Bone formation by *Escherichia coli*-expressed rhBMP-2 using MBCP block carrier in rat calvarial defect and ectopic subcutaneous models

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A Dissertation Thesis
Submitted to the Department of Dental Science,
the Graduate School of Yonsei University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy of Dental Science

Sung Soo So

June 2009

This certifies that the dissertation thesis
of Sung-Soo So is approved.

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June 2009

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감사합니다.

2009년 6월

저자 씀

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Abstract

Bone formation by *Escherichia coli* -derived rhBMP-2 using MBCP block carrier in rat calvarial defect and ectopic subcutaneous models

E.coli-expressed rhBMP-2 (bone morphogenic protein) is currently being studied as an alternative to mammalian rhBMP-2. MBCP is a useful carrier for BMPs as its porous structure, low resorption rate, and favorable osteoconductive and bioactive properties allow it to serve as a scaffold for bone forming cells.The purpose of the present study was to evaluate bone formation by *E.coli*-derived rhBMP-2 (ErhBMP-2) using a macroporous biphasic calcium phosphate (MBCP) block carrier in a male Sprague-Dawley rat calvarial defect and ectopic subcutaneous models. Rats were divided into two groups, a MBCP carrier control and a ErhBMP-2/MBCP group and were evaluated by histologic and histometric analysis following a 2- or 8- week healing interval. For the calvarial defect model, enhanced new bone formation was observed in ErhBMP-2/MBCP group. For the ectopic subcutaneous model, few new bone formation was evident in the ErhBMP-2/MBCP group. The MBCP block was effective as a carrier for *E.coli*-expressed rhBMP-2 for new bone formation, which

was stably maintained throughout the healing period. The MBCP block system could prove to be a useful carrier system for tissue engineering using *E.coli*-expressed rhBMP-2.

Key Words: Bone morphogenetic protein; Ectopic bone formation; Bone regeneration; Bone tissue engineering; *Escherichia coli ;* Macroporous biphasic calcium phosphate

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I. INTRODUCTION

Bone morphogenic proteins (BMPs) belong to the transforming growth factor-ß (TGF-13) superfamily. Urist, in 1965, observed that ectopic bone formed around a demineralized bone matrix when transplanted hypodermically or intramuscularly in a rat model, and extracted a substance called Bone Morphogenetic Proteins (BMPs)[1)]. It was not until Wozney and his colleagues succeeded in cloning a BMP in 1988 for the first time that serious study on BMPs began. BMP-2 was produced from Chinese hamster ovary (CHO) cells[2,3)], and human BMP-4 was produced from a mouse

myeloma cell line (NSO) and a human embryonic kidney cell line (292). Human BMP-7 (OP-1) was produced from a CHO cell and a primate cell line[4].

These BMPs have proven to be capable of bone formation but have been limited in their clinical application due to their high production costs. Many researchers are now directing their efforts towards improving extraction and purification methods or towards devising new methods to produce BMPs artificially for clinical application. Thus far, BMP-2 and BMP-4 have been successfully produced using bacterial systems such as *E. coli* and their activities have been verified both *in vivo* and *in vitro*[5-8]. The production of rhBMP using an *E.coli* expression system has greatly reduced the cost of BMP production, thus making the clinical application of BMP more feasible.

In order to use this new source of BMPs, it is necessary to use an appropriate carrier; one that will not be harmful to surrounding tissue, that will be absorbed as the new bone forms, and that is simple to use[9]. A number of studies have investigated bone matrix[10], absorbable collagen sponge (ACS)[11-15], fibrin sealant[16-18], synthetic polymers[19], β-tricalcium phosphate (β-TCP)[13,14,18,20,21], and acellular dermal matrix (ADM), but a carrier that perfectly satisfies these requirements has yet to be discovered. One potential candidate is macroporous biphasic calcium phosphate (MBCP), which has a porous structure, a low resorption rate, and favorable osteoconductive and bioactive properties[22,23]. Ease of manipulation and ready

formation into required shapes, especially for block types, are additional merits of this biomaterial.

The purpose of this study was to evaluate the bone formation capacity of ErhBMP-2 and the potential for a MBCP block to act as a carrier in rat calvarial defect and ectopic subcutaneous models.

