramic (HA/β-TCP, 30/70) incorporating with type I collagen produced more effective and more reliable bone regeneration in rabbit calvarial model under a rhBMP2 (0.05 mg/ml) treatment. These results implicate that the rhBMP2-treated CBCP might be the practical and convenient biomaterial for bony defect reconstructions.

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FIGURES

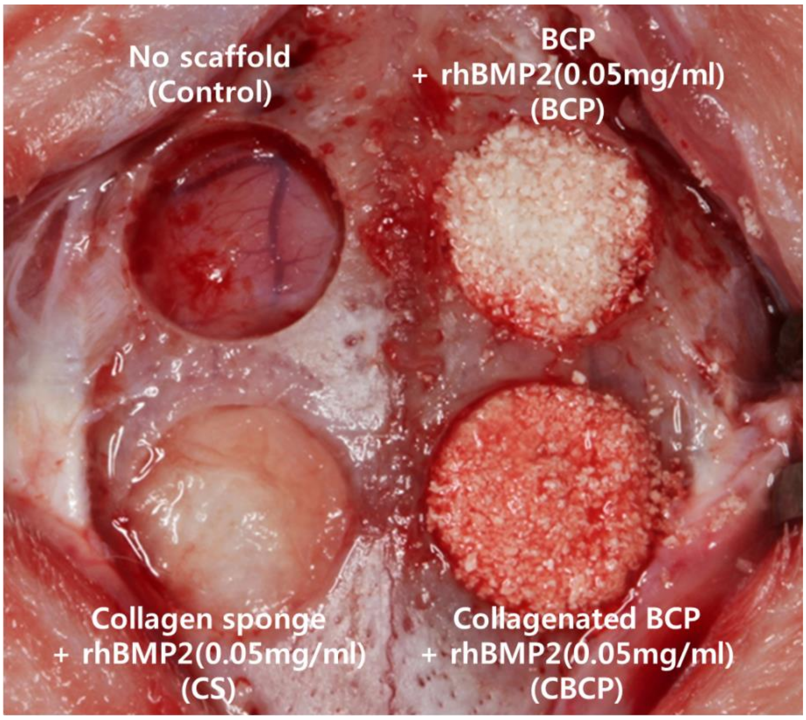

Figure 1. Clinical photograph illustrating the experiment.

