# Effect of Fibrin-Fibronectin Sealing System in combination with ß-Tricalcium Phosphate as a Carrier for Recombinant Human Bone Morphogenetic Protein-2 on Bone Formation in Rat Calvarial Defects

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A Dissertation Thesis
Submitted to the Department of Dental Science
and the Graduate School of Yonsei University
In partial fulfillment of the
Requirements for the degree of
Doctor of Philosophy of Dental Science

Sung-Jae Hong

June 2005

This certifies that the dissertation thesis

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June 2005

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2005년 6월

저자 씀

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Abstract

## Effect of Fibrin-Fibronectin Sealing System in combination with ß-Tricalcium Phosphate as a Carrier for Recombinant Human Bone Morphogenetic Protein-2 on Bone Formation in Rat Calvarial Defects

Bone morphogenetic proteins (BMPs) are being evaluated as potential candidates for periodontal and bone regenerative therapy. In spite of good prospects for BMP applications, an ideal carrier system for BMPs has not yet been identified. The purpose of this study was to evaluate the osteogenic effect of a fibrin-fibronectin sealing system (FFSS) combined with ß-tricalcium phosphate (ß-TCP) as a carrier system for rhBMP-2 in the rat calvarial defect model.

Eight-mm critical-size calvarial defects were created in 100 male Sprague-Dawley rats. The animals were divided into 5 groups of 20 animals each. The defects were treated with rhBMP-2/FFSS, rhBMP-2/FFSS/ß-TCP, FFSS and FFSS/ß-TCP carrier control or were left untreated as a sham-surgery control. Defects were evaluated by histologic and histometric parameters following a 2- and 8-week healing interval (10 animals/group/healing intervals).

The FFSS/ß-TCP carrier group was significantly greater in new bone area at 2

weeks ($p < 0.05$) and augmented area at 2 and 8 weeks ($p < 0.01$) relative to the FFSS carrier group. New bone area and augmented area in the rhBMP-2/FFSS/ß-TCP group were significantly greater than in the rhBMP-2/FFSS group at 8 weeks ($p < 0.01$). On histologic observation, FFSS remnants were observed at 2 weeks, but by 8 weeks, the FFSS appeared to be completely resorbed. rhBMP-2 combined with FFSS/ß-TCP produced significantly more new bone formation and augmentation in this calvarial defect model. In conclusion, FFSS/ß-TCP may be considered as an available carrier for rhBMP-2.

**Key Words:** Osteogenic effect; bone morphogenetic protein-2; fibrin-fibronectin sealing system; ß-tricalcium phosphate; rat calvarial defect model

# Effect of ß-Tricalcium Phosphate in combination with Fibrin-Fibronectin Sealing System as a Carrier for Recombinant Human Bone Morphogenetic Protein-2 on Bone Formation in Rat Calvarial Defects

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## I. Introduction

In 1965, Urist observed that implanting demineralized bone matrix in extraskeletal (e.g., subcutaneous) sites generated osseous tissue in rats (Urist, 1965). The factors within the matrix responsible for this effect were named bone morphogenetic proteins ((BMPs) Urist et al., 1971). BMPs are growth and differentiation factors that act on mesenchymal cells, causing them to differentiate into mature osteoblasts and build new bone. Advances in DNA technology permitted cloning and characterization of several BMPs, yielding larger quantities of purified

recombinant human BMPs (Wozney et al., 1988; Wang et al., 1988). Today, more than 20 BMPs have been identified and several including BMP-2, -4, -5, -6, and -7 have been shown to be osteoinductive (Sampath et al., 1992; Wikesjo et al., 1999;Kim et al., 2004).

Implantation of BMPs alone does not induce bone formation because the protein rapidly diffuses from the site of implantation. Therefore, a carrier is needed to deliver and release substantial quantities of BMPs to the specific location for osteoinduction. Such a carrier material should be biocompatible to minimize local tissue response and biodegradable to allow replacement by newly formed bone (Aldinger et al., 1991). Addition, the requirements for an ideal carrier system include simplicity in application and manufacture. Studies of such carriers to date include biological materials such as bone matrix (Toriumi et al., 1991; Yassco et al., 1992), absorbable collagen sponge ((ACS) Sampath et al., 1981; King et al., 1998; Barboza et al., 2000), fibrin sealant (Kawamura et al., 1988), synthetic polymers (poly(glycolic acid-co-lactic)acid) (Miyamoto et al., 1993; Miki et al., 1996), and ceramic materials, such as tricalcium phosphate (Urist et al., 1984; Urist et al., 1987; Gao et al., 1996; Alam et al., 2001). Although various carriers for BMPs have been investigated, an ideal carrier system has not yet been determined.

A series of investigations have been conducted utilized ACS, ß-tricalcium phosphate (ß-TCP), and a fibrin-fibronectin sealing system (FFSS) as carriers for

BMPs delivery (Kim et al., 2002; Choi et al., 2002; Ahn et al., 2003; Kim et al., 2004; Pang et al. 2004; Han et al., 2005; Hyun et al., 2005). In space-providing skeletal defects, ACS appears to be an effective carrier. However, in cases in which space could not be provided, compressive forces acted unfavorably on ACS, resulting in compromised effectiveness. A ß-TCP carrier showed sufficient resistance to compressive force and resulted in successful augmentation. However, delayed resorption of the particles appeared to hinder new bone formation and it was difficult to manipulate.