II. MATERIALS AND METHODS

1. Animals

Sixty four male Sprague-Dawley rats, ranging in weights from 250-300g, were used in this study. The rats were maintained in plastic cages in a 12 h-day/night cycle room at a temperature of 21°C. They had *ad libitum* access to water and standard feed pellets. Animal selection, management, surgical protocols, and preparation were in conformance with the guidelines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea.

2. Expression and Purification of rhBMP-2 in *E.coli*

2.1 Cloning of Human BMP-2 gene

U2OS cell was cultivated in DMEM (Gibco BRL, NY, USA) medium including 10% fetal bovine serum, 100unit/ml penicillin, and 100ug/ml streptomycin at 37℃ in CO2incubator (Thermo Electron Co, Ohio, USA) supplying 5% CO_2. Reverse transcription was performed after extraction of total cellular RNA from the U2OS cell using Trizol (Gibco BRL, NY, USA) solution to acquire human BMP-2 gene. With

cDNA as a primer, 5'-AGAAGAACATATGCAAGCCAAACACAAA CAGCG G-3' as a sense primer, and 5'-AATTTTACAGCTTCTAGCGACACCCA CAACCCT-3' as an antisense primer, polymerase chain reaction (PCR) was conducted. Then, the product of the PCR was separated and inserted into the pGEM-T vector (Promega, USA), followed by cloning employing E.coli (DH5α) cell. Transgenic material was selected and rhBMP-2 gene was inserted into the expression vector (pRSET(A)), which was named as pRSET(A)/hBMP-2. BL21(DE3) was inoculated in 50 ug/ml ampicillin added LB medium after transformed into pRSET(A)/hBMP-2, and its expression was confirmed having going through cultivation at 37℃.

2.2 The high-cell-density cultivation

The high-cell-density cultivation was accomplished using the method described by Fatemeh and others[25,26] in the fermenter (KoBioTec, Incheon, Korea). Then, a single colony was inoculated in 300 ml of ampicillin (200 ug/ml) added LB liquid medium, and cultured for 16~18 hours at 37℃ with the cycle of 200 rpm, which resulted seed culture as a product. We inoculated the seed culture into the 10% batch culture (yeast extract 1 g/L, peptone 2g/L), which was then agitated in 250rpm for 24 hours at 30℃ in the fermenter. The absorbance at 600nm was measured throughout the cultivation by periodically attaining samples. When A600 became 3~4, we cultured it for 24 hours adding sterilized nutrient medium (Glucose 33.3 g/L, peptone

10 g/L, yeast extract 5 g/L, MgSO4 1 g/L, CaCl2 0.048 g/L, ZnSO4 0.0176 g/L, CuSO4 0.008 g/L).

2.3 Protein Purification

The medium cultured for 24 hours was then collected. After going through centrifuge (Sovall, CA, USA) for 10 minutes at 4℃, 8,000xg, the resulted cell bodies were precipitated and supernatant was removed. The cell precipitation was washed two times with 50mM sodium phosphate buffer (pH 7.0), and emulsified into the concentration of 220 mg (weight of wet matter)/L. The suspension was stored in deepfreezer (Nihon Freezer, Japan) at -80℃. Then, we broke the cells by applying pressure after thawing the frozen suspension, and centrifuged for 45 minutes at 5,500xg, 4℃. After centrifugation, the precipitation was suspended again with 50mM sodium phosphate buffer (pH 7.0), and centrifuged again. The resulted precipitation was stored at -80℃.

After that, the frozen precipitation was re-suspended in buffer A (20mM Tris-HCl (pH 8.5), 0.5 mM EDTA, 1% (v/v) TritonX-100) in concentration of 2 mg (weight of wet matter)/L. The suspension was centrifuged at 26,000xg, 4℃ for 30 minutes and the inclusion bodies were collected. It is followed by the addition of solubilization buffer (4M Guanidine-HCl, 0.1 M Tris-HCl (pH 8.5), 0.1 M dithiothreitol,1 mM EDTA) and dissolution of protein with 16~18 hours of slow

agitation.

