Effect of Fibrin-Fibronectin Sealing System as a Carrier for Recombinant Human Bone Morphogenetic Protein-4 on Bone Formation in Rat Calvarial Defect

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감사의 글

이 작은 결실을 맺을 수 있도록 부족한 저를 항상 따뜻한 관심과 지도로 격려해 주시고 이끌어 주신 채중규 교수님께 깊은 감사를 드립니다. 그리고, 많은 조언과 격려를 해주신 김종관 교수님, 조규성 교수님, 최성호 교수님, 이용근 교수님께 진심으로 감사드립니다. 또한, 본 연구에 많은 관심과 도움을 주신 김창성 교수님께도 감사의 마음을 전합니다.

본 연구 내내 많은 도움을 아끼지 않은 정의원, 윤정호 연구강사 선생님들과 치주과 의국원 여러분께 고마움을 전합니다.

항상 곁에서 든든하게 후원해주시고, 언제나 끝이 없는 사랑으로 저를 감싸주시는 아버지, 어머니, 장인, 장모님께 감사드립니다.

마지막으로, 늘 아낌 없는 사랑으로 나를 복돋아 주고 헌신적인 도움으로 따뜻한 버팀목이 되어준 사랑하는 나의 아내 민경이에게 다시 한번 감사하며 진정으로 고마움을 담아 이 논문을 드립니다.

모든 분께 진심으로 감사 드립니다.

2004년 12월

저자 씀

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Abstract

Effect of Fibrin-Fibronectin Sealing System as a Carrier for Recombinant Human Bone Morphogenetic Protein-4 on Bone Formation in Rat Calvarial Defect

Bone morphogenetic proteins (BMPs) have been shown to play an important role in bone formation during development and healing. In spite of there being good prospects for BMP applications, the ideal carrier system of BMPs has not yet been determined. The fibrin-fibronectin sealing system (FFSS) has the potential to promote wound healing, hemostasis and tissue adhesion. The purpose of this study was to evaluate the possibility of FFSS as a carrier system for rhBMP-4 and the osteogenic effect of FFSS in the rat calvarial defect model.

An 8-mm, calvarial, critical-size osteotomy defect was created in each of 30 male Sprague-Dawley rats. Three groups of 10 animals each received either rhBMP-4 (0.025mg/ml) in an FFSS carrier, FFSS only, or negative surgical control. The groups were evaluated by histologic and histometric parameters following a 2(5rats) and 8(5rats) weeks healing interval.

The results were as follows:

1. The augmented area and new bone area of the FFSS group were significantly greater than that of the surgical control group at both time points ($P<0.05$). The new bone area and bone density of FFSS at 8 weeks were greater than those of FFSS at 2 weeks ($P<0.05$).

2. The new bone area and bone density of the rhBMP-4/FFSS group were

significantly greater than those of the FFSS group at both time points ($P<0.05$). The augmentation area of rhBMP-4/FFSS was not significantly different compared with that of FFSS at either time point ($P>0.05$).

The results of this controlled animal study indicated that FFSS served as a scaffold to maintain room for new bone formation without any adverse reaction and that it released rhBMP-4 in the proper way. Therefore, we conclude that FFSS has an osteoconductive activity and that it is a candidate as a carrier system for rhBMP-4

KEY WORD : Recombinent human bone morphogenetic protein-4; Fibrin-fibronectin sealing system; Carrier; Bone regeneration; Tissue augmentaion; Rat calvarial defect

Effect of Fibrin-Fibronectin Sealing System as a Carrier for Recombinant Human Bone Morphogenetic Protein-4 on Bone Formation in Rat Calvarial Defect

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I. Introduction

Bone morphogenetic proteins are regarded as members of the transforming growth factor superfamily according to characteristic features in their amino acid sequences. In 1965, Urist demonstrated ectopic bone and cartilage formation following intramuscular implantation of demineralized bone matrix in rats (Urist et al., 1965). The factor within the matrix responsible for this effect was later named bone morphogenetic protein(BMP). After recombinant human BMP (rhBMP) was successfully synthesized by Wozney et al. in 1988, a number of studies have demonstrated the biologic activities of rhBMPs which include the induction of ectopic cartilage and bone formation at implanted sites in vivo and the stimulation of osteoblastic phenotype expression during the course of osteoblastic differentiation in various types of cells in vitro. Now more than

20 BMPs have been identified, several of which have been shown to be significantly osteoinductive, including BMP-2, -4, -5, -6, and -7. Therefore, many trials using BMPs for bone tissue engineering have been well attempted.

