Immediate versus delayed application of bone morphogenetic protein-2 solution in damaged extraction sockets: A preclinical in vivo investigation

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Immediate versus delayed application of bone morphogenetic protein-2 solution in damaged extraction sockets: A pre-clinical in vivo investigation

Directed by Professor Ui-Won Jung

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타지에서 공부하는 딸을 위해 사랑과 기도로 뒷받침 해주신 사랑하는 부모님과, 언제나 변함없는 명해 언니 그리고 동생 명은이 에게도 진심으로 감사의 말을 전하고 싶습니다.

마지막으로, 지금까지 저를 인도하시고 보살펴 주신 하나님께 감사를 드리며, 이 모든 영광 올려드립니다.

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김명지 드림

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Abstract

Immediate versus delayed application of bone morphogenetic protein-2 solution in damaged extraction sockets: A pre-clinical in vivo investigation

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Purpose: To compare the clinical, radiographic and histological healing patterns between the immediate and delayed application of bone morphogenetic protein-2 (BMP-2) in damaged extraction sockets in dogs.

Materials and Methods: The distal roots of the fourth premolars of the mandible were extracted bilaterally in five beagle dogs, and buccal bone defects (4 mm wide and 9 mm high) were surgically created. Collagenated biphasic calcium phosphate (CBCP) soaked for 10 minutes in 100 μl of BMP-2 solution was applied immediately to the defect site in the control group. In the test group, the BMP-2 solution of same dose was injected

into the grafted site 2 weeks after grafting with a saline-soaked CBCP. The dogs were sacrificed 2 weeks later. Clinical, histological and radiographic analyses were followed.

Results: Swelling and inflammatory reactions were predominantly observed in the control group at 2 weeks. The area of new bone formation was significantly larger in the control group compared to the test group (10.8±7.0 mm^2 [mean±SD] and 6.3±3.1 mm^2, respectively; p=0.043). No significant difference was found in ridge width at 2 mm, 4 mm and 6 mm below the lingual bone crest between the control (2.6±1.0 mm, 3.2±0.9 mm and 4.5±0.5 mm, respectively) and test group (3.3±1.0 mm, 3.7±1.3 mm and 4.2±1.0 mm; all p>0.05).

Conclusions: Delayed application of BMP-2 2 weeks after surgery did not show any advantage over immediate application of BMP-2 in terms of new bone formation.

Keywords: Bone morphogenetic protein 2; Alveolar ridge augmentation; Bone substitutes; Animal experimentation; Tooth extraction; Damaged extraction socket; Extraction socket regeneration

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I. INTRODUCTION

Numerous studies investigated the dimensional changes of the alveolar ridge following tooth extraction, thereby reporting a consistent partial shrinkage of the buccal bone. These resorptive remodeling processes are notable at the initial stage of healing, during which marked inflammatory responses and osteoclastic activities occur (Araujo et al., 2010; Araujo et al., 2005; Cardaropoli et al., 2003).

Alveolar ridge preservation has been performed to counteract both horizontal

and vertical dimensional changes of the alveolar ridge which spontaneously follow the extraction, and has been reported to be effective in minimizing the physiological remodeling process (Araujo and Lindhe, 2009; Avila-Ortiz et al., 2014; Barone et al., 2013; MacBeth et al., 2017; Vittorini Orgeas et al., 2013). However, most of the previous studies focused on the efficacy of ridge preservation in cases of partially intact buccal plates and did not include extensively damaged buccal bone wall. In addition, new bone formation seemed to be slower in the sockets where alveolar ridge preservation was performed, compared to the natural spontaneous healing due to the grafted biomaterials which might delay the new bone formation (Cardaropoli et al., 2003; Carmagnola et al., 2003; Hong et al., 2014). These led to the further researches to find a high predictable method to accelerate the regenerative process and improve the bone quality.

Considering the lack of an osteogenic healing potential in damaged extraction sockets, the application of a growth factor such as bone morphogenetic protein-2 (BMP-2) might enhance the bone regenerative potential, thereby accelerating new bone formation (Choi et al., 2012; Jung et al., 2008; Yon et al., 2015). Previous *in vivo* studies found that adding a growth factor increases the osteogenic potential and minimizes the dimensional alterations in the ridge profile of compromised extraction sockets (Jung et al., 2015; Lee et al., 2015; Schwarz et al., 2008). However, local and systemic side effects of BMP-2 have also been reported such as severe post-operative swelling and local inflammation during the early healing period, which can increase the discomfort experienced by patients and make clinicians avoid applying it in their clinics (James et al., 2016; Kim et al., 2015).

The initial inflammatory stage after ridge preservation is characterized by granulation tissue, inflammatory cells and large number of vessels occupying the socket as a result of tissue repair reactions (Lee et al., 2018). This initial inflammatory response could be amplified by the burst release of BMP-2 that induces considerable initial swelling and inflammation in the surrounding tissues, with a dose-dependent inflammatory effect (James et al., 2016; Lee et al., 2011; Tazaki et al., 2009; Wong et al.,

2008). Moreover, the applied BMP-2 could be lost due to the constant inflammatory fluid flowing during the initial healing periods. It has therefore been hypothesized that a delayed application of BMP-2 subsequently to the initial inflammatory reaction (early healing period) induces a comparable osteogenic effect whilst reducing clinical complications compared with the conventional method of an immediate BMP-2 application, however, the previous publication that verifies the hypothesis is still scarce.

The objective of the present preclinical study was to compare the clinical, radiographic and histological healing patterns following an immediate or a delayed application of BMP-2 in damaged extraction sockets in conjunction with ridge preservation procedures.

