Accelerated Bone Formation in Distracted Alveolar Bone after Injection of recombinant human Bone Morphogenetic Protein-2

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Accelerated Bone Formation in Distracted Alveolar Bone after Injection of recombinant human Bone Morphogenetic Protein-2

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This certifies that the dissertation thesis of
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2015년 12 월

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ABSTRACT

Accelerated Bone Formation in Distracted Alveolar Bone after Injection of recombinant human Bone Morphogenetic Protein-2

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Department of Dentistry

(Directed by Professor Jung-Yul Cha, D.D.S., M.S., Ph.D)

This study was done to evaluate the effect of recombinant human bone morphogenic protein-2 (rhBMP-2) on enhancing the quality and quantity of regenerated bone when injected into distracted alveolar bone.

Sixteen adult beagle dogs were assigned to either the control or rhBMP-2 group. After distraction was completed, an rhBMP-2 dose of 330 μg in 0.33 ml was injected slowly into the distracted alveolar crest of the mesial, middle, and distal parts of the alveolar bone in the experimental group. Histological and micro-computed tomography analyses of regenerated bone were done after 2 and 6 weeks of consolidation.

After 6 weeks of consolidation, the vertical defect height of regenerated bone was statistically lower in the rhBMP-2 group (2.2 mm) than in the control group (3.4 mm) ($P < 0.05$). Additionally, the width of the regenerated bone was significantly greater in the rhBMP-2 group (4.3 mm) than in the control group (2.8 mm) ($P < 0.05$). The bone density and volume of regenerated bone in the

rhBMP-2 group were denser and greater, respectively, than in the control group after 6 weeks of consolidation ($P < 0.001$).

Injection of rhBMP-2 into regenerated bone after a distraction osteogenesis procedure, significantly increased bone volume in the dentoalveolar distraction site, and improved both the width and height of the alveolar ridge and increased the bone density.

Key words: rhBMP-2, Bone regeneration, Distraction osteogenesis, Alveolar bone distsraction osteogenesis.

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I. Introduction

The possibility of bone lengthening by means of distraction osteogenesis (DO) was first described by Codivilla 1905(Codivilla, 1905). Distraction osteogenesis is a surgical process used to reconstruct skeletal deformities and lengthen the long bones of the body(Ilizarov, 1989a, b).

Distraction osteogenesis regenerates new bone by the gradual separation of bony segments and the maturation of bone processed during the consolidation period, which makes the new bone strong enough to support the bone structure (Paley et al.,1997). The technology of DO has been used mainly in the field of orthopedics (Yen, 1997).

In dental fields, DO have been applied to alveolar bone and the anterior maxillary complex and is known as interdental distraction or premaxillary DO (Tong et al., 2003). These are the treatment choices for patients with cleft palate or constricted dentition (Choi et al., 2013). With this method, new

dentoalveolar bone structures are regenerated by transportation distraction of alveolar bone. The created alveolar bone provides space for aligning crowded dentition (Tong et al., 2003), or the dentition can be restored by further rehabilitative treatments such as implant placement (Terbish et al.,2014).

Recently, alveolar bone DO has been applied to atrophic mandibular and maxillary alveolar ridges, and the alveolar segment can be distracted in the vertical and horizontal directions according to morphologic features of the atrophic ridge (Bianchi et al.,2008). Compared to bone grafts for the atrophic alveolar ridge, alveolar DO has been applied successfully for the augmentation of the height of the alveolar bone ridge where the amount of soft tissue uncovered may be limited, and the bone defect is complicated (Perry et al., 2012). Alveolar bone can be distracted in conjunction with the surrounding soft tissues. These adaptive changes reduce the risk of recurring infection in the bone defect and promote regeneration of the alveolar bone (Uckan et al., 2008).

However, the relapses after DO of the maxillofacial bone are still a major concern to clinicians (Choi et al., 2012). After completing DO, the distractor should be stabilized as an anchorage unit to reduce postoperative relapse. For this purpose, an appliance needs to be maintained for long periods, but is often uncomfortable for the patient and may cause infection in the anchorage area (Choi et al., 2012). The appliances can also fracture. A relapse rate of 10% to 25% was reported for premaxillary distraction, and bone height relapse after alveolar distraction varies from 8.5% to 18% depending on the appliance type and surgical technique (Herford et al., 2007). Therefore, a distracted segment necessitates an over correction of 15% to 20%. As a result of relapse, the height of the alveolar bone distraction site is often not sufficient for dental implantation, leading to additional surgery to augment the alveolar bone height, such as bone grafts or guided bone regeneration (Bianchi et al., 2008; Cortese et al., 2011).

For this reason, there have been many attempts to accelerate the orthogenesis of the distraction to reduce both relapse and shorten consolidation period (Francis et al., 2013). Previous studies report acceleration of bone formation in DO and bone healing by applying demineralized bone matrix (Hatzokos et al.,2011; Song et al., 2004), growth factors (Ai-Aql et al., 2008; Huet al., 2007; Moore et al., 2009) and marrow-derived progenitor cells (Verseijden et al., 2010) to the distraction site.

Various carrier systems for recombinant human bone morphogenetic protein-2 (rhBMP-2) have been reported for distraction of long bones and maxillofacial bones, including a collagen sponge (Cochran et al.,2000; Sailhan et al., 2010) and chitosan hydrogel (Konas et al., 2009) during the surgical operation; sequential injection (Li et al., 2002) of rhBMP-2 at the site of distracted bone during the distraction period has also been reported. These approaches showed acceleration of the osteogenic potential of bone formation and increased the stability of the regenerated bone structure (Rihn et al., 2009; Sailhan et al., 2010). However, the carrier delivery system required an additional flap to cover the surrounding tissue, and sequential injections can increase patients discomfort during the distraction period.

