# Bone regenerative efficacy of limited-dose Escherichia Coli-derived rhBMP-2 with biphasic calcium phosphate carrier in rabbit calvarial defect model

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# Bone regenerative efficacy of limited-dose Escherichia Coli-derived rhBMP-2 with biphasic calcium phosphate carrier in rabbit calvarial defect model

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This certifies that the Doctoral Dissertation
of Hoon You is approved.

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# 감사의 글

부족하지만 이번 박사학위 논문으로 학위과정의 결실을 얻게 되었습니다. 그간 수련 및 대학원 과정을 돌이켜 보면, 앞으로도 30 년 이상 수행할 치과의사로서의 제 삶에 가능성과 자신감을 더 할 수 있는 시간들이었습니다.

이제까지 길러주신 부모님, 대학원과정 시작 즈음부터 만나 지금까지 옆에서 많은 응원을 해준 아내, 처음 치주과에 들어간 날부터 지도교수님, 임상가, 때로는 인생 선배로서 많은 가르침을 주신 최성호 교수님, 세심하며 명철한 제안과 도움을 주신 정의원 교수님, 이중석 교수님, 김영택 교수님, 임현창 교수님께 깊은 감사의 마음을 전합니다.

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저 자 씀

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Abstract

# Bone regenerative efficacy of limited-dose Escherichia Coli-derived rhBMP-2 with biphasic calcium phosphate carrier in rabbit calvarial defect model

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**Purpose**: The aim of this study was to characterize the healing in rabbit calvarial bone defects after delivery of limited-dose (1.5 μg) Escherichia Coli-derived recombinant human bone morphogenetic protein-2 (ErhBMP-2), and evaluate biphasic calcium phosphate (BCP) as a carrier.

**Materials and methods**: Four 8-mm-diameter circular calvarial bone defects were made in 16 rabbits, and filled with one of the following: (1) blood-filled (control), (2) BCP alone, (3) ErhBMP-2-loaded absorbable collagen sponge (ACS), or (4) ErhBMP-2-loaded BCP. The animals were allowed to heal for either 2 or 8 weeks and were evaluated in clinical, micro-computed tomographic, histological, and

histomorphometric analyses.

**Results**: Micro-computed tomography revealed extensive new bone formation in both of the limited-dose ErhBMP-2-loaded groups. However, bony collapse of the upper defect borders was found in the ErhBMP-2-loaded ACS group. Histomorphometric examination revealed significantly greater new bone formation at 8 weeks than at 2 weeks in all four groups ($p<0.05$). Both new bone formation and the size of the augmented area differed significantly between the ErhBMP-2-loaded BCP group (6.88±0.74mm$^2$, 19.62±0.77 mm$^2$) and the ErhBMP-2-loaded ACS group (3.04±0.27 mm$^2$, 5.41±0.43 mm$^2$) at 8 weeks of healing.

**Conclusion**: ErhBMP-2 promotes bone regeneration in rabbit calvarial defects, even at a limited-dose (1.5 μg). The results of this study suggest that BCP is the more effective carrier for this protein than ACS.

**Keywords:** Biphasic calcium phosphate, bone morphogenetic protein 2, bone regeneration, bone substitutes, rabbit calvaria

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## I. Introduction

Bone morphogenetic protein (BMP) was first identified in 1965 by Urist[1], and it has since been manufactured by protein purification and cloning[2], with many studies having established its osteoinductive properties as a bone substitute. The results of these studies have shown that carriers of BMP, such as collagen[3-5], hydroxyapatite[6], allogenic[7], xenogenic[8], and alloplastic[9,10] materials, improve the bone-regenerative efficacy of this protein compared with control findings.

However, despite the huge number of studies on BMP involving various biomaterials, there remain several barriers to the clinical application of this protein.

For example, BMP induces concentration-dependent adverse effects such as swelling at the surgical site and seroma formation. Leknes et al.[11] and Wikesjo et al.[12] reported the occurrence of implant displacement as a result of seromal swelling when BMP was applied at high concentrations. Furthermore, although no definitive association between BMP and the promotion of tumorigenesis or metastasis has been established[13], some researchers have raised the possibility that it promotes invasion[14] and rapid local growth of tumor cells[15]. BMP also has other adverse effects, including osteolysis and ectopic bone formation[16].

It is therefore important to determine whether bone regeneration can be induced using only small amounts of BMP in order to minimize its side effects. In our previous studies, the rat calvarial defect model was used to evaluate bone formation following implantation of recombinant human BMP-2 (rhBMP-2) at doses of 2.5, 5.0, 10.0, and 20.0 μg using absorbable collagen sponge (ACS) and biphasic calcium phosphate (BCP) as carriers and at 2 and 8 weeks of healing[17,18]. No remarkable dose-dependent bone-regenerative effects were observed. However, few studies have investigated the regenerative capacity of lower doses of BMP.

The optimum carrier system for BMP also remains to be determined. The United States Food and Drug Administration (US FDA) has approved the use of BMP with an ACS carrier for maxillofacial bone grafting procedures, since collagen carriers have a remarkable ability to promote the formation of new bone in the healing phase[5,19]. However, collagen is rapidly resorbed (i.e., within 2 weeks), and rhBMP-2 becomes separated from the collagen under physical pressure[20]. Moreover,

due to the limitations in its physical space-maintaining properties, it cannot conserve the space required for future bone regeneration. We therefore hypothesized that the ability to maintain space and a slow resorption rate are critical properties for BMP carriers, and particularly so when applying a limited-dose of BMP. Moreover, Escherichia Coli-derived recombinant human BMP-2 (ErhBMP-2) was chosen over rhBMP-2 derived from Chinese hamster ovary cells because it is cheaper to produce due to the improved cell-expression rate[21].