In spite of there being good prospects for BMP applications, there remainsa gap between research results and the clinical use of BMPs, due to the lack of accessible delivery systems with bioactivity. Therefore, to achieve the osteoinductive effect of BMPs, carrier systems are essential their delivery. Carrier systems for delivering BMPs should be biocompatible and biodegradable to minimize local tissue response and to allow replacement by newly formed bone (Aldinger et al., 1991). In addition, the ideal carrier system will retain and release the BMP in a controlled fashion, not interfere with normal bony healing, be easy to apply and be easy to manufacture. Carriers studies to date include inorganic materials such as calcium phosphate ceramics, natural materials such as collagen, fibrin and synthetic polymers such as polyglycolic acid (Howard et al., 2002; Dan et al., 2002; King et al., 1998; Kenley et al., 1994). In our previous studies, we have shown that rhBMP, when incorporated in absorbable collagen sponges (ACS) and beta tricalcium phosphate (ß-TCP) , promoted a significant increase in new bone formation in the rat model (Kim et al., 2004; Pang et al., 2004; Ahn et al., 2003). However, ACS was not biomechanically strong enough to maintain the space for new bone formation. Although ß-TCP exhibited sufficient biomechanical strength to maintain space, resorption of ß-TCP was slow. Therefore, new carrier systems that have proper biomechanical strength and resorption rate are needed.

The fibrin-fibronectin sealing system (FFSS) mimics the last step of the

coagulation cascade, resulting in a fibrin clot independent of the patient's coagulation pathway. FFSS has the potential to promote wound healing, hemostasis and tissue adhesion. So, FFSS is currently used in various medical treatments (Jackson et al., 2001; Whiteman et al., 1997; Davis et al., 1998). FFSS seems to have an osteogenic effect on bone healing. Isogai et al. reported that fibrin clot supports the growth, adhesion, migration, differentiation of osteoblasts in vitro (Isogai et al., 2000). In a clinical study, fibrin matrix maintains room for new bone formation (Pini Prato et al., 1988). Since FFSS is an absorbable glue type, it is possible that FFSS retained rhBMPs and released them in the proper way. Thus, it is possible to suggest that the delivery of rhBMPs with FFSS possessing osteogenic potential may promote wound healing and bone regeneration.

The purpose of this study was to evaluate the osteogenic effect of FFSS and the potential of FFSS as a carrier system for rhBMP-4 in the rat calvarial defect model.

II. Materials and Methods

1. Animals

Thirty male Sprague-Dawley rats (body weight 250-300g) were used in this study. They 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 followed the routines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea.

2. rhBMP-4/FFSS and FFSS implants construct

A rhBMP-4/FFSS implant was made by the same volume of FFSS† and rhBMP-4‡ in buffer at a concentration of 0.05 mg/ml. The final concentration of rhBMP-4 in the rhBMP-4/FFSS implant was 0.025 mg/ml, which was based on our previous study (Pang et al., 2004). For the positive control experiments, FFSS was used alone. The rhBMP-4/FFSS and FFSS implants were made by clotting in a cylinder type mold (Figure 1 A). For the peripheral seal, additional rhBMP/FFSS or FFSS solution was injected into the implant margin(Figure 1 B). The final implant's block form was 3 mm height and 8 mm diameter disc type (Figure 1 C). The implants were placed into the calvarial defects.