For this reason, trials of rhBMP-2 injections without a carrier system have been done at the end of the distraction phase. These trials reported that rhBMP-2 injections could accelerate bone healing compared with conventional DO, suggesting that the consolidation period can be reduced. However, these trials were performed for distraction osteogenesis (DO) of the long bone or mandible (Cheung et al., 2006). There are no reports as yet on the anatomical characteristics of the alveolar structures created when rhBMP-2 injections are applied after distraction osteogenesis (DO). This study evaluated the bone quality and quantity when rhBMP-2 was injected into distracted alveolar bone compared with the conventional alveolar DO procedure.

II. Materials and methods

A. Animals and Surgery

Sixteen 16-to-18 month-old beagle dogs (weighing: 15-16 kg) were used. The dogs were caged individually and fed liquid food and water for two months. The dogs were divided into the control and rhBMP-2 groups. In each dog, a horizontal crestal incision was made, and a mucoperiosteal flap was raised in order to extract a maxillary canine tooth before the main surgical operation. All animal procedures were approved by the Institutional Review Board of the Committee of the Department of Laboratory Animal Medicine, Medical Research Center, Yonsei University College of Medicine (Y 09-120), Seoul, South Korea.

The dogs were divided into the following 2 groups: control group (n=8) and experimental group (n=8). The experimental protocol is shown in (Fig. 1). After a latency period of 7 days, distraction was started gradually at a rate of 0.8 mm twice daily until the dentoalveolar segment reached the opposite edge of the cleft by day 10. The alveolar distractor was fabricated with an orthodontic hyrax screw (Hyrax® Ispringen, Dentaurum, Germany), which allowed a maximum distraction of 8 mm at an expansion rate of 0.20 mm/quarter turns.

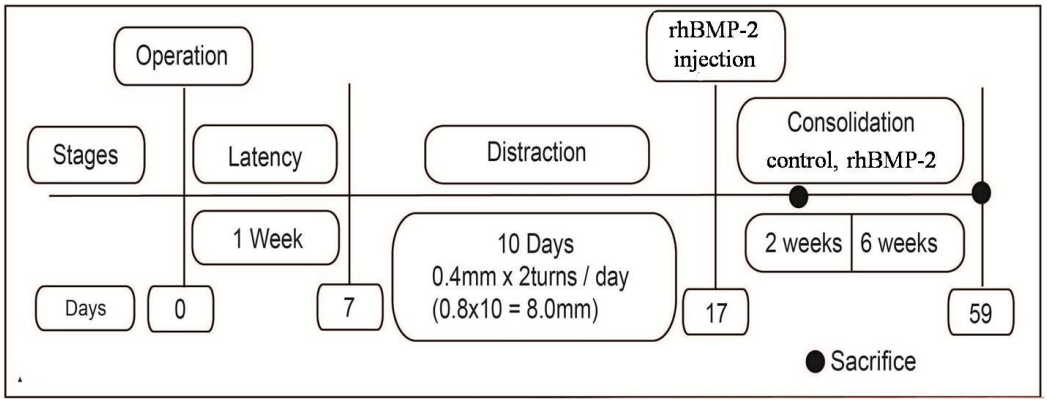

Fig. 1. Experimental protocol for the sequential stages of the maxillary alveolar DO and the rhBMP-2 injection time.

Die stone models from alginate impressions were used to fabricate the distraction device consisted of an orthodontic hygienic-type Hyrax screw (Dentaurum, Ispringen, Germany). The vertical and horizontal osteotomies were performed to allow distraction of the first premolar segment into a bony defect at the canine site and creation of a dentoalveolar transport segment containing the second premolar. A complete horizontal subapical osteotomy was performed 7 mm apical to the cementoenamel junction (CEJ) of the first and second premolars, and a complete vertical interdental osteotomy was created between second and third premolars.

The maxillary first, second premolars and first molar were etched with 37% phosphoric acid gel for 30 seconds. The crowns were filled with the 3M Filtek Supreme restorative composite resin (3M ESPE, St Paul, Minn) (Fig. 2).