The aim of this study was to characterize the healing in rabbit calvarial bone defects in response to delivery of a limited-dose (1.5 μg) of ErhBMP-2, and evaluate BCP (hydroxyapatite/β-tricalcium phosphate at a ratio of 30/70) as a carrier compared with ACS.

# II. Materials and methods

## Experimental animals

Sixteen 16-week-old male New Zealand white rabbits, each weighing 3.0~3.5 kg, were used in this study. The animal selection, management, preparation, and surgical protocol were approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, South Korea (approval number 2013-0323).

## Study design

Four standardized, 8-mm-diameter circular bone defects were formed in the calvarial bone of each rabbit. These defects were allocated randomly to one of the following (Fig. 1):

1. Blood-filled (control) group—the defect was filled with nothing.

2. BCP group—the defect was filled with 0.1 g of BCP (30% wt hydroxyapatite granules and 70% wt β-tricalcium phosphate).

3. BMP+ACS group—the defect was filled with 1.5 μg of ErhBMP-2-loaded ACS (1/4 of CollaCote).

4. BMP+BCP group—the defect was filled with 1.5 μg of ErhBMP-2-loaded BCP (0.1 g).

The animals were allowed to heal with access to food and water ad libitum for either 2 weeks (n=8) or 8 weeks (n=8).

## ErhBMP-2 and carriers

The ErhBMP-2 used in this study (Novosis, Daewoong Pharmaceutical, Gyeonggi-do, South Korea) was supplied in bottles containing 0.1 mg of the protein, which was diluted with 10 ml of sterilized distilled water (to a concentration of 10 μg/ml). The assigned carrier (i.e., ACS or BCP) was then soaked for 10 min in 0.15 ml of this diluted ErhBMP-2 solution, thus achieving an ErhBMP-2 dose of 1.5 μg. The loaded carrier was subsequently applied to the planned rabbit calvarial defect.

The ErhBMP-2 carriers tested in the present study were BCP (BoncelOS, BioAlpha, Gyeonggi-do, South Korea) and ACS (CollaCote, Zimmer Dental, Carlsbad, CA, USA). The BCP had a hydroxyapatite/β-tricalcium phosphate composition of 30%/70% wt, and a ceramic particle size in the range 0.6–1.0 mm.

## Surgical procedure

The surgical sites were shaved and disinfection was achieved using povidone iodine under general anesthesia induced with 10 mg/kg tiletamine/zolazepam (Zoletil, Virbac Laboratories, Carros, France) and 5 mg/kg xylazine (Rompun, Bayer, Pittsburgh, PA, USA) by intravenous injection. An incision was made in the sagittal plane across the cranium, from the frontal to the occipital bone, and a full-thickness flap was lifted to expose the calvarial bone under localized infiltration anesthesia (2% lidocaine HCl; Huons, Seoul, South Korea). Four standardized circular 8-mm-diameter bone defects were formed around the sagittal plane using a trephine bur under saline irrigation, and the minimum distance between each bone defect was set

to at least 3 mm (Fig. 1). Three of the calvarial bone defects were filled with one of the following: BCP (0.1 g; BCP group), ErhBMP-2-loaded ACS (BMP+ACS group), or ErhBMP-2-loaded BCP (BMP+BCP group). The remaining calvarial bone defect was left unfilled and was set as a control group.

The experimental animals were sacrificed under general anesthesia with 10 mg/kg tiletamine/zolazepam (Zoletil, Virbac Laboratories) and 5 mg/kg xylazine (Rompun, Bayer) by intravenous injection at either 2 or 8 weeks postsurgery. Samples of calvarium including the defect site were harvested, fixed with 10% neutral formalin for 10 days, demineralized with 5% nitric acid for 5 days, embedded in paraffin, and then sectioned serially at a thickness of 7 μm. The sections were mounted onto glass slides, deparaffinized, and then stained with hematoxylin–eosin to enable histological examination.

## Evaluation

### Clinical findings

The animals were evaluated during the dressing and stitch-removal procedures, and during the 2- and 8-week postsurgery periods for possible complications such as inflammation, allergic reaction, and exposure of the graft material.

### Micro-computed tomography findings

All harvested specimens were scanned with a high-resolution micro-

computed tomography system (SkyScan 1173, SkyScan, Aartselaar, Belgium) at a resolution of 13.85 μm (achieved using settings of 130 kV and 60 μA), and with a 1.0-mm aluminum filter and an exposure time of 500 ms. Radiographic images were produced with the aid of reconstruction software (Nrecon, SkyScan; DataViewer, Brucker, Kontich, Belgium). The color range of the cross-sectional radiographic view was set in the range of 56–255 to avoid scan noise.

**Histological and histomorphometric findings**

For the histological and histomorphometric analyses, all of the specimens were observed using a binocular microscope (Leica DM LB, Leica Microsystems, Wetzlar, Germany) equipped with a video camera (DC300F, Leica Microsystems), which was used to capture images.

The structures between the defect margins in the images, including new bone, residual materials, and fibrovascular, adipose, and marrow tissues, were selected and layered separately using image-processing software (Photoshop CS6, Adobe Systems, San Jose, CA, USA), and the following three histomorphometric parameters (Fig. 2) were measured using image-analysis software (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA):

1. Augmented area—the area including new bone, residual material, and fibrovascular, adipose, and marrow tissues in the bone defect.
2. New bone area—the area of newly formed bone in the bone defect.
3. Residual materials—the area of residual, unresorbed graft materials.

