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In vivo evaluation of commercially available gel-type polyethylene glycol membrane for carrier of recombinant human bone morphogenetic protein-2

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In vivo evaluation of commercially available gel-type polyethylene glycol membrane for carrier of recombinant human bone morphogenetic protein-2

Directed by Professor Kyoo-Sung Cho

A Doctoral Dissertation

submitted to the Department of Dentistry

the Graduate School of Yonsei University

in partial fulfillment of the requirements for the degree of

Ph.D. in Dental Science

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December 2016

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This certifies that the Doctoral Dissertation
of Ji-Woong Jang is approved.

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감사의 글

본 논문이 완성되기까지 부족하기만 한 저를 항상 격려해 주시고 참된 스승의 의미를 일깨워 주신 조규성 교수님께 깊은 감사를 드립니다. 그리고 진심 어린 조언과 따뜻한 관심으로 지켜봐 주신 김종관 교수님, 채중규 교수님, 최성호 교수님, 김창성 교수님, 정의원 교수님, 이중석 교수님께 감사드립니다.

연구 내내 많은 도움을 준 연구원을 비롯한 치주과 선배님들과 의국원들에게도 감사와 애정을 전합니다.

그리고 제가 이 자리까지 올 수 있도록 격려해주시고 도와주신 양가 부모님과 사랑하는 아내 김희정에게 감사의 마음을 전합니다. 이 지면을 통해 인사드리지 못한 다른 모든 분들께도 진심으로 감사드립니다.

2016년 12월

저자 씀

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Abstract

In vivo evaluation of commercially available gel-type polyethylene glycol membrane for carrier of recombinant human bone morphogenetic protein-2

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Objective: This study evaluated a commercially available, 3-dimensional gel-type polyethylene glycol (PEG) membrane as a carrier for rhBMP-2 using a rat calvarial defect model. Another gel-type carrier, fibrin-fibronectin system (FFS) was used as a positive control.

Material and Methods: Critical-sized defects were made in rat calvarium, which were then allocated to ten groups comprising two healing periods and biomaterial conditions: (1) sham control, (2) FFS only, (3) FFS/BMP-2, (4) PEG only, (5) PEG/BMP-2. Radiographic and histologic analyses were performed at 2 and 8 weeks after surgery.

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Results: After 2 weeks, some parts of FFS were biodegraded and extensive cellular infiltration was observed at sites that received FFS or FFS/BMP-2. PEG membrane retained its augmented volume without cellular infiltration at sites that received PEG or PEG/BMP-2. After 8 weeks, FFS was completely degraded and replaced by new bone and connective tissues. On the other hand, the volume of residual PEG was similar to that at 2 weeks, with slight cellular infiltration. In particular, there was progressed bone regeneration around micro-cracks and resorbed outer surface in the PEG/BMP-2. Although PEG/BMP-2 showed increased area and percentage of new bone, there is no statistical significance after 2 weeks and 8 weeks in histomorphometric analyses. However, the appearance of the healing differed (with new bone formation along microcracks in PEG/BMP-2), and further studies with longer healing periods are needed to draw conclusions about clinical applications.

Conclusion: Evidences of mechanical stability and new bone formation along micro-cracks within PEG/BMP-2 might support PEG membrane as a candidate carrier material for rhBMP-2.

KEYWORDS: bone morphogenetic protein-2, polyethylene glycols, bone regeneration, bone substitutes, hydrogel, fibrin

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In vivo evaluation of commercially available gel-type polyethylene glycol membrane for carrier of recombinant human bone morphogenetic protein-2

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I. Introduction

Various barrier membranes have been developed and used for periodontal and bone regeneration during the past 3 decades. Among these, expanded polytetrafluoroethylene (ePTFE) membrane was recommended as the gold standard until its commercial use was discontinued. Even though ePTFE membrane could produce more predictable bone regeneration in many cases, it has disappeared from dental use due to manageability difficulties and the necessity for additional surgery to

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remove the membrane. In addition, membrane exposure can frequently occur in cases using nonresorbable membranes, and this complication may be associated with the loss of soft tissue and bacterial infection at the augmented site.

Resorbable collagen membrane is the most widely used barrier membrane for bone regeneration in dental applications, due to its favorable clinical manageability. Moreover, previous studies have demonstrated that collagen and ePTFE membranes exhibit comparable suitability for guided bone regeneration,(Coulthard et al., 2003; Esposito et al., 2006) which has lead to nonresorbable membrane being replaced by resorbable membrane.(Buser et al., 1993; Hammerle and Karring, 1998) However, these studies were restricted to favorable defect types such as dehiscence, fenestration, and other horizontal defects. The insufficient mechanical stability of a resorbable membrane can make it difficult to apply to unfavorable types of defects such as vertical ridge deficiency. Since ePTFE membranes are no longer commercially available, the use of various other treatment options has been suggested, such as allogenous/synthetic bone block, titanium mesh, and a newly developed gel-type membrane.

A gel-type membrane was developed based on hydrogel technology using a complex of polyethylene glycols (PEGs), which can be degraded by water with a minimal adverse response. Previous studies found that the hydrolysis of a gel-type membrane is a significantly slower process than the biodegradation of resorbable collagen membrane, with most of the morphologic structure being maintained over a

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2-month period.(Herten et al., 2009) In subcutaneous transplantation it was demonstrated that cells could penetrate the gel-type membrane even after 4 months.(Wechsler et al., 2008) Randomized clinical trials using this gel-type and collagen membranes revealed comparably successful bone formation in the experimental and control groups, and a significantly reduced time for membrane application due to its easy clinical manageability.(Jung et al., 2009a; Ramel et al., 2012) These properties of long-term mechanical stability and favorable clinical manageability have made the gel-type membrane a candidate material for use in guided bone regeneration with vertical augmentation. However, high rates (up to 60%) of clinical complications occurred in the vertically augmented sites, such as delayed healing, wound dehiscence, and limited bone regeneration within the grafted area, although several case reports showed successful regeneration of alveolar ridge.(Esposito et al., 2009) These complications can even occur in vertical augmentation cases using an ePTFE membrane or autogenous block bone graft due to the restricted healing potential at the recipient site.