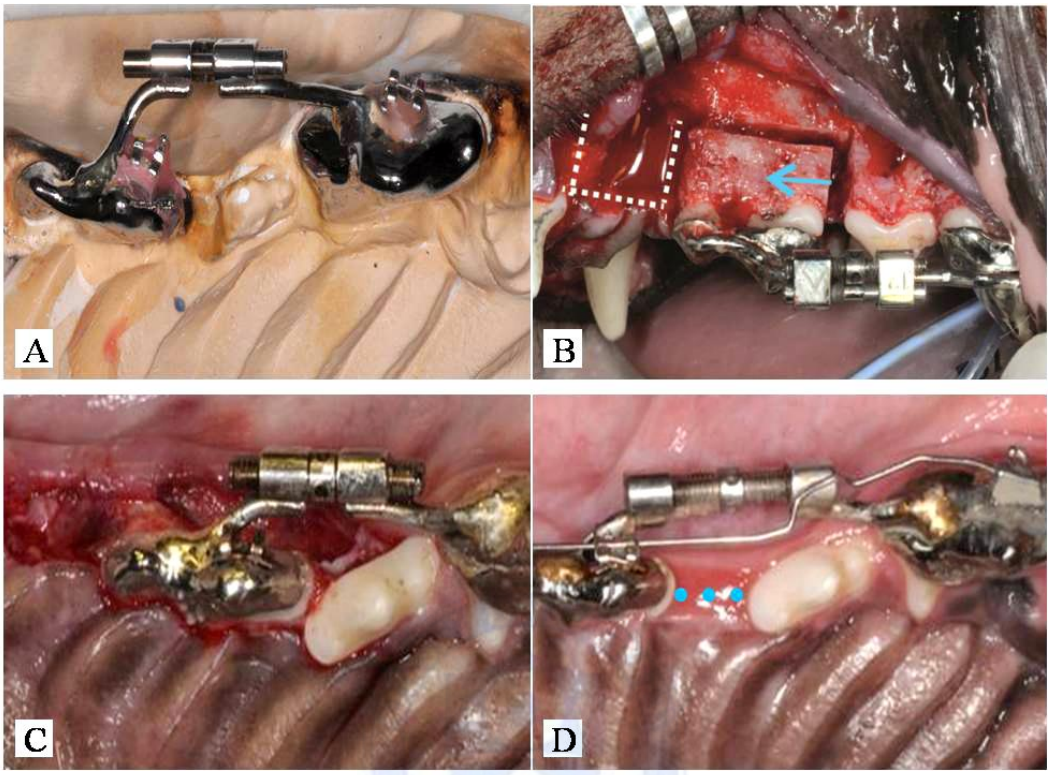

Fig. 2. Surgical procedure using a customized alveolar distractor on the maxillary arch, and quantitative analysis of osteogenesis in the regenerated bone. A. Model distraction device. B. The osteotomy procedure for alveolar bone distraction. The removed bone is displaced in the extraction socket of the maxillary canine (white dotted box). The direction of movement of the segmental alveolar bone (blue arrow). C. Latency period maxillary alveolar segments including first and second premolars and first molar. D. After distraction period. The space created in the alveolar bone after distraction was completed. rhBMP-2 was injected into the alveolar crest (blue point).

After the distraction was completed, a 1 ml syringe was loaded with 330 μg rhBMP-2 (Cowellmedi, Busan, Korea) in 0.33 ml phosphate buffered saline (PBS). In the experimental group, 0.11 ml of the rhBMP-2 solution was injected slowly into each part (mesial, middle, and distal, respectively) of the distracted alveolar crest of the alveolar bone (Fig. 1D). The sedative analgesics Zolazepam with tiletamine (5 mg/kg) (Zoletil 50, Virbac Laboratories, Carros, France) and Xylazine (0.2 mg/kg) (Rompun 2%, Bayer Healthcare Korea, Korea) were administered during rhBMP-2 injection through an intravenous line placed in the brachial vein.

B. Micro-computed Tomography Analysis

After 2 or 6 weeks of consolidation, the animals were sacrificed and the alveolar segments were scanned by micro-computed tomography (SkyScan micro-CT 1076, Bruker, Kontich, Belgium) at a voltage of 100 kV and a current of 100 mA with 36 μm resolution (Fig. 3) (Cha et al., 2009). The frame averaging was set at 3 with rotational imaging of 360 degrees. Scanning data were reconstructed using NreconVer 1.5 (Nrecon v.1.5, Bruker).

	Pre-set values
Filter	Al 0.5mm
Resolution	18㎛
Voltage	100kV
Current	100mA
Exposure time	1180mS
Rotation step	0.5 °

Fig. 3. Skyscan micro-CT

The bone parameters were analyzed by CT-An (CTAn v.1.13, Bruker) to estimate bone density, bone volume (BV/TV) fraction, trabecular number (Tb.N), trabecular separation (Tb.Sp), and trabecular thickness (Tb.Th) for each consolidation period. Regenerated bone was divided into 3 volumes (mesial, middle, and distal), with each volume including 50 slices. The alveolar bone height and width of the regenerated bone were measured with Data Viewer Version 1.3.2 (DataViewer v.1.3.2, Bruker) (Fig. 4).

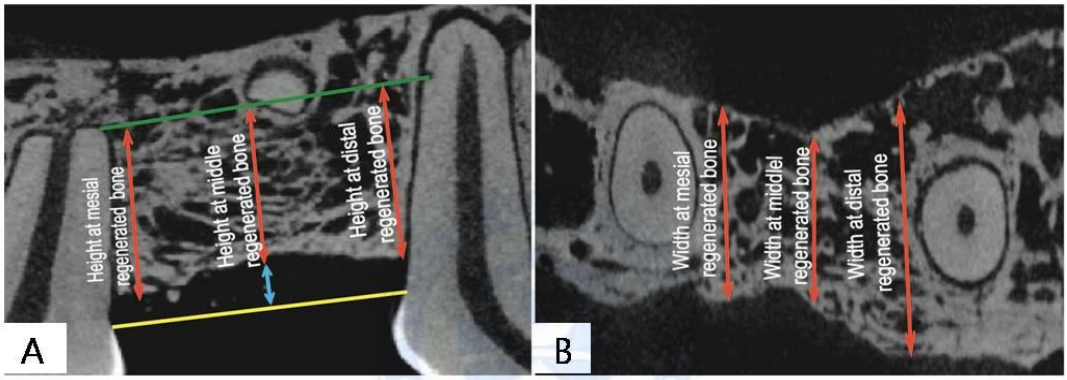

Fig. 4. Alveolar bone height and width of regenerate bone was measured at the mesial, middle and distal part of the regenerate. A. The height of the regenerated alveolar bone was measured from the osteotomy line (green line) to the alveolar crest of the mesial, middle, and distal parts of the regenerated bone area, respectively (orange arrows). Vertical bone defects after DO were measured in the middle of the regenerated alveolar ridge to the connecting line (yellow line) at the cementoenamel junction of the second premolar, and third premolar vertical lateral height (green line, osteotomy line). B. The width of the regenerated alveolar bone was measured at the mesial, middle, and distal parts of the regenerated bone, respectively (orange arrows), and in the middle.

C. Tissue Preparation

The specimens obtained after sacrifice were fixed in 4% paraformaldehyde for 24 h and decalcified with Rapid-Cal immune (Rapid Cal Immuno, BBC Biochemical Mount Vernon, WA) for 2 weeks. Each maxillary alveolar bone was divided axially into 2 segments then embedded in paraffin. Sections that were 9 microns thick were mounted on the SP 1600 microtome (SP 1600 microtome, Leica DFC 290, Leica, Nussloch, Germany) after staining with hematoxylin and eosin (H&E) (Figs. 7A through 7H). The histological examination was done using picrosirius red birefringence (PicroSirius, Sigma-Aldrich, St. Louis, MO) to visualize collagen type I under polarized light (Figs. 7I through 7L) (Plate et al., 2014).