Then, insoluble matters were precipitated and removed after 45 minutes of centrifugation at 26,000xg, 4℃. The solution with dissolved protein was diluted in renaturation buffer (0.5 M Guanidine-HCl, 50 mM Tris-HCl(pH 8.5), 0.75 M 2-(cyclohexylamino) ethanesulfonic acid (CHES), 1 M NaCl, 5mM EDTA, 5 mM total Glutathione) and stored for 72 hours, which is followed by separation of soluble and insoluble material with 10 minutes of centrifugation at 38,000xg, 4℃. If the mature rhBMP-2 gone through re-denaturation comes to have its original tertiary structure, the N-terminus attains heparin-binding site[24]. Utilizing such characteristic, we purified refolded rhBMP-2 dimer separating it from monomer using Heparin Sepharose 6 Fast Flow column (GE healthcare, USA). The most of the proteins which went through re-denaturation bonded with heparin column, and we eluted them varying the concentration of NaCl. Finally, we isolated them with 3 levels of concentration gradient after confirming the elution of rhBMP-2 with constructing the continuous concentration gradient of NaCl.

3. ErhBMP-2 Implant Construction

Disc-shaped MBCP implants (3mm ht. /8mm dia.) were manufactured (Biomatlante, Vigneux de Bretagne, France). ErhBMP-2 (Cowellmedi Co. LTD,

Pusan, Korea) was produced and diluted in a buffer to a concentration of 25μg/ml. For ErhBMP-2/MBCP implants, MBCP implants were loaded an hour before surgery with 0.2 ml of ErhBMP-2 solution. MBCP implants are shown in Figure 1.

4. Experimental and Control Groups

Sixty four male Sprague-Dawley rats were used. The animals were divided into 2 groups of 16. One set was allowed to heal for 2 weeks, and the second set for 8 weeks. Each animal received either MBCP carrier alone (control) or ErhBMP-2/MBCP as an experimental treatment.

5. Surgical Procedures

The animals were anaesthetized by an intramuscular injection (5 mg per kg body wt.) of a 4:1 solution of ketamine hydrochloride (Ketalar®, Yuhan Co., Seoul, Korea) : Xylazine (Rompun®, Bayer Korea, Seoul, Korea). At the surgical site, routine infiltration of anaesthesia was used (2% lidocaine, 1:100,000 epinephrine, Kwangmyung Pharm., Seoul, Korea).

5.1 Calvarial defect model

An incision was made in the sagittal plane across the cranium. A full thick flap was reflected to expose the calvarial bone. A standardized, circular, transosseous defect, 8 mm in diameter, was created on the cranium using a saline-cooled trephine drill (3i, Palm Beach Gardens, FL, USA). The periosteum and skin were closed with an absorbable monofilament suture (Monosyn®, Aesculap AG Co. KG., Tuttlingen, Germany) for primary intention healing.

5.2 Ectopic subcutaneous model

A vertical incision was made in the skin of the back. After flap reflection, a subcutaneous pocket was prepared by a blunt instrument. Each animal received either a MBCP carrier (control) or ErhBMP-2/MBCP as an experimental treatment. The periosteum and skin were closed with absorbable monofilament suture.

6. Evaluation methods

6.1 Histology Procedures

Two and eight weeks after surgery, the animals were sacrificed by CO_2 asphyxiation. The block sections, including the experimental sites, were removed and fixed in a 10% neutral buffered formalin solution for 10 days. The samples were

decalcified using 5% formic acid for 14 days, then embedded in paraffin. Serial sections, 7μm thick, were prepared at 80 μm intervals, stained with hematoxylin/eosin (H-E), and examined by optical microscopy. The most central sections from each block were selected for the histology and histometric evaluation.

6.2 Histometric Procedures

Computer-assisted histometric measurements were acquired by an automated image analysis system (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA), coupled with a video camera, attached to an optical microscope. The sections were examined at magnifications of x 25 and x 100. The histometric parameters are shown in Figure 2.

- Augmented area (mm^2): all tissues within the boundaries of the MBCP carrier, i.e. new bone, fatty marrow, fibrovascular tissue/marrow and residual biomaterial.
- New bone area (mm^2): the area of newly formed bone within the total augmented area.

7. Statistical Analysis

The histometric recordings from the samples were used to calculate the means and standard deviations (mean ± SD). The interactions between the healing interval and treatment condition were examined using a two-way analysis of variance (two-way ANOVA). A unpaired t-test was used for the comparisons between the two groups. A p-value <0.01 was considered significant.