**Statistical analysis**

Data analyses were performed using SPSS software (version 20, SPSS, IBM, Chicago, IL, USA). The measured parameters are summarized within the same experimental group in terms of mean values and standard error of the mean (SEM). The relationship between the healing obtained at 2 and 8 weeks within the same group was compared using the Mann-Whitney test, while differences between the four groups within the same healing period were assessed using the Friedman test and Bonferroni post-hoc procedure. However, the area of residual materials for a given healing period was compared using the paired Wilcoxon signed-ranks test. The cut-off for statistical significance was set at $\alpha=0.05$.

# III. Results

## Clinical observations

No specific adverse findings (including infection, inflammatory response, or pus formation) were observed for any of the calvarial defect sites during the postoperative period. Furthermore, no complications were evident on histological sections. Ultimately, 64 specimens from 16 rabbits were investigated histologically and histomorphometrically.

## Micro-computed tomography findings

The defect coverage was minimal at 2 weeks of healing, and almost all of the margins remained, clearly surrounding the defects when compared to the 8-week healing case (Fig. 3). Nevertheless, the amount of new bone formation was markedly greater in the ErhBMP-2-treated groups than in the other groups at both 2 and 8 weeks of healing.

Cross-sectional radiographic views of the BMP+ACS group at 2 weeks revealed extension of the radiopaque phase from both sides (Fig. 4C), and by 8 weeks bone had formed on both sides to form a bony bridge (Fig. 5C). However, that bony bridge lay at the bottom of the defect. In contrast, this bony collapse was only rarely found in the BCP and BMP+BCP groups (Figs. 4 and 5).

The healing observed at 2 weeks did not differ significantly between the BCP and BMP+BCP groups (Fig. 4). However, by 8 weeks postsurgery there were increased yellow spots histologically, indicating ossification, in the BMP+BCP group, and a greater bone density and defect coverage than in the BCP group (Fig. 5B and 5D).

**Histological findings**

A small amount of new bone could be detected in the control group, which was due to volumetric shrinkage of the defect area. The collapsed defect was filled with adipose tissue, muscle ingrowth, and dense and neat collagen fibers running parallel to the bone defect (Figs. 6A and 7A).

There was no noticeable volume shrinkage in the BCP group at either 2 or 8 weeks of healing. An increase in new bone formation was observed at 8 weeks compared to 2 weeks, with an opposing alteration in the amount of residual materials. However, most of the residual BCP particles remained in a nonabsorbed state, without surrounding osteoid tissue, at the 8-week healing time point (Figs. 6B and 7B).

Good defect closure was observed in the BMP+ACS and BMP+BCP groups. In particular, there was extensive new bone formation in the BMP+BCP group (Figs. 6D and 7D). In the BMP+BCP group the residual BCP particles were surrounded by new bone, and especially so at 8 weeks of healing. All defects exhibited marked bone

bridging, and the new bone had matured compared to that observed at 2 weeks (Fig. 7D, left). Calcified cartilage had formed around the newly formed, immature bone in this group (Fig. 7D, right). However, due to the inability of ACS to conserve space, structural collapse could be observed in the BMP+ACS group (Figs. 6C and 7C).

## Histomorphometric findings

The defect volume differed significantly between the 2- and 8-week healing periods only in the BMP+ACS group ($p<0.05$; Table 1, Fig. 8A). The size of the augmented area appeared to be slightly increased in the control group, and slightly decreased in the BCP, BMP+BCP groups. Statistically significant differences were observed between the control and the BCP, the control and the BMP+BCP groups at 2 weeks, and between the control and the BCP, the control and the BMP+BCP groups, the BMP+ACS and the BMP+BCP groups at 8 weeks ($p<0.05$).

Quantitative histomorphometry revealed that in all groups there was significantly more newly formed bone at 8 weeks than at 2 weeks postsurgery ($p<0.05$; Table 1, Fig. 8B). Statistically significant differences were observed between the control and the BCP, the control and the BMP+BCP groups at 2 weeks, and between the control and the BMP+BCP, the BMP+ACS and the BMP+BCP groups at 8 weeks ($p<0.05$). Despite the presence of denser and more well-differentiated new bone formation in the BMP+ACS group compared to the BCP group, collapse of the defect site in the BMP+ACS groups ultimately meant that the

amount of new bone formation in that group at both 2 and 8 weeks postsurgery was slightly less than in the BCP group at both healing periods.

The BMP+ACS group was not included in the analysis of 'residual materials' because the histological properties of ACS are difficult to differentiate from the natural connective tissue. The amount of residual materials differed significantly between 2 and 8 weeks of healing in both the BCP and BMP+BCP groups ($p<0.05$; Table 1, Fig. 8C), but it did not differ significantly between these two groups at the same healing time point.

# IV. Discussion

The objective of this study was to determine the bone-regenerative capacity of a limited-dose (1.5 μg) of ErhBMP-2, and the effectiveness of BCP carrier systems for ErhBMP-2 in the rabbit calvaria defect model. Previous studies have found no significant differences in rat calvarial bone regeneration among different BMP doses, and that a low (2.5 g) dose of rhBMP-2 significantly induced new bone formation[17,18] Pelaez et al.[22] noticed that the threshold bone-regeneration capacity may already be reached at an rhBMP-2 dose of 1.25–2.5 μg in the rat calvarial critical-size-defect model. The findings of other studies support this theoretical background[23-25]. However, a few studies have revealed a dose-dependent response[26-28].