D. Statistical Analysis

The height and width of the regenerated bone were compared between controls and the rhBMP-2 group. The bone parameters between the control and rhBMP-2 groups were also compared. Statistical analyses were performed by using SPSS software (SPSS v.16, IBM. Armonk, NY). Nonparametric Wilcoxon signed rank tests were used to analyze differences between the control and rhBMP-2 groups.

III. Results

A. Animal data

Among the 16 adult beagle dogs, inflammation was observed in one dog in each of the control and rhBMP-2 groups. Inflammation occurred during the distraction period prior to the injection of rhBMP-2, and was controlled after the distraction was complete in both dogs.

B. Micro-Computed Tomography Results

Differences in new bone height and width were observed after 2 and 6 week of consolidation between the control and rhBMP-2 groups (Figs. 5 and 6).

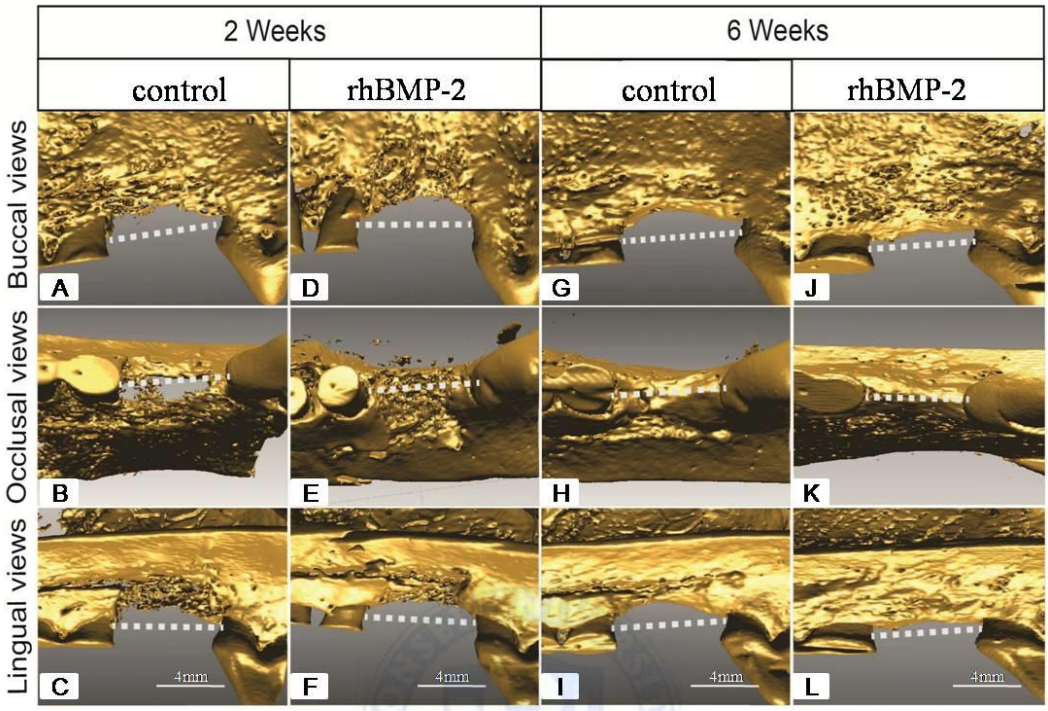

Fig. 5. Three-dimensional features of the alveolar bone regenerated from the buccal, occlusal and lingual perspectives for the control and rhBMP-2 groups after 2 and 6 weeks of the consolidation. Vertical bone defects in the regenerated bone were comparable between the control and rhBMP-2 groups (white dotted line). Scale bars: 4 mm

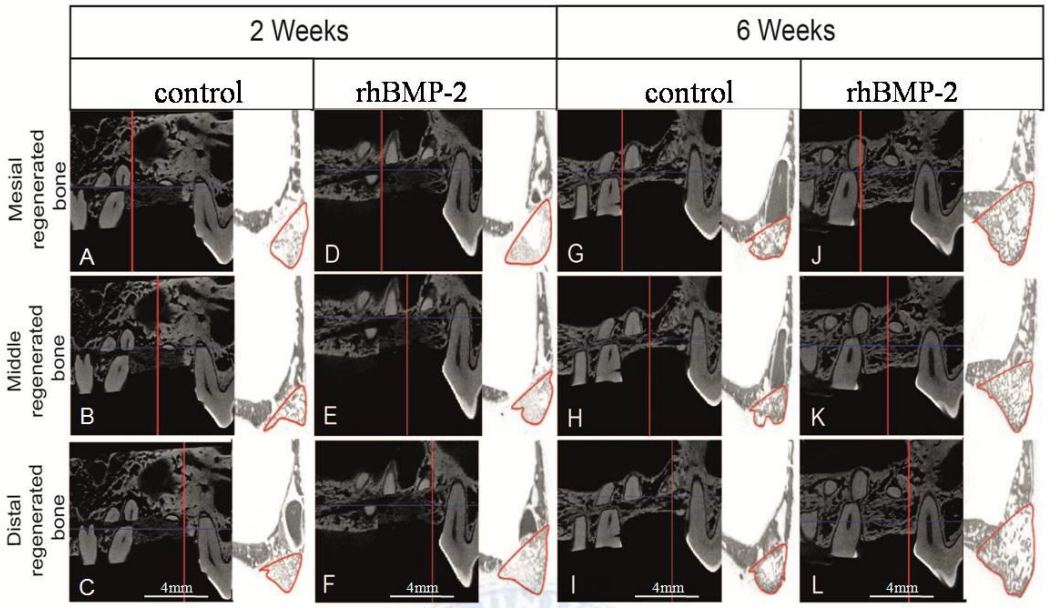

Fig.6. Representative sagittal and coronal images at the mesial, middle, and distal segments of the regenerated bone in the control and rhBMP-2 groups after 2 and 6 weeks of consolidation. The region of interest for the 3-dimensional bone parametric analysis was defined as a red box. Scale bars: 4 mm.