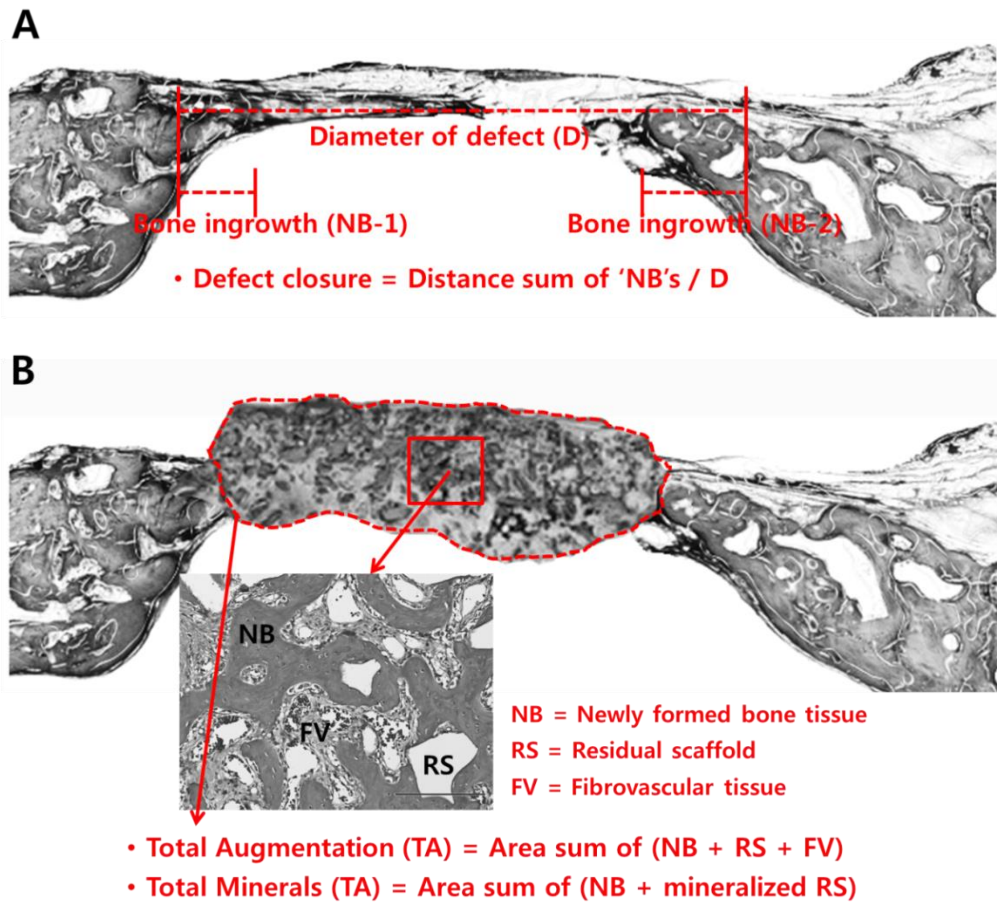

Figure 2. Schematic drawings for histomorphometric analysis

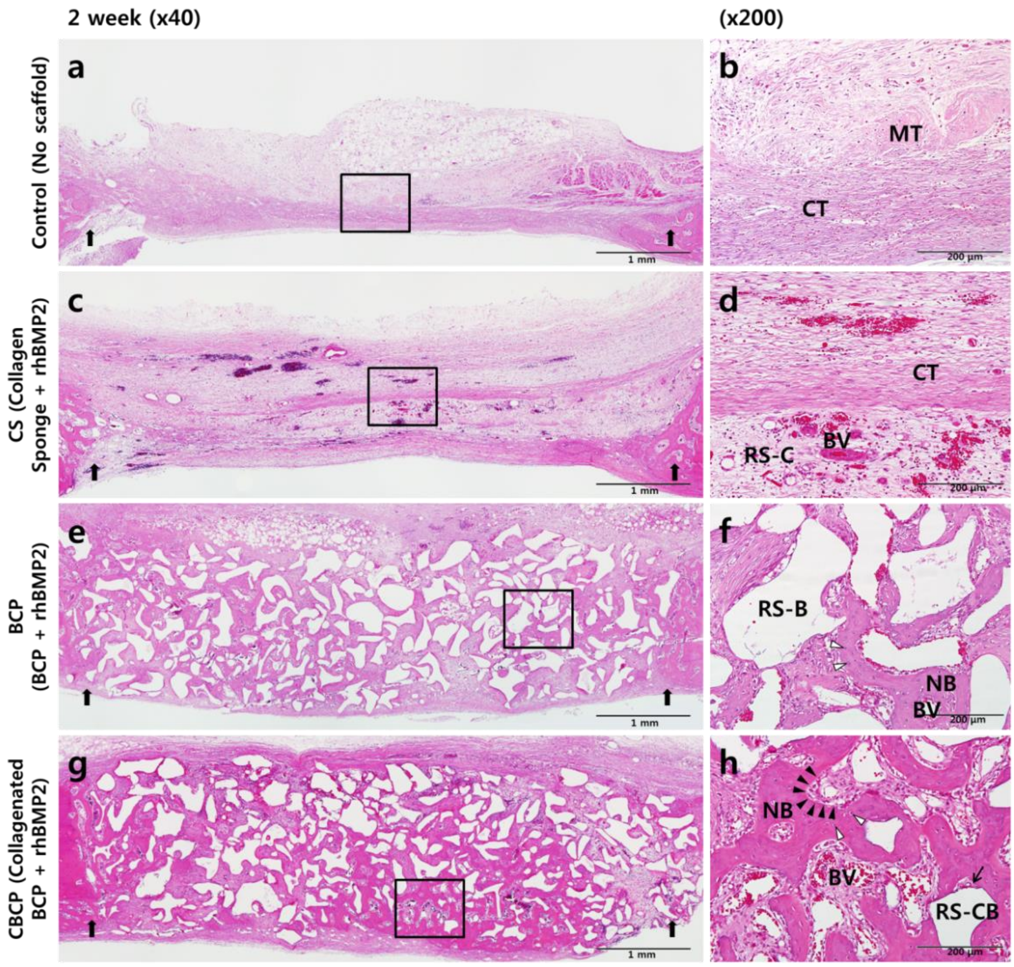

Figure 3. Histological overview of 2week postsurgery

MT, muscle tissue; CT, connective tissue; BV, blood vessel; RS-C, residual collagen sponge; RS-B, residual BCP; RS-BC, residual collagenated BCP; NB, newly formed bone tissue; Thick arrow set in left figure, margins of defect; Black rectangle, area of interest for magnification; Closed arrowhead, Osteoblast; Open arrowhead, Osteocyte; Small arrow in right figure, Osteoclast.