III. RESULTS

1. A rat calvarial defect model

1.1 MBCP control group

Histological and histometric results are shown in Figure 3 and Tables 1 and 2. After 2 weeks, there were no significant histological indications of inflammation or foreign body reactions. A minimal amount of new bone formation was present adjacent to the margins of the defect and below the MBCP block. Along the surface of the macropores in the lower part of the implant, there was evidence of osteogenic activity in the form of a dense osteoblast-like cell lining, osteoids, and bone apposition. The macropores on the upper surface were typically filled with loose fibrous connective tissue. Bone-forming activity was rare on this surface.

At 8 weeks, the majority of the macropores in the lower part of the block had filled with newly formed bone. The specimens showed a more advanced stage of regeneration and consolidation. Loose or dense fibrous connective tissue was observed in the central and upper part of the block.

There was no significant absorption of the MBCP block over this healing period.

1.2 ErhBMP-2/ MBCP group

Histological and histometric results are shown in Figure 4 and Tables 1 and 2. At 2 weeks, the bone healing results were similar to those seen for the MBCP control group. The macropores in the lower part of the block were predominantly filled with new bone. The majority of the defect sites below the block were bridged with new bone. Bone forming activity in the central and upper part of the block was still minimal. In the case of macropores with bone forming activity, new bone that was associated with cemental lines was in direct contact with the surface of the MBCP. In the central area, an osteoblast-like cell lining was present. This suggests that bone formation and mineralization were proceeding inwards from the implant surface.

At 8 weeks, the quantity of new bone below the block and in the macropores in the lower part of the block was greater than that observed at 2 weeks. Overall, the specimens showed a more advanced form of regeneration and consolidation. The macropores in the upper part of the block were predominantly filled with new bone. Macropores with fibrous connective tissue were noted in the central part of the block, as were macropores with newly formed bone. This newly formed bone was comprised of woven and lamellar bone and showed cemental lines that were separated from the more recently deposited bone. There was no evidence of cartilage formation. However, fatty marrow was present in the center of the newly formed bone.

2. A rat ectopic subcutaneous model

2.1 MBCP control group

Histological and histometric results are shown in Figure 5 and Tables 3 and 4. At 2 weeks, there was minimal histological evidence of inflammation or foreign body reactions. MBCP blocks were enclosed by loose connective tissue. The block macropores were permeated with connective tissue. No indication of osteogenic activity could be discerned histologically. At 8 weeks post-surgery, the implanted block was encompassed with dense fibrous connective tissue. Adjacent to the margins of the block, a minimal amount of new bone formation was observed. There had been insignificant absorption of the MBCP block over the healing period.

2.2 ErhBMP-2/ MBCP group

Histological and histometric results are shown in Figure 6 and Tables 3 and 4. At 2 weeks, the macropores in the perimeter of the block were filled with new bone. Along the surface of the macropores, evidence of osteogenic activity, such as a dense osteoblast-like cell lining, osteoids, and bone apposition was present. Macropores in the center were often filled with loose fibrous connective tissue, but bone forming activity was rare.

At 8 weeks, the quantity of new bone was slightly greater than that observed at 2

weeks. Some macropores with fibrous connective tissue were also found in the center of the block. The extent of bone formation was significantly less than that seen for the calvarial defect model. There was no indication of cartilage formation.

IV. DISCUSSION

The objective of this study was to evaluate the ectopic bone formation of ErhBMP -2 using a MBCP block as a carrier in a rat calvarial defect model and in a subcutane --ous model. In the present study, ErhBMP-2 was clearly biologically active and osteo -inductive *in vivo*, as well as being quantitatively efficient. In addition, the MBCP lock proved to be an effective new carrier and clearly could substitute for currently used carriers.

Many studies have been carried out since Wozney first succeeded in cloning BMPs in 1988. The most significant finding was that rhBMP-2 and rhBMP -7 had the best capacity for osteoinduction[3,4], which resulted in many additional studies on these proteins. In terms of clinical studies, however, there has not been much significant progress because of the prohibitive costs of production and purification of BMPs. Moreover, a higher concentration of rhBMP is required to achieve effective bone formation in higher order species[24-26].