After 2 weeks of consolidation, the median vertical defect height was 4.0 mm and 2.2 mm for the control and rhBMP-2 groups, respectively. After 6 weeks of consolidation, the median vertical defect height was 3.4 mm and 2.2 mm for the control and rhBMP-2 groups, respectively, with significant differences over each period. The median alveolar width in the middle of the regenerated bone after 6 weeks of consolidation was 2.8 mm and 4.3 mm for the control and rhBMP-2 groups, respectively, with significant differences between the two groups. The alveolar height ratio of the regenerated bone after 6 weeks of consolidation was 55.7% and 82.2%, and the alveolar width ratio was 61.9% and 78.1% for the control and rhBMP-2 groups, respectively, with significant differences between the two groups (P <0.05) (Table 1).

Table 1. The height of vertical defect and width of regenerated bone in the control and rhBMP-2 groups after 2 and 6 weeks of consolidation.

	2 weeks consolidation							6 weeks consolidation						
	control(n=4)			rhBMP-2(n=4)				control(n=4)			rhBMP-2(n=4)			
	Med	Min	Max	Med	Min	Max	*P*	Med	Min	Max	Med	Min	Max	*P*
Vertical defect (mm)	4.0	3.8	4.0	2.2	1.8	2.8	*	3.4	3.2	5.0	2.2	1.5	2.9	*
Middle Height (mm)	1.1	1.0	2.1	2.7	1.1	2.8	*	1.8	1.5	2.1	3.9	3.6	4.4	*
Distal Height (mm)	2.8	2.6	3.9	3.1	1.4	4.0	*	3.6	3.6	4.6	5.5	5.2	5.9	NS
Mesial Height (mm)	1.8	1.2	3.1	2.9	2.0	2.9	NS	2.9	2.8	4.6	4.4	3.7	4.5	NS
Alveolar Height ratio (%)	59.8	44.3	60.4	79.2	65.4	94.4	*	55.7	32.8	65.9	82.2	73.8	88.0	*
Middle Width (mm)	2.7	2.4	2.7	2.9	1.4	3.2	*	2.8	1.5	3.4	4.3	3.1	5.4	*
Distal Width (mm)	3.0	2.9	3.8	3.2	1.3	3.5	NS	3.2	3.0	4.2	5.1	3.9	5.83	*
Mesial Width (mm)	3.5	3.2	3.6	3.8	2.1	3.9	NS	3.7	2.8	5.9	6.5	4.0	7.7	*
Alveolar Width ratio (%)	79.4	73.9	81.9	81.5	80.5	86.2	*	61.9	42.2	67.7	78.1	74.6	80.1	*

NS, not significant; Med, Median. *Significant difference between the control and rhBMP-2.

Table 2. Three-dimensional histomorphometric analyses of the middle and distal aspects of the regenerated alveolar bone in the control and rhBMP-2 groups after 2 and 6 weeks consolidation

	Regenerated Bone												
	Middle Aspect							Distal Aspect					
	control (n=4)			rhBMP-2 (n=4)				control (n=4)			rhBMP-2 (n=4)		
	Med	Min	Max	Med	Min	Max	*P*	Med	Min	Max	Med	Min	Max	*P*
After 2 weeks of consolidation														
Bone density (mg HA/cm^3)	283.2	275.3	295.2	336.8	305.2	396.3	NS	312.7	301.6	326.9	365.1	313.0	400.6	NS
Bone volume (BV/TV, %)	10.9	10.3	16.0	13.8	13.1	14.3	*	15.8	9.6	18.4	15.2	11.8	21.3	*
Trabecular number (1/mm)	1.1	0.9	1.7	1.2	1.0	1.4	NS	1.2	1.2	2.0	1.3	0.8	1.6	*
Trabecular thickness (mm)	0.08	0.07	0.10	0.11	0.07	0.16	*	0.11	0.11	0.12	0.14	0.10	0.16	NS
Trabecular separation (mm)	0.28	0.24	0.32	0.35	0.31	0.38	NS	0.31	0.23	0.35	0.36	0.17	0.50	NS
After 6 weeks of consolidation														
Bone density (mg HA/cm^3)	283.5	209.9	338.9	621.8	559.6	674.5	*	308.3	271.8	326.6	673.2	656.4	702.2	*
Bone volume (BV/TV, %)	28.1	24.4	31.7	42.9	36.3	48.0	*	30.8	27.5	33.6	51.1	49.4	54.3	*
Trabecular number (1/mm)	1.8	1.9	2.1	1.9	1.7	2.1	NS	2.2	2.1	2.6	2.6	2.5	2.8	NS
Trabecular thickness (mm)	0.13	0.12	0.15	0.23	0.29	0.25	*	0.18	0.17	0.19	0.29	0.28	0.31	*
Trabecular separation (mm)	0.3	0.29	0.34	0.3	0.22	0.36	NS	0.41	0.33	0.49	0.41	0.37	0.44	NS

NS, not significant; Med, Median. *Significant difference between the control and rhBMP-2 group (P <0.05).

The bone density, BV/TV, Tb.N, Tb.Sp, and Tb.Th in the middle of the regenerated bone were lower than those of the distal segments of the regenerated bone in both groups and after each consolidation period (Table 2). After 6 weeks, the bone density in the middle of the regenerated bone was 283.5 mg/cm^3 and 621.8 mg/cm^3, the BV/TV was 28.1% and 42.9 %, and the Tb.Th was 0.13 mm and 0.23 mm for the control and rhBMP-2 groups, respectively, showing significant differences between the two groups (P <0.001) (Table 2).

C. Histological Results

After 2 weeks of consolidation in the control group, the histologic section demonstrated new bone formation in the host bone, with margins of fibrous tissue in the center of the gap. Picrosirius red stained images showed dense fibrous tissue (green color) (Fig. 7I). For the rhBMP-2 group after 2 weeks of consolidation, bone trabeculae could be seen in the distraction area with spindle-shaped fibroblasts surrounding them. The distracted bone gap was almost completely filled by newly formed bone. However, some fibrous tissue was observed in the center of the distracted bone gap and between the newly formed bone islands. Some osteoblasts were observed in the new bone, which looked like a reticular structure (Fig. 7J). After 6 weeks of consolidation, osteoblasts and osteoclasts in the rhBMP-2 group were seen around the area of angiogenesis (Fig. 7L).