Most studies using the rabbit calvarial defect model have applied doses of BMP higher (5–10 μg)[29-31] than in those using the rat calvarium. In the present study, although the applied dose of ErhBMP-2 was lower than in previous studies, new bone formation with active angiogenesis was observed. In particular, as demonstrated in Figs. 6B, 6D, 7B, and 7D, BCP particles in contact with new bone could be observed in the ErhBMP-2-loaded groups. Even in Fig. 7D, calcified cartilage— cartilage in which calcium salts are deposited in the matrix, a characteristic of endochondrial ossification—can be found. Although the skull is formed by intramembranous ossification, BMP is involved in the formation of stem cells via the processes of proliferation, maturation, and hypertrophy of chondroblasts[32]. This may explain the observation of endochondrial ossification in the calvarial bone in the present study. In

addition, large amounts of blood vessels and red blood cells can be observed in Figs. 6C, 6D, 7C, and 7D, and the hard tissue was more mature than in the non-ErhBMP-2-loaded group.

Histomorphometric analysis revealed that administration of 1.5 μg of ErhBMP-2 on a BCP carrier produced significantly more new bone formation than the control, and BCP alone showed similar or slightly more new bone formation than when the protein was delivered using ACS. The use of BCP as a carrier for ErhBMP-2 appears to prevent the upper structural collapse that occurs due to soft-tissue pressure, and to result in a larger bone regenerative field due to the wider augmented area than with ACS (Fig. 8A).

The possibility that the rhBMP-2 loaded onto either BCP or ACS affected the surrounding non-BMP-loaded site must be addressed. Lee et al.[33] set the distance between defects at 2 mm and then analyzed the sites histologically and histomorphometrically to determine whether rhBMP-2 affects nearby bone defects after 2 and 8 weeks healing. Those authors found no differences in either the healing pattern or the characteristics of each variable in these sites, indicating that a control defect is unlikely to be affected by rhBMP-2 applied to an adjacent site at this separation distance. The distance between the defects was therefore set to be at least 3 mm in the present study.

Despite the finding that the critical size of defects made in rabbit calvaria is >10 mm, and critical sizes of 17 mm have even been reported[34,35] circular 8-mm diameter defects were chosen for evaluating the bone healing in the present study.

Sohn et al.[36] reported that 8-mm defects do not fully heal within 8 weeks, but that a closure of approximately 65% is observed, and 39% of the defect area was filled with new bone following spontaneous bone healing. However, the 8-mm rabbit calvaria defect model can show the early phase of healing at postsurgery time points of 2–4 weeks, and at 8 weeks the late phase of healing can be observed, as reflected by resorption of materials, new bone formation, and bone remodeling, for example.

rhBMP-2-loaded ACS has been approved by the US FDA for the promotion of bone formation in maxillofacial applications since March 2007[37], and Lee et al.[38] suggested that the quality of the new bone generated using rhBMP-2-loaded ACS is significantly better than that achieved with autogenous bone grafts. However, rhBMP-2 separates from ACS rapidly in the presence of compressive forces, and it may be resorbed within 2 weeks[20], or even (according to Yamamoto et al.[39]) within 7 days in vivo. Furthermore, uncontrolled rhBMP-2 release from ACS has been demonstrated[40], and it is difficult to maintain volumetric stability under the pressure of the surrounding tissues. Kim et al.[41] compared ACS and BCP as BMP carriers and found that in the ACS groups there was a failure to increase the amount of new bone at 8 weeks over that generated at 2 weeks due to the inability of ACS to conserve space in the defects.

Finally, structural collapse of the bone defect site was observed in the BMP+ACS group in the present study, and there was a significant reduction in the augmented area between 2 and 8 weeks postsurgery (Fig. 8A). This particular disadvantage of ACS as a BMP carrier would be greater with limited-doses of

ErhBMP-2. However, ACS confers positive effects such as retention of blood clots, absorption of BMP, and adaptability[42]. Therefore, applying the collagen mixture together with calcium phosphate as a combined carrier for BMP may be worth considering[31].

# V. Conclusion

ErhBMP-2 accelerates bone regeneration in rabbit calvarial defects even at a limited-dose (1.5 μg). BCP seems to be a more effective carrier than ACS for ErhBMP-2.

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# Figure Legends

**Figure 1.** Surgical procedure. (A) Calvarial defects (8 mm in diameter) were created. The minimum distance between each bone defect was set to 3 mm. (B) The four defects were filled with different materials: (1) BCP, (2) ErhBMP-2 loaded onto ACS (BMP+ACS), (3) ErhBMP-2-loaded onto BCP (BMP+BCP), or (4) blood-filled (control) group.

**Figure 2.** Scheme for the histomorphometric analysis of the calvarial defect model.

**Figure 3.** 3-D-reconstructed radiographic views at (A) 2 weeks of healing and (B) 8 weeks of healing. The four defects were filled, as labeled, with BCP, BMP+ACS, or BMP+BCP, or blood-filled (control).

**Figure 4.** Cross-sectional radiographic views obtained at 2 weeks of healing in (A) the blood-filled (control) group, (B) the BCP group, (C) the BMP+ACS group, and (D) the BMP+BCP group. Residual material is a noticeable blue color in the picture.

**Figure 5.** Cross-sectional radiographic views obtained at 8 weeks of healing in (A) the blood-filled (control) group, (B) the BCP group, (C) the BMP+ACS group, and (D) the BMP+BCP group.

**Figure 6.** Histological views of rabbit calvarial defects at 2 weeks of healing in (A) the blood-filled (control) group, (B) the BCP group, (C) the BMP+ACS group, and (D) the BMP+BCP group (hematoxylin and eosin stain). The boxed areas in the left panels (magnification ×40) are magnified in the corresponding panels on the right (×100). The defect margin is labeled with an arrowhead. BV, blood vessels; FT, fibrous tissue; NB, new bone; RM, residual material.