II. MATERIALS AND METHODS

1. Ethical statement

The selection and management of animals, surgical protocol and preparation were approved by the Animal Care and Use Committee, Yonsei Medical Center, Seoul, South Korea (Permission no. 2013-0317-3).

2. Experimental animals

Due to the pilot nature of this study and the absence of any prior reference studies, sample size calculation was not conducted. Five male beagle dogs aged 12 months and weighing 10-15 kg were selected for this study. A 2-week period of isolation was allowed prior to performing any procedure in order to determine their general health condition. An oral examination was performed to detect the possible presence of oral and dental diseases that could interfere with the interventions. One day before the surgery, all dogs received supragingival scaling and plaque control to avoid possible infections during the experimental period.

3. Experimental materials

Collagenated biphasic calcium phosphate (CBCP) is a synthetic bone graft based on bovine type I collagen (Osteon III Collagen® , Genoss, Suwon, South Korea) and is supplied in samples of 6 mm in diameter and of 5 mm in height. The concentration of BMP-2 (Cowellmedi, Busan, South Korea) used in the present study (0.025mg/mL) was calculated based on the findings of a previous *in vivo* study (Cha et al., 2014). The bone graft material was soaked in 100 μL of either BMP-2 solution (control group) or saline

(test group) for 10 minutes prior to being applied to the damaged socket. In the test group, the same dose of BMP-2 solution was injected by a 1 mL syringe (26 gauge) at 2 weeks post-operatively. Both treatment groups were randomly assigned to the bilateral lower-fourth premolars. The allocation of each group was performed using a computer randomization program (version 23, SPSS, Chicago, IL, USA).

4. Surgical procedure

On the day of surgery, inhalation general anaesthesia was induced (Isoflurane; Forane® , Choongwae Pharmaceutical, Seoul, South Korea) after an intravenous injection of xylazine (2 mg/kg; Rompun® , Bayer Korea, Seoul, South Korea) and Zoletil® (5mg/kg; Zolazepam + Tiletamine, Zoletil, Virbac, France). Local infiltrative anaesthesia was carried out at the surgical site using 2% lidocaine HCL with epinephrine 1:80,000 (Kwangmyung Pharm., Seoul, Korea).

A crevicular incision was made along the fourth premolar and two vertical releasing incisions were also made before elevating full-thickness flaps. The fourth premolars were hemisected, and then their distal root was extracted carefully. The pulp chamber of the remaining mesial root was removed using a round bur, and it was then capped with calcium hydroxide paste (Dycal® , Dentsply Caulk, Milford, DE, USA). Subsequently, the buccal bone plate was surgically removed to form a bone defect that was 9 mm high and 3 mm wide (Figure 1a). CBCP that had been soaked in BMP-2 solution for 10 minutes was immediately applied to the surgical site in the control group. In the test group, a CBCP sample of the same size that had been soaked in saline was grafted at the extracted site (Figure 1b). A buccal flap was positioned coronally and primary closure was performed using absorbable sutures (4-0 glyconate monofilament; Monosyn® , B. Braun, Tuttlingen, Germany). The dogs received post-surgical treatment by antibiotics (20 mg/kg intramuscular cefazolin sodium, Yuhan, Seoul, Korea) and anti-inflammatory drugs for 3 days, and analgesic medications (Ketorolac, Hana Pharm.,

Gyeonggi-do, Korea; Meloxicam, Boehringer Ingelheim, Bogota D.C., Colombia) for 7 days. After 2 weeks of healing, the same dose of BMP-2 was injected into the surgical sites in the test group. In brief, the needle penetrated from the buccal side until its tip touched the lingual bone wall, and then it was moved back slightly before the BMP-2 solution was injected twice: once in the center and once in the coronal zone (Figure 1c). The dogs were fed a soft diet during 4 weeks of healing period. Meanwhile, plaque control by the use of a toothbrush and wound cleaning by 0.2% chlorhexidine solution (Hexamedin, Bukwang Pharmaceutical, Seoul, Korea) were performed twice or thrice per week.

The dogs were sacrificed, after another 2 weeks of healing, by administering an intravenous overdose of pentobarbital sodium.

5. Radiographic analyses

Scan data were obtained using a micro computed tomography system (SkyScan 1072, SkyScan, Aartselaar, Belgium) at a resolution of 35 μm (achieved at 100 kV and 100 μA), and visualized using a 3D software (OnDemand3D, Cybermed, Seoul, South Korea). Bucco-lingual sectioning was performed in the middle of each mesial root and the grafted site of the fourth premolar and the ridge widths were measured. The two obtained images were superimposed with the aid of a computer software (Adobe Photoshop CS5, Adobe Systems, San Jose, CA, USA), and three horizontal lines were drawn perpendicular to the mesial root axis at 2 mm, 4 mm and 6 mm below the most-coronal point of the augmented ridge and the corresponding mesial root ridge to compare the bone widths after bone regeneration (Figure 2). The differences of values (in millimeters) were calculated as the augmented bone widths minus the corresponding pristine bone widths in each group.

6. Histological and histomorphometric analyses

The center-most part of each grafted area was sectioned and stained with Masson's trichrome. Histological observations were performed using a microscope (BX-50 Olympus Optical, Tokyo, Japan) and computer software (CaseViewer, 3DHISTECH, Budapest, Hungary). The histomorphometric analyses involved using a software program (Adobe Photoshop CS5, Adobe Systems, San Jose, CA, USA) to measure the parameters.