For these reasons, *E coli* expression systems for production of BMP-2 and BMP-4 have been developed and their activities have been verified *in vivo* and *in vitro* [5-8]. Because the production of rhBMP-2 using these systems has greatly reduced the overall costs of producing BMP, the clinical application of BMP is now feasible. The

results of the current study showed that the osteoinduction capacity of ErhBMP-2 is equal to that of the BMP produced by CHO cell expression systems [27-29], which is consistent with result obtained by Bessho30). The clinical application of the ErhBMP-2 used in this study is promising, especially as it can be produced at a lower cost. The fact that it is effective in osteoinduction at a low concentration provides not only an economical advantage but also increases its clinical safety.

Since rhBMP-2 is a water soluble substance, its efficacy may be reduced unless it is delivered efficiently to the application site[18]. This means that it is important to maintain consistent amounts, concentrations, application times and so on, in order to maximize its efficacy[31]. It is essential, therefore, to use an appropriate carrier that will be able to deliver rhBMP-2 effectively and maintain it at the site during the bone-forming process[9,13,18,37]. From a clinical point of view, a carrier for the delivery of BMPs should also be able to act as a scaffold for bone forming cells, providing a space where bone formation can occur. Additionally, this space should be maintained for a relatively long period of time, to allow proper maturation of the newly formed bone. For clinical bone tissue engineering, a pertinent factor in successful treatment is the groundwork behind the volume and shape of the bone tissue.

MBCP has a structure similar to that of human bone, in that it has a porosity of almost 70% and the mixture ratio between HA (hydroxyapatite) and ß-TCP is 6:4 [32-34]. HA is absorbed slowly, providing scaffolding, whereas ß-TCP is absorbed rapidly,

opening up spaces for bone formation. At present, MBCP is available in blocks, particulates, and as an injectable material in a polymer carrier[22,35]. MBCP has shown exceptional biocompatibility and bioactivity, on account of its porous structure. Since the micropore is less than 10㎛, it still allows for adequate fluid circulation, but rhBMPs are trapped within its pores. Thus, normally mobile rhBMP can be retained and its activity prolonged.

The porous structure of MBCP is also believed to allow cells and newly formed tissues to permeate into the block. The observation of new bone formation in the macropores of the MBCP block in the present study supports this suggestion. The macropores of more than 100㎛ act as a scaffold for bone cells, which then allows centripetal bone ingrowth[33]. The newly formed bone has direct contact with the biomaterial surface, which makes this biomaterial osteoconductive. Although this osteoconductive effect has been well-documented, the osteoinductive effect of MBCP makes it valuable for use as an alternative or additive to autogenous bone for orthopedics, traumatology, odontology, and dental applications[34,36]. Thus, the MBCP block should be recommended as a carrier for rhBMPs.

There is as yet no standard titer for rhBMP-2 treatment because the osteoinduction depends on the animal, the application body part, and the type and nature of the carrier used. The concentration-dependency of osteoinduction of rhBMP-2 is controversial because this is found to be reduced when the concentration

exceeds a threshold value. Pang and his colleagues studied the concentration-dependency in a rat calvarial defect model and reported no difference in osteanagenesis between concentrations of 5mg/ml and 25mg/ml[37]. In the current study, bone formation in the ectopic subcutaneous model was not observed as clearly as it was in the calvarial model. It is likely that a higher concentration of EhrBMP-2 is required in tissues such as the subcutaneous layer that have poor osteoinduction capabilities. Additional in depth study is needed on this aspect.

V. Conclusion

1. For the calvarial defect model, the experimental group showed significantly more osteoinduction than did the control group. In the experimental group, the majority of the defect sites below the block were bridged with new bone within 2 weeks. By 8 weeks, the macropores in the upper part of the block were also predominantly filled with new bone.

2. For the ectopic subcutaneous model, the experimental group showed slight bone formation, but less than that seen for the calvarial defect model. Further experimentation using higher BMP concentrations is needed to validate this model.

3. The bone formation at the inner face of the MBCP block was shown to be less than that at the outer one. This problem may be solved by improving the nature of the carrier.

4. ErhBMP-2 showed osteoinduction capacity similar to that of rhBMP-2 expressed by CHO cells.

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Legends

Figure 1. Block type MBCP implant used in this study.