**Figure 7.** Histological views of rabbit calvarial defects at 8 weeks of healing in (A) the blood-filled (control) group, (B) the BCP group, (C) the BMP+ACS group, and (D) the BMP+BCP group (hematoxylin and eosin stain). The boxed areas in the left panels (magnification ×40) are magnified in the corresponding panels on the right (×100). The defect margin is labeled with an arrowhead. AT, adipose tissue; BV, blood vessels; CC, calcified cartilage; FT, fibrous tissue; MI, muscle ingrowth; NB, new bone; RM, residual material.

**Figure 8.** Histomorphometric analysis of various parameters at 2 and 8 weeks of healing following calvaria defect filling with BCP, BMP+ACS, or BMP+BCP, and unfilled (control). (A) Augmented area; (B) area of new bone; (C) area of residual materials. Data are mean and SEM values at each time point. *Statistically significant at the given time ($p<0.05$).

# Table

**Table 1.** Augmented area, new bone area, and residual materials at 2 and 8 weeks postsurgery.

| Group | Augmented area | | New bone area | | Residual materials | |
|---|---|---|---|---|---|---|
| | 2 weeks ($mm^2$) | 8 weeks ($mm^2$) | 2 weeks ($mm^2$) | 8 weeks ($mm^2$) | 2 weeks ($mm^2$) | 8 weeks ($mm^2$) |
| Control | 3.43±0.39 | 4.28±0.75 | 0.73±0.10 | 1.99±0.46* | - | - |
| BCP | 19.12±1.24† | 16.92±0.87† | 2.14±0.40† | 3.39±0.30* | 4.65±0.30 | 3.58±0.32* |
| BMP+ACS | 8.66±1.04 | 5.41±0.43* | 1.94±0.27 | 3.04±0.27* | - | - |
| BMP+BCP | 20.33±1.01† | 19.62±0.77†‡ | 3.87±0.40† | 6.88±0.74*†‡ | 4.43±0.17 | 3.60±0.28* |

Data are mean±SEM values for each group. Note that BMP+ACS group was not included in this analysis because the histological properties of ACS are difficult to differentiate from the natural connective tissue.

* Statistically significant difference compared to 2weeks ($P<0.05$).

† Statistically significant difference compared to control group ($P<0.05$).

‡ Statistically significant difference compared to BMP+ACS group ($P<0.05$).

## Figures

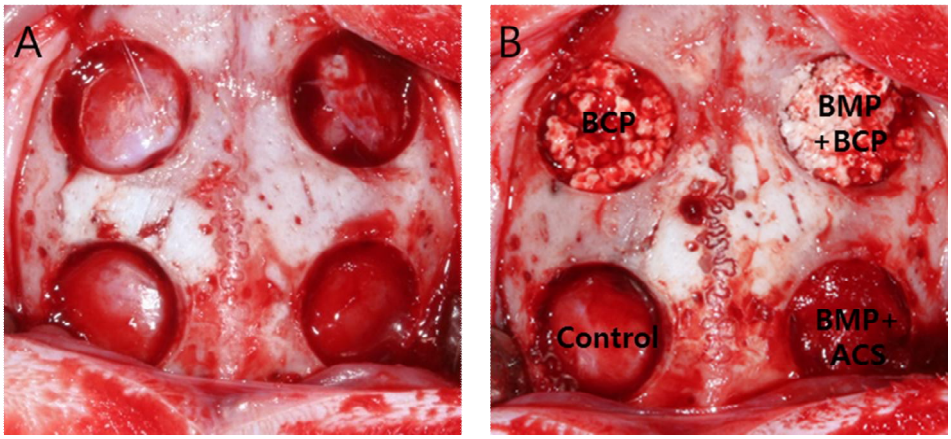
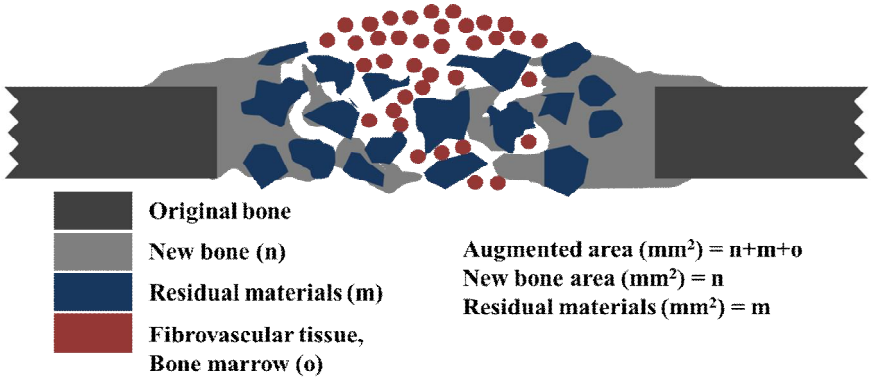