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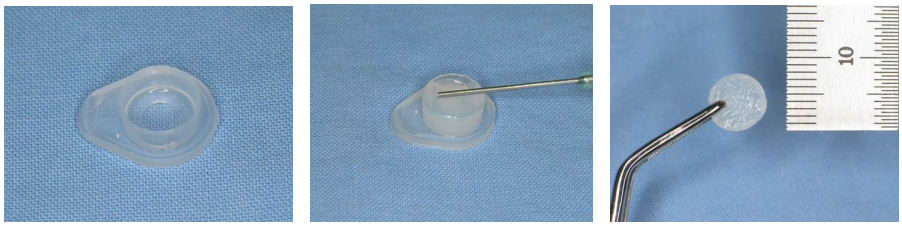
† Tisseel®, Immuno AG, Vienna, Austria.
‡R&D Systems Inc., Minneapolis, MN



A B C

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

3. Surgical protocol

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 reflected, exposing the calvarial bone. A standardized, circular, transosseous defect, 8 mm in diameter, was created on the cranium using a saline-cooled trephine drill. The animals were divided into 3 groups of 10 animals each and allowed to heal for 2 (5 rats) and 8 (5 rats)weeks. Each animal received one of three experimental conditions: surgical control that did not receive any materials into the defect, FFSS (Tisseel) only and rhBMP-4(0.025mg/ml)/FFSS. The periosteum and skin were then closed and sutured with 4-0 coated Vicryl violet†† .

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§ Ketalar®, Yuhan Co., Seoul, Korea
** Rompun®, Bayer Korea, Seoul, Korea
†† Polyglactin 910, braided absorbable suture, Ethicon, Johnson & Johnson Int., Edinburgh, UK

4. Histologic procedures

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

5. Histometric procedures

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 magnification of 20x.

The following measurements were made (Figure 2):

1. Defect closure (%): the distance between the defect margin and the ingrowing bone margin
2. New bone area (mm^2): the area of newly formed bone within the total augmented area
3. Bone density (%): new bone area / total augmented volume * 100
4. 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.

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‡‡ Image-Pro Plus®, Media Cybernetics, Silver Spring, M.D.
§§ Olympus BX50, Olympus Optical co., Tokyo, Japan.

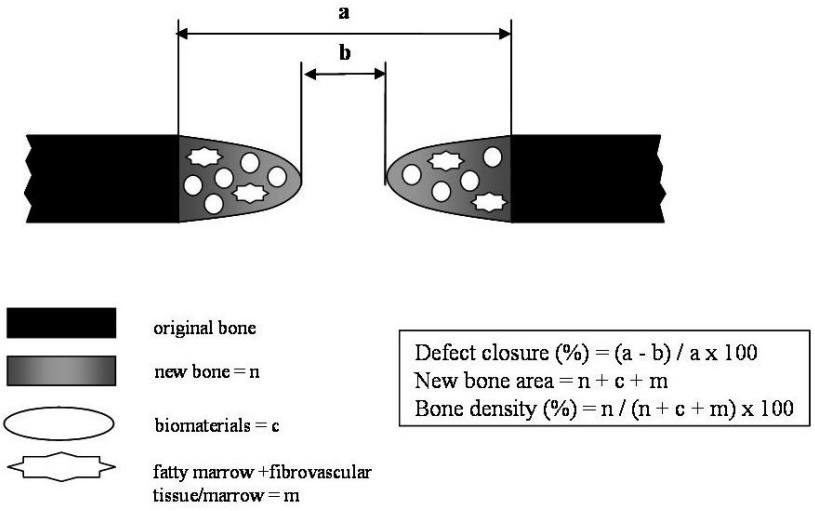

Figure 2. Schematic drawings of calvarial osteotomy defect showing histomorphometric analysis

6. Statistical analysis

Histomorphometric recordings from the samples were used to calculate means and standard deviations (m±SD). To analyze the effect of both time and condition and to detect the interaction effect between time and condition, two-way analysis of variance was used. ANOVA and post hoc t-test were used to analyze differences between groups at both time points. For the comparison between 2- and 8-week healing in the same group, statistical significance was determined by paired t-test. Statistical significance was set at $P<0.05$

III. Results

1. Clinical Observations

Wound healing was generally uneventful and appeared similar for all groups.