Three lines were drawn to determine the region of interest (ROI): the first line was drawn horizontally along the basal bone, then a perpendicular line coinciding with the highest lingual crest point and a third line following the contour of the augmented area. The following measurements were obtained from the ROI (Figure 3):

1. Histological ridge width (in millimeters): horizontal lines perpendicular to the lingual bone were drawn at 2 mm, 4 mm and 6 mm below the lingual crest, where the augmented width was measured.
2. The area of new bone (NBA), area of residual bone substitute (RBS) and fibrovascular area (FVA) were determined within the ROI (all in square millimeters).

7. Experimental outcomes

The primary outcome of this study was the area of new bone formation in the histological analysis. Radiographic and histomorphometric parameters were considered secondary outcomes.

8. Statistical analysis

The statistical analysis was performed using SPSS software (version 23.0). As

the measurements were not distributed normally, non-parametric Wilcoxon's test was therefore used to evaluate the differences between the test and control groups ($p<0.05$).

III. RESULTS

1. Clinical outcomes

No eventful event was observed in both groups in terms of severe post-operative complications such as wound dehiscence or local inflammation throughout the experimental period. After 2 weeks of healing, clinical signs such as redness, swelling and inflammation were predominantly observed in the surgical sites of the control group, however all of the experimental sites were fully healed with a favourable soft tissue status at 4 weeks after the surgery (Figure 4).

2. Radiographic analyses

Radiographic measurements for the ridge width are presented in Table 1. The subtraction of values between mesial root ridge width and augmented ridge width in the control group were -0.2±1.5 mm (mean±SD), 0.8±1.0 mm and 1.5±0.5 mm at 2 mm, 4 mm and 6 mm, respectively; the corresponding values in the test group were 0.2±0.8 mm, 1.1±1.0 mm and 1.4±0.8 mm. There were no significant differences between any of these values.

3. Histological and histomorphometric analyses

Representative histological healing patterns and histomorphometric measurements are presented in Figure 5 and Table 2. All specimens did not demonstrate any sign of inflammatory cell infiltration at 4 weeks of healing. The pattern of new bone formation was differed between the two groups. In the control group, newly formed bone extended from the basal bone, occupying the entire augmented region. In higher-

magnification, new bone was mainly observed in the crest and lateral wall of the socket with the presence of osteoblasts (Figure 5b and 5d). The central region of the socket was predominantly occupied by residual biomaterial, with a small amount of mineralized tissue in the middle third of the socket (Figure 5c). A particularly interesting finding was observed in the test group. In three out of five specimens mineralized bone formation extended along the periosteum following the outline of the buccal wall (Figure 5e). In histologic photomicrographs the most-coronal region of the socket was covered by new woven bone, extending to the buccal bone wall along the outline of the alveolar bone (Figure 5f and 5g). The central region of the socket mainly comprised of residual particles which dense connective tissue surrounded (Figure 5h).

The NBA of the control group was significantly larger than that of the test group (10.8±7.0 mm^2 and 6.3±3.1 mm^2, respectively; p=0.043). The values of the other parameters including the total augmented area, area of RBS and FVA were higher in the test group than the control group, but the differences were not statistically significant. There were no intergroup differences in the widths at 2 mm, 4 mm and 6 mm below the lingual bone crest: 2.6±1.0 mm, 3.2±0.9 mm and 4.5±0.5 mm, respectively, in the control group, and 3.3±1.0 mm, 3.7±1.3 mm and 4.2±1.0 mm in the test group, (all p>0.05).

IV. DISCUSSION

The aim of this preclinical study was to compare the healing pattern between two timings for applying BMP-2 (immediate and delayed) to damaged extraction sockets augmented with a bone substitute. Although more swelling and inflammatory reactions were observed in the control group at 2 weeks, it showed more amount of newly formed bone compared to the delayed application group. The greater new bone formation in the control group compared to the test group (10.8±7.0 mm^2 and 6.3±3.1 mm^2, respectively) was probably due to the longer time of action of the growth factor in the control group.

A 4-week experimental period was designed in order to observe the acceleration of osteogenesis induced by BMP-2 during the early phase of healing, allowing the growth factor to exert its effects at the target site during 2 and 4 weeks in the test and control groups, respectively. This was based on Araujo et al. (2005) finding that cortical bone formation in canines (known as corticalization) appeared between 4 and 8 weeks around marginal compartments of extracted sockets. Terbish et al. (2015) evaluated the quality and quantity of distracted alveolar bone when BMP-2 was experimentally injected after the distraction was completed. That study found significant increases in the volume and density of the alveolar bone after 6 weeks and a prolonged residence time of BMP-2 in the experimental group compared to the conventionally distracted group. It might be speculated that the 2 weeks-healing period allowed after the delayed application of BMP-2 was not long enough to observe the full effects of the growth factor in the present study.

The radiographic ridge widths did not differ between the two groups. This is consistent with a previously published *in vivo* study that compared alveolar bone defect grafted with either a bone substitute alone or rhBMP-2-loaded bone substitute, and found that the radiographic ridge widths at 2 and 4 mm below the bone crest did not differ significantly (Lee et al., 2015). Limited comparisons with the present results can be

established due to the paucity of studies that have evaluated the dimensional changes of ridge augmentation in buccal-bone-damaged sockets with and without BMP-2. Further studies having longer observational periods are needed to evaluate the histological and volumetric changes in ridge augmentation in this particular clinical condition.

The present study tested the hypothesis that the delayed application of BMP-2 induces smaller tissue reactions and increases the osteogenic potential due to the BMP-2 solution being injected directly into the augmented alveolar ridge after the initial inflammatory phase has finished. From a clinical viewpoint, notable clinical complications such as swelling and redness were observed at 2 weeks of healing in the control group, while at 4 weeks, full healing had occurred in both control and test groups without any tissue reactions. The least inflammatory reaction in the test group at 2 weeks after the injection might be for applying BMP-2 after the early healing period. These findings are important to consider for clinical applications, since they indicate that patient discomfort related to the soft tissue complications could be decreased. However, it is necessary to conduct more studies to confirm greater results in terms of early new bone formation.