**Figure 1**

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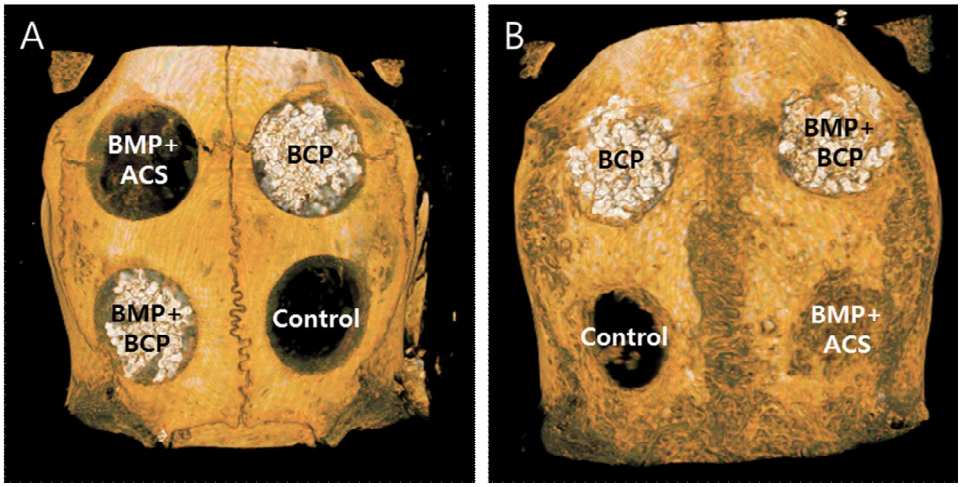


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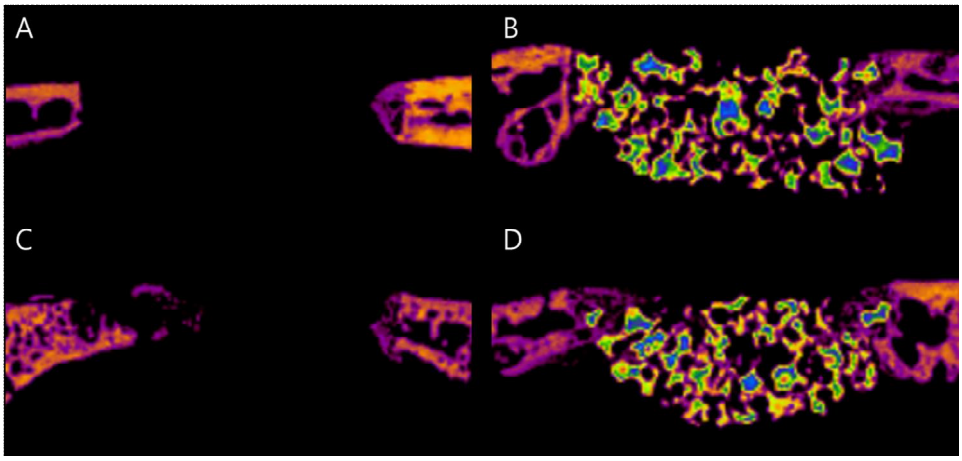


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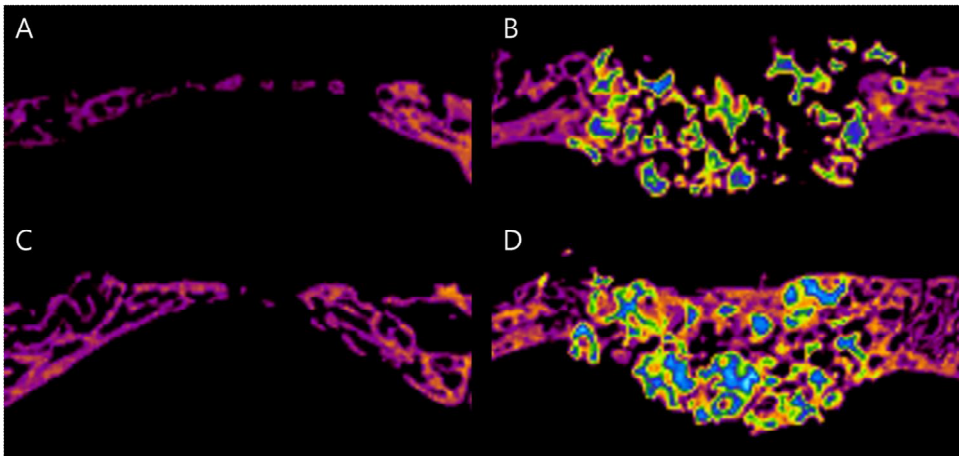