FFSS, also called “fibrin sealant” or “fibrin glue,” is a human plasma derivative that mimics the final stages of blood coagulation, forming a fibrin clot. FFSS has a potential for promoting wound healing, hemostasis and tissue adhesion. Therefore, FFSS has been used as an adjunct in a wide variety of surgical procedures (Whiteman et al., 1997; Davis et al., 1998; Jackson et al., 2001; Tribod et al., 2004).

In this present study, FFSS was mixed with particulated ß-TCP to serve as a carrier for BMPs. The rationale behind this was to utilize the sticky properties of FFSS, which is resorbable glue, and to mix it with ß-TCP to create a new carrier system that was easy to mold and manipulate. It was expected that the synergic effect of the two osteoconductive materials would result in different resorption rates and release kinetics.

The purpose of this study was to evaluate the osteogenic effect of fibrin-fibronectin sealing system combined with ß-tricalcium phosphate (FFSS/ß-TCP) as a carrier system for rhBMP-2 in the rat calvarial defect model.

# II. Materials & methods

## 1. Animals

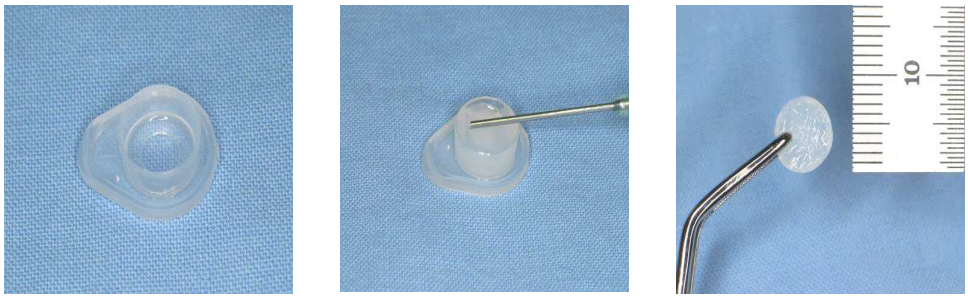
One-hundred male Sprague-Dawley rats (weight 250-300 g) were used. Rats were maintained in plastic cages in a room with a 12 h-day/night cycle and an ambient temperature of 21°C, with *ad libitum* access to water and standard laboratory pellets. Animal selection and management, surgical protocol, and preparation ere in accordance with the routines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea.

## 2. rhBMP-2 Implant Construction

FFSS[‖] are available as vapor-heated, freeze-dried 2-component preparation: a fibrinogen/fibronectin/factor XIII concentrate and a thrombin concentrate. The fibrinogen concentrate is dissolved in an antifibrinolytic solution (aprotinin), and the thrombin concentrate is dissolved in dilute calcium chloride.

rhBMP-2[§] was reconstituted and diluted in buffer to produce a concentration of 0.05 mg/ml. The rhBMP-2/FFSS implants were made by mixing equal volumes of FFSS and rhBMP-2 (0.05 mg/ml) in buffer. The final rhBMP-2 concentration in rhBMP-2/FFSS implants was 0.025 mg/ml.

For the rhBMP-2/FFSS/ß-TCP implants, ß-TCP¶ particles were placed in a sterile cylinder type mold and loaded with a rhBMP-2/FFSS solution. For the FFSS/ß-TCP implants, ß-TCP particles were loaded with FFSS solution only. All implants were made by clotting in a cylinder type mold. The final disc-shaped implant was 3 mm in height and 8 mm in diameter. Following a 5-minute binding time, the implant was placed into the calvarial defects (Figure1).




A B C

Figure 1. Cylinder type mold (8mm in diameter) used in this study (A). For peripheral seal, additional solution was injected into the block margin (B). The final disc-shaped implant was 3 mm in height and 8 mm in diameter (C).

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‖ Tisseel®, Immuno AG, Vienna, Austria

§ R&D Systems Inc., Minneapolis, MN, USA

¶ Cerasorb®, 150-500 ㎛, Curasan, Kleinotheim, Germany

### 3. Surgical Procedures

The animals were anaesthetized by an intramuscular injection (5 mg/kg body wt.) of a 4:1 solution of ketamine hydrochloride[**]:Xylazine[††]. Routine infiltration anaesthesia[‡‡] was used at the surgical site. An incision was made in the sagittal plane across the cranium and a full thickness flap was reflected, exposing the calvarial bone. A standardized, circular, transosseous defect, 8 mm in diameter, was created on the cranium using saline-cooled trephine drill [#]. The animals were divided into 5 groups of 20 animals each and allowed to heal for 2 (10 rats) or 8 (10 rats) weeks. Each animal received 1 of 5 experimental treatments: sham-surgery control in which no material was applied to the defect, FFSS carrier control, FFSS/ß-TCP carrier control, rhBMP-2/FFSS and rhBMP-2/FFSS/ß-TCP. The periosteum and skin were closed and sutured with 4-0 coated Vicryl sutures[§§] for primary intention healing.