On the other hand, the limitation of this delayed application method is that the control of the BMP-2 distribution within the carrier is unclear. While the BMP-2 was soaked overall graft materials before being applied in the socket in control group, the concentrated doses of BMP-2 could be applied locally at the injected site in test group. The BMP-2 injection was carried out 2 weeks after the surgical intervention, when the augmented area was composed by immature granulation tissue and provisional matrix (Arau´jo MG, 2005). According to this regenerative process, an even distribution of injected BMP-2 solution was presumed by the repairing tissue diffusion; however, confirming whether the grafted material was completely soaked by injected BMP-2 solution could not be conducted and BMP-2 solution might not spread evenly. One particularly interesting histological feature in the test group was that the mineralized bone

formation extended along the outline of the periosteum where the buccal bone originally existed. This phenomenon was observed in three of the five test group specimens. Injecting into the central and peripheral regions of the augmented ridge was attempted in this study with the hope that the injected BMP-2 would spread homogeneously inside the carrier, and therefore the osteogenic potential of the basal bone and periosteum would be appropriately stimulated. A standardized method of injection into the augmented region should be included in further study.

According to an *in vivo* study in dogs, extensive new bone formation with high osteogenic activity was found even when BMP-2 was applied at the low concentration of 0.1 mg/mL (Cha et al., 2014). The study of damaged extraction socket by (Lee et al., 2015) compared the new bone formation in ridge regeneration among four experimental groups (buccal-bone-deficient group without any treatment, grafted group, grafted and rhBMP-2-loaded group, and grafted and covered with membrane group), and showed that both total augmented and new bone areas in groups used rhBMP-2 and membrane were 1.6 to 1.7-fold greater than control and only grafted group at 8 weeks of healing. The observation period was shorter in the present study, and the BMP-2 solution used in the present study was 0.1 mL of 0.025 mg/mL, 20 times lower than that of the previous study (0.2ml of 0.25 mg/mL), thus may have restricted the effectiveness of BMP-2 on the amount of newly formed bone. It should also be taken into consideration no negative control group was utilized, therefore, the effect of the growth factor could not be completely determined.

There were some limitations in this study. Firstly, as mentioned above, negative control group (sham surgery or ridge augmentation only) was not included. Secondly, the sample number used in this study was small (n=5) and therefore the study with increased sample size might be needed for further verification before applying in clinics. Thirdly, the direction and distribution of BMP-2 fluid were uncontrolled even though the injection was tried as much as possible to soak whole residual biomaterial. Furthermore, this study

only focused the observations in early healing period. Studies with both early and late healing periods are needed to compare the healing pattern in terms of new bone formation between two different time points. Within the limitations of this study, it may be concluded that the immediate application of BMP-2 in a grafted socket following tooth extraction increases the amount of new bone formation compared to the delayed application of BMP-2. Further studies are needed with longer healing periods and higher concentrations of BMP-2 to observe the quantity and quality of augmented alveolar bone in damaged sockets for these two methods of applying BMP-2.

V. CONCLUSION

Within the limitations of this study, it may be concluded that the immediate application of BMP-2 in a grafted socket following tooth extraction increases the amount of newly formed bone compared to the delayed application of BMP-2. Further studies are needed with longer healing periods and higher concentrations of BMP-2 to observe the quantity and quality of augmented alveolar bone in damaged sockets for these two methods of applying BMP-2.

REFERENCE

Arau´jo MG LJ (2005). Dimensional ridge alterations following tooth extraction. An experimental study in the dog. *J Periodontol* 32: 212-218.

Araujo MG, Liljenberg B, Lindhe J (2010). Dynamics of Bio-Oss Collagen incorporation in fresh extraction wounds: an experimental study in the dog. *Clin Oral Implants Res* 21(1): 55-64.

Araujo MG, Lindhe J (2009). Ridge preservation with the use of Bio-Oss collagen: A 6-month study in the dog. *Clin Oral Implants Res* 20(5): 433-440.

Araujo MG, Sukekava F, Wennstrom JL, Lindhe J (2005). Ridge alterations following implant placement in fresh extraction sockets: an experimental study in the dog. *J Clin Periodontol* 32(6): 645-652.

Avila-Ortiz G, Elangovan S, Kramer KW, Blanchette D, Dawson DV (2014). Effect of alveolar ridge preservation after tooth extraction: a systematic review and meta-analysis. *J Dent Res* 93(10): 950-958.

Barone A, Ricci M, Tonelli P, Santini S, Covani U (2013). Tissue changes of extraction sockets in humans: a comparison of spontaneous healing vs. ridge preservation with secondary soft tissue healing. *Clin Oral Implants Res* 24(11): 1231-1237.

Cardaropoli G, Araujo M, Lindhe J (2003). Dynamics of bone tissue formation in tooth extraction sites. An experimental study in dogs. *J Clin Periodontol* 30(9): 809-818.

Carmagnola D, Adriaens P, Berglundh T (2003). Healing of human extraction sockets filled with Bio-Oss. *Clin Oral Implants Res* 14(2): 137-143.

Cha JK, Lee JS, Kim MS, Choi SH, Cho KS, Jung UW (2014). Sinus augmentation using BMP-2 in a bovine hydroxyapatite/collagen carrier in dogs. *J Clin Periodontol* 41(1): 86-93.