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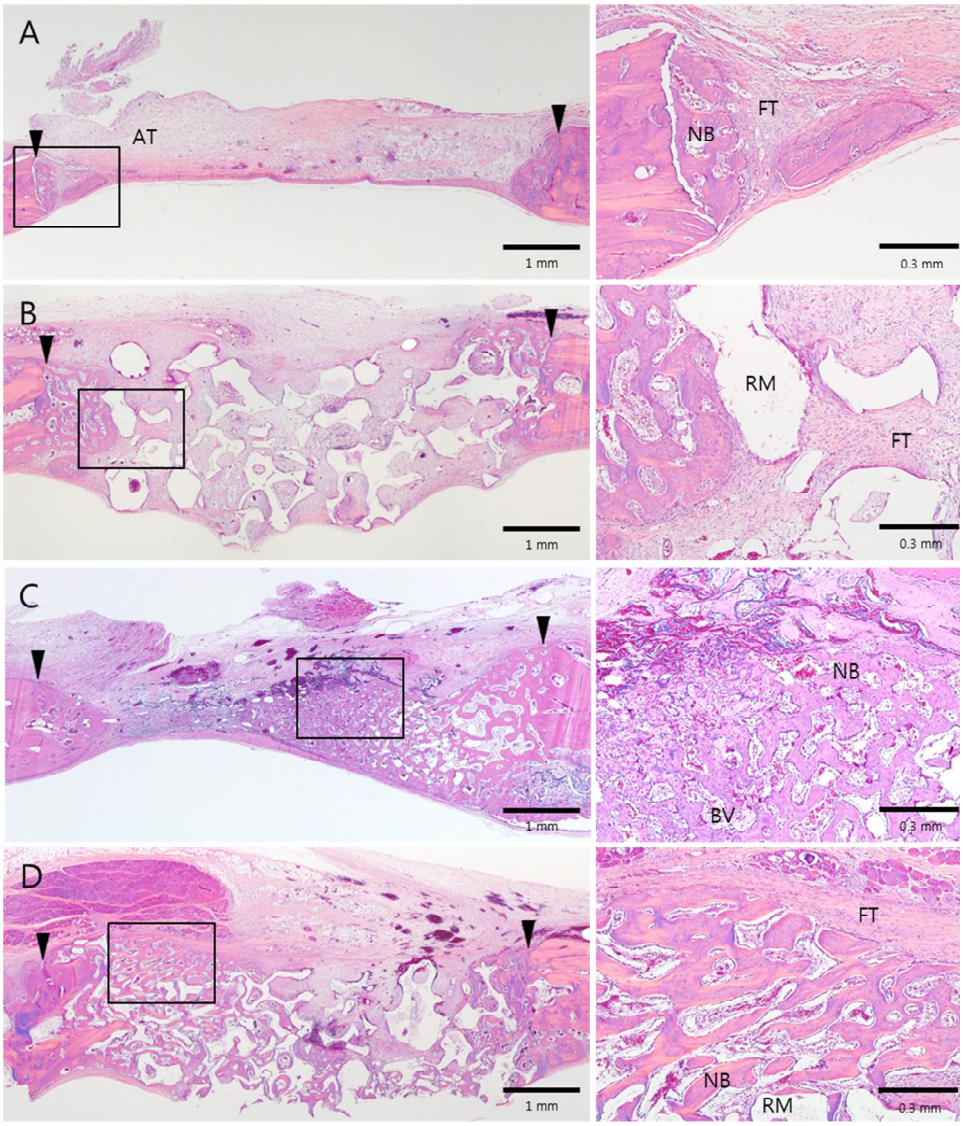


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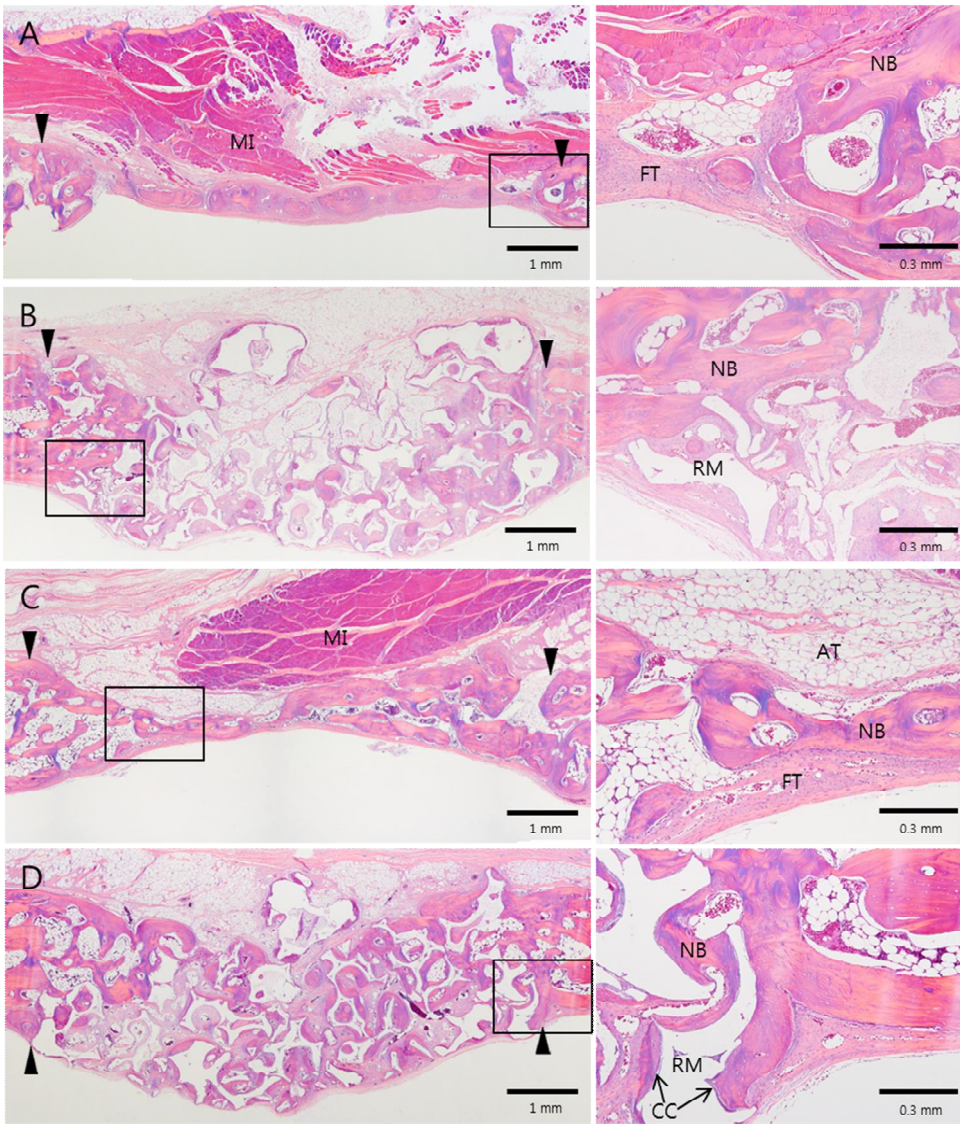