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** Ketalar®, Yuhan Co., Seoul, Korea

†† Rompun®, Bayer Korea, Seoul, Korea

‡‡ 2% lidocaine, 1:100,000 epinephrine, Kwangmyung Pharm., Seoul, Korea

# 3i, Palm Beach Gardens, FL, USA

§§ Polyglactin 910, braided absorbable suture, Ethicon, Johnson & Johnson Int., Edinburgh, UK

**4. Histologic and Histometric Procedures**

The animals were sacrificed by $CO_2$ asphyxiation at 2 and 8 weeks post-surgery. Block sections, including the experimental sites, were removed and fixed in a 10% neutral buffered formalin solution for 10 days. Samples were decalcified by 5% formic acid for 14 days and embedded in paraffin. Serial sections 5um in thickness were prepared at intervals of 80 um, stained with hematoxylin/eosin (H-E), and examined using a light microscope. The most central sections from each block were selected for histological evaluation.

Computer-assisted histometric measurements were obtained using an automated image analysis system‖ coupled with a video camera on a light microscope¶¶. Sections were examined at magnifications of x 20 and x 100. Histometric parameters were defined as follows (Figure 2). :

Defect closure (%): the distance between the defect margin and ingrown bone margin in mm. The percent defect closure was calculated by subtracting this figure from the total defect diameter, divided by the total defect diameter x 100.

New bone area ($mm^2$): the area of newly formed bone within the total augmented area.

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‖ Image-Pro Plus®, Media Cybernetics, Silver Spring, MD, USA

¶¶ Olympus BX50, Olympus Optical Co., Tokyo, Japan

Augmented area ($mm^2$): all tissues within the boundaries of newly formed bone, i.e. mineralized bone and fatty marrow and fibrovascular tissue/marrow and residual biomaterial.

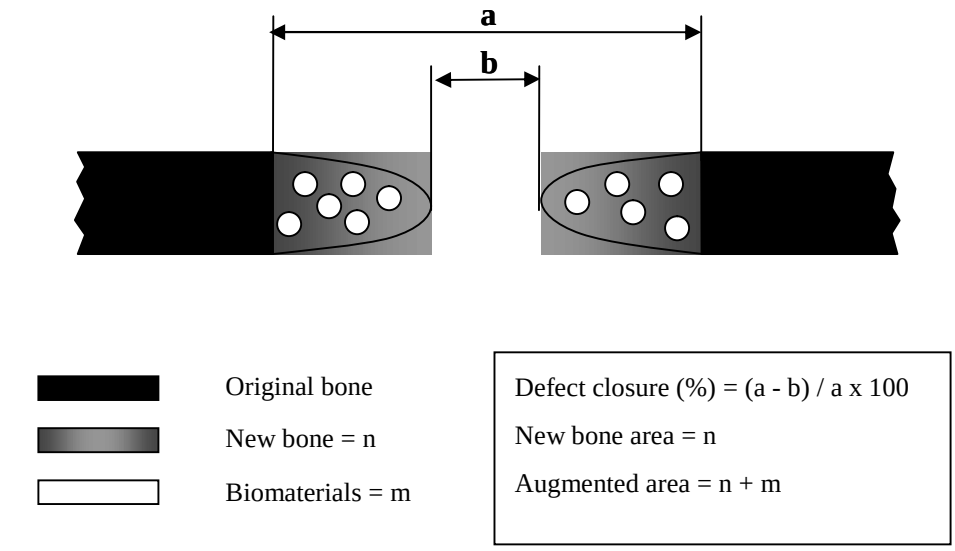


Figure 2. Schematic drawing of calvarial osteotomy defect showing the histometric analysis

**5. Statistical Analysis**

Histometric recordings from the samples were used to calculate means and standard deviations (m±SD). To detect interactions between the healing interval and treatment condition, a two-way analysis of variance was used (two-way ANOVA). ANOVA and post hoc t-tests were used to analyze the differences between treatment groups at each healing interval. For the comparisons between the 2- and 8-week healing interval within the same group, a paired t-test was used. A p-value $<0.05$ was considered significant.

# III. Results

## 1. Clinical Observations

Wound healing was generally uneventful and was similar for all groups. No material exposure or other complications were observed at the surgical sites.

## 2. Histologic Observations

Sham-surgery control group: At 2 and 8 weeks post-surgery, defects were filled with thin loose connective tissue. Also, minimal new bone formation originating from the defect margins was observed. The defect center appeared to be collapsed, possibly due to tissue compression (Figure 3).

FFSS carrier control group: At 2 weeks post-surgery, the defect sites were filled with dense connective tissue and small particles of residual FFSS. Macrophages and inflammatory round cells were observed around the FFSS remnants. Minimal new bone formation was observed. At 8 weeks, defect sites exhibited more bone formation from the defect margin to the center than was seen at 2 weeks. The FFSS appeared to be resorbed completely (Figure 4).

FFSS/ß-TCP carrier control group: At 2 weeks post-surgery, a large number of residual ß-TCP particles were present within dense connective tissue at the defect site.