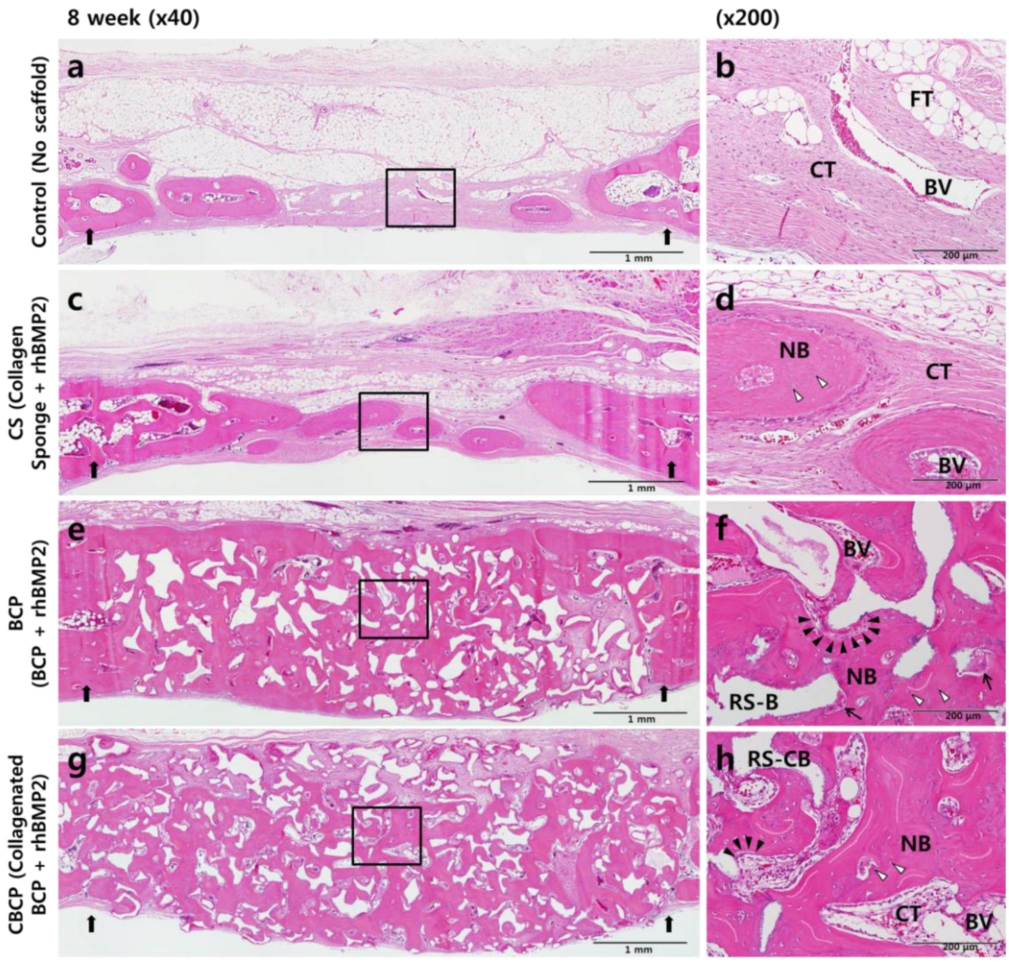

Figure 4. Histological overview of 8week postsurgery

FT, fat tissue; CT, connective tissue; BV, blood vessel; RS-B, residual BCP; RS-BC, residual collagenated BCP; NB, newly formed bone tissue; Thick arrow set in left figure, margins of defect; Black rectangle, area of interest for magnification; Closed arrowhead, Osteoblast; Open arrowhead, Osteocyte; Small arrow in right figure, Osteoclast.