Using picrosirius red stained images and polarized light microscopy, a collagen matrix was evident in the new bone. These were highlighted to distinguish the lamellar and woven bone. Sections stained with picrosirius red indicated that significant woven bone formation had occurred after 2 weeks of consolidation in both the control and rhBMP-2 groups (Fig. 7I and 7J). The orientation of the collagen was mature, showing the increased organization of new bone.

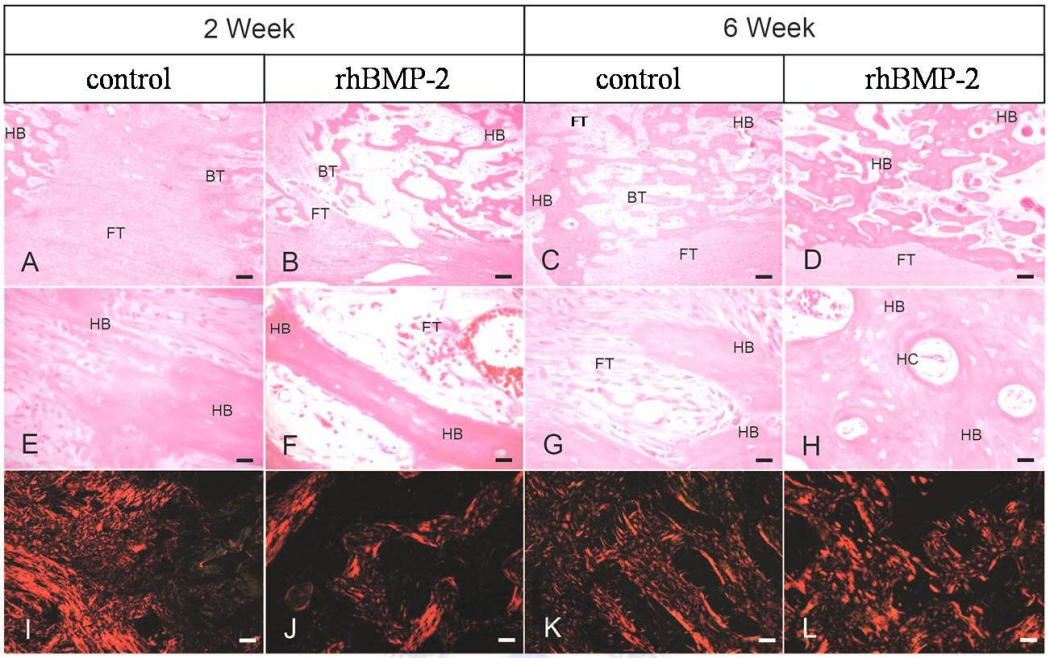

Fig. 7. Alveolar distraction site in the maxilla after 2 and 6 weeks of consolidation. Slides were stained with H&E and picrosirius red. A. Control group after 2 weeks of consolidation. Histologic sections show new bone (NB) formation located in the host bone (HB) and margins with fibrous tissue (FT) in the center of the gap. BT=bone trabeculae. B. rhBMP-2 group after 2 weeks of consolidation. The distracted bone gap is almost completely occupied by newly formed bone. C. Control group after 6 weeks of consolidation. D. rhBMP-2 group after 6 weeks of consolidation. E through H. High magnification of H&E stained images showing osteoblasts, osteocytes, and fibrous tissue (FT), Haversian canal (HC). I through L. High magnification of picrosirius red stained images under polarized light microscopy. A collagen fiber was evident in the new bone. Magnification bars=A through D, ×4 objective: 500 μm; E through L, ×40 objective: 50 μm.

Ⅵ. Discussion

In this study, the height and width of the regenerated alveolar bone differed depending on the location (middle, distal, or medial) of the regenerated alveolar bone. Both the control and rhBMP-2 groups showed narrower width (61.9%-78.1%) and shorter vertical height (55.7%-82.2%) in the middle part of the regenerated bone compared with the mesial and distal segments after 6 weeks of consolidation (Table 1). This characteristic of the regenerated bone resulted in an alveolar ridge that was of insufficient width for prosthetic orthodontic implantation, which would require a bone graft in the regenerated site.

However, a previous study reported that bone regeneration in the dentoalveolar distraction of the mandible showed no differences in healing pattern between the mesial, middle, and distal segments (Moore et al.,2011). Alveolar bone distractions in the maxilla seem to be difficult compared with those of the mandible; but the difference has not yet been reported.

The rhBMP-2 group showed significantly higher BV/TV in the regenerated bone compared with the control group after 6 weeks of consolidation. The BV/TV in the middle of the regenerated bone was 28.1% and 42.9 %, and the trabecular thickness was 0.13 mm and 0.23 mm in the control and rhBMP-2 groups, respectively, with significant differences between the two groups (Table 2). As these results suggest, rhBMP-2 increases the total amount of newly-formed bone (Ozdemir et al., 2014; Zheng et al., 2006). In a femoral fracture model in rats, a single, local, percutaneous injection of rhBMP-2 accelerated fracture healing (Einhorn et al., 2003). rhBMP-2 injection into distracted alveolar bone showed similar results in a previous study (Yasko et al., 1992). The bone mineral density of the two groups was significantly different at 283.5 mg/cm^3 and 621.9 mg/cm^3 for the control and rhBMP-2 groups, respectively, after 6 weeks of consolidation (Table 2). These results suggest that a more

mature pattern of bone density was present in the rhBMP-2 group after 6 weeks of consolidation.