2. Histologic observations

Surgical control group

At 2 and 8 weeks postsurgery, thin connective tissue was present at the defect site. Also, there was a minimal amount of new bone formation originating from the defect margins. The defect center appeared to be collapsed due to tissue compression(Figure 3 A and B).

FFSS group

At 2 weeks postsurgery, the defect sites were filled with FFSS remnants and loose connective tissue including minimal new bone formation at the defect margins. There was no evidence of adverse reaction (Figure 4 A and B). At 8 weeks, defect sites exhibited more bone formation from the defect margin to center compared to at 2 weeks. FFSS appeared completely resorbed at 8 weeks (Figure 4 C and D).

rhBMP-4/FFSS group

All defects 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 exhibiting a dense arrangement adjacent to the newly formed bone were suggestive of

continued bone apposition at the initial healing stage. However, less bone formative activity was found at the central aspects of the defects. Thus, healing of rat calvarial defect following treatment of rhBMPs initiated from the defect margin. At 2 weeks postsurgery, marked bone regeneration, as well as a consolidation of lamellar bone along the dural aspect, was observed. There was no evidence of adverse reaction (Figure 5 A and B).

At 8 weeks, the quantity of the new bone was greater than that observed at 2 weeks and the specimens showed a more advanced stage of remodeling and consolidation. The newly formed bone consisted of woven bone and lamellar bone, and showed cement lines separated earlier from more recently deposited bone and concentric rings of Haversian system. Fatty marrow was seen in the new bone area. No remnants of rhBMP-4/FFSS were detected (Figure 5 C and D).

3. Histomorphometric analysis

The results of the histomorphometric analysis are shown in Table 1-4. The augmented area of the FFSS group was significantly greater than that of the surgical control group at both time points ($P<0.05$). The new bone area and bone density of FFSS at 8 weeks were greater than those of FFSS at 2 weeks ($P<0.05$), thereby demonstrating the osteoconductive activity of FFSS. The new bone area and bone density of the rhBMP-4/FFSS group were significantly greater than those of the FFSS group at both time points ($P<0.05$). The rhBMP-4/FFSS implant appeared to release rhBMP-4 in normal bone healing procedure. These results support the role of FFSS as the proper carrier for rhBMP-4.

Two-way ANOVA revealed that there was an interaction between time

and condition in new bone area and bone density (P<0.05). Condition had an influence on defect closure, new bone area, augmented area and bone density (P<0.05), whereas time had an influence on new bone area and bone density (P<0.05). These results indicate that FFSS had an influence on total augmentation area, and that rhBMP-4 had an influence on bone formation.

Table 1. Defect closure(group means ± SD; n=5, %)

	2 weeks	8 weeks
surgical control	12.1 ± 3.9	13.5 ± 4.6
FFSS	39.6 ± 26.9¶	66.8 ± 30.8*¶
rhBMP4/FFSS	93.1 ± 15.3¶₸	94.0 ± 13.4¶₸

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

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

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

Table 2. New bone area (group means ± SD; n=5, mm^2)

	2 weeks	8 weeks
surgical control	0.2 ± 0.1	0.4 ± 0.1*
FFSS	0.4 ± 0.3	2.3 ± 0.7*¶
rhBMP4/FFSS	2.1 ± 0.5¶₸	3.4 ± 0.5*¶₸

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

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

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

Table 3. Augmented area (group means ± SD; n=5, mm^2)

	2 weeks	8 weeks
surgical control	0.2 ± 0.1	0.4 ± 0.2*
FFSS	5.8 ± 3.1¶	4.9 ± 1.5¶
rhBMP4/FFSS	5.5 ± 1.3¶	4.7 ± 0.5¶

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

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

Table 4. Bone density (group means ± SD; n=5, %)