The above-mentioned considerations led the present authors to hypothesize that combining clinical technology with recombinant human bone morphogenetic protein-2 (rhBMP-2) can enhance bone regeneration within the protected site by the use of a gel-type membrane. The specific aim of this study was to evaluate a commercially available gel-type PEG membrane as a carrier for rhBMP-2 using a rat calvarial defect model and with healing periods of 2 and 8 weeks.

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II. Materials and Methods

1. Animals

One hundred male Sprague-Dawley rats (body weight 200–300 g) were used in ten groups, covering five types of materials and two healing periods. Animals were kept in plastic cages in a room at an ambient temperature of 21°C with ad libitum access to water and a standard laboratory pellet diet. Animal selection, care, and the surgical protocol were approved by the Institutional Animal Care and Use committee at Yonsei Medical Center, Seoul, Korea (2011-0322).

2. Materials

Gel-type membrane: Commercially available, synthetic PEG membrane (MembraGel®, Straumann, Basel, Switzerland) was used as the gel-type membrane. Two sterile PEG components were packed in separate syringes; this allowed the membrane to be applied in a liquid state, with mixing of the components resulting in hydrogel solidification shortly thereafter.

Fibrin-fibronectin system: A commercially available fibrin-fibronectin sealing system (FFS; Tisseel®, Immuno, Vienna, Austria) was used as a positive control for the gel-type carrier. In our previous study, this FFS carrying rhBMP-2 induced bone formation and produced significantly enhanced bone healing within critically sized

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calvarial defects. (Han et al., 2005)

rhBMP-2: A commercial institute (Korea Bone Bank, Seoul, Korea) provided rhBMP-2 that had been expressed by Chinese hamster ovary cells, and this was reconstituted and diluted in a buffer solution to a concentration of 0.05 mg/ml.

3. Study design

Surgically produced, critically sized, rat calvarial defects were grafted with biomaterials according to the experimental design. Experimental groups were allocated to ten groups covering two healing periods (2 and 8 weeks) and five biomaterial conditions as follows:

- Sham control: standardized calvarial defect produced but no grafted biomaterials used.
- FFS: calvarial defect grafted with a prefabricated, standardized disc comprising FFS only.
- FFS/BMP-2: calvarial defect grafted with a disc comprising an FFS mixture containing 5 μg of rhBMP-2.
- PEG: calvarial defect grafted with a prefabricated, standardized disc comprising gel-type PEG membrane only.
- PEG/BMP-2: calvarial defect grafted with a disc comprising a PEG membrane mixture containing 5 μg of rhBMP-2.

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4. Prefabrication of FFS and PEG discs

The four types of discs were prefabricated according a previously described protocol.(Han et al., 2005) Briefly, a gel-type FFS and PEG membrane was made by mixing each solution set according to the manufacturer's recommendation. rhBMP-2 solution (100 μl) at a concentration of 0.05 mg/ml was then added to the FFS and PEG mixture. The mixture was injected into a custom-made circular mold with a diameter of 8 mm, which yielded standardized 8-mm-diameter discs after solidification.

5. Surgical procedures

The animals were anesthesized by an intramuscular injection (5 mg/kg body weight) of ketamine hydrochloride (Ketalar®, Yuhan, Seoul, Korea) and xylazine (Rompun®, Bayer Korea, Seoul, Korea). The surgical site was shaved and disinfected with iodine. A linear incision was made midsagitally and a full-thickness flap was reflected. A standardized, circular, transosseous defect with a diameter of 8 mm was prepared on the calvarium using a trephine drill (3i Implant Innovation, Palm Beach Gardens, FL, USA). The trephined calvarial disk was removed carefully to avoid injury to the brain, and the treatments were then applied to the defect area according to the group allocations. All surgical sites were sutured with 4-0 Monosyn® (glyconate absorbable monofilament, B-Braun, Aesculap, PA, USA) for primary wound closure. The animals were sacrificed by CO_2 suffocation at either 2 or 8 weeks after surgery.

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6. Micro-computed tomography

The specimens were fixed in 10% neutralized-buffered formalin for 10 days and scanned using a micro-computed tomography (micro-CT) system (Skyscan® 1072, Skyscan, Aartselaar, Belgium) at a resolution of 35 μm (100 kV and 100 μA) before processing for the histology analysis. The site of interest was reconstructed and analyzed using OnDemand 3D® software (Cybermed, Seoul, Korea). Mineralized tissues were distinguished based on a previously described threshold of 275 mg cm^{-3}.(Aghaloo et al., 2006) Segmentation of mineralized tissue from the total augmented area and exclusion of native calvarial bone were performed. Segmented newly formed mineralized tissue is coded in red in Fig. 1. The total augmented volume and newly formed mineralized tissue volume were measured for quantitative analysis. The degree of defect resolution was quantified as the percentage of newly formed mineralized tissue volume relative to the calvarial defect volume.

7. Histology analysis and histometric measurements

The specimens were decalcified in 5% formic acid for 14 days, and then embedded in paraffin. Serial 5-μm-thick sections were prepared through the center of the calvarial defects. The two central-most sections were selected from each block and stained with Masson's trichrome. Each slide was examined, and digital images were obtained using a light microscope equipped with a digital camera (BX50, Olympus, Tokyo, Japan). Computer-assisted histometric measurements were carried

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out using an automated image analysis system (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA). The areas and percentages of new bone, residual material, and fibrovascular tissue were measured. The augmented area bordered by an outline of grafted biomaterials including newly formed bone was measured, and residual biomaterial or newly formed bone was measured separately within the augmented area. The amount of fibrovascular tissue was calculated by subtracting the area of newly formed bone and residual biomaterials from the augmented area. The proportion of each component within the augmented area was calculated. The measured parameters and the calculation methods are illustrated in Fig. 1.

8. Statistical analysis

Statistical software was used to analyze the results from micro-CT and histomorphometry analyses (SPSS 15.0, SPSS, Chicago, IL). One-way ANOVA and the post-hoc Scheffe test were used to analyze the differences between groups at each healing period. The independent *t*-test was carried out to evaluate the effect of time. The cutoff for statistical significance was set at p=0.05.

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III. RESULTS

1. Clinical Findings

The entire study period passed uneventfully for all animals, with no postoperative infections and normal wound healing observed during the experimental period.

2. Histology and radiography findings

Two-week healing period: The histology and radiography results for the sham controls showed that a very small amount of new bone had filled the defect margin and that most of the defect contained loose connective tissue (Figs. 2 and 3). In both the PEG and PEG/BMP-2 groups, after 2 weeks of healing, the PEG membrane maintained the augmented area and there was no inflammatory reaction. Cell infiltration within the PEG shell and the resorption process of biomaterials were barely detectable, and the cell-occlusion property persisted. Some fragmentation was detected within the inner part of the PEG membrane during histology analysis. There was a limited amount of newly formed bone at the periphery of the defect toward the calvarium. In particular, new bone ingrowth in clear space between the PEG membrane and original bone was observed in the PEG/BMP-2 group (Fig. 4). In contrast to the PEG membrane, the adapted FFS at sites that received FFS or FFS/BMP-2 exhibited considerable biodegradation, and extensive cell infiltration was

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observed. The defect contained a small amount of residual FFS, fibrovascular tissue, and new bone. Although there was a small amount of new bone at the periphery of the defect in the FFS-only group, the use of FFS/BMP-2 increased the amount of newly formed woven bone at the defect margin (Fig. 5). The radiography findings were similar to the histology findings. The filling of the radiopaque defect was slightly increased in the PEG/BMP-2 than the PEG-only group, but both PEG and PEG/BMP-2 produced small degrees of defect filling. The radiography images indicated that FFS/BMP-2 produced enhanced radiodense tissues compared to the other groups (Fig. 2).

Eight-week healing period: After 8 weeks of healing, the dimensions of the adapted PEG membrane were well maintained, and slight cell invasion was detectable in the groups that received PEG or PEG/BMP-2. Outline irregularities and slight resorption of the outer surface were evident. While bone regeneration had slightly increased and most of the defect contained residual PEG membrane and fibrous tissues in the PEG-only group, progressed bone regeneration was detected at microcracks and at the resorbed outer surface area in the PEG/BMP-2 group (Fig. 4). Microcracks in the PEG shell facilitated cell invasion and subsequent matrix remodeling.(Wechsler et al., 2008) rhBMP-2 released around the microcracks induced effective new bone formation at sites that received PEG/BMP-2. Moreover, rhBMP-2 around the resorbed outer surface of PEG membrane also enhanced the formation of new bone. Micro-CT revealed that the defect filling was still restricted in the PEG-only group, whereas radiopaque defect filling was increased around the defect borders in the

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PEG/BMP-2 group (Fig. 2). The adapted FFS was completely degraded and had been replaced by new bone or connective tissues at sites that received FFS or FFS/BMP-2. There was a small amount of new bone formation toward the center of the defect at sites that received FFS only, whereas increased amounts of mature lamellar bone and bone marrow were seen throughout the implantation site in the FFS/BMP-2 group (Fig. 5). The radiographic images showed marked radiopacity, with the defect being almost completed covered with mineralized tissues in the FFS/BMP-2 group. The FFS-only group still showed restricted radiopaque defect filling (Fig. 2). In the sham controls, as at 2 weeks postsurgery, there was a minimal amount of new bone at the defect margin and most of the defect still contained loose connective tissue (Fig. 3).

3. Histomorphometry measurements and quantitative micro-CT analyses.

At sites that received PEG or PEG/BMP-2, the area and percentage of residual PEG were maintained, and there were no significant differences between 2 and 8 weeks. On the other hand, at sites that received FFS or FFS/BMP-2, the adapted FFS was quite degraded after 2 weeks, and there was no residual FFS after 8 weeks (Table 1). The percentage and area of new bone were significantly greater for FFS/BMP-2 than for the other groups after 2 and 8 weeks. Although sites with PEG/BMP-2 showed increased area and percentage of new bone, the differences were not statistically significant except relative to sham surgery after 2 and 8 weeks (Table 1).

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The quantitative micro-CT results were similar to histomorphometry measurements. The mineralized volume and defect resolution were significantly greater in the FFS/BMP-2 group than in the other groups. On the other hand, PEG/BMP-2 did not induce significantly increased mineralized volume or defect resolution (Table 2).

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IV. DISCUSSION

This study evaluated the use of a gel-type PEG membrane as a carrier for rhBMP-2, with the specific objective of radiographically and histologically observing the bone healing process within rat calvarial defects grafted with PEG membrane carrying rhBMP-2, in comparison to the use of FFS with rhBMP-2. To focus on the osteoinductive effects of rhBMP-2 carried by a gel-type PEG membrane, no other bone substitutes were included in this study, and FFS was used for a positive control carrier based on previous results.(Hong et al., 2006) However, little bone formation was observed within defects grafted with either PEG/BMP-2 or PEG irrespective of the healing period (2 or 8 weeks) and despite extensive augmentation based on the volume of the PEG membrane. In contrast, bone formation was induced at sites grafted with FFS/BMP-2, and the defect was almost complete covered by newly formed bone with complete degradation of biomaterials after 8 weeks of healing. This is consistent with the results of a previous study that evaluated FFS as a carrier for rhBMP-2 in the same animal model.(Han et al., 2005)

At 2 weeks after surgery, residual biomaterials could be observed at all experimental sites, but with the appearance differing according to the grafted biomaterials (i.e., FFS and PEG membrane). While only small remnants of FFS remained and extensive cellular infiltration could be found around/within residual biomaterials at sites that received FFS or FFS/BMP-2, most of the augmented volume of PEG membrane remained, with limited evidence of cellular infiltration or

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resorption processes of biomaterials at sites that received PEG or PEG/BMP-2. Even at the 8-week healing period, the volume of residual PEG membrane was similar to that after 2 weeks, and the membranes separated the defect area from the covering mucocutaneous flap with small amounts of cellular infiltration into the membrane. These observations may be related to cell occlusiveness and mechanical stability as an ideal property of a barrier membrane, and these properties might also explain previous clinical results.(Jung et al., 2009b; Jung et al., 2006; Thoma et al., 2009; Thoma et al., 2011)

Previous studies have found that the use of a PEG matrix delivering rhBMP-2 accelerated or enhanced bone regeneration in various animal defect models.(Hanseler et al., 2012; Jung et al., 2008; Wehrhan et al., 2012; Wen et al., 2011) However, in the present results, the use of a PEG membrane carrying rhBMP-2 did not enhance bone healing within critically sized rat cavarial defects compared to the healing at sites that received PEG membrane only. This difference between the studies might be due to the methods used to apply the PEG membrane carrier—while the previous studies used a mixture of PEG matrix and bone substitute particles, the present study used a PEG membrane only as a carrier of rhBMP-2. rhBMP-2 that is entrapped within the PEG membrane after gelation cannot participate in the *in vivo* healing processes, and so any effects of rhBMP-2 carried by the PEG membrane would depend on the presence of degradation or mechanical cracking.(Lutolf et al., 2003) This idea is supported by the present results for the 8-week samples, in which new bone formation was evident along microcracks within the PEG membrane or on resorbed outer

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surfaces at sites that received PEG/BMP-2, whereas only fibrous tissue formed around and within the biomaterials at sites that received PEG membrane only. In the previous studies that used mixtures of PEG matrix and bone substitutes, mechanical stability would have been significantly decreased in the presence of increased interfaces between heterogeneous biomaterials, and these could influence the release of rhBMP-2 from the PEG matrix. These features could finally produce clinical and histologic enhancements of bone regeneration within the defects even for the same observational period. Considering these previous results, rhBMP-2 carried by PEG membrane can also be expected to enhance bone formation over a longer healing period, with greater biodegradation of the biomaterials. The present authors hypothesized that clinical application of rhBMP-2 carried by PEG membrane can enhance bone regeneration within the augmented area in guided bone regeneration. This study therefore histologically evaluated the osteoinductivity when using a gel-type PEG membrane containing rhBMP-2. While the histomorphometric results indicated that the use of PEG/BMP-2 failed to enhance bone formation after 2 and 8 weeks of healing, evidences of mechanical stability and new bone formation along micro-cracks within PEG/BMP-2 might support PEG membrane as a candidate carrier material for rhBMP-2. However, further studies with longer healing periods are needed to draw conclusions about clinical applications. In addition, various methodologies for applying PEG membrane should be developed and studied in order to control the degradation of biomaterials.

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Figure Legends

Figure 1. Schematic diagrams of the methods used for histomorphometry measurements (a) and quantitative micro-CT analyses (b). The histology diagram shows defect margins, in which the augmented area (light gray) with residual biomaterials, newly formed bone (brown), and fibrovascular tissues (blue) were measured. The augmented area bordered by an outline of grafted biomaterials including newly formed bone was measured, and residual biomaterial or newly formed bone was measured separately within the augmented area. The amount of fibrovascular tissue was calculated by subtracting the area of newly formed bone and residual biomaterials from the augmented area. In micro-CT analysis, segmentation of mineralized tissue from the total augmented area and exclusion of native calvarial bone were performed. Segmented newly formed mineralized tissue is indicated in red.

Figure 2. Three-dimensionally reconstructed micro-CT views demonstrating marked radiopaque defect filling in the FFS/BMP-2 group compared to the other groups, regardless of the experimental period. The use of PEG/BMP-2 produced a larger mineralized volume than in the PEG-only group, but both groups showed restricted radiopaque mineralized tissue after 2 and 8 weeks.

Figure 3. Overview of histology results after 2 and 8 weeks. After 2 weeks, there was

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no histologically detected cell invasion at sites that received PEG or PEG/BMP-2, and the PEG membrane was well maintained. In contrast, at sites that received FFS or FFS/BMP-2, the adapted FFS was quite biodegraded and cell infiltration was observed. After 8 weeks, the adapted FFS was completely degraded and had been replaced by new bone and connective tissues. However, the dimensions of the PEG membrane were still maintained and there was a small amount of cellular infiltration. In particular, there was enhanced bone regeneration around microcracks and a resorbed outer surface in the PEG/BMP-2 group. The PEG-only group showed fibrovascular tissue formation around areas with microcracks. (Masson trichrome stain, original magnification ×40.)

Figure 4. Representative photomicrographs of the PEG (a, c) and PEG/BMP-2 (b, d) groups. After 2 weeks, the PEG membrane was maintained in the augmented area and intact with an absence of inflammation. Cell infiltration within the PEG membrane was barely detectable, and the cell-occlusion property persisted (a, b). In particular, new bone ingrowth in clear space between PEG membrane and original bone was observed (b), whereas there was a restricted amount of new bone at the periphery of the defect (a). After 8 weeks, the augmented area was still maintained and the PEG membranes were able to provide sufficient physical strength. Irregularities formed in the outline, and slight cell invasion was detectable (c, d). Progressed bone regeneration was detected at the defect periphery, center area, and resorbed outer surface (d). This could reflect that microcracking in the PEG shell facilitated cell

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invasion and subsequent matrix remodeling. rhBMP-2 that is entrapped within the PEG membrane after gelation cannot participate in the healing process. However, rhBMP-2 released from around microcracks can induce new bone formation. There was limited bone regeneration and most of the defect still contained PEG membrane and fibrovascular tissue (c). (▲= new bone; Masson trichrome stain, low magnification ×40; high magnification ×100.)

Figure 5. Representative photomicrographs of the FFS (a, c) and FFS/BMP-2 (b, d) groups. Only small remnants of FFS remained after 2 weeks, and extensive cellular infiltration could be found around residual biomaterials (a, b). Newly formed woven bone was deposited at the defect margin (b), whereas there was a restricted amount of new bone (a). After 8 weeks the adapted FFS had completely degraded and was replaced by new bone and connective tissues (c, d). Mature lamellar bone and bone marrow were evident throughout (d), whereas newly formed bone remained limited (c). (▲ = new bone, ⬆ = bone marrow; Masson trichrome stain, low magnification ×40; high magnification ×100.)

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Figures

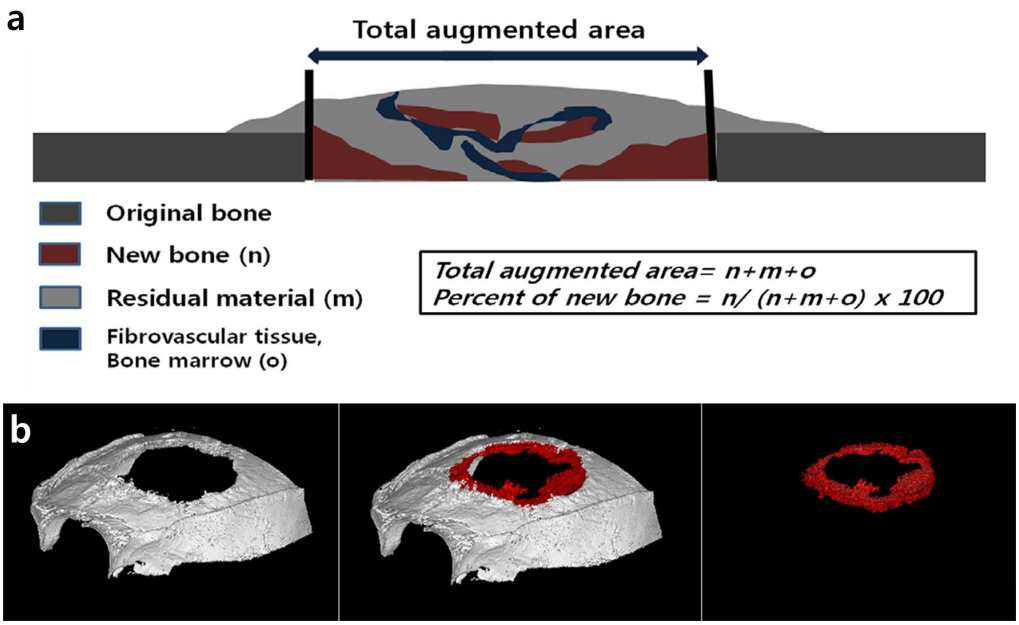

Figure 1.

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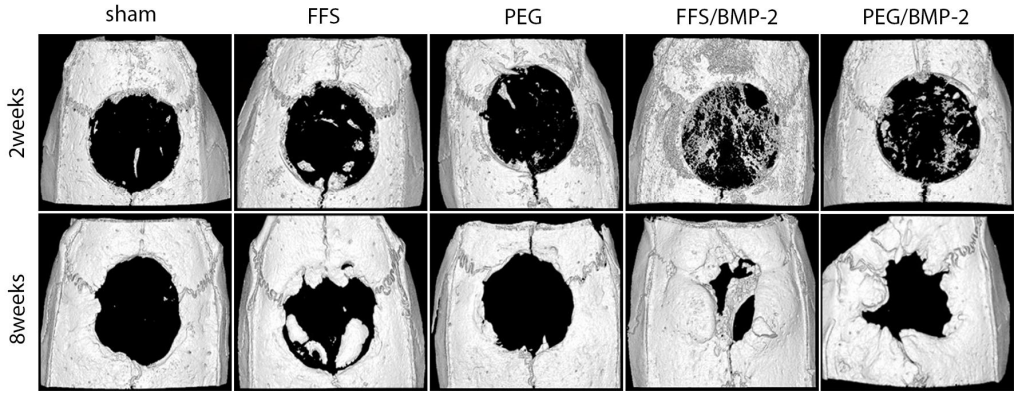

Figure 2.

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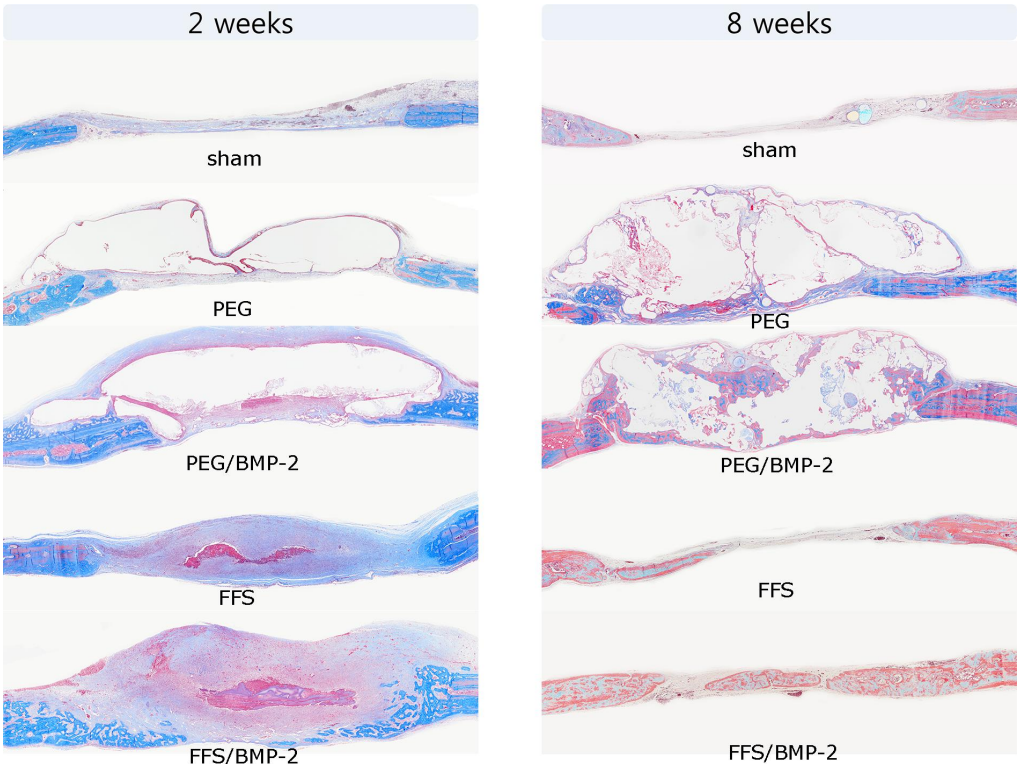

Figure 3.

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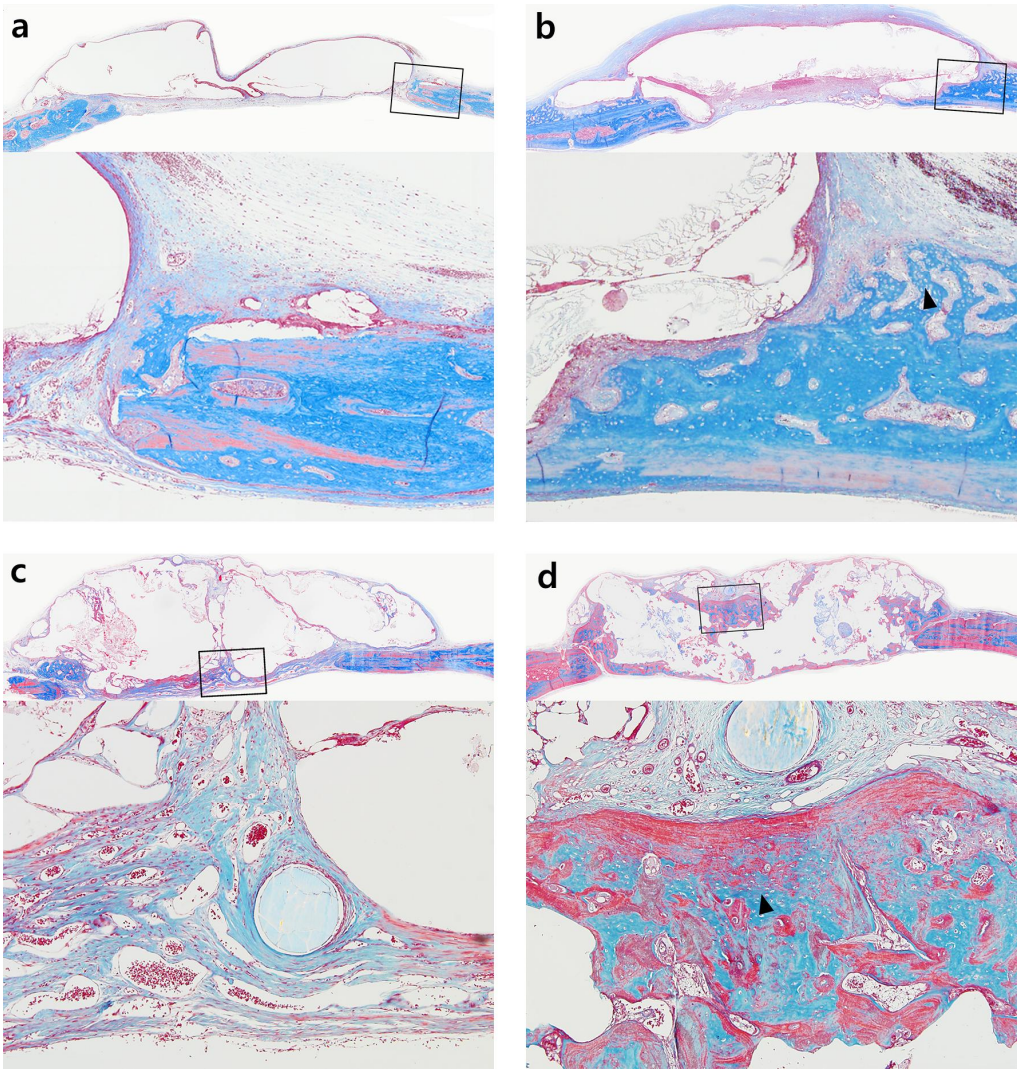

Figure 4.

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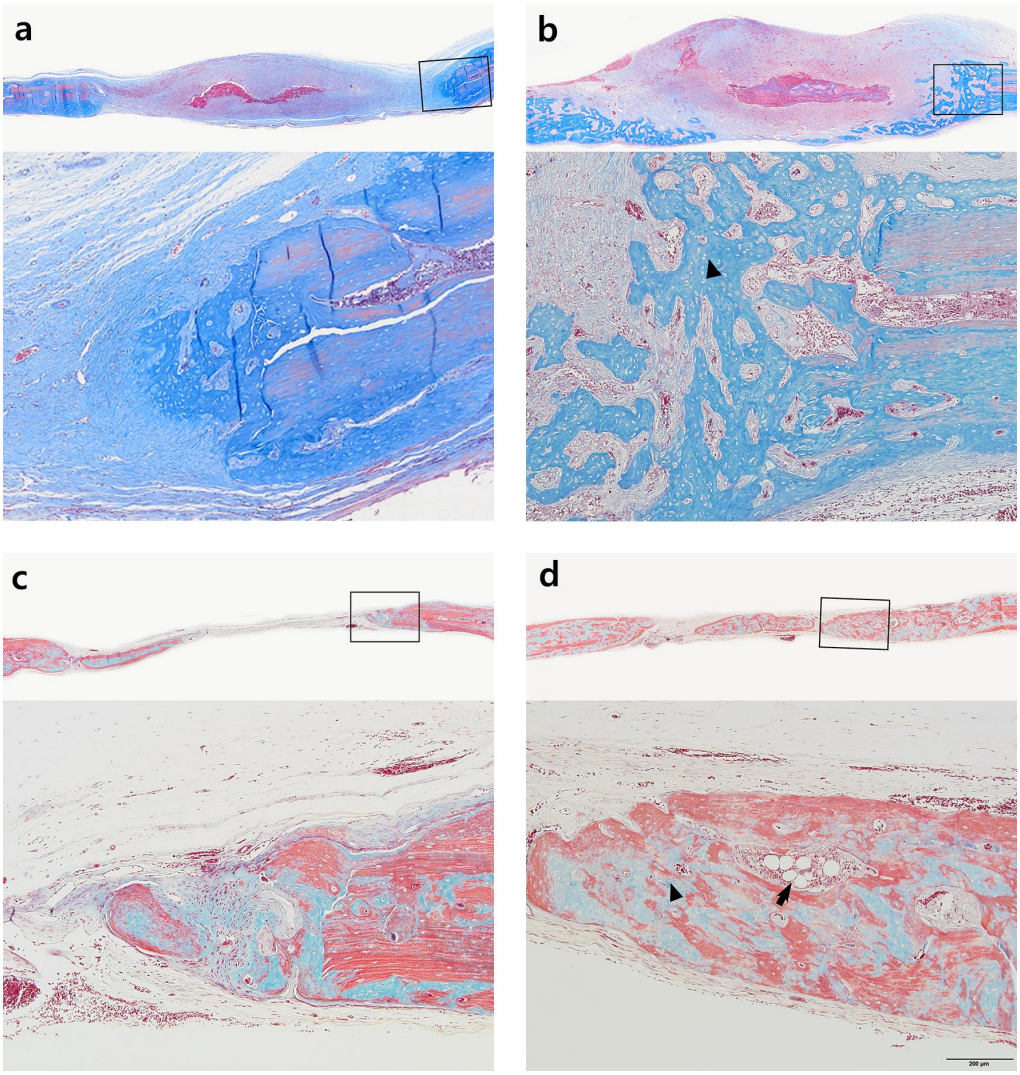

Figure 5.

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Tables

Table I. Composition of total augmented area (mm^2; and proportion; %) in histomorphometric analysis. (mean ± SD values; n = 10)

		Sham Surgery	FFS	FFS/ BMP-2	PEG	PEG/ BMP-2
Augmented area	2 weeks	0.95 ± 0.31 (100)	11.61 ± 2.27 [a] (100)	12.85 ± 1.85 [a] (100)	34.08 ± 3.82 [abc] (100)	37.58 ± 3.48 [abc] (100)
	8 weeks	0.76±0.25 (100)	11.54 ± 3.20 [a] (100)	10.80 ± 3.58 [a] (100)	30.22 ± 6.28 [abc] (100)	27.75 ± 5.44 *[abc] (100)
New bone	2 weeks	0.09 ± 0.05 (11.53 ± 10.21)	1.71 ± 0.88 [a] (16.67 ± 13.48)	3.76 ± 1.37 [abd] (30.62± 15.07 [abde])	1.53 ± 0.90 [a] (4.43 ± 2.23)	2.61 ± 1.14 [a] (6.86 ± 2.86)
	8 weeks	0.19 ± 0.08 * (25.29 ± 7.02 *)	3.98 ± 0.91 *[a] (36.50± 11.60 *[de])	6.23 ± 1.93 *[abde] (64.80± 24.24*[abde])	2.94 ± 0.82 *[a] (9.77 ± 2.36 *)	3.91 ± 1.12 *[a] (15.21 ± 6.53 *)
Residual material	2 weeks	-	4.01 ± 2.40 (33.82 ± 18.86)	4.79 ± 2.41 (36.73 ± 14.2)	23.93 ± 4.40 [bc] (69.75 ± 6.37 [bc])	25.54 ± 3.48 [bc] (67.39 ± 7.49 [bc])
	8 weeks	-	0.00 * (0.00 *)	0.00 * (0.00 *)	20.63 ± 6.19 [bc] (67.38 ± 6.31 [bc])	14.55 ± 7.31 [bc] (50.81 ± 16.31 [bc])
Fibrovascular tissue	2 weeks	0.86 ± 0.35 (88.47 ± 10.21)	5.89 ± 2.91 [a] (49.52 ± 20.12 [a])	4.30 ± 2.08 [a] (32.65 ± 14.74 [a])	8.62 ± 1.80 [a] (25.83 ± 7.30 [a])	9.43 ± 3.19 [a] (25.75 ± 7.55 [a])
	8 weeks	0.57 ± 0.19 (74.71 ± 7.02)	7.56 ± 3.06 [a] (63.50 ± 11.60)	4.57 ± 3.66 [a] (36.79 ± 21.53 [a])	6.65 ± 1.28 [a] (22.84 ± 6.54 [a])	9.29 ± 3.07 [a] (33.99 ± 12.51[a])

[a]Significant difference from sham control group ($P < .05$)
[b]Significant difference from FFS group ($P < .05$)
[c]Significant difference from FFS/BMP-2 group ($P < .05$)
[d]Significant difference from PEG group ($P < .05$)
[e]Significant difference from PEG/BMP-2 group ($P < .05$)
*Significant difference between at 2 and 8 weeks postsurgery ($P < .05$)

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Table II. Results of radiographic analyses using micro-CT

(mean ± SD values; n = 10)

		Sham Surgery	FFS	FFS/ BMP-2	PEG	PEG/ BMP-2
Augmented volume (mm^3)	2 weeks	53.57 ± 13.22	92.53 ± 20.28 [a]	86.07 ± 19.71 [a]	120.54 ± 25.52 [ac]	136.33 ± 21.60 [abc]
	8 weeks	41.56 ± 7.65 *	80.41 ± 13.95 [a]	81.96 ± 20.86 [a]	112.28 ± 15.05 [abc]	110.92± 11.77*[abc]
Mineralized volume (mm^3)	2 weeks	2.32 ± 0.43	4.52 ± 1.72	14.24 ± 3.44 [abde]	3.84 ± 1.29	6.97 ± 3.34 [a]
	8 weeks	3.46 ± 0.36 *	7.72 ± 1.56 *	23.63 ± 10.58 *[abde]	4.64 ± 1.12 *	8.38 ± 5.81 *
Defect resolution (%)	2 weeks	9.67 ± 1.57	16.65 ± 7.02 [a]	51.19 ± 6.28 [abde]	11.75 ± 3.42	22.20 ± 9.45 [a]
	8 weeks	10.53 ± 1.84	24.87 ± 4.88 [ad]	65.07 ± 24.59 [abde]	14.12 ± 3.93	21.86 ± 11.06 [ad]

[a]Significant difference from sham control group ($P < .05$)
[b]Significant difference from FFS group ($P < .05$)
[c]Significant difference from FFS/BMP-2 group ($P < .05$)
[d]Significant difference from PEG group ($P < .05$)
[e]Significant difference from PEG/BMP-2 group ($P < .05$)
*Significant difference between at 2 and 8 weeks postsurgery ($P < .05$)

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국문요약

골형성 유도 단백질의 전달체로서 폴리에틸렌글리콜 막 (gel-type polyethylene glycol membrane)의 평가

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장 지 웅

겔 타입 막은 흡수성 막(resorbable membrane)보다 느리게 분해(biodegradation)되며 2달 이상 그 형태학적 구조를 유지할 수 있다. 뿐만 아니라 시술 부위에 빠르게 적용할 수 있는 임상적 편리함때문에 성공적인 골재생을 보이는 것으로 알려져 있다. 이 연구의 목적은 시판되어 임상에 사용되고 있는 겔 타입 폴리에틸렌글리콜 막을 골형성유도단백질(bone morphogenetic protein)의 전달체로서 그 효과를 평가하는 것이다. FFS(fibrin-fibronectin system)가 양성대조군으로 사용되었다.

총 100마리의 백서를 5군으로 나누어 실험을 시행하였으며, 모든 동물에서 두개골 부위 직경 8mm의 골결손부를 형성하였다. 각각의 군은 다음과 같으며 (1) sham-surgery control, (2) FFS only, (3) FFS plus BMP-2, (4) PEG only, (5) PEG plus BMP-2 2주와 8주의 치유 기간 후

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희생하여 조직학적 및 조직계측학적 정량분석을 시행하였다.

2주 후 FFS only 및 FFS plus BMP-2 군에서 FFS의 일부분이 분해되고 광범위한 세포 침윤이 관찰된다. PEG only 및 PEG plus BMP-2 군은 거상된 부피가 잘 유지되고 있으며 세포 침윤은 거의 관찰되지 않았다. 8주 후 FFS only 및 FFS plus BMP-2군에서는 잔존 FFS가 거의 분해되고 신생골로 대체된 반면 PEG 그룹에서는 그 부피가 2주와 거의 비슷하고 약간의 세포 침윤이 관찰되었다. 특히 PEG plus BMP-2 그룹에서 미세균열(micro-crack) 및 resorbed outer surface에서 많은 신생골 재생을 보였음에도 불구하고 통계학적 유의성은 관찰되지 않았다. 그러나 치유되는 양상이 다르므로 (micro-crack 주위로 신생골 형성) 정확한 평가를 위해 더 긴 치유기간에서 재평가가 필요하다.

PEG plus BMP-2는 미세균열을 따라 신생골이 잘 형성되었고 충분한 구조적 안정성을 보였으므로 BMP-2의 전달체로서 효과적으로 사용될 수 있을 것으로 여겨진다.

핵심되는 말: 골 형성 단백질, 폴리에틸렌글리콜, 골 재생, 하이드로겔