In addition, some new bone formation adjacent to defect margins was observed. Histological observations at 8 weeks were similar to observations at 2 weeks. Compared with the 2 week observations, residual ß-TCP particles were fewer in number and the quantity of newly formed bone was greater (Figure 5).

rhBMP-2/FFSS group: All defect sites were almost completely bridged at 2 and 8 weeks. At 2 weeks, newly formed bone with osteocytes was evident mainly at the periphery of the defects, and osteoblast-like cells exhibited a dense arrangement adjacent to the newly formed bone. At 8 weeks, the quantity of newly formed bone was greater than that observed at 2 weeks and the specimens showed more advanced stages of remodeling and consolidation. Cement lines, concentric rings of the Haversian system and fatty marrow were observed in the new bone area (Figure 6).

rhBMP-2/FFSS/ß-TCP group: At 2 weeks postsurgery, a large number of residual ß-TCP particles were observed within the newly formed bone at the defect site. Occasionally, ß-TCP particles were surrounded by woven bone. The newly formed bone was found not only in direct contact with ß-TCP particles, but also throughout the defect. At 8 weeks, fewer residual ß-TCP particles were observed compared to the 2 week observations. The quantity of the newly formed bone was greater than that observed at 2 weeks, and the appearance of the new bone was more lamellar than that

at 2 weeks. Cement lines and fatty marrow were observed in the new bone area (Figure 7).

### 3. Histometric Analysis

Ten animals were excluded from the histometric analysis due to technical complications in the histologic processing (one animal/group/healing intervals).

Tables 1-3 show the results of histometric analysis. The FFSS/ß-TCP carrier group was significantly greater than the FFSS carrier group in terms of new bone area at 2 weeks ($p < 0.05$) and augmented area at 2 and 8 weeks ($p < 0.01$). Defect closure, new bone area, and augmented area in the rhBMP-2/FFSS and rhBMP-2/FFSS/ß-TCP groups were significantly greater than in the sham-surgery control group at each healing interval ($p < 0.01$). The defects were almost completely closed in the rhBMP-2/FFSS and rhBMP-2/FFSS/ß-TCP groups. In terms of new bone area, there was no significant difference between these two rhBMP-2 groups at 2 weeks. However at 8 weeks, the rhBMP-2/FFSS/ß-TCP group was significantly greater than the rhBMP-2/FFSS group ($p < 0.01$). The augmented area in the rhBMP-2/FFSS/ß-TCP group was significantly greater than in the rhBMP-2/FFSS group at 2 and 8 weeks ($p < 0.01$).

A two-way ANOVA revealed that there was an interaction between the healing interval and treatment condition in defect closure and new bone area ($p < 0.01$). Treatment had a strong influence on defect closure, new bone area and augmented

area ($p < 0.01$), whereas healing interval had an influence on defect closure and new bone area ($p < 0.01$).

**Table 1. Defect closure (group means ± SD; n=9, %)**

| | 2 weeks | 8 weeks |
|---|---|---|
| Sham-surgery control | 11.7 ± 2.9 | 13.7 ± 4.6 |
| FFSS | 38.1 ± 26.4* | 68.9 ± 30.0*ϕ |
| FFSS/ ß-TCP | 55.9 ± 25.4*¶ | 86.2 ± 10.5*¶ϕ |
| rhBMP-2/FFSS | 89.4 ± 11.7*¶† | 95.4 ± 8.1*¶ |
| rhBMP-2/FFSS/ ß-TCP | 91.4 ± 8.7*¶† | 96.9 ± 2.5*¶ |

*: Statistically significant difference compared to surgical control group (P<0.01)

¶: Statistically significant difference compared to FFSS group (P<0.05)

†: Statistically significant difference compared to FFSS/ß-TCP group (P<0.01)

ϕ : Statistically significant difference compared to 2 weeks (P<0.05)

**Table 2. New bone area (group means ± SD; n=9, $mm^2$)**

| | 2 weeks | 8 weeks |
|---|---|---|
| Sham-surgery control | 0.2 ± 0.1 | 0.4 ± 0.1ϕ |
| FFSS | 0.4 ± 0.2 | 2.3 ± 0.7*ϕ |
| FFSS/ ß-TCP | 1.4 ± 1.2*¶ | 2.1 ± 0.3*ϕ |
| rhBMP-2/FFSS | 2.6 ± 0.6*¶† | 3.4 ± 0.5*¶†ϕ |
| rhBMP-2/FFSS/ ß-TCP | 2.8± 1.4*¶† | 5.3 ± 2.1*¶†‡ϕ |

*: Statistically significant difference compared to surgical control group (P<0.05)

¶: Statistically significant difference compared to FFSS group (P<0.05)

†: Statistically significant difference compared to FFSS/ß-TCP group (P<0.05)

‡: Statistically significant difference compared to rhBMP-2/FFSS (P<0.01)

ϕ : Statistically significant difference compared to 2 weeks (P<0.05)