	2 weeks	8 weeks
surgical control	97.8 ± 3.2	94.0 ± 3.3
FFSS	6.7 ± 3.0¶	47.1 ± 9.2*¶
rhBMP4/FFSS	39.1 ± 10.6¶₸	72.5 ± 6.3*¶₸

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

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

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

IV. Discussion

The purpose of this study was to evaluate the osteogenic effect of the fibrin-fibronectin sealing system (FFSS) and the possibility of FFSS as a carrier system for rhBMP-4 in the rat calvarial defect model. An 8-mm, calvarial, critical-size osteotomy defect was created in each of 30 male Sprague-Dawley rats. Three groups of 10 animals each received either rhBMP-4 (0.025mg/ml) in an FFSS carrier, FFSS only carrier control, or negative surgical control. The groups were evaluated by histologic and histometric parameters following a 2- and 8-week healing interval (10 animals/group/healing interval). Histologic and histometric results revealed that FFSS has an osteoconductive activity and that it is a candidate as a delivery system for rhBMP-4.

The beneficial effect of FFSS in wound healing has been well documented. Several animal studies have demonstrated positive results of FFSS on bone healing. Warrer et al. evaluated the regenerative effects of FFSS in dog periodontal defect model (Warrer et al., 1992). New attachment and bone regrowth after flap surgery with and without FFSS were evaluated in 4 beagle dogs. The results indicated that the most favorable results were obtained in flap surgery with FFSS. Bosch et al. have also shown that fibrin sealant produced an early enhancement of bone repair in rabbits (Bosch et al., 1980). The physical properties of the bony callus seemed to also be enhanced by fibrin sealants (Keller et al., 1985). In contrast, other studies have produced conflicting results on the bone healing and regenerative effectiveness of FFSS. In an experiment with dogs, Carmagnola et al. examined the healing of self-contained bone defects after

the placement of Bio-Oss particles alone or mixed with FFSS (Carmagnolaet al., 2002). The results showed that Bio-Oss treated defect had a higher percentage of contact between graft particles and bone tissue than defects treated with Bio-Oss + FFSS. The authors concluded that fibrin treatment inhibited the natural bone healing. Another study also reported similar results in rabbits (Brittberg et al., 1997). Thus, the effects of FFSS on bone healing remain controversial.

In our present study, the augmentation area of the FFSS group was significantly greater than that of the surgical control group (Table 3). When FFSS is clotted, the fibrin forms cross-linking which increases the mechanical strength of the clot and reduces its susceptibility to proteolytic cleavage (Soffer et al., 2003). It seems that the biomechanically stable fibrin clot was strong enough to maintain sufficient space for new bone formation. This conclusion was supported by the following results; 1) new bone area of the FFSS group was significantly greater than that of the surgical control group at both time points, 2) new bone area and bone density of the FFSS group at 8 weeks being greater than those at 2 weeks (Tables 2 and 4). Therefore, it could be suggested that FFSS acts like osteoconductive materials on bone healing.

New bone area and bone density of the rhBMP-4/FFSS group were significantly greater than those of the FFSS group at both time points (Tables 2 and 4). And those of rhBMP-4/FFSS at 8 weeks were greater than at 2 weeks ($P<0.05$). These results indicate that the FFSS clot successfully retained and released rhBMP-4 and that new bone derived from the rhBMP-4/FFSS clot had been formed in the normal bone healing procedure. Carrier systems for BMPs must have proper releasing kinetics.

FFSS used in this study was a gel type. The effects of gel type carrier for BMPs on bone regeneration were evaluated in the rat model (Talwar et al., 2001). The study showed that a composite of gelatin and rhBMP-2 released rhBMP-2 in the proper way and showed the most bone formation. Thus, a gel type substance is a suitable carrier for BMPs. Carrier systems for BMPs must also be biomechanically strong enough to maintain the space for new bone formation. The augmentation area of rhBMP-4/FFSS was statistically greater than that of the control group($P<0.05$). There were no differences between FFSS and rhBMP-4/FFSS at either time point. These results indicate that rhBMP-4/FFSS was biomechanically strong enough to maintain the space for new bone formation and that rhBMP-4 does not change the space maintaining property of FFSS. In histological analysis, rhBMP-4/FFSS appeared to be completely absorbed without adverse reaction at 8 weeks. Thus, FFSS seems to have a biodegradable property.