**Figure 7**

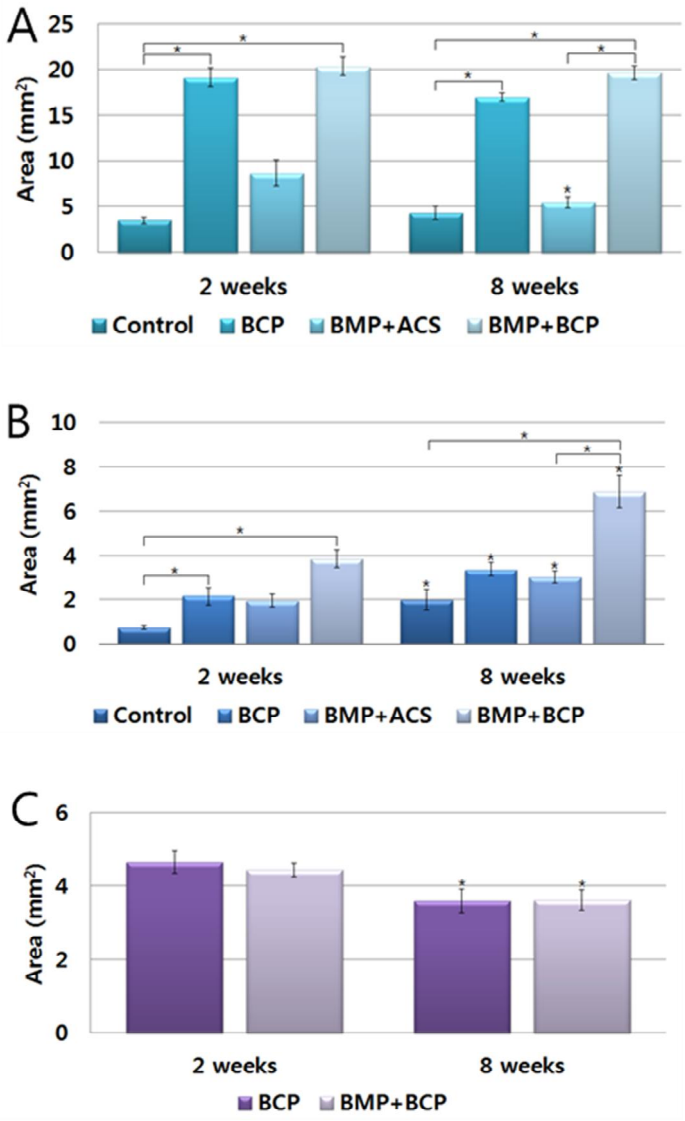


**Figure 8**

국문요약

# 토끼 두개골에서 제한된 용량의 골형성 단백질과 이상인산칼슘 전달체를 적용한 후의 골재생능 평가

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**유 훈**

지금까지 많은 연구들을 통하여 골형성 단백질의 골유도능은 다각도로 입증되었지만, 위 단백질의 적용으로 인한 부작용이 여러 연구들에서 용량 의존적으로 보고된 바 있다. 또한, 콜라겐은 FDA 에서 승인받은 골형성 단백질의 전달체이지만, 신생골이 형성되기 전에 조직내에서 용해되는등 공간 유지능에 대한 의문이 제기되어 왔다.

따라서, 본 연구의 목적은 이상인산칼슘 (30/70 비율로 혼합된 hydroxyapatite/β-tricalcium phosphate) 또는 콜라겐 전달체를 이용하여, 제한된 용량(1.5 ㎍)의 골형성 단백질을 적용하였을 때 토끼 두개골 재생능을 평가하는 것이다.

16 마리 토끼의 두개골에 각각 4 개의 8mm 크기의 원형 결손부를 형성하였다. 각각의 결손부는 (1) 아무것도 처리하지 않은 군 (대조군), (2)

이상인산칼슘 군 (BCP 군), (3) 골형성 단백질 적용 콜라겐 군 (BMP+ACS 군), (4) 골형성 단백질 적용 이상인산칼슘 군 (BMP+BCP 군) 으로 구성되었다. 실험동물들은 2 주 또는 8 주 동안 치유기간을 가졌고 임상적, 방사선학적, 조직학적, 그리고 조직형태계측학적 분석을 통해 평가되었다.

미세 컴퓨터 단층촬영을 분석한 결과 BMP+ACS 군과 BMP+BCP 군에서 새로운 골 형성이 광범위하게 이루어진 것이 확인되었다. 그러나 BMP+ACS 군에서는 상단의 결함부위 경계에서 골의 함몰이 발생했다. 조직형태계측학적 결과 모든 군에서 8 주 치유기간을 가졌을 때 2 주에 비해 통계학적으로 유의차 있게 더 많은 신생골이 형성됨을 볼 수 있었다 ($p<0.05$). 또한, 8 주의 치유기간을 가진 BMP+BCP 군 ($6.88\pm0.74mm^2$, $19.62\pm0.77mm^2$) 에서는 신생골 형성 및 골부피 증강에 있어서 BMP+ACS 군 ($3.04\pm0.27mm^2$, $5.41\pm0.43mm^2$) 과 통계학적으로 유의미한 차이가 있었다.

이상의 연구를 통해, 토끼 두개골 결손부에서 제한된 용량의(1.5 $\mu g$) 골형성 단백질을 적용하였음에도 불구하고, 골형성 단백질은 골재생을 촉진하는 것으로 보인다. 그리고 본 연구에서는 콜라겐보다는 이상인산칼슘이 더 효과적인 골형성 단백질의 운반체임을 확인할 수 있었다.

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**핵심되는 말** : 이상인산칼슘, 골형성 단백질, 골재생, 골 이식재, 가토 두개골