Figure 2. Schematic diagram of the calvarial osteotomy defect showing histometric analysis

Figure 3. Representative photomicrographs of calvarial defect sites receiving MBCP at 2 weeks (A,B) and 8 weeks (C,D). (H&E stain, original magnification A and C×25, B and D ×100)

Figure 4. Representative photomicrographs of calvarial defect sites receiving ErhBMP-2/ MBCP at 2 weeks (A,B) and 8 weeks (C,D). (H&E stain, original magnification A and C×25, B and D X100)

Figure 5. Representive photomicrographs of ectopic site receiving MBCP at 2 weeks (A,B) and 8 weeks (C,D). (H&E stain, original magnification A and C×25, B and D ×100)

Figure 6. Representive photomicrographs of ectopic site receiving ErhBMP-2/ MBCP at 2 weeks (A,B) and 8 weeks (C,D). (H&E stain, original magnification A and C×25, B and D×100)

Tables

Table-1. Total augmented area of calvarial defect sites (group means ±SD,mm^2 , n=8)

	2 weeks	8 weeks
MBCP	20.8±2.5	20.1±2.1
ErhBMP-2/MBCP	23.1±1.0¶	24.0±1.4¶

¶: Statistically significant difference compared to MBCP group ($p < 0.01$)

Table-2. New Bone area of calvarial defect sites (group means±SD,mm^2, n=8)

	2 weeks	8 weeks
MBCP	1.1±0.6	2.8±0.8*
ErhBMP-2/MBCP	2.5±1.1 ¶	6.1± 1.5*¶

*: Statistically significant difference compared to 2 weeks ($p < 0.01$)

¶: Statistically significant difference compared to MBCP group ($p < 0.01$)

Table-3. Total augmented area of ectopic site (group means ±SD,mm^2 , n=8)

	2 weeks	8 weeks
MBCP	21.1±2.3	19.6±2.8
ErhBMP-2/MBCP	22.3±2.0	22.6±1.8

No significant difference when compared to all groups ($P>0.01$)

Table-4. New Bone area of ectopic site (group means±SD,mm^2, n=8)

	2 weeks	8 weeks
MBCP	0.5±0.2	0.8±0.5
ErhBMP-2/MBCP	1.0±0.5	1.7±0.8*¶

*: Statistically significant difference compared to 2 weeks ($p < 0.01$)

¶: Statistically significant difference compared to MBCP group ($p < 0.01$)

Figures

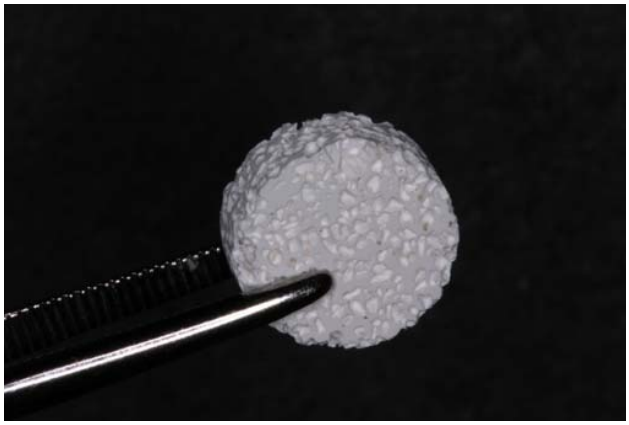

Figure 1.

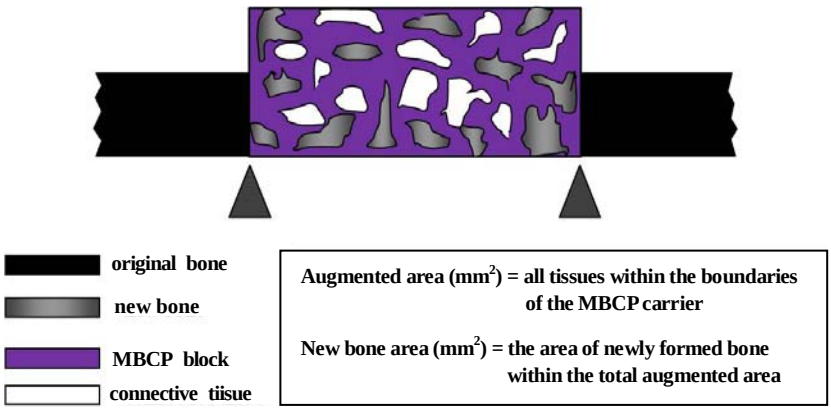

Figure 2.