Kawamura and Urist evaluated FFSS as a carrier of BMPs in mouse quadriceps muscle pouch and determined that the composite of FFSS and rhBMPs promotes an osteogenic activity of rhBMPs (Kawamura et al., 1988). Their composite induced interaction of mesenchymal cells with BMP in the early healing stage and they concluded that the composite a synergic effect on BMP activity compared to BMPs alone. In our study, it was observed that rhBMP-4/FFSS specimens showed more osteocytes and osteoblast-like cells than FFSS (Figure 4 and 5). Thus, rhBMP-4/FFSS appears to promote the growth and differentiation of osteogenic cells.

In conclusion, the results of this controlled animal study indicated that FFSS served as a scaffold that maintained room for new bone formation

without adverse reaction and released rhBMP-4 in the proper way. It was concluded that FFSS has an osteoconductive activity and that it is a candidate as a carrier system for rhBMP-4. However, further studies are required to clarify the effect of FFSS on the bone formation and delivery of rhBMP-4.

V. Conclusion

The purpose of this study was to evaluate the osteogenic effect of FFSS and the possibility of FFSS as a carrier system for rhBMP-4 in the rat calvarial defect model. An 8-mm, calvarial, critical-size osteotomy defect was created in each of 30 male Sprague-Dawley rats. Three groups of 10 animals each received either rhBMP-4 (0.025mg/ml) in an FFSS (Tisseel) carrier, FFSS only, or negative surgical control. The groups were evaluated by histologic and histometric parameters following a 2- and 8-week healing interval (10 animals/group/healing interval). The results were as follows:

1. The augmented area and new bone area of the FFSS group were significantly greater than that of the surgical control group at both time points ($P<0.05$). The new bone area and bone density of FFSS at 8 weeks were greater than those of FFSS at 2 weeks ($P<0.05$).

2. The new bone area and bone density of the rhBMP-4/FFSS group were significantly greater than those of the FFSS group at both time points ($P<0.05$). The augmentation area of rhBMP-4/FFSS group was not significantly different compared with that of FFSS group at either time point ($P>0.05$).

The results of this controlled animal study indicated that FFSS served as a scaffold to maintain room for new bone formation without any adverse reaction and that it released rhBMP-4 in the proper way. Therefore, we conclude that FFSS has an osteoconductive activity and that it is a candidate as a carrier system for rhBMP-4.

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Legends

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

Figure 2. Schematic drawings of calvarial osteotomy defect showing histomorphometric analysis.

Figure 3. Representative photomicrographs of the surgical control group at 2 weeks (A) and 8 weeks (B). Thin loose connective tissues were observed between the margins. The middle of the defects appears collapsed (arrow head=defect margin; H-E stain; original magnification ×20).

Figure 4. Representative photomicrographs of the FFSS group at 2 weeks (A and B) and 8 weeks (C and D). FFSS remnants were observed at 2 weeks. At 8 weeks, FFSS had been replaced by connective tissues and new bone (arrow head=defect margin; asterisk=FFSS remnants; NB=new bone; H-E stain; original magnification A and C ×20; B and D ×100).

Figure 5. Representative photomicrographs of the rhBMP-4/FFSS group at 2 weeks (A and B) and 8 weeks (C and D). FFSS remnants and newly formed bone were observed at 2 weeks. At 8 weeks, FFSS had

been replaced by new bone and fatty marrow and adipose cells were observed. (arrow head=defect margin; asterisk=FFSS remnants; X=fatty marrow and adipose cells; NB=new bone; H-E stain; original magnification A and C ×20; B and D ×100).