Choi Y, Yun JH, Kim CS, Choi SH, Chai JK, Jung UW (2012). Sinus augmentation using

absorbable collagen sponge loaded with Escherichia coli-expressed recombinant human bone morphogenetic protein 2 in a standardized rabbit sinus model: a radiographic and histologic analysis. *Clin Oral Implants Res* 23(6): 682-689.

Hong JY, Lee JS, Pang EK, Jung UW, Choi SH, Kim CK (2014). Impact of different synthetic bone fillers on healing of extraction sockets: an experimental study in dogs. *Clin Oral Implants Res* 25(2): e30-37.

James AW, LaChaud G, Shen J, Asatrian G, Nguyen V, Zhang X, et al. (2016). A Review of the Clinical Side Effects of Bone Morphogenetic Protein-2. *Tissue Eng Part B Rev* 22(4): 284-297.

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

Jung UW, Lee IK, Park JY, Thoma DS, Hammerle CH, Jung RE (2015). The efficacy of BMP-2 preloaded on bone substitute or hydrogel for bone regeneration at peri-implant defects in dogs. *Clin Oral Implants Res* 26(12): 1456-1465.

Kim JS, Cha JK, Cho AR, Kim MS, Lee JS, Hong JY, et al. (2015). Acceleration of Bone Regeneration by BMP-2-Loaded Collagenated Biphasic Calcium Phosphate in Rabbit Sinus. *Clin Implant Dent Relat Res* 17(6): 1103-1113.

Lee JS, Choe SH, Cha JK, Seo GY, Kim CS (2018). Radiographic and histologic observations of sequential healing processes following ridge augmentation after tooth extraction in buccal-bone-deficient extraction sockets in beagle dogs. *J Clin Periodontol* 45(11): 1388-1397.

Lee JS, Jung JS, Im GI, Kim BS, Cho KS, Kim CS (2015). Ridge regeneration of damaged extraction sockets using rhBMP-2: an experimental study in canine. *J Clin Periodontol* 42(7): 678-687.

Lee KB, Taghavi CE, Song KJ, Sintuu C, Yoo JH, Keorochana G, et al. (2011). Inflammatory characteristics of rhBMP-2 in vitro and in an in vivo rodent model.

Spine (Phila Pa 1976) 36(3): E149-154.

MacBeth N, Trullenque-Eriksson A, Donos N, Mardas N (2017). Hard and soft tissue changes following alveolar ridge preservation: a systematic review. *Clin Oral Implants Res* 28(8): 982-1004.

Schwarz F, Rothamel D, Herten M, Ferrari D, Sager M, Becker J (2008). 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.

Tazaki J, Murata M, Akazawa T, Yamamoto M, Ito K, Arisue M, et al. (2009). BMP-2 release and dose-response studies in hydroxyapatite and beta-tricalcium phosphate. *Biomed Mater Eng* 19(2-3): 141-146.

Terbish M, Yoo SH, Kim HJ, Yu HS, Hwang CJ, Baik HS, et al. (2015). Accelerated Bone Formation in Distracted Alveolar Bone After Injection of Recombinant Human Bone Morphogenetic Protein-2. *J Periodontol* 86(9): 1078-1086.

Vittorini Orgeas G, Clementini M, De Risi V, de Sanctis M (2013). Surgical techniques for alveolar socket preservation: a systematic review. *Int J Oral Maxillofac Implants* 28(4): 1049-1061.

Wong DA, Kumar A, Jatana S, Ghiselli G, Wong K (2008). Neurologic impairment from ectopic bone in the lumbar canal: a potential complication of off-label PLIF/TLIF use of bone morphogenetic protein-2 (BMP-2). *Spine J* 8(6): 1011-1018.

Yon J, Lee JS, Lim HC, Kim MS, Hong JY, Choi SH, et al. (2015). Pre-clinical evaluation of the osteogenic potential of bone morphogenetic protein-2 loaded onto a particulate porcine bone biomaterial. *J Clin Periodontol* 42(1): 81-88.

TABLES

Table 1. Difference between the augmented ridge width and the corresponding pristine ridge by the radiographic linear measurements (mm; mean ± standard deviation).

	Control group ($\triangle AB_{dp}$-PB_{mp})	**Test group ($\triangle AB_{dp}$-PB_{mp})**
2mm	-0.2 ± 1.5	0.2 ± 0.8
4mm	0.8 ± 1.0	1.1 ± 1.0
6mm	1.5 ± 0.5	1.4 ± 0.8

AB_{pd}, Augmented bone widths in distal premolar; PB_{pm}, Pristine bone widths in mesial premolar.

No significant difference ($p > 0.05$)

Table 2. Histomorphometric measurements

	Control group	**Test group**
Total area (mm^2)	32.1 ± 8.1	32.6 ± 11.3
New bone area (mm^2)	10.8 ± 7.0*	6.3 ± 3.1*
Bone graft area (mm^2)	4.1 ± 0.7	5.3 ± 7.4
2mm	2.6 ± 1.0	3.3 ± 1.0
4mm	3.2 ± 0.9	3.7 ± 1.3
6mm	4.5 ± 0.5	4.2 ± 1.0

(*) : Significantly different ($p<0.05$)