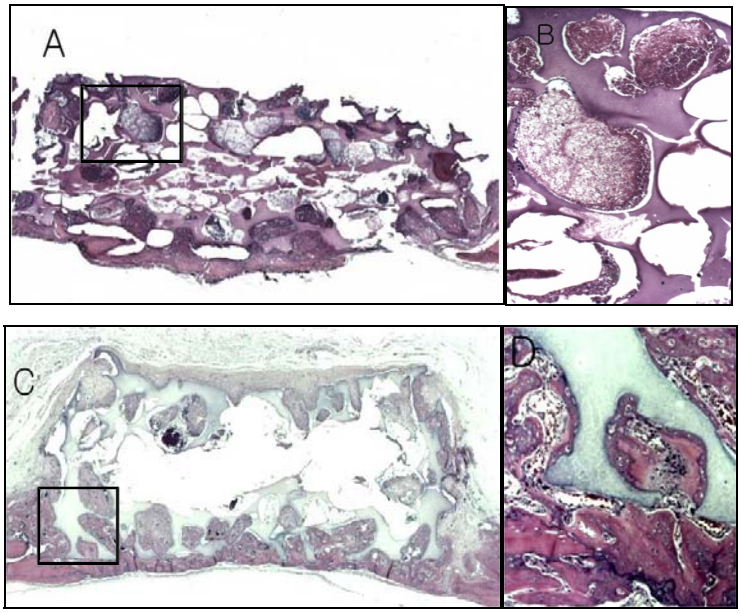

Figure 3.

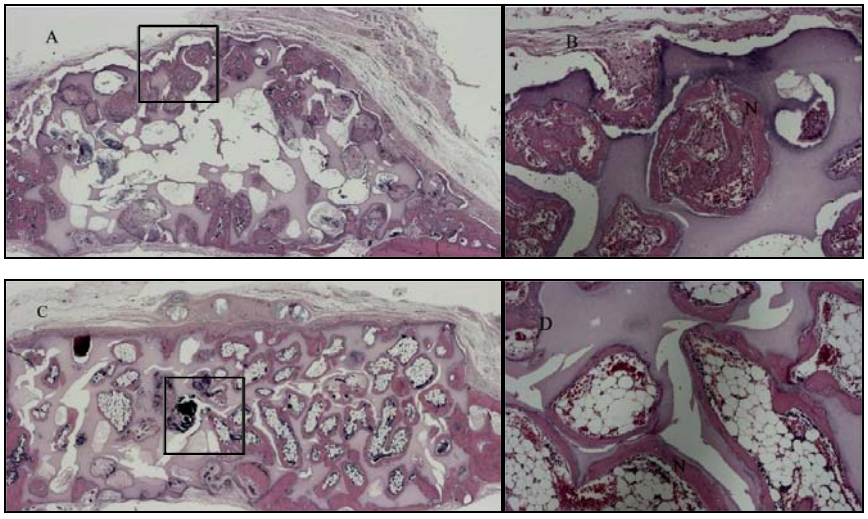

Figure 4.

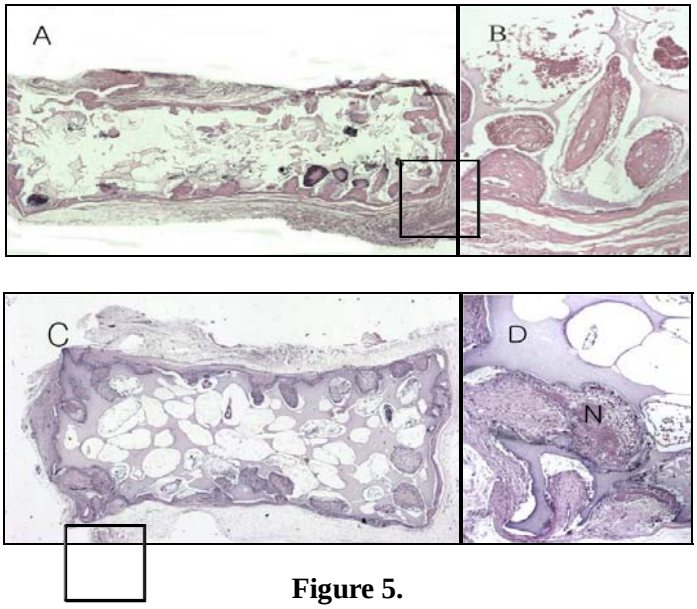

Figure 5.