Figures

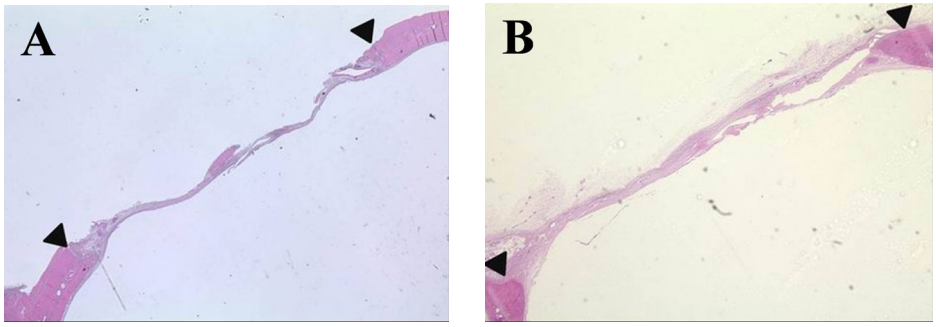

Figure 3

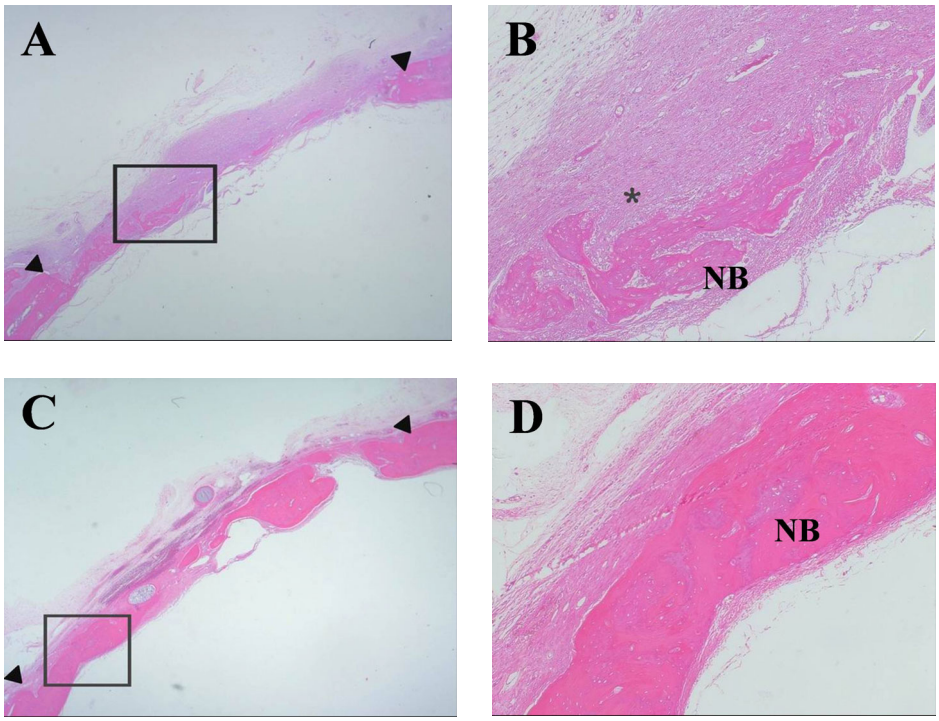

Figure 4

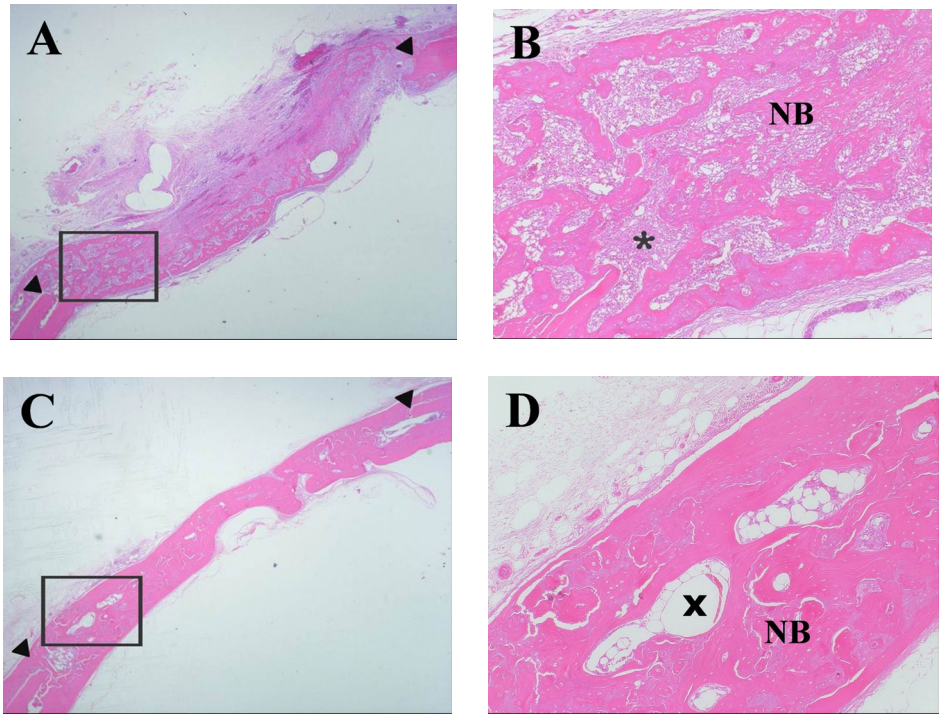

Figure 5

국문 요약

백서 두개골 결손부에서 bone morphogenetic protein-4의 전달체로서 fibirn-fibronectin sealing system의 골재생효과

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한 동 관

Bone morphogenetic protein(BMP)는 발육이나 상처치유 기간에 골형성과정에 있어서 중요한 역할을 한다. 그러나 아직까지 BMPs의 이상적인 운반체는 발견되지 않았다. Fibrin-fibronectin sealing system(FFSS)은 상처치유를 증진시키고, 국소지혈 및 조직접착등의 효과가 있다. 이연구의 목적은 백서 두개골에서 FFSS를 운반체로 사용하여 rhBMP-4를 백서 두개골 결손부에 적용하였을때 골형성 효과를 평가하고, FFSS 자체의 골형성능을 평가하는 것이다.