**Table 3. Augmented area (group means ± SD; n=9, $mm^2$)**

| | 2 weeks | 8 weeks |
|---|---|---|
| Sham-surgery control | 0.2 ± 0.1 | 0.4 ± 0.1$^{\oint}$ |
| FFSS | 5.6 ± 3.0$^{*}$ | 5.0± 1.4$^{*}$ |
| FFSS/ ß-TCP | 11.0 ± 1.7$^{*¶}$ | 10.2± 1.6$^{*¶}$ |
| rhBMP-2/FFSS | 4.9± 0.5$^{*†}$ | 4.8 ± 1.2$^{*†}$ |
| rhBMP-2/FFSS/ ß-TCP | 13.2± 1.3$^{*¶†‡}$ | 12.1 ± 1.9$^{*¶†‡}$ |

*: Statistically significant difference compared to surgical control group (P<0.01)

¶: Statistically significant difference compared to FFSS group (P<0.01)

†: Statistically significant difference compared to FFSS/ß-TCP group (P<0.01)

‡: Statistically significant difference compared to rhBMP-2/FFSS (P<0.01)

$\oint$ : Statistically significant difference compared to 2 weeks (P<0.01)

# IV. Discussion

The objective of this study was to evaluate the osteogenic effects of fibrin-fibronectin sealing system combined with ß-tricalcium phosphate (FFSS/ß-TCP) as a carrier system for rhBMP-2 in the rat calvarial defect model. Five groups of 20 animals each received one of the following: sham-surgery control, FFSS and FFSS/ß-TCP carrier control, rhBMP-2/FFSS and rhBMP-2/FFSS/ß-TCP. These groups were evaluated by histologic and histometric parameters following a 2- and 8-week healing interval. The experimental defects receiving rhBMP-2 underwent extensive bone formation following each healing interval.

The experimental model used in this study was based on that described by Takagi and Urist (Takagi et al., 1982). The critical-size rat calvarial defect, compared with other experimental bone defects, is a convenient model for evaluating bone regenerative effects of biomaterials because of its relative accessibility, simplicity and reproducibility because spontaneous healing does not occur in the control specimens (Frame, 1980; Schmitz et al., 1986).

It has been reported that rhBMPs alone is sufficient to induce bone formation; however, if not delivered properly to the effecter site, water-soluble rhBMP may be diffused, resulting in a reduced osteoinductive effect (Nakahara et al., 1989). It has been shown that the therapeutic outcome of rhBMP-2 depends on its quantity,

concentration, and time of application (King et al., 2002). Therefore, an appropriate carrier system is critical for the delivery, retention, and release of BMPs at the implantation site in order to achieve an osteoinductive effect (Urist et al., 1984, 1987; Kenley et al., 1994; Wikesjö et al., 2001).

In this study, FFSS and FFSS combined with ß-TCP (FFSS/ß-TCP) were used as the carrier system for rhBMP-2. ß-TCP has been developed as an osteoconductive, biodegradable bone substitute. Its porous structure enables it to entrap rhBMPs within its micropores, and intrinsically diffusible rhBMPs can be retained and its action consequently prolonged (Urist et al. 1984). Although the porous structure of ß-TCP allows cells and newly formed tissues to migrate into it, it also provides sufficient firmness against soft tissue pressure.

FFSS is an organic, biodegradable material derived from human plasma. It has angiotropic, hemostatic, and osteoconductive properties. The beneficial effect of FFSS in wound healing has been well-documented, but studies of its direct influence on bone healing and effectiveness in augmenting bone graft healing have produced conflicting results. A clinical study showed the fibrin matrix maintains room for new bone formation (Pini prato et al., 1998). FFSS produced an early enhancement of bone repair in rabbits (Bosch et al., 1980), and enhancement of physical properties of the bony callus in dogs (Keller et al., 1985). Isogai et al. (2000) reported that fibrin clot support the growth, adhesion, migration and differentiation of osteoblasts in vitro.

In contrast, Carmagnola et al. (2002) reported that FFSS may have impaired the early vascularization of biomaterials and, as a consequence, prevented growth of host bone into the grafted defect. Brittberg et al. (1997) observed that FFSS treatment inhibited the natural repair of osteochondral defects in rabbits. Even though the effect of FFSS on bone healing remains controversial, FFSS is considered effective for BMPs carriers. Kawamura et al. (1988) reported that FFSS may enhance cell proliferation and improve contact between BMPs and the surrounding cells.

In this study, the FFSS/ß-TCP carrier group was significantly greater than the FFSS carrier group in new bone area at 2 weeks ($p < 0.05$) and augmented area at 2 and 8 weeks ($p < 0.01$). The control group of ß-TCP alone was not included since extensive evaluation was previously conducted in a series of research studies. Pang et al. (2004) evaluated ß-TCP alone on bone formation using this type of model and found new bone area of $0.9mm^2$ at 2 weeks and 1.2 $mm^2$ at 8 weeks. Although statistical analysis was not performed, these findings are comparable with the result in our FFSS/ß-TCP carrier group ($1.4mm^2$ of new bone area at 2 weeks and 2.1 $mm^2$ at 8 weeks). Mixing FFSS with ß-TCP particles may have a positive effect on bone regeneration

New bone area in the rhBMP-2/FFSS group at 8 weeks was significantly greater than at 2 weeks ($p < 0.05$). However, in the previous study, there were no significant differences between the healing interval and new bone area in the rhBMP-4/ß-TCP

group. It may be that, when soaking BMPs in ß-TCP particles, BMPs are released quickly. On the contrary, when BMPs are mixed with FFSS, BMPs are released slowly as resorption of FFSS progresses.