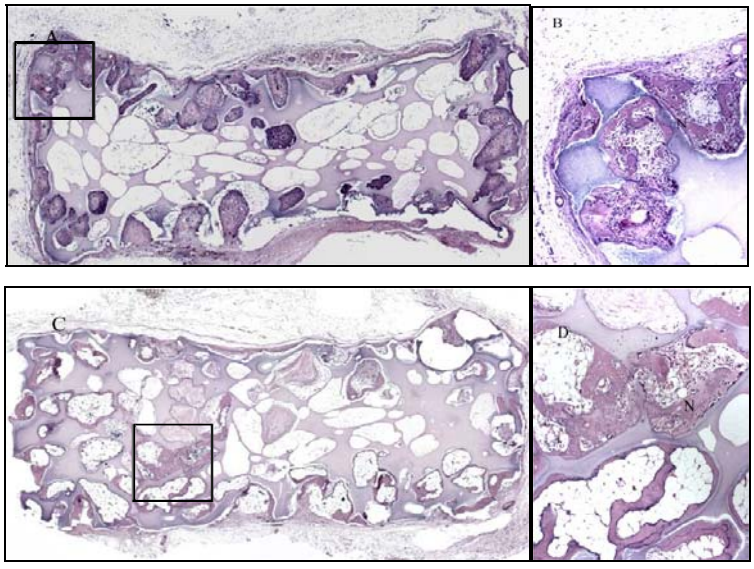

Figure 6.

국문요약

백서 두개골 결손부와 피부하조직에서, *Escherichia coli*에서 생산된 **recombinant human bone morphogenetic protein-2**의 **Macroporous Biphasic calciumphosphate-block** 운반체에 의한 골형성효과

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소 성 수

대부분의 골형성유도단백질 (Bone morphogenetic protein:BMP)은 동물 세포에서 만들어져 왔으나 비용 문제 등의 한계로 인해 *E.coil*에서 생산된 rhBMP가 그 대안으로 많은 연구가 이루어졌다. 이러한 rhBMP의 효과적인 적용을 위해서는 운반체가 필요한데, 운반체는 골형성세포를 위한 골격 역할을 해야 하며, 다공성 구조, 낮은 흡수율, 안정적인 생체친화성을 가져야한다. 이러한 조건을 충족시키는 운반체 중의 하나가 Macroporous biphasic calcium phosphate (MBCP)라 할 수 이다. 이 연구의 목적은 *E.coli*에서 생산된 rhBMP (ErhBMP-2)와 MBCP 운반체에 의한 골형성 효과를 백서 두개골 결손부와 피부하 조직에서 관찰하는 것이다.

총 64마리의 웅성백서를 2군으로 나누었다. 두개골 부위 직경 8mm의

골결손부를 형성하고, 동물의 등에 피부하 주머니를 형성해 MBCP block만 이식한 군, MBCP block을 운반체로 사용하여 농도 0.025mg/ml ErhBMP-2를 이식한 군으로 나누어 술 후 2주와 8주에 치유결과를 조직학적, 조직계측학적으로 비교 관찰 하였다.

두개골 결손부의 경우, 뚜렷한 신생골 형성이 대조군에 비해 실험군에서 나타났다. 각 군에서도 8주째가 2주째보다 골밀도가 유의성있게 증가하였다. 피부하 결손부의 경우 실험군에서 신생골 형성이 관찰되었으나 그 양은 미미하였다. 이에 좀더 높은 농도에서의 추가적인 관찰이 필요하다.

ErhBMP-2/MBCP block에 의한 신생골은 관찰 기간 내내 안정되게 유지됨을 볼 수 있었다. 이러한 결과로 MBCP block을 이용한 ErhBMP-2의 사용은 조직공학분야에 유용하리라 사료된다.

핵심되는 말 : 골형성유도단백질; 골재생; *E.coli* expression system; 이소성골형성; Macroporous biphasic calcium phosphate; 백서 두개골결손부; 백서 피하조직낭;