FIGURES

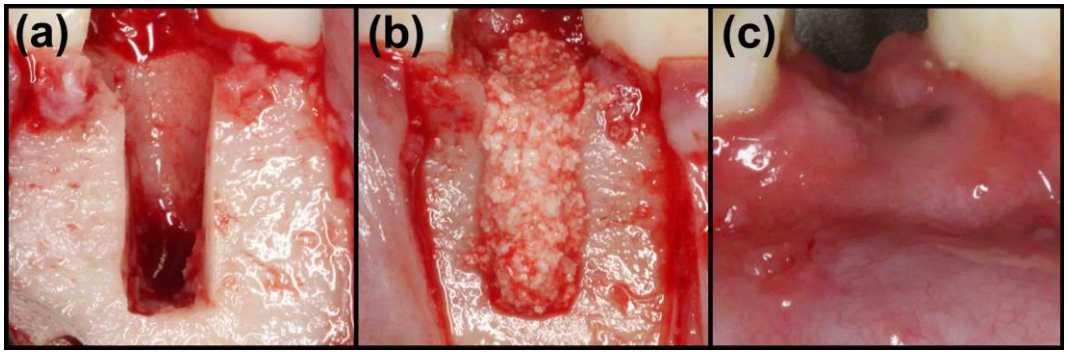

Figure 1. Clinical photographs of surgical intervention.

Bone defect (a), bone graft (b), and BMP-2 injection at the test site after 2 weeks of healing (c)

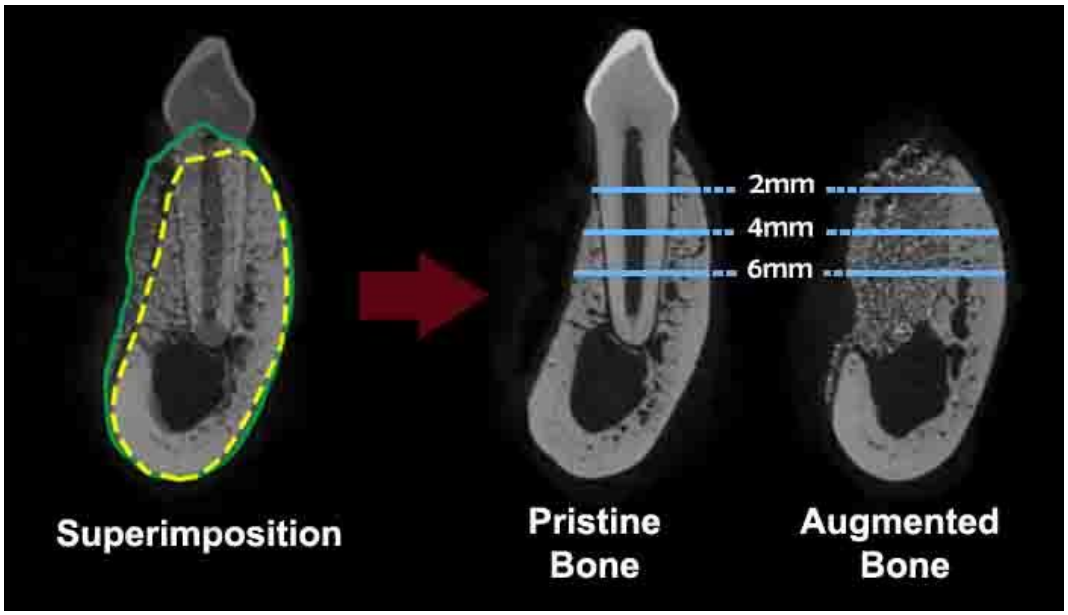

Figure 2. Radiographic measurements.

Radiographic measurements of the ridge width in micro computed tomography images. Bucco-lingual sectioning was performed in the most-central parts of a pristine tooth (yellow line) and at the experimental site (green line). Both images were superimposed and linear measurements of the width were made at 2 mm, 4 mm and 6 mm from the crest perpendicular to the mesial root axis.

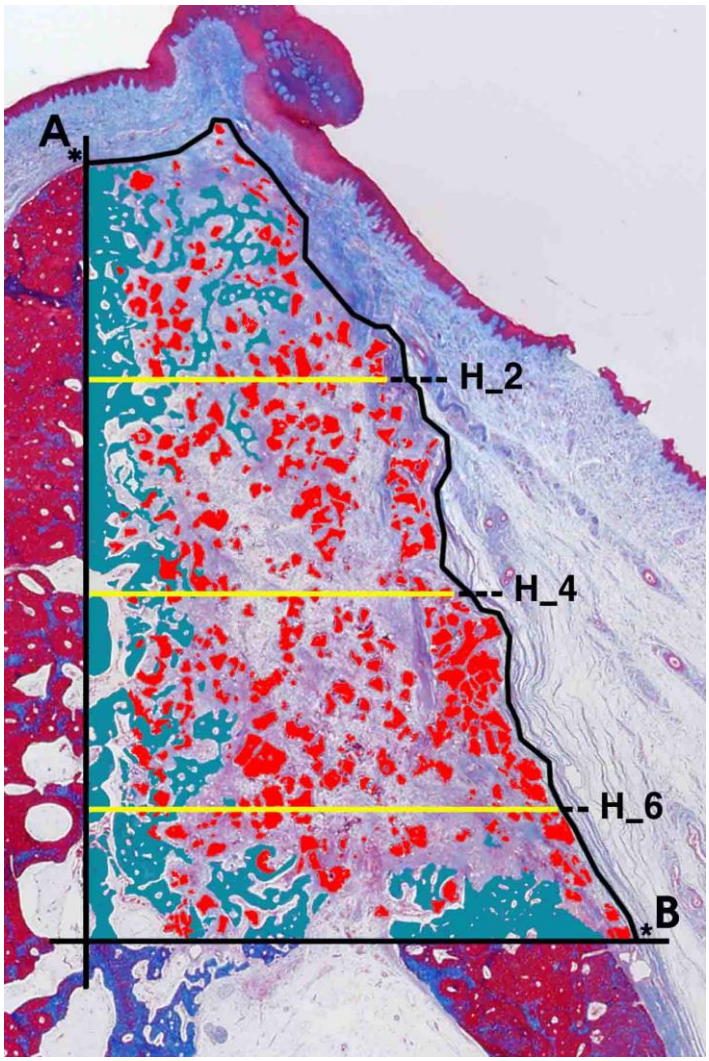

Figure 3. Histomorphometric measurements.

The ROI was drawn based on two points: (A) the crest of the lingual plate point and (B) the basal bone point. Histomorphometric analysis was performed within the ROI (blue area, new bone; red area, bone material; yellow lines, augmented tissue thickness) and widths were measured at 2 mm, 4 mm and 6 mm below the lingual crest (H_2, H_4 and H_6, respectively).

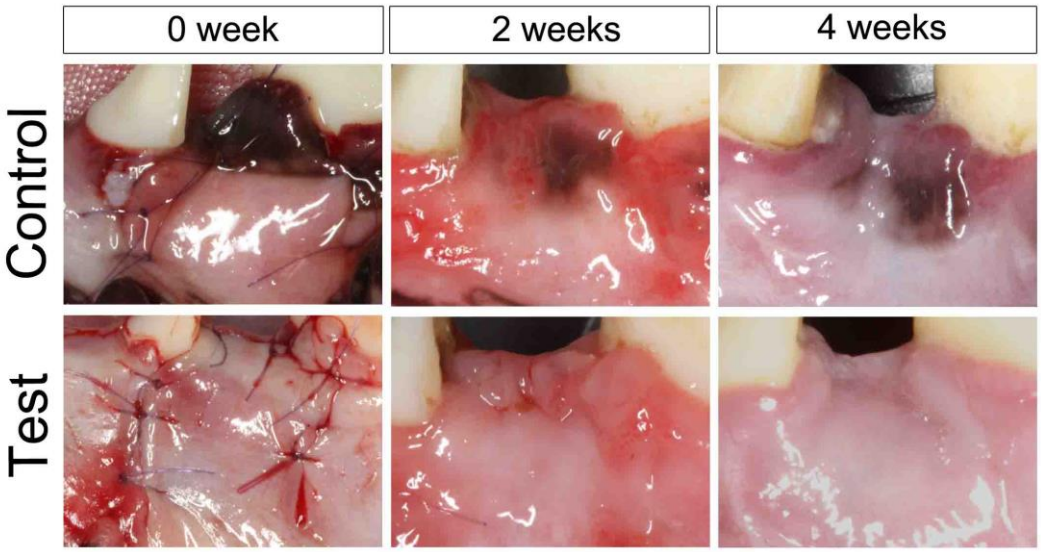

Figure 4. Clinical outcomes.

After 2 weeks of healing, the soft tissue reactions induced by BMP-2 including swelling and redness were more prominent in the control group than in the test group, which showed normal soft tissue healing. Both groups had completely healed at 4 weeks without any clinical complications.

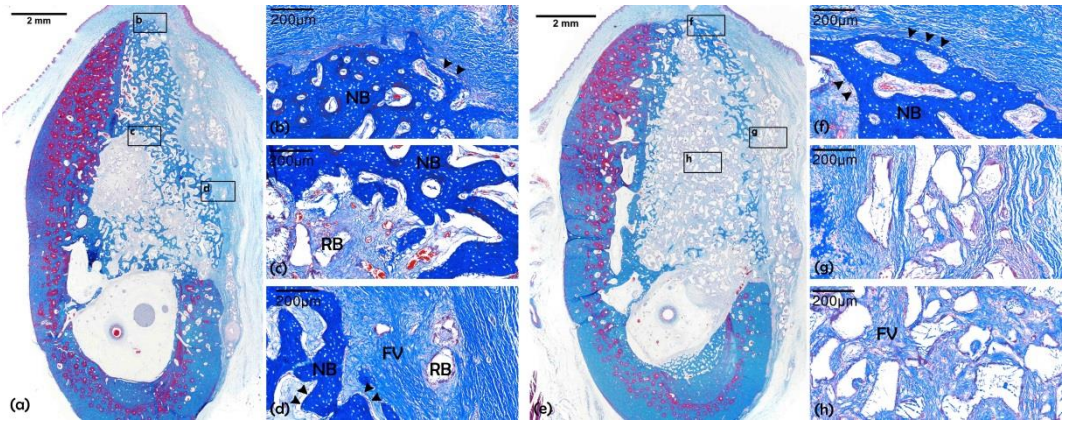

Figure 5. Descriptive histology.

Representative histological lower-magnification photographs of the control (a) and test (e) groups (scale bar = 2 mm). (b) and (f): The bone crest in each group is compounded by new bone with osteoblasts on its surface (scale bar = 200 μm). In high-magnified views of the control group, (c) periosteum is observed in the lateral bone and, (d) the innermost residual particles are surrounded by newly formed mineralized tissue (scale bar = 200 μm). In the test group, (g) bone was formed around the bone ridge outline and biomaterial particles remain with the periosteum, and (h) the central part was completely filled by bone materials (scale bar = 200μm). NB, new bone; RB, residual biomaterials; CT, connective tissue; arrowhead, osteoblasts.