The augmented area of the rhBMP-2/FFSS/ß-TCP group was significantly greater than that of the other groups at each healing interval. Bone augmentation is necessary in many clinical cases. Over the years, autogenous grafting has been considered the "gold standard" in this form of treatment. Autogenous grafting has limitations, however, including an inadequacy of supply and surgical morbidity, including donor site pain and infection. Moreover, graft resorption poses a severe problem (Schallhorn. 1972). rhBMP-2/FFSS/ß-TCP is considered an effective substitute that overcomes these problems. Furthermore, FFSS combined with ß-TCP allows easy clinical manipulation.

These results indicate that using FFSS as a carrier for rhBMP-2 is effective in new bone formation. However, FFSS was resorbed too quickly to produce sufficient augmentation. On the other hand, rhBMP-2 combined with FFSS/ß-TCP produced significant new bone formation and augmentation in the calvarial defect model. In conclusion, FFSS/ß-TCP may be considered an available carrier for rhBMP-2.

# V. Conclusion

The purpose of this study was to evaluate the osteogenic effect of a fibrin-fibronectin sealing system (FFSS) combined with ß-tricalcium phosphate (ß-TCP) as a carrier system for rhBMP-2 in the rat calvarial defect model.

Eight-mm critical-size calvarial defects were created in 100 male Sprague-Dawley rats. The animals were divided into 5 groups of 20 animals each. The defects were treated with rhBMP-2/FFSS, rhBMP-2/FFSS/ß-TCP, FFSS and FFSS/ß-TCP carrier control or were left untreated as a sham-surgery control. Defects were evaluated by histologic and histometric parameters following a 2- and 8-week healing interval (10 animals/group/healing intervals).

The FFSS/ß-TCP carrier group was significantly greater in new bone area at 2 weeks ($p < 0.05$) and augmented area at 2 and 8 weeks ($p < 0.01$) relative to the FFSS carrier group. New bone area and augmented area in the rhBMP-2/FFSS/ß-TCP group were significantly greater than in the rhBMP-2/FFSS group at 8 weeks ($p < 0.01$). On histologic observation, FFSS remnants were observed at 2 weeks, but by 8 weeks, the FFSS appeared to be completely resorbed. rhBMP-2 combined with FFSS/ß-TCP produced significantly more new bone formation and augmentation in this calvarial defect model. In conclusion, FFSS/ß-TCP may be considered as an available carrier for rhBMP-2.

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# Legends

**Figure 1.** Cylinder type mold (8mm in diameter) used in this study.

**Figure 2.** Schematic drawing of calvarial osteotomy defect showing histometric analysis.

**Figure 3.** Representative photomicrographs of the sham-surgery control at 2 (A) and 8 weeks (B). Thin loose connective tissues were observed between margins. The center of the defect appears collapsed (arrow head: defect margin; H-E stain; original magnification x20).

**Figure 4.** Representative photomicrographs of FFSS carrier control at 2 weeks (A and B) and 8 weeks (C and D). At 2 weeks, the defect sites were filled with dense connective tissue and small particles of residual FFSS. Minimal new bone formation was observed. At 8 weeks, more bone formation compared to 2 weeks (arrow head: defect margin, NB: new bone; H-E stain; original magnification A and C x20; B and D x100).

**Figure 5.** Representative photomicrographs of FFSS/ ß-TCP carrier control at 2 weeks (A and B) and 8 weeks (C and D). Residual ß-TCP particles were still present

within dense connective tissue at the defect site at 8 weeks. Compared with the 2 weeks observations, residual ß-TCP particles were fewer in number, and the quantity of newly formed bone was greater than observed at 2 weeks. (arrow head: defect margin, +: ß-TCP; H-E stain; original magnification A and C x20; B and D x100).

**Figure 6**. Representative photomicrographs of rhBMP-2/FFSS group at 2 weeks (A and B) and 8 weeks(C and D). Newly formed bone was observed at 2 weeks. At 8 weeks, FFSS had been replaced by new bone and cement lines were observed. The quantity of the new bone was greater and the appearance of the new bone was more lamellar compare to 2 weeks. (arrow head: defect margin, arrow: cement lines, NB: new bone; H-E stain; original magnification A and C x20; B and D x100).

**Figure 7.** Representative photomicrographs of rhBMP-2/FFSS/ ß-TCP group at 2 weeks (A and B) and 8 weeks(C and D). A large number of residual ß-TCP particles were observed within the new bone at 2, 8 weeks. Compare to 2 weeks, the quantity of the new bone was greater and the appearance of the new bone was more lamellar at 8 weeks. Cement lines was seen in the new bone area (arrow head: defect margin, arrow: cement lines, NB: new bone; H-E stain; original magnification A and C x20; B and D x100).