Bone morphogenic proteins (BMPs) exhibit osteoinductive ability that can enhance bone formation or consolidationduring the consolidation period. rhBMP-2 is reported to affect the rate of callus formation and mineralization, exhibiting the strongest osteoinductive ability among these proteins (Campisi et al., 2003; Li et al., 2002). The micro-computed tomography data showed that the quality of the regenerated bone in the rhBMP-2 group was much better, both quantitatively and qualitatively, compared with the control group.

rhBMP-2 has been administered to osteogenesis sites with a polymer-coated gelatin sponge, collagen sponge, chitosan hydrogel, or by injection at the distraction site of orthogenesis in long bones (Sailhan et al., 2010). Collagen and gelatin promote tissue regeneration, so the effect of pure rhBMP-2 could not be assessed in previous reports (Rihn et al., 2009). In the current study, rhBMP-2 was injected directly into the distracted side after distraction was finished. The direct injection method after the distraction procedure as the advantage of simplicity, easy dose calculation, and increased predictability. It was expected that the rhBMP-2 material would diffuse into the bony callus, which is composed of mineralized extracellular matrix generated during the DO procedure, and that it would act as a reservoir for the injection material. However, a limitation of this study was that the diffusion and clearance of rhBMP-2 into the target tissue area after injection was not investigated. When a collagen carrier system was used, retention of rhBMP-2 in a rat model was reported as less than 5% 14 days after implantation (Kempen et al., 2008; Uludag et al., 1999). Therefore, when it is released without controlled diffusion, rhBMP-2 clearance might be more rapid than the bone-induction response of the host. Furthermore, the optimal release profile may vary in different animal species (Zhang et al.,2009). Nevertheless, the rhBMP-2 group in our present model showed significantly increased bone volume,

including increased bone density, after 6 weeks of consolidation, which supports the notion that retention of rhBMP-2 in distracted alveolar bone tissue is prolonged and within an expected residence time.

Local rhBMP-2 injection was done directly after the distraction phase. In previous studies applying rhBMP-2 delivery, the BMP-2 concentration was not maintained in the area of the bone defect for a period of time sufficient to recruit osteoprogenitor cells to the target site and allow them to differentiate into osteoblasts because of its short biological half-life (Uludag et al., 1999). For this reason, use of a carrier system or synthetic polymer coating for sustained delivery of BMP-2 was introduced to achieve prolonged osteogenic induction in the target area (Kempen et al., 2008). In our present model, the area where the rhBMP-2 was injected into the distracted alveolar bone tissue was composed of chondrocyte like cells and fibroblasts as well as differentiating osteoblasts that deposit osteoid along collagen bundles (Ai-Aql et al., 2008). Therefore, we speculated that the local injection of rhBMP-2 could immediately affect the differentiation of osteogenic cells within tissue that is abundant in collagen.

In the maxillary bone of the beagle dog, the sinus is elongated to the inter root space. Therefore, we had to cut the root tip during the surgery because the horizontal cutting line under the sinus level was ovoid-shaped. In humans, the sinus is in a different location than that of the animal model, so horizontal osteotomy could be performed 3 to 5 mm away from the dental root apex with a cutting saw for the DO procedure (Liou et al.,2000). Root damage was intended in our study model, but it is easy to control inflammation in an experimental animal without perforating the sinus. For this reason, no complications were observed in our model during or after the DO procedure.

In this study, an osteoinductive effect was achieved at a lower dosage of rhBMP-2 (Sciadini et al., 2000). Previous studies showed a wide range of doses of BMP, ranging from 20-3000 μg/kg depending on the size of the defect, animal

species, and location of the distraction (Carreira et al., 2014; Zheng et al., 2006). Some studies have shown that the amount of bone induced by BMPs depends on the dose of BMP and the length of the consolidation period. However, excessive doses of BMP can cause swelling, inflammation, and a higher cancer risk, emphasizing the need for refined guidelines when using BMP clinically (Carreira et al., 2014; Zheng et al., 2006). In this study, an osteoinductive effect was achieved at a lower dose of rhBMP-2. We speculate that inflammation was not observed because we did not use carriers to deliver the rhBMP-2 and because we reduced the amount of rhBMP-2 injected compared with previous studies.

In the present study, the amount of rhBMP-2 injected (330 μg) represents about 1/15 of the total volume of the regenerated bone, with a small dosage of rhBMP-2 relative to previous studies. This dose activated significant bone formation at the regeneration site. Future experiments should include a comparative study of bone formation after direct injection of rhBMP-2 to determine if the regenerative activity is dependent on the dose of rhBMP-2.

The bone parameter analysis of the new bone trabeculae agreed with the histologic examination. The bone remodeling process, characterized by osteoclasts and osteoblasts on the newly formed bone surfaces, was evident through the consolidation period. After 2 and 6 weeks of consolidation, there was active bone formation within the distracted gap in the rhBMP-2 group. New bone volume was significantly higher in the rhBMP-2 group after 6 weeks of consolidation compared with the control group, and the vertical defect of new bone in the middle of the regenerated bone was significantly lower in the rhBMP-2 group after 6 weeks of consolidation compared with the control group. Histologic slides stained with picrosirius red indicated that significant mature bone formation had occurred after 6 weeks of consolidation in the BMP-2 group. This finding reflects vigorous bony regeneration in the alveolar bone DO after treatment with rhBMP-2.

The results of the present study after alveolar bone DO coincide with the results found in a prospective study by Chiapasco et al. and Jensen et al (Chiapasco et al., 2004; Jensen et al., 2002). These authors reported on the quality and quantity of regenerated bone after the consolidation period directly related to bony relapse, suggesting that an increase in the consolidation period could reduce the rate of relapse. The accelerated bone formation that occurs after treatment with rhBMP-2, as found in our study, could improve the stability of the distracted alveolar bone and decrease the rate of relapse, without increasing the length of the consolidation period.