국문요약

성견의 파괴된 발치와에서의 치조제 보존술 시 제2형 골형성 단백질의 적용시기에 따른 효과에 대한 비교 연구

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발치 후 발생하는 대부분의 골 흡수는 주로 염증반응과 파골세포의 활성이 활발한 치유 초기에 일어난다. 치조제 보존술은 발치 후 일어나는 생리적 골 흡수를 줄일 수 있다고 알려져 있지만, 대부분의 연구들은 임상에서 주로 마주하는 치주질환 등으로 인해 파괴된 발치와가 아닌 건전한 발치와에서 진행되었다. 파괴된 발치와에는 건전한 발치와에 비해 골 형성능이 낮다는 것을 고려하였을 때, 성장인자인 제 2 형 골형성 단백질을 적용할 경우 골 재생을 촉진시켜 신생골 형성을 증진시킬 가능성이 제기되었다. 실제로 여러 선행 동물 실험에서는, 발치와에 성장인자를 사용하였을 때 골 형성능이 증가하고 치조제의 부피 위축이 최소화 된다고 보고되었다. 하지만 제 2 형 골형성 단백질의 경우, 술 후 심한 염증과 붓기가 동반되어, 이로 인한 환자의 불편감이 초래된다는 점이 단점으로 지적되어 왔다. 치조제 보존술 후 초기 치유 단계에서는 과립 조직과 염증 세포들에 의한 염증 반응이 나타나는데, 이 염증 반응이 제 2 형 골형성 단백질로 인해 더욱 증폭 될 수 있다고 보고되었다. 또한 제 2 형 골형성 단백질의 일부가 초기 염증 반응

단계에서 염증성 삼출액의 흐름에 의해 소실 될 수 있다는 보고도 있었다. 이에 따라, 제 2 형 골형성 단백질을 초기 치유 단계 이후에 적용 시 그 효능이 더 증진 될 것이라는 가설이 수립되었다.

따라서 본 연구는, 파괴된 발치와에 대한 치조제 보존술 시, 제 2 형 골형성 단백질의 적용시기를 달리하였을 때의 효능을 임상적, 방사선학적 그리고 조직학적으로 비교 고찰하는 데에 목적을 두었다.

치조제 보존술을 위한 골 이식 재료로는 소 유래 1 형 콜라겐이 함유된 합성골 (Collagenated biphasic calcium phosphate, CBCP) 6mm (직경) x 5mm (높이)과 제 2 형 골형성 단백질 0.025mg/mL 이 사용되었다. 성견 5 마리를 대상으로 양측 4 번 소구치를 편측 절제하여 원심 치근을 발거하였고, 9mm (높이) x 3mm (넓이)의 골 결손부를 발치와 협측에 외과적으로 형성 하였다. 대조군 (즉시 적용)의 경우, 이식 10 분 전 제 2 형 골형성 단백질을 골 이식재에 흡수시켜 발치와에 적용하였다. 실험군 (지연 적용)의 경우, 식염수를 흡수시킨 골 이식재를 발치와에 이식 한 뒤, 2 주 후 동일한 양의 제 2 형 골형성 단백질을 이식 부위에 주사기를 이용하여 주입하였는데, 치조제 재건 부위의 중앙과 상방에 나눠 적용하였다. 발치와에 골 이식을 시행한 시점으로부터 4 주 후 실험 동물을 희생하였고, 조직학적 분석과 방사선학적 분석을 통해 두 군의 결과를 비교하였다. 방사선학적으로는 치근을 발거하지 않은 근심 측과 치조제 보존술을 시행한 원심 측을 중첩하여, 치조정 하방 2, 4 그리고 6mm 의 위치에서의 치조제 폭 변화량을 계측하였다. 조직학적으로는 광학 현미경을 통한 고배율 관찰과 더불어, 신생골의 면적과 치조정 하방 2, 4 그리고 6mm 위치에서의 치조제 폭 증강량을 계측하였다. 시험군과 대조군의 정규 분포가 확인되지 않아

조직학적과 방사선학적 계측치의 통계적 비교는 Wilcoxon 검정을 통해 시행하였다 (p<0.05).

임상적으로, 대조군의 경우 치조제 보존술 및 제 2 형 골형성 단백질 적용 2 주 후 붓기와 염증이 두드러지게 관찰된 반면, 실험군의 경우 제 2 형 골형성 단백질 주입 2 주 뒤 심한 붓기와 염증 소견 없이 치유되었음을 확인하였다. 방사선학적 계측 결과, 치조제 폭 변화량에 대한 두 군 간의 유의한 차이는 확인되지 않았다. 조직학적 관찰 결과, 두 군 모두 신생골 바깥쪽으로 조골세포가 관찰 되었고, 중앙 부위에는 주로 잔존 골 이식재가 관찰되었다. 신생골 분포 양상은 두 군에서 서로 다른 양상이 나타났는데, 대조군에서는 신생골이 기저골에서부터 형성되는 양상이 관찰된 반면, 실험군에서는 협측의 골막을 따라 신생골이 형성되는 양상이 관찰 되었다. 조직학적 계측 결과, 치조제 폭의 증강량은 두 군 사이에서 유의한 차이가 확인되지 않았으나, 신생골의 면적은 대조군이 실험군에 비해 유의하게 큰 것으로 확인되었다 (10.8±7.0 mm^2 vs 6.3±3.1 mm^2; p=0.043).

결론적으로, 파괴된 발치와에서의 치조제 보존술 시 제 2 형 골형성 단백질의을 즉시 적용 하였을 때, 지연 적용한 경우에 비해 더 많은 신생골이 형성 되었다. 후속 연구에서 치유기간을 길게 하고, 단백질의 농도를 다르게 하여 두 적용 시기에서의 골질과 골양을 연구해야 할 것이다.

핵심되는 말: 제 2 형 골 형성 단백질; 치조제 보존술; 골 이식재; 동물 실험; 발치; 파괴된 발치와; 발치와 재생술