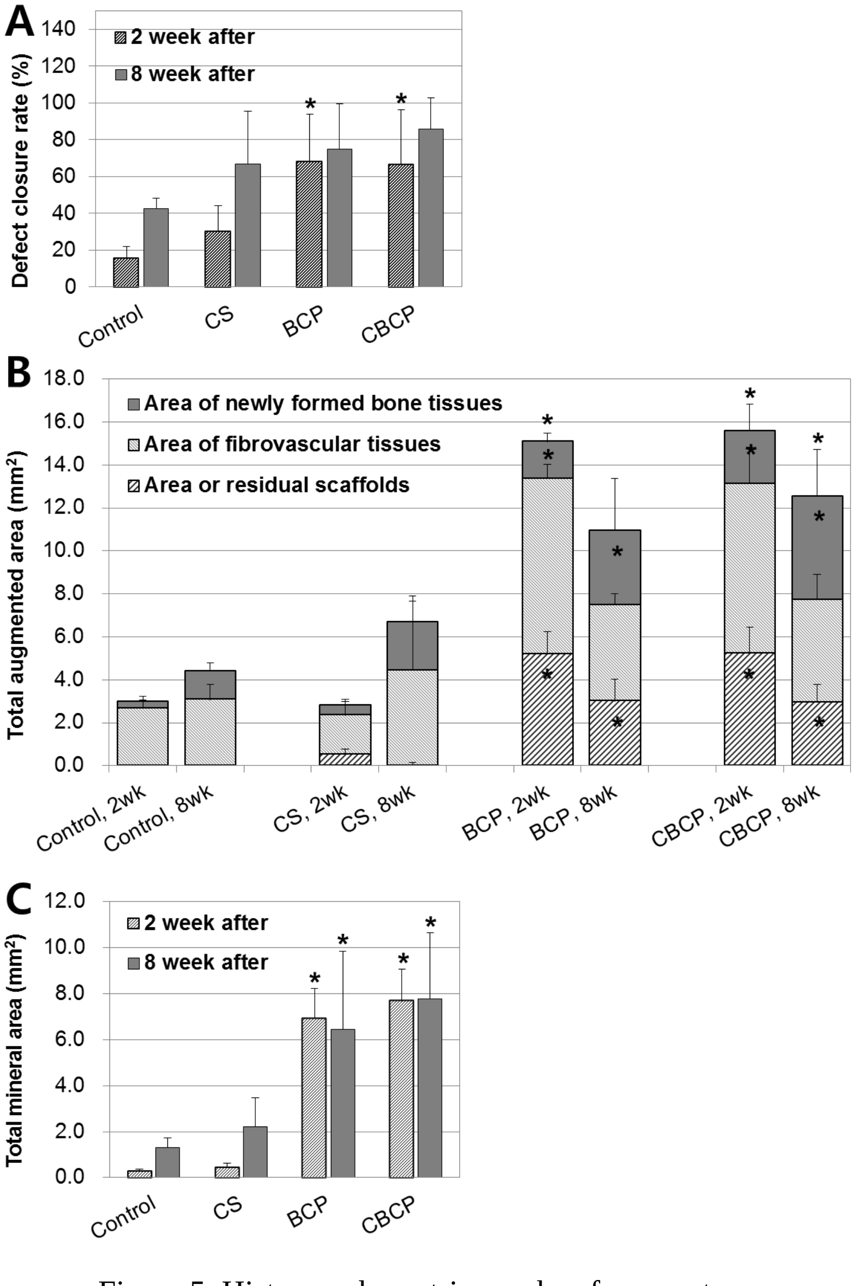

Figure 5. Histomorphometric graphs of parameters

*, Statistically significant difference in comparison to Control (p<0.05)

TABLES

Table 1. Histomorphometric measurement of DC (Mean±SD)

		Defect closure (DC)
Group	2 week	8 week
Control	15.811±6.00[¶,¥]	42.733±5.51
CS	30.219±14.08[¶]	66.762±28.80
BCP	68.276±25.71[¤,§]	74.781±24.81
CBCP	66.813±29.48[¤,§]	85.756±16.85

Results are written in % within total augmentation;
Control (No scaffold); [¤], statistically significant difference in comparison to Control (p<0.05);
CS (Collagen sponge + rhBMP2); [§], statistically significant difference in comparison to CS (p<0.05);
BCP (BCP + rhBMP2); [¶], statistically significant difference in comparison to BCP (p<0.05);
CBCP (Collagenated BCP + rhBMP2); [¥], statistically significant difference in comparison to CBCP (p<0.05).

Table 2. Histomorphometric measurements of NB, RS, TA and TM (Mean±SD)

	Newly formed bone (NB)		Residual scaffold (RS)		Total augmentation (TA)		Total minerals (TM)	
Group	2 week	8 week	2 week	8 week	2 week	8 week	2 week	8 week
Control	0.298±0.07[¶,¥] (10.18±2.8)	1.320±0.40 (29.9±7.3)	-	-	2.974±0.57[¶,¥]	4.396±0.89[¥]	0.298±0.07[¶,¥] (10.18±2.8[¶,¥])	1.320±0.40[¶,¥] (29.96±7.3[¶,¥])
CS	0.460±0.18[¶,¥] (14.11±5.3)	2.226±1.23 (32.54±12.6)	0.517±0.27[¶,¥] (31.54±22.7)	0.050±0.11[¶,¥] (5.27±10.5)	3.326±0.69[¶,¥]	6.767±4.03	0.600±0.18[¶,¥] (14.11±5.3[¶,¥])	2.226±1.23[¥] (32.54±12.6[¶,¥])
BCP	1.721±0.41[¤,§] (11.36±2.1)	3.457±2.43 (29.11±11.6)	5.208±1.02[¤,§] (34.38±4.6)	3.000±1.02[¤,§] (27.27±1.8)	15.079±1.33[¤,§]	10.943±3.56	6.929±1.28[¤,§] (45.73±5.3[¤,§])	6.457±3.39[¤] (56.38±11.6[¤,§])
CBCP	2.456±1.25[¤,§] (16.23±9.4)	4.817±2.17 (36.94±8.1)	5.232±1.22[¤,§] (33.31±4.9)	2.949±0.85[¤,§] (23.79±2.1)	15.586±1.79[¤,§]	12.546±3.88[¤]	7.688±1.37[¤,§] (49.54±8.3[¤,§])	7.766±2.88[¤,§] (60.73±6.1[¤,§])

Results are written in mm^2 (% within the TA, for NB, RG and TM);
Control (No scaffold); [¤], statistically significant difference in comparison to Control (p<0.05);
CS (Collagen sponge + rhBMP2); [§], statistically significant difference in comparison to CS (p<0.05);
BCP (BCP + rhBMP2); [¶], statistically significant difference in comparison to BCP (p<0.05);
CBCP (Collagenated BCP + rhBMP2); [¥], statistically significant difference in comparison to CBCP (p<0.05)

ABSTRACT (IN KOREAN)

골 형성 유도단백질 함유 콜라겐-이상인산칼슘 복합 골 대체제의 토끼 두개골 결손부 모델 적용

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정 임 희

연구 배경 및 목적

골 대체재로서, 하이드록시아파타이트 (HA)와 베타인산삼칼슘(β-TCP)을 혼합한 이상인산칼슘이 주목 받고 있다. 보다 높은 골형성 효과와 보다 용이하게 이용할 수 있는 골 대체재를 위해, HA/β-TCP 비율이 30/70인 이상인산칼슘에 제1형 콜라겐을 도입한 재료가 최근 새로 개발되었다. 본 연구에서는 이 신개발 골 대체제 (collagenated bicalcium phosphate, CBCP)의 골 형성 촉진 효과를 시험하고자 하였고, 보다 효과적인 골 재생을 위하여 골 형성 유도 단백질과 조합한 후, 토끼 두개골 결손부 모델에서의 치유효과를 관찰하였다.

연구 재료 및 방법

총 10마리의 건강한 토끼를 사용하였고, 각 토끼 두개골에 지름 8mm의 골 결손부를 형성한 후, 다음과 같은실험군에 따라 재료를 이식하였다; 이식재료 없음 (Control), 콜라겐 스폰지 + 골형성유도단백질 (0.05mg/ml)

(CS), 이상인산칼슘 (HA/β-TCP, 30/70) + 골형성유도단백질 (0.05mg/ml) (BCP), 콜라겐 코팅 이상인산칼슘 (HA/β-TCP, 30/70) + 골형성유도단백질 (0.05mg/ml) (CBCP). 2주와 8주 후, 각각 5마리씩의 토끼를 희생하여 조직을 적출하고, 헤마톡실린 & 에오신 염색 후, 조직학적 및 조직계측학적 분석을 시행하였다..

결과

2주 및 8주 후의 조직 및 조직계측학적 분석 결과, 잔존된 이식재료의 양은 실험기간에 따라 줄어들었고, 잔존 이식재료를 제외한 다른 모든 계측치 (신생골량, 결손부 회복량, 기능부량) 에서 BCP 와 CBCP가 Control과 CS보다 유의하게 높은 수치를 보였다. 주요 실험군인 CBCP는 통계학적으로 유의하진 않았으나 가장 많은 신생골량 및 골밀도, 결손부 회복량, 기능부량 및 기능부율을 보였다.

결론

본 연구는 토끼 두개부 결손부 모델을 이용, CBCP가 효과적이고 보다 실용적인 골 결손부 회복을 유도할 수 있음을 확인하였다. BMP2와 조합된 CBCP는 조작이 용이하고 골 재생 유도 효과가 뛰어난 신 골 대체재가 될 수 있을 것이다.

핵심되는 말: 골재생, 이상인산칼슘, 콜라겐, 골형성유도단백질-2,
토끼 두개골 결손부 모델