Bioresorbable collagen membrane as a carrier for recombinant human bone morphogenetic protein-2 on lateral onlay grafts in dogs

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감사의 글

본 논문이 완성되기까지 부족한 저를 항상 격려해 주시고 사랑과 관심으로 이끌어 주신 채중규 교수님께 깊은 감사를 드립니다. 그리고, 많은 조언과 따뜻한 관심으로 지켜봐 주신 김종관 교수님, 조규성 교수님, 최성호 교수님, 김창성 교수님, 정의원 교수님, 김성태 교수님께 진심으로 감사 드립니다.

연구 내내 많은 도움을 주신 치주과 교실원 여러분들께도 고마움을 전합니다.

그리고, 늘 아낌없는 사랑과 헌신적인 도움으로 든든하고 따뜻한 버팀목이 되어준 사랑하는 부모님과 동생에게 진정으로 사랑과 고마움의 마음을 전합니다.

인생에 힘든시기와 어려운 일들이 닥칠 때마다 부족하지만 이 논문을 준비하고 작성했던 마음가짐으로 배우고 노력하며 용기를 얻어가며 살아나가겠습니다.

마지막으로, 믿음과 사랑으로 이해해 주고 항상 곁에서 용기가 되며 든든하게 후원해 주신 저희 가족과 친구들에게 모든 감사의 마음을 담아 이 논문을 드립니다.

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ABSTRACT

Bioresorbable collagen membrane as a carrier for recombinant human bone morphogenetic protein-2 on lateral onlay grafts in dogs

The objective of this study was to evaluate bone regenerative effect of bioresorbable collagen membrane (CM) as a carrier for recombinant human bone morphogenetic protein-2 (rhBMP-2) when performing lateral onlay graft using bovine hydroxyapatite incorporated with collagen matrix (BHC) in combination with CM in dogs. A guided bone regeneration procedure was performed at the buccal aspect of edentulous maxillary alveolar ridges using two treatment modalities in dogs (*N*=5): (1) BHC group, in which rhBMP-2-loaded BHC was covered by a CM, and (2) CM group, in which BHC was covered by an rhBMP-2-loaded CM. A histologic and histometric analysis was performed after 8 weeks of healing. Both the BHC and CM groups exhibited substantial new bone formation that was well integrated with the native bone. More newly formed bone was found in the CM group than in the BHC group without statistical significance. Most of the newly formed bone was in direct contact with the residual bone substitute in the BHC group, whereas the projections and islands of newly formed bone were observed in the spaces between the residual bone

substitute clusters in the CM group. The bone-to-residual biomaterial contact ratio was significantly lower in the CM group than in the BHC group ($p<0.05$). Within the limitations of the present study, it was found that rhBMP-2-loaded CM was effective for inducing the formation of new bone in the lateral onlay graft procedure. This implies that soaking CM with rhBMP-2 solution could simplify the surgical procedure.

Key Words: bone regeneration; bone morphogenetic protein 2; collagen; Bio-OSS; histology

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I. Introduction

Various treatment regimens have been used to overcome horizontal and vertical defects in atrophied alveolar ridges, and the introduction of the guided bone regeneration (GBR) technique has resulted in successful bone regeneration in intrabony defect models in both preclinical and clinical studies (De Boever and De Boever, 2005; Hammerle and Jung, 2003; Hammerle and Karring, 1998). However, several studies have shown that GBR exhibits very limited regenerative efficacy at defects that have limited healing sources, such as the vertically resorbed ridge (Caplanis et al., 1997; Huang et al., 2008; Wikesjo et al., 2004). The autogenous

block bone graft may be considered the first choice for resolving such a challenging defect (McAllister and Haghighat, 2007; Simion et al., 2007). However, autogenous bone cannot be used routinely due to unavoidable problems such as donor-site morbidity, limited quantity of available bone, unpredictable resorption and, most importantly, the need for a high level of surgical skill.

Tissue engineering technologies using growth factors have been rapidly developed for the regeneration of bone tissue, and much of the researches have focused on the use of recombinant human bone morphogenetic protein (rhBMP) to enhance the osteogenic potential of the bone substitute (Sigurdsson et al., 2001; Tatakis et al., 2002; Wikesjo et al., 2002). RhBMP-2 has received much attentions for alveolar bone augmentation, and has been extensively studied under various experimental conditions including onlay and vertical grafts (Huang et al., 2008; Jung et al., 2008; Wikesjo et al., 2002).

The characteristics of bovine hydroxyapatite (BH) have been widely documented, and has been proven to be biocompatible and osteoconductive (Hammerle and Karring, 1998; Hockers et al., 1999). It was reported that rhBMP-2-loaded BH enhances the bone-to-graft contact ratio (Jung et al., 2003) as well as the osteogenic healing potential (Schwarz et al., 2009). Recently, incorporation of BH with collagen matrix (BHC) was used in previous study and showed improved manageability without dissipation of the graft particles during surgery (Jung et al., 2011). The BHC

has a three-dimensional porous structure which could provide space for both ingrowth of endothelial cells and proliferation of osteogenic progenitor cells. Molecules of bone morphogenetic protein incorporated with the collagen matrix of BHC can be rapidly released at the initial stage of healing. These have prompted evaluations of BHC as a suitable carrier for rhBMP-2 (Kim et al., 2010; Schwarz et al., 2008a).

The use of a bioresorbable collagen membrane (CM) as a barrier membrane in combination with grafting bone substitute as a scaffold is a well-documented GBR procedure (Chiapasco and Zaniboni, 2009; Hammerle et al., 2002). Previous studies combining GBR with rhBMP-2 have used conventionally bone substitute or absorbable collagen sponge as a carrier material for the rhBMP-2 (Jovanovic et al., 2007; Schwarz et al., 2009; Schwarz et al., 2008a). Bone substitute supports the CM and provides space for osteoinduction. However, the carrier should be absorbable, otherwise it will require additional processing, such as lyophilization to enable rhBMP-2 loading (Jung et al., 2003).

CM could be considered as a carrier of rhBMP-2 if it stably covers the augmented volume while allowing the continuous release of rhBMP-2. Covering of the bone substitute with an rhBMP-2-loaded CM may enhance the osteogenic potential of the bone substitute, since it is in direct contact with the periosteum of the overlying mucogingival flap. It has been reported that the periosteum is highly osteogenic and rich in the necessary mesenchymal cells that can differentiate into osteoblastic cells

(Cho et al., 2011; Hayashi et al., 2008; Zhu et al., 2006). Furthermore, it has been documented that a certain type of CM permits early transmembranous anastomosis between the reflected mucogingival flap and the inferior tissue (Schwarz et al., 2009; Schwarz et al., 2006). Therefore, it is anticipated that new bone induced by an rhBMP-2-loaded CM may form from the upper part of the onlay graft, and subsequently surround the bone substitute located inside the onlay graft with native bone. The bone substitute will ultimately be surrounded by multiple osteogenic sources and thus undergo gradual osteogenesis. In other words, the osteoinductive potential of rhBMP-2-loaded CMs placed over a bone substitute will alter the osteogenic environment of onlay grafts in self-contained defects such as in the sinus cavity, and influence the regenerative capacity of the bone substitute. In addition, previous studies have demonstrated that the addition of rhBMP-2 clinically enhances soft-tissue healing compared to the control condition (i.e. no addition of rhBMP-2) (Misch, 2011; Wikesjo et al., 2003a). RhBMP-2-loaded CM that is in direct contact with the overlying mucoperiosteal flap can also exert a positive effect on the soft-tissue healing during GBR.

The aim of this study was to evaluate the bone regenerative effect of CM as a carrier for rhBMP-2 when performing lateral onlay grafts using BHC in combination with CM in dogs.

II. Materials and Methods

1. Animals

Five male mongrel dogs aged 20–24 months and weighing approximately 15 kg were used. All animals had a full, healthy permanent dentition and were allowed a period of adaptation of at least 1 week before the surgical procedure. The animal selection and management, surgical protocol, and preparation followed routines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea (no. 09-007).

2. Graft materials

A 2.0-mg of rhBMP-2 (Cowellmedi, Pusan, Korea) was reconstituted with 1.34 ml of sterile water and further diluted with 2.68 ml of buffer to produce a stock solution with an rhBMP-2 concentration of 0.5 mg/ml. For experimental constructs, BHC (BioOss collagen: width, 8 mm; thickness, 4 mm; height, 5 mm;Geistlich Biomaterials, Wolhusen, Switzerland) was formed into a rectangular shape of dimensions 4 × 5 × 4 mm (length × width × height), and the CM (BioGide, 25 × 25-mm squares, Geistlich Biomaterials) was cut into 12.5 × 12.5-mm squares. The

rhBMP-2 solution (0.2 ml of the 0.5 mg/ml stock) was withdrawn using a sterile 1-ml syringe and uniformly dispensed over the entire surface of both the BHC and CM on a sterile dish. Following a 15-minutes loading time at room temperature, the rhBMP-2-loaded BHC or CM was applied to the experimental site (according to the experimental group to which the site had been assigned).

3. Surgical procedure

The surgical procedure was performed under general anesthesia induced by an intravenous injection of atropine (0.05 mg/kg; KwangMyung Pharmaceutical, Seoul, Korea) and an intramuscular injection with a combination of xylazine (2 mg/kg; Rompun, Bayer Korea, Seoul, Korea) and ketamine (10 mg/kg; Ketalar, Yuhan, Seoul Korea), followed by maintenance via inhalation anesthesia (Gerolan, Choongwae Pharmaceutical, Seoul, Korea). Routine local infiltrative anesthesia (2% lidocaine HCl with epinephrine 1:100,000, KwangMyung Pharmaceutical) was used at the surgical sites. Oral prophylaxis including scaling and plaque control were performed before the surgical procedure.

The left maxillary first, second, and third premolar teeth were carefully extracted to create an edentulous alveolar ridge;the extraction sites were allowed to heal for 2 months. A separate experiment that did not form part of the present study was

performed on the right upper jaw (Jung and Lee et al., submitted). After the healing period, complete extraction-socket healing was confirmed under general anesthesia (as described above), and a midcrestal incision was made from the first molar to the canine. Two vertical incisions were then made at the mesial aspect of the first molar and the distal aspect of the canine on the left upper jaw quadrant. The mucoperiosteal flaps were elevated buccally and the experimental site was completely exposed (Fig. 1a).

Six intramarrow perforations were made with a #330 carbide bur at two sites under copious sterile saline irrigation. RhBMP-2-loaded BHC was applied at the first site and then covered with an untreated CM (BHC group), while untreated BHC was applied and then covered with rhBMP-2-loaded CM at the second site (CM group; Fig. 1b). The CMs at each site (i.e. treated and untreated) were fixed by pin on the mesiodistal side of the CM (Membrane Pin, Dentium, Seoul, Korea). Periosteal releasing incisions were made at the base of the flaps to obtain primary wound closure with tension-free adaptation. The buccal flaps were then sutured with 4-0 resorbable nylon (Monosyn 4.0 Glyconate Monofilament, B. Braun,Tuttlingen, Germany).

All of the animals were given antibiotics intramuscularly and fed a soft diet for 14 days postsurgery. Daily topical application of chlorhexidine was performed until suture removal, which took place 7–10 days postsurgery. The animals were killed

after 8 weeks by an intravenous injection of concentrated sodium pentobarbital. The experimental sites, including soft tissues, were removed and then fixed in 10% buffered formalin for 10 days.

4. Histological processing

The resected specimens were decalcified in 5% formic acid for 10 days. The experimental site of each specimen was cut apicocoronally, and one of the sections was embedded in paraffin. The central-most section of the experimental site was chosen for histologic and histometric analysis. Cross-sectioned specimens were further cut to a final thickness of 5 μm in the apicocoronal direction and stained with hematoxylin and eosin (H-E) and Masson's trichrome (MTC). Each section was observed with the aid of a light microscope (Olympus Research System Microscope BX51, Olympus, Tokyo, Japan) equipped with a camera.

5. Histometric analysis

Histometric analysis was performed using an automated image-analysis computer program (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA) by a single

experienced investigator who was blinded as to the experimental group to which the specimens belonged. The following histometric parameters were measured (Fig. 2):

- **Augmented area (AA) and height (AH)** were determined by measuring the area and vertical height, respectively, between the inferior border of the CM and the native bone.
- **Newly formed bone (NB) area (NBA) and height (NBH)** were determined by measuring the area and vertical height, respectively, of NB between the inferior border of the CM and the native bone.
- **Bone-to-residual biomaterial contact ratio (BRC; %)** was measured as a proportion of residual BH (RB) in direct contact with bone for entire circumferences of RB.
- **Ratio of the components (%)** were compared by calculating the proportions of NB, RB, and fibrovascular connective tissue (FV) within the AA.

6. Statistical analysis

Mean and standard deviation values of each histometric parameter were calculated for each experimental group. The significance of differences between the groups was

determined using the Wilcoxon signed-rank test. The level of statistical significance was set at $p < 0.05$.

III. Results

1. Clinical observations

All of the animals healed uneventfully, and neither of the experimental sites (i.e. receiving either rhBMP-2-loaded BHC or rhBMP-2-loaded CM) in any of the animals exhibited wound dehiscence or signs of infection.

2. Histological observations

Dome-shaped AAs were observed in both groups within the space circumscribed by the CM and native bone, well-integrated into the native bone. The AAs were composed of NB, RB, and FV which included newly formed vascular tissue, fatty bone marrow, and dense connective tissue. The NB projected from the recipient bone bed into the AA and was present beneath the CM. The NB was composed of woven bone and mature lamellar bone with Haversian systems. Most of the RBs were located at the central portion of the grafted site.

In the BHC group, the cortical bone layer of the recipient bed was resorbed and the bone marrow space was in communication with the AA (Fig. 3a). The RB was in direct contact with the NB (Fig. 3b). The intact CM containing many capillaries was

clearly observed at 8 weeks of healing (Fig. 3c). Multinucleated osteoclast-like cells were found at the resorption lacunae on the surface of the NB and RB (Fig. 3d,e).

The overall histologic features in the CM group were similar to those of the BHC group (Fig. 4a). However, the configuration of the NB differed slightly between the two groups. Finger- or column-like projections of NB that were directed toward or lying alongside the rhBMP-2-loaded CM were commonly observed underneath the CM in the CM group, and the NB seemed to be closer to the CM than in the BHC group (Fig. 4b, c), with newly formed vascular tissues penetrating the rhBMP-2-loaded CM (Fig. 4e). While most of the RB was in direct contact with the NB in the BHC group, it was clustered into large or small areas and embedded within FV in the CM group. The projections and islands of NB were frequently observed in the spaces between the RB clusters in most specimens of the CM group (Figs. 4d and 5).

3. Histometric analysis

The results of the histometric analysis are summarized in Table 1. The mean values of AA and NBA were slightly higher in the CM group than in the BHC group; however, the difference between the two groups was not statistically significant. The AH and NBH also appeared to be greater in the CM group than in the BHC group; however, the difference did not reach statistical significance. Each histologic

component that occupied AAs showed similar distribution ratio between both groups (Fig. 6). The percentage of NBH of the original BHC height did not differ significantly between the BHC group (41.8%) and the CM group (46.8%). The BRC ratio was significantly lower for the CM group than for the BHC group (43.4 ± 17.7 and 10.1 ± 5.4, respectively; $p < 0.05$; Fig. 7).

IV. Discussion

This study evaluated the potential of CM as a carrier for rhBMP-2, since most of the previous studies on growth factors and their clinical use have focused on the biomaterials for the scaffold as a carrier of the growth factors. However, there may be several difficulties when using the conventional carrier system and growth factors in certain clinical cases, especially for non-contained defects. It is not easy to manage and apply rhBMP-2-loaded bone substitute to non-contained defects without dissipation. A certain amount of rhBMP-2-loaded bone substitute could be lost throughout the healing process, which will adversely influence the outcome of GBR. The use of CM as a carrier for rhBMP-2—as in the present study—might minimize this issue. In addition, rhBMP-2-loaded CM may stimulate the osteogenic potential of the periosteum, which may lead to the formation of a new bone shell surrounding the augmented bone substitute. The rhBMP-2-loaded CM has not been evaluated in previous animal or clinical studies.

In the present study, both the BHC and CM groups exhibited substantial NB that was well integrated with native bone. The NBA and NBH appeared to be slightly better in the CM group than in the BHC group, but the difference was not statistically significant. The percentage of NBA within the AA also appeared to be greater in the CM group than in the BHC group, but again the difference was not significant. The

percentage of NBA obtained in the CM group (29.5%) was comparable with that obtained in previous studies in a dehiscence defect augmented with rhBMP-2-coated BH (37%) (Jung et al., 2003) and in an onlay defect augmented with 10 μg/ml rhBMP-2-loaded bone substitute (26.3 ± 8.5%) (Jung et al., 2008). The NBH of 1.87 ± 0.66 mm (46.8%) obtained in our CM group is consistent with that of 1.89 ± 0.55 mm (47.3%) found in a recent evaluation of vertical augmentation using rhBMP-2-loaded BHC in rabbit calvaria (Kim et al., 2010). Therefore, it was confirmed that the application protocol of loading CM with rhBMP-2 could be an effective treatment option for lateral onlay grafting.

CMs are convenient for soaking up rhBMP-2 and are easily manipulated at surgical sites in the clinical context (Jung et al., 2003). In the present context, the use of CM as a carrier of rhBMP-2 could simplify surgical procedures while simultaneously obtaining comparable NB. Furthermore, it would help to reduce the amount of rhBMP-2 required to load particulate or block-type bone substitutes. Minimizing the dose of rhBMP-2 would provide both cost and safety benefits.

The necessity of an osteoinductive agent is being increasingly emphasized in defects with a restricted healing source. In our previous study (Jung and Lee et al., 2013), we evaluated lateral onlay grafts treated using BHC with/without CM under the same conditions as in the present study, and demonstrated that NB was observed only at the experimental sites that were covered with a CM. Both sites of the BHC and CM

groups in the present study exhibited significantly greater NB than those that received the same biomaterials without rhBMP-2 in the previous study (3.26 ± 1.04 mm^2). These findings are highly consistent with those of Wikesjö et al. (Wikesjo et al., 2004; Wikesjo et al., 2003b), who reported that rhBMP-2 increased the regenerative efficacy of GBR at onlay grafts.

Most of the RB particles were in direct contact with the NB in BHC group, which was consistent with a previous report that rhBMP-2-coated BH enhanced the NB-to-graft-materials contact ratio (Jung et al., 2003). The rhBMP-2-loaded bone substitute protocol has been reported to improve the osteoinductive properties of other bone substitutes as well as BHC (Jung et al., 2008). However, the RB particles that were not loaded with rhBMP-2 in CM group did not show increased BRC, instead being clustered and embedded within the dense FV. Finger- or columnar-like projections of NB were observed in the spaces between the clusters of RB particles, communicating with each other or the native bone. In addition, NB appeared to be directed toward or lying alongside the rhBMP-2-loaded CM. The histometric analysis indicated that the BRC ratio was significantly lower in the CM group than in the BHC group. This specific feature of RB has been found in previous studies (Jung et al., 2011; Stavropoulos and Wikesjo, 2010) investigating surgical implantation of BHC with no rhBMP-2 loading in one-wall intrabony defects. In particular, the histologic configuration of NB and RB was also confirmed when lateral onlay grafts were

performed using only BHC and CM, without rhBMP-2 (Jung and Lee et al., 2013). The rhBMP-2 may have been highly concentrated on the CM in the CM group at the early stage of healing. Thus rhBMP-2 loaded CM had more chemotatic and mitogenic potential from periosteum-like connective tissue than the untreated CM of the BHC group. It is suggested that the CM allowed the penetration of small blood vessels from the periosteum-like dense connective tissue (Jung and Lee et al., 2013). The histologic results of the present study also indicate that the newly formed vascular tissues pass through the rhBMP-2-loaded CM. Schwarz et al. (2008b) demonstrated that enhanced bone formation was observed just below the barrier membrane covering a dehiscence defect. They attributed this formation of peripheral bone underneath the barrier membrane to the early transmembranous angiogenesis of the barrier membrane, permitting the migration of pre-osteoblastic cells from the periosteum to the submembranous tissue via newly formed vascular tissues (Schwarz et al., 2009; Schwarz et al., 2008b). In addition, the rhBMP-2-loaded CMs persisted after 8 weeks of healing, and prevented ingrowth of the epithelium and the outer connective tissue (Moses et al., 2008). Thus, enhanced transmembranous angiogenesis by the rhBMP-2 and the barrier function of the CM may have affected the histologic morphogenesis of NB and RB.

V. Conclusion

Within the limitations of the present study, it can be concluded that rhBMP-2-loaded CM performed lateral onlay grafts as effectively as rhBMP-2-loaded BHC in dogs. The loading of CMs with rhBMP-2 might therefore be a recommendable treatment option for indication demanding to minimize the loss of bone substitute, to increase soft tissue healing, and to simplify the surgical procedure associated with lateral onlay grafts.

References

Caplanis N, Sigurdsson TJ, Rohrer MD, Wikesjo UM: Effect of allogeneic, freeze-dried, demineralized bone matrix on guided bone regeneration in supra-alveolar peri-implant defects in dogs. *Int J Oral Maxillofac Implants* 12(5): 634-642, 1997.

Chiapasco M, Zaniboni M: Clinical outcomes of GBR procedures to correct peri-implant dehiscences and fenestrations: a systematic review. *Clin Oral Implants Res* 20(Suppl 4): 113-123, 2009.

Cho EH, Park JC, Cha JK, Kim YT, Jung UW, Kim CS, et al.: Dimensional change of the healed periosteum on surgically created defects. *J Periodontal Implant Sci* 41(4): 176-184, 2011.

De Boever AL, De Boever JA: Guided bone regeneration around non-submerged implants in narrow alveolar ridges: a prospective long-term clinical study. *Clin Oral Implants Res* 16(5): 549-556, 2005.

Hammerle CH, Jung RE: Bone augmentation by means of barrier membranes. *Periodontol 2000* 33: 36-53, 2003.

Hammerle CH, Jung RE, Feloutzis A: A systematic review of the survival of implants in bone sites augmented with barrier membranes (guided bone regeneration) in partially edentulous patients. *J Clin Periodontol* 29(Suppl 3): 226-231; discussion 232-223, 2002.

Hammerle CH, Karring T: Guided bone regeneration at oral implant sites. *Periodontol 2000* 17: 151-175, 1998.

Hayashi O, Katsube Y, Hirose M, Ohgushi H, Ito H: Comparison of osteogenic ability of rat mesenchymal stem cells from bone marrow, periosteum, and adipose tissue. *Calcif Tissue Int* 82(3): 238-247, 2008.

Hockers T, Abensur D, Valentini P, Legrand R, Hammerle CH: The combined use of bioresorbable membranes and xenografts or autografts in the

treatment of bone defects around implants. A study in beagle dogs. *Clin Oral Implants Res* 10(6): 487-498, 1999.

Huang YH, Polimeni G, Qahash M, Wikesjo UM: Bone morphogenetic proteins and osseointegration: current knowledge - future possibilities. *Periodontol 2000* 47: 206-223, 2008.

Jovanovic SA, Hunt DR, Bernard GW, Spiekermann H, Wozney JM, Wikesjo UM: Bone reconstruction following implantation of rhBMP-2 and guided bone regeneration in canine alveolar ridge defects. *Clin Oral Implants Res* 18(2): 224-230, 2007.

Jung RE, Glauser R, Scharer P, Hammerle CH, Sailer HF, Weber FE: Effect of rhBMP-2 on guided bone regeneration in humans. *Clin Oral Implants Res* 14(5): 556-568, 2003.

Jung RE, Weber FE, Thoma DS, Ehrbar M, Cochran DL, Hammerle CH: Bone morphogenetic protein-2 enhances bone formation when delivered by a synthetic matrix containing hydroxyapatite/tricalciumphosphate. *Clin Oral Implants Res* 19(2): 188-195, 2008.

Jung UW, Lee JS, Lee G, Lee IK, Hwang JW, Kim MS, Choi SH, Chai JK: Role of collagen membrane in lateral onlay grafting with bovine hydroxyapatite incorporated with collagen matrix in dogs. *J Periodontal Implant Sci* 43(2):64-71, 2013.

Jung UW, Lee JS, Park WY, Cha JK, Hwang JW, Park JC, et al.: Periodontal regenerative effect of a bovine hydroxyapatite/collagen block in one-wall intrabony defects in dogs: a histometric analysis. *J Periodontal Implant Sci* 41(6): 285-292, 2011.

Kim SJ, Shin HS, Shin SW: Effect of bone block graft with rhBMP-2 on vertical bone augmentation. *Int J Oral Maxillofac Surg* 39(9): 883-888, 2010.

McAllister BS, Haghighat K: Bone augmentation techniques. *J Periodontol* 78(3): 377-396, 2007.

Misch CM: Bone Augmentation of the Atrophic Posterior Mandible for Dental Implants Using rhBMP-2 and Titanium Mesh: Clinical Technique and

Early Results. *Int J Periodontics Restorative Dent* 31(6): 581-589, 2011.

Moses O, Vitrial D, Aboodi G, Sculean A, Tal H, Kozlovsky A, et al.: Biodegradation of three different collagen membranes in the rat calvarium: a comparative study. *J Periodontol* 79(5): 905-911, 2008.

Schwarz F, Ferrari D, Sager M, Herten M, Hartig B, Becker J: Guided bone regeneration using rhGDF-5- and rhBMP-2-coated natural bone mineral in rat calvarial defects. *Clin Oral Implants Res* 20(11): 1219-1230, 2009.

Schwarz F, Rothamel D, Herten M, Ferrari D, Sager M, Becker J: Lateral ridge augmentation using particulated or block bone substitutes biocoated with rhGDF-5 and rhBMP-2: an immunohistochemical study in dogs. *Clin Oral Implants Res* 19(7): 642-652, 2008a.

Schwarz F, Rothamel D, Herten M, Sager M, Becker J: Angiogenesis pattern of native and cross-linked collagen membranes: an immunohistochemical study in the rat. *Clin Oral Implants Res* 17(4): 403-409, 2006.

Schwarz F, Rothamel D, Herten M, Wustefeld M, Sager M, Ferrari D, et al.: Immunohistochemical characterization of guided bone regeneration at a dehiscence-type defect using different barrier membranes: an experimental study in dogs. *Clin Oral Implants Res* 19(4): 402-415, 2008b.

Sigurdsson TJ, Nguyen S, Wikesjo UM: Alveolar ridge augmentation with rhBMP-2 and bone-to-implant contact in induced bone. *Int J Periodontics Restorative Dent* 21(5): 461-473, 2001.

Simion M, Fontana F, Rasperini G, Maiorana C: Vertical ridge augmentation by expanded-polytetrafluoroethylene membrane and a combination of intraoral autogenous bone graft and deproteinized anorganic bovine bone (Bio Oss). *Clin Oral Implants Res* 18(5): 620-629, 2007.

Stavropoulos A, Wikesjo UM: Influence of defect dimensions on periodontal wound healing/regeneration in intrabony defects following implantation

of a bovine bone biomaterial and provisions for guided tissue regeneration: an experimental study in the dog. *J Clin Periodontol* 37(6): 534-543, 2010.

Tatakis DN, Koh A, Jin L, Wozney JM, Rohrer MD, Wikesjo UM: Peri-implant bone regeneration using recombinant human bone morphogenetic protein-2 in a canine model: a dose-response study. *J Periodontal Res* 37(2): 93-100, 2002.

Wikesjo UM, Lim WH, Thomson RC, Cook AD, Wozney JM, Hardwick WR: Periodontal repair in dogs: evaluation of a bioabsorbable space-providing macroporous membrane with recombinant human bone morphogenetic protein-2. *J Periodontol* 74(5): 635-647, 2003a.

Wikesjo UM, Qahash M, Thomson RC, Cook AD, Rohrer MD, Wozney JM, et al.: rhBMP-2 significantly enhances guided bone regeneration. *Clin Oral Implants Res* 15(2): 194-204, 2004.

Wikesjo UM, Sorensen RG, Kinoshita A, Wozney JM: RhBMP-2/alphaBSM induces significant vertical alveolar ridge augmentation and dental implant osseointegration. *Clin Implant Dent Relat Res* 4(4): 174-182, 2002.

Wikesjo UM, Xiropaidis AV, Thomson RC, Cook AD, Selvig KA, Hardwick WR: Periodontal repair in dogs: rhBMP-2 significantly enhances bone formation under provisions for guided tissue regeneration. *J Clin Periodontol* 30(8): 705-714, 2003b.

Zhu SJ, Choi BH, Huh JY, Jung JH, Kim BY, Lee SH: A comparative qualitative histological analysis of tissue-engineered bone using bone marrow mesenchymal stem cells, alveolar bone cells, and periosteal cells. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 101(2): 164-169, 2006.

Figure Legends

Figure 1. Clinical photograph of an experimental site after tooth extraction. (b) The rhBMP-2 loaded BHC was covered with untreated CM on the left side (BHC group), while untreated BHC was covered with an rhBMP-2-loaded CM on the right side (CM group).

Figure 2. Schematic drawing illustrating histometric landmarks. The augmented area (AA) was demarcated by the native bone (black dotted line) and the inferior border of the CM (blue line). The areas of newly formed bone (NBA), residual BH (RB), and fibrovascular connective tissue (FV) were measured within the AA. Augmented height (AH) was measured according to the vertical distance between the native bone and the highest point of the AA. NB height (NBH) was measured according to the vertical distance between the native bone and the highest point of NB.

Figure 3. (a) Low-magnification view of the histologic presentation in the BHC group (H-E staining;scale bar = 1 mm). (b) The NB was in direct contact

with the RB (H-E staining;scale bar = 500 μm). (c) The intact CM containing many capillaries could be clearly observed (H-E staining;scale bar = 200 μm). (d, e) Osteoclast-like cells (arrowhead) were observed on the surface of the NB and RB [H-E staining;scale bar = 100 μm (d) and 200 μm (e)]. (BHC, bovine hydroxyapatite incorporated with collagen matrix; CM, collagen membrane; FV, fibrovascular connective tissue; NB, newly formed bone; RB, residual bovine hydroxyapatite)

Figure 4. (a) Low-magnification views of the histologic presentation in the CM group (MTC staining;scale bar = 1 mm). (b) and (c) Finger- or column-like projections of NB were observed underneath the CM (arrow). The NB was directed toward or lying alongside the CM [MTC staining;scale bar = 1 mm (b) and 500 μm (c)]. (d) NB was not integrated with the RB, instead existing as islands of bone within the FV (MTC staining;scale bar = 500 μm). (e) Newly formed vascular tissues (arrowhead) were observed passing through the CM (MTC staining;scale bar = 100 μm). (CM, collagen membrane; MTC, Masson's trichrome; NB, newly formed bone; RB, residual bovine hydroxyapatite)

Figure 5. High-magnification views of the histologic presentation in other specimens of the BHC groups (a) and CM (b). (a) Most of the RBs were in direct contact with the NB (H-E staining;scale bar = 1 mm). (b) Numerous clusters of RB particles were embedded within the FV. NB that was not direct contact with the RB was observed in the spaces between the RB clusters (H-E staining;scale bar = 1 mm). (BHC, bovine hydroxyapatite incorporated with collagen matrix; CM, collagen membrane; FV, fibrovascular connective tissue; NB, newly formed bone; RB, residual bovine hydroxyapatite)

Figure 6. Ratios of NB (%NB), RB (%RB), and FV (%FV) in the BHC and CM groups.

Figure 7. Bone-to-residual biomaterial contact ratio (BRC) in the BHC and CM groups. BRC differed significantly between the two groups (*$p < 0.05$)

Table

Table 1. Histometric data (n = 5; Means ± SD)

	BHC Group	CM Group
AA (mm^2)	20.21 ± 10.24	25.95 ± 12.71
NBA (mm^2, %)	5.32 ± 1.97 (26.4)	7.77 ± 3.26 (29.5)
RB (mm^2, %)	1.17 ± 1.43 (5.8)	0.85 ± 1.12 (3.4)
FV (mm^2, %)	13.68 ± 6.97 (67.8)	17.43 ± 9.49 (67.3)
AH (mm)	2.17 ± 0.76	2.27 ± 0.94
NBH (mm)	1.67 ± 0.65	1.87 ± 0.66

AA, augmented area; NBA, newly formed bone; RB, residual bovine hydroxyapatite; FV, fibrovascular connective tissue; AH, augmented height; NBH, newly formed bone height.

Figures

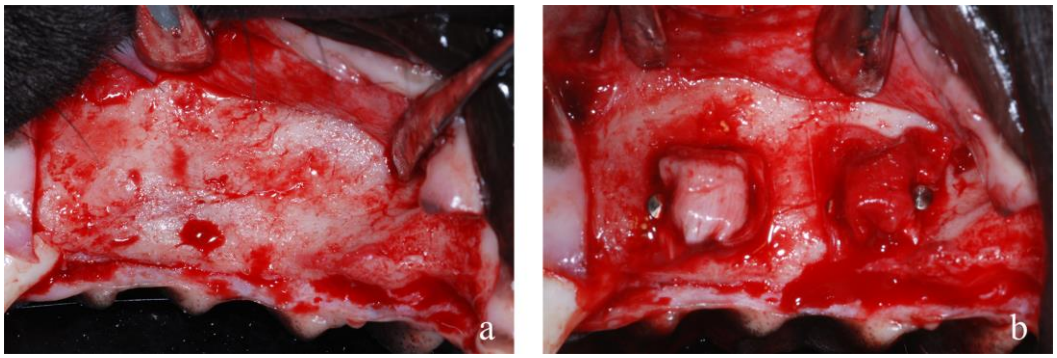

Figure 1

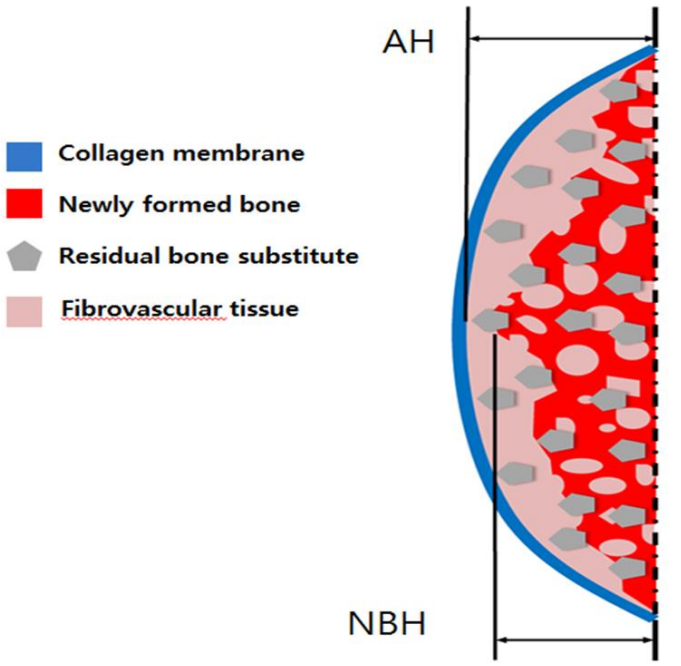

Figure 2

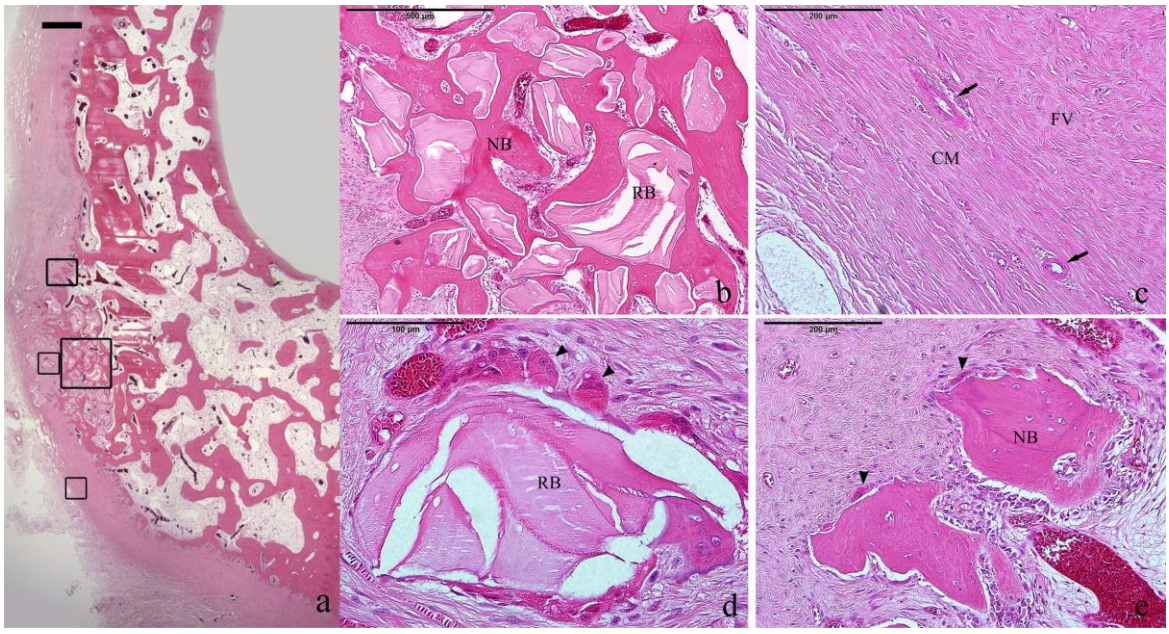

Figure 3

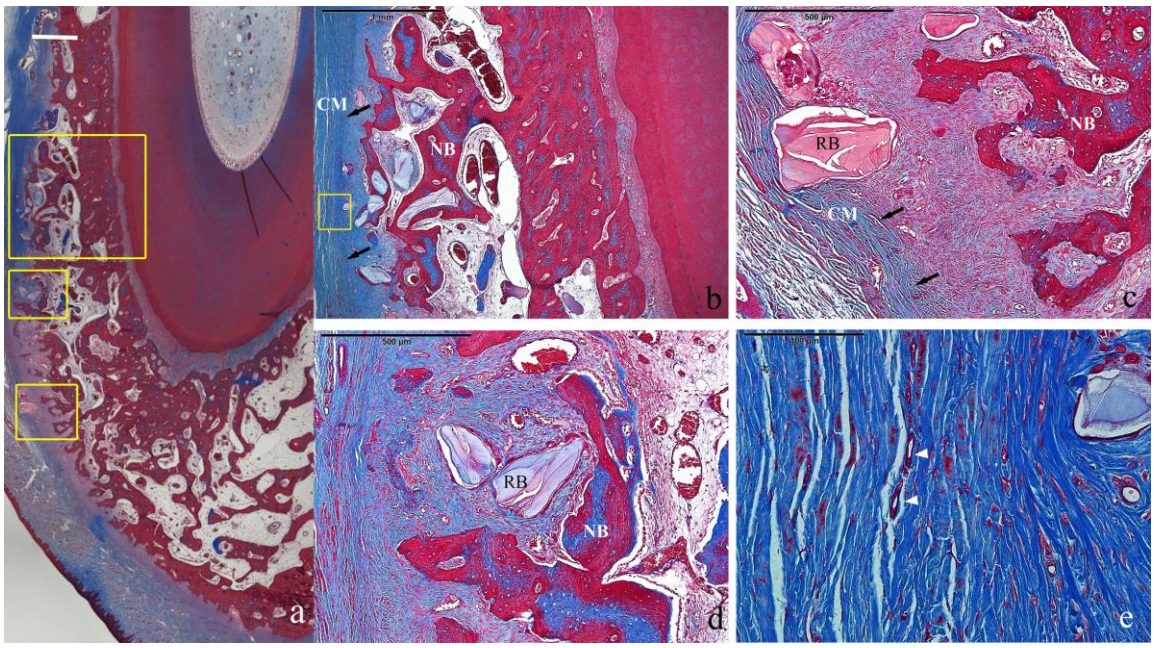

Figure 4

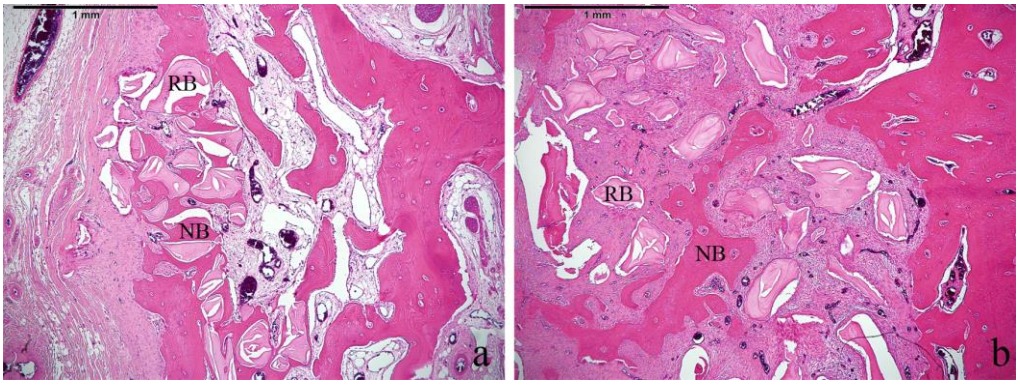

Figure 5

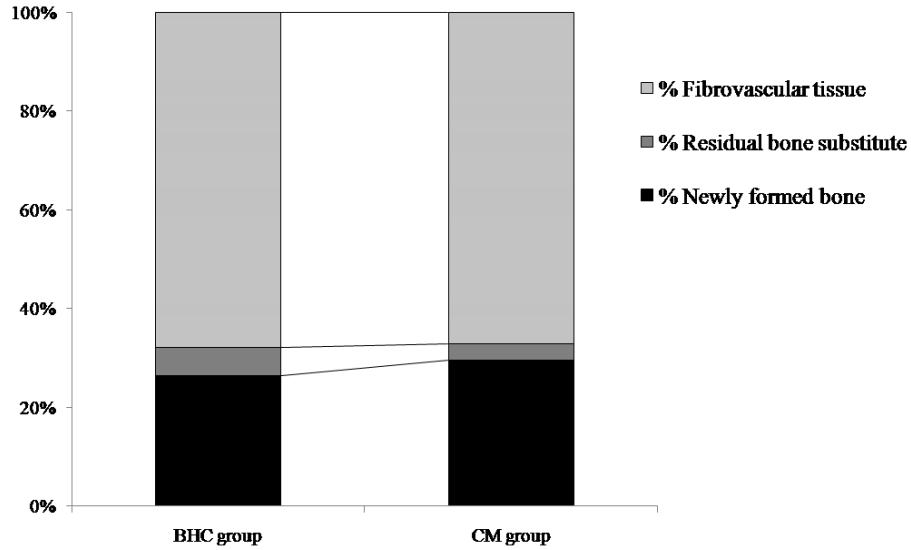

Figure 6

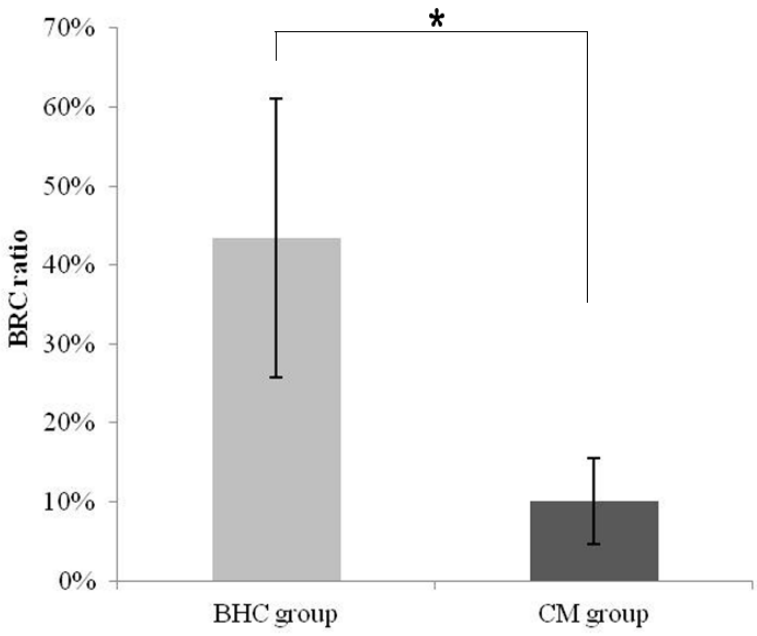

Figure 7

국문 요약

성견 측방 온레이 골이식시 재조합 인간 골형성 유도 단백질-2 의 전달체로서의 흡수성 콜라겐 차단막의 골재생 효과

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장 윤 영

본 연구는 성견에서 흡수성 콜라겐 차단막, 콜라겐 결합 하이드록시아파타이트, 그리고 재조합 인간 골형성 유도 단백질-2 을 이용한 측방 온레이 골이식시 흡수성 콜라겐 차단막을 재조합 인간 골형성 단백질-2 의 전달체로 이용하여 그 골재생 효과를 평가하였다.

총 5 마리의 잡견에서 상악 좌측 제 1,2,3 소구치를 발거하고 8 주 동안 치유시킨 후 무치악 치조제 외측에 두가지 수술방법으로 온레이 골이식을 시행하였다. 먼저 콜라겐 결합 하이드록시아파타이트군 (BHC group)은 재조합 인간 골형성 유도 단백질-2 을 콜라겐 결합 하이드록시아파타이트에 흡수시킨후 수술부위에 위치시켜 흡수성 콜라겐 차단막으로 피개하였다. 흡수성 콜라겐 차단막군 (CM group)은 콜라겐

결합 하이드록시아파타이트를 수술부위에 위치시킨후 재조합 인간 골형성 단백질-2 을 흡수시킨 흡수성 콜라겐 차단막으로 피개하였다. 8 주의 치유기간 후 동물들을 희생하였고 조직학 및 조직계측학적으로 평가하였다.

실험결과, 흡수성 콜라겐 차단막군 (CM group)에서 콜라겐 결합 하이드록시아파타이트군 (BHC group)보다 높은 신생골 형성량을 보였으나 통계적 유의성은 없었다. 콜라겐 결합 하이드록시아파타이트군 (BHC group)에서는 대부분의 신생골이 잔존이식재와 직접 접촉하면서 형성되었으나 흡수성 콜라겐 차단막군 (CM group)에서는 신생골이 잔존이식재와 접촉없이 잔존이식재 군집(clusters) 사이 공간을 차지하면서 형성되었다.

이상의 실험을 통해, 재조합 인간 골형성 유도 단백질-2 을 이용한 외측 온레이 골이식시 흡수성 콜라겐 차단막을 전달체로서 이용하는 방법은 효과적으로 신생골을 형성하였으며 이는 수술과정을 보다 간단히 할 수 있는 좋은 대안이라 사료된다.

핵심되는 말 : 골재생, 재조합 인간 골형성 단백질-2, 콜라겐, Bio-OSS, 조직학