# Figures I

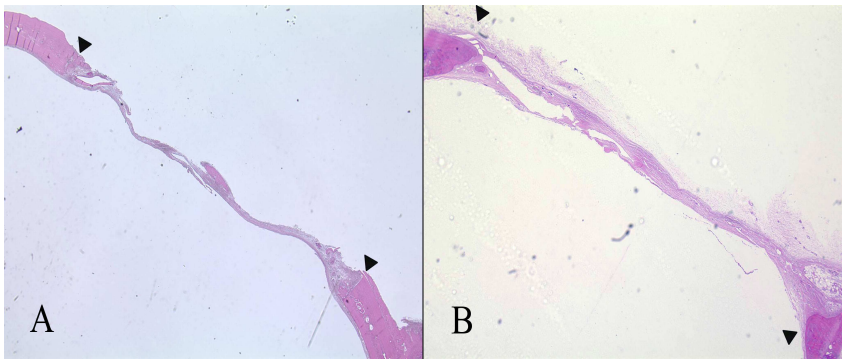


**Figure 3**

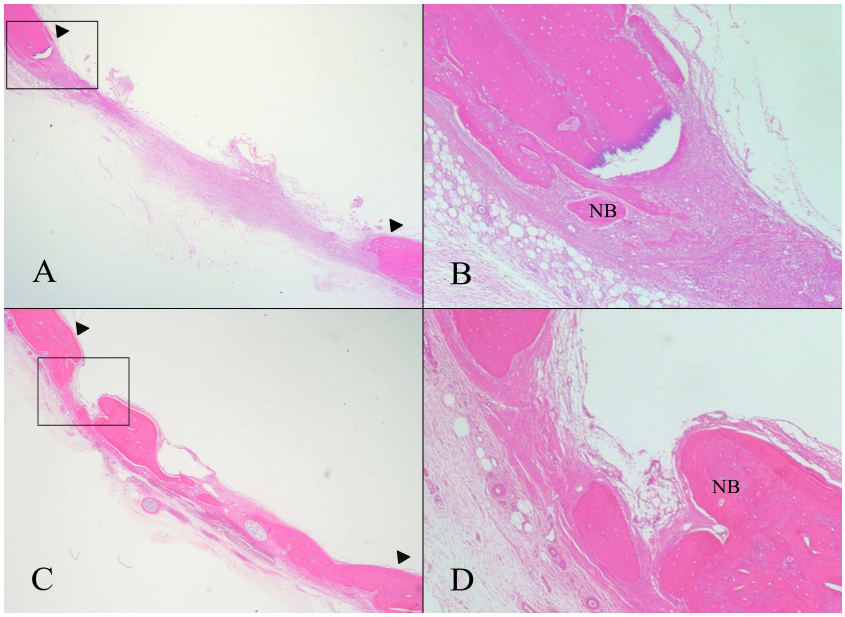


**Figure 4**

# Figures II

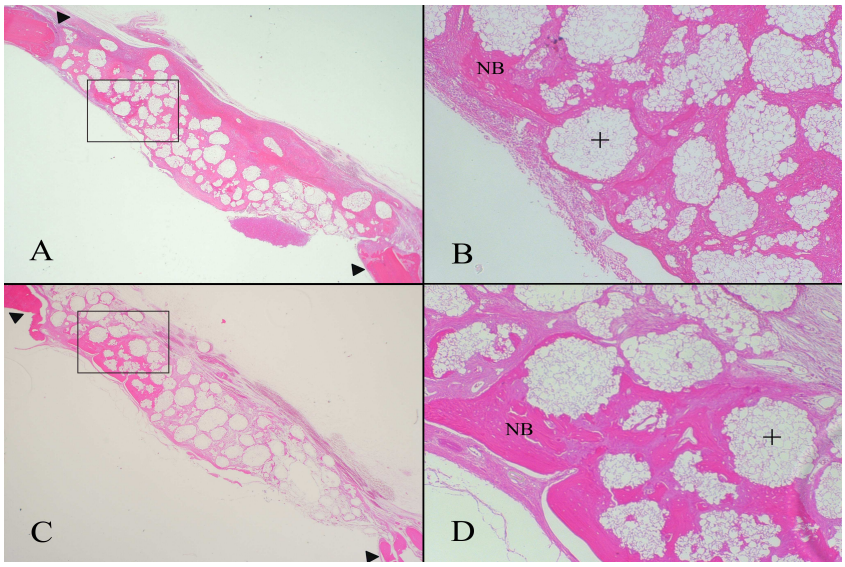


**Figure 5**

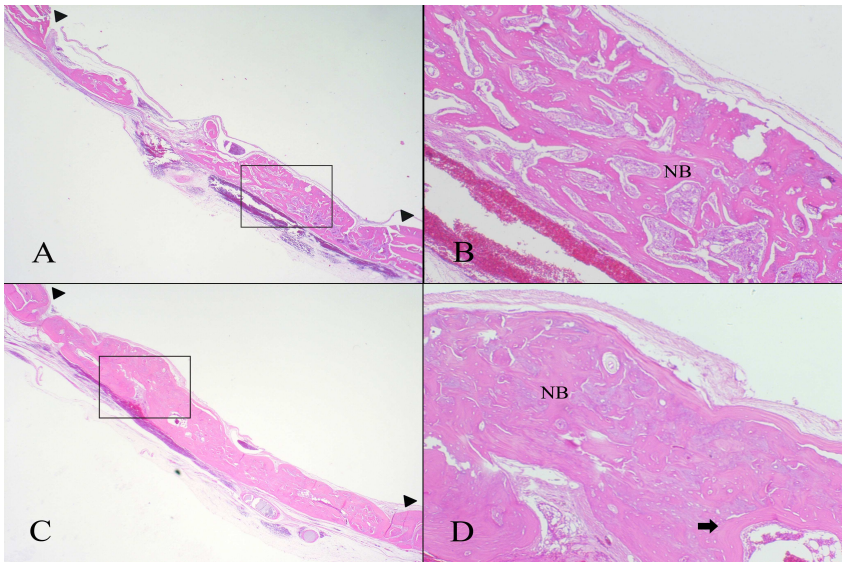


**Figure 6**

# Figures III

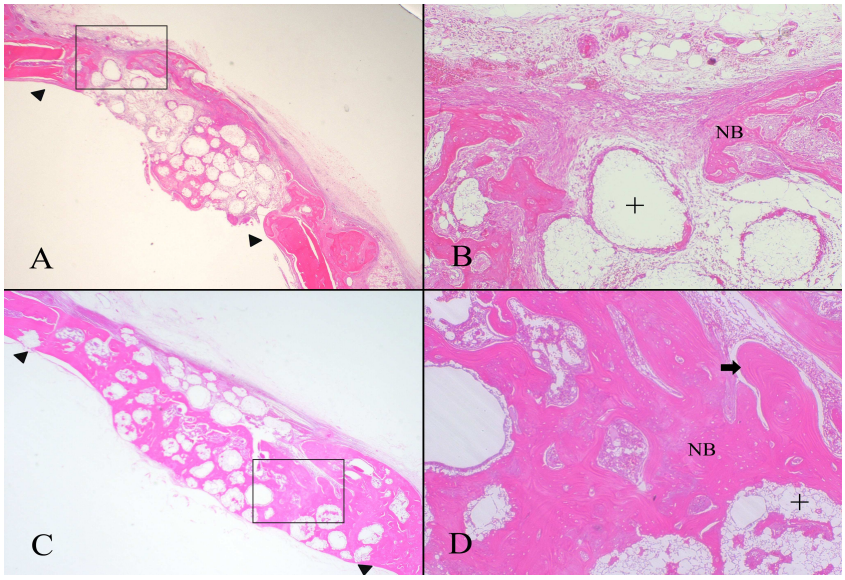


**Figure 7**

국문요약

# 백서 두개골 결손부에서 rhBMP-2의 전달체로서 fibirn-fibronectin sealing system 과 beta tricalcium phosphate 복합체의 골재생 효과

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골형성 유도 단백질 (bone morphogenetic protein, BMPs)은 치주치료와 골 재생 치료를 위한 효과적인 골 대체 물질로 평가되어왔다. BMPs 골형성 능력에도 불구하고, 아직 이상적인 전달체는 존재하지 않는다. 이 연구의 목적은 fibrin-fibronectin sealing system 과 ß-TCP (FFSS/ß-TCP) 복합체를 골형성 유도 단백질(rhBMP-2) 운반체로 사용하여 백서 두개골 결손부에 적용하였을 때, 골형성 효과를 평가하는 것이다.

100 마리의 웅성 백서에서 8mm 지름을 갖는 임계크기의 두개부 결손을 형성하였다. 20 마리씩 5 개의 군으로 나누고, 각 군은 아무것도 이식하지 않은 대조군, FFSS 를 이식한 군, FFSS/ß-TCP 를 이식한 군, FFSS 와 FFSS/ß-TCP 를 전달체로 사용하여 농도 0.025mg/ml rhBMP-2 를 이식한 군으로 나누어 술 후 2 주와 8 주에 치유 결과를 조직학적, 조직계측학적으로 비교 관찰하였다.

조직계측학적 관찰 결과, FFSS/ß-TCP군에서 FFSS군보다 2주째 신생골 형성량 (new bone area) 및 2, 8주째 총조직 형성량 (augmented area)이 유의성 있게 증가하였다. 2주째 신생골 형성량은 rhBMP-2/FFSS, rhBMP-2/FFSS/ß-TCP 군간의 유의 차는 없었다. 그러나 8주째에는 두 군간에도 유의 차가 관찰되었다 ($P < 0.01$). 총조직 형성량은 rhBMP-2/FFSS/ß-TCP군에서 rhBMP-2/FFSS군 보다 2, 8주째 모두에서 유의 차 있는 증가 하였다 ($P < 0.01$).

백서 두개골 결손부에서 FFSS/ß-TCP 결합체를 rhBMP-2 전달체로 사용하였을 때 신생골 형성 및 총조직 형성에 유의한 효과가 있었다. 결론적으로, FFSS/ß-TCP는 rhBMP-2의 전달체로서의 가능성 있다고 사료된다.

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**핵심되는 말**: 골형성 효과, 골형성 유도 단백질, fibirn-fibronectin sealing system, 베타삼화인산칼슘염(ß-TCP), 백서 두개골 결손부