30마리의 웅성 백서에서 8mm 임계크기의 두개부 결손을 형성하였다. 10마리씩 3개의 군으로 나누고, 각 군은 아무것도 이식하지 않은 대조군, FFSS를 이식한 군, FFSS를 전달체로 사용하여 농도 0.025mg/ml rhBMP-4를 이식한 군(rhBMP-4/FFSS)으로 나누어 술 후 2주와 8주에 치유 결과를 조직학적, 조직계측학적으로 비교 관찰하였다.

결과는 다음과 같다.

1. FFSS 군의 조직 증대양과 신생골형성양은 대조군보다 통계학적으로 유의성 있게 큰 값을 보였다($P<0.05$). FFSS군에서 신생골형성양과 골밀도는 8주에서 2주보다 유의성 있게 큰 값을 보였다.($P<0.05$)

2. rhBMP-4/FFSS군의 신생골형성양과 골밀도는 2, 8 주에서 모두 FFSS군보다 유의성 있게 큰 값을 보였다($P<0.05$). 2, 8주에서 rhBMP-4/FFSS군의 조직증대양은 모두 FFSS군과 유의성 있는 차이를 보이지 않았다($P>0.05$).

이와 같은 결과로 미루어보아 FFSS는 신생골형성시 공간 유지를 하는 지지체(scaffold)로서의 역할을 하며, 적절하게 골형성단백(rhBMP-4)을 유리시킴을 알 수 있다. 따라서 FFSS는 골형성능이 있는 것으로 사료되며, 골형성단백(rhBMP-4)의 전달체로서의 가능성이 있다.

핵심되는 말 : Bone morphogenetic protein(BMP); Fibrin-fibronectin sealing system; 운반체; 골재생; 조직증대; 백서 두개 결손부