It was an interesting finding that the histological characteristic at 8 weeks of consolidation period in previous study related with mandibular DO using experimental dogs, was similar to histological finding at the 6 weeks of consolidation period in the present study (Cope JB et al., 2000). Meanwhile, after 2 weeks of consolidation, rhBMP-2 group showed classic 3 zones which were composed of fibrous tissue (FT) bounded by bony trabeculae (BT) originating from the host bone (HB) margins. The distracted bone gap was almost completely occupied by newly formed bone at 2 weeks of consolidation period for rhBMP-2 group. Surprisingly after 6 weeks of consolidation, histological section of regeneration was observed with almost complete absence of interzone, and showing harvasian canal in newly formed bone, which might indicate that the consolidation period can be reduced with rhBMP-2 injection compared with conventional methods.

This study supports that rhBMP-2 is effective in enhancing the consolidation of regenerated alveolar bone. However, we did not directly compare the relapse rates between the control and rhBMP-2 groups. In addition, our experimental model was limited to the maxillary bone. A previous study using mandibular bone showed smaller vertical defects compared with our results. This difference may result from different bone formation activity

depending on the location of the distraction site (Cope et al.,2002). Therefore, in the future the rhBMP-2 injection method will need to be applied to mandibular bone to evaluate whether rhBMP-2 can enhance bone formation in different bone areas.

Ⅴ. Conclusion

rhBMP-2 injection after a DO procedure significantly increases bone volume in regenerated dentoalveolar structures after 6 weeks of consolidation and improved both the width and height of the alveolar ridge as well as increasing the bone density. Therefore, rhBMP-2 injection accelerates bone formation, and results in adequate bone morphology and volume.

References

Ai-Aql ZS, Alagl AS, Graves DT, Gerstenfeld LC, Einhorn TA (2008). Molecular mechanisms controlling bone formation during fracture healing and distraction osteogenesis. *J Dent Res*, 87(2): 107-118.

Bianchi A, Felice P, Lizio G, Marchetti C (2008). Alveolar distraction osteogenesis versus inlay bone grafting in posterior mandibular atrophy: a prospective study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 105(3): 282-292.

Campisi P, Hamdy RC, Lauzier D, Amako M, Rauch F, Lessard ML (2003). Expression of bone morphogenetic proteins during mandibular distraction osteogenesis. *Plast Reconstr Surg*, 111(1): 201-21.

Carreira AC, Lojudice FH, Halcsik E, Navarro RD, Sogayar MC, Granjeiro J M (2014). Bone morphogenetic proteins: facts, challenges, and future perspectives. *J Dent Res*, 93(4): 335-345.

Cha JY, Lim JK, Song JW, Sato D, Kenmotsu M, Inoue T, Park YC (2009). Influence of the length of the loading period after placement of orthodontic mini-implants on changes in bone histomorphology: microcomputed tomographic and histologic analysis. *Int J Oral Maxillofac Implants*, 24(5): 842-849.

Cheung LK, Zheng LW (2006). Effect of recombinant human bone morphogenetic protein-2 on mandibular distraction at different rates in an experimental model. *J Craniofac Surg*, 17(1): 100-110.

Chiapasco M, Romeo E, Casentini P, Rimondini L (2004). Alveolar distraction osteogenesis vs. vertical guided bone regeneration for the correction of vertically deficient edentulous ridges: a 1-3-year prospective study on humans. *Clin Oral Implants Res*, 15(1): 82-95.

Chin M, Toth BA (1996). Distraction osteogenesis in maxillofacial surgery using internal devices: review of five cases. *J Oral Maxillofac Surg*, 54(1): 45-54.

Choi HY, Hwang CJ, Kim HJ, Yu HS, Cha JY (2012). Maxillary anterior segmental distraction osteogenesis with 2 different types of distractors. *J Craniofac Surg*, 23(3): 706-711.

Choi YK, Park SB, Kim YI, Son WS (2013). Three-dimensional evaluation of midfacial asymmetry in patients with nonsyndromic unilateral cleft lip and palate by cone-beam computed tomography. *Korean J Orthod*, 43(3): 113-119.

Cochran DL, Jones AA, Lilly LC, Fiorellini JP, Howell H (2000). Evaluation of recombinant human bone morphogenetic protein-2 in oral applications including the use of endosseous implants: 3-year results of a pilot study in humans. *J Periodontol*, 71(8): 1241-1257.

Codivilla A (1905). On the means of lengthening, in the lower limbs, the muscles and tissues which are shortened through deformity. J Bone Joint Surg Am, 1905;s2(2):353-69.

Cope JB, Samchukov ML (2000). Regenerate bone formation and remodeling during mandibular osteodistraction . *Angel Orthod, 70*: 99-111.

Cope JB, Samchukov ML, Muirhead DE (2002). Distraction osteogenesis and histogenesis in beagle dogs: the effect of gradual mandibular osteodistraction on bone and gingiva. *J Periodontol*, 73(3): 271-282.

Cortese A, Savastano M, Savastano G, Claudio PP (2011). One-step transversal palatal distraction and maxillary repositioning: technical considerations, advantages, and long-term stability. *J Craniofac Surg*, 22(5): 1714-1719.

Einhorn TA, Majeska RJ, Mohaideen A, Kagel EM, Bouxsein ML, Turek TJ, Wozney JM (2003). A single percutaneous injection of recombinant

human bone morphogenetic protein-2 accelerates fracture repair. *J Bone Joint Surg Am*, 85-A(8): 1425-1435.

Francis CS, Mobin SS, Lypka MA, Rommer E, Yen S, Urata MM, Hammoudeh JA (2013). rhBMP-2 with a demineralized bone matrix scaffold versus autologous iliac crest bone graft for alveolar cleft reconstruction. *Plast Reconstr Surg*, 131(5): 1107-1115.

Hatzokos I, Stavridis SI, Iosifidou E, Karataglis D, Christodoulou A (2011). Autologous bone marrow grafting combined with demineralized bone matrix improves consolidation of docking site after distraction osteogenesis. *J Bone Joint Surg Am*, 93(7): 671-678.

Herford AS, Tandon R, Stevens TW, Stoffella E, Cicciu M(2013). Immediate distraction osteogenesis: the sandwich technique in combination with rhBMP-2 for anterior maxillary and mandibular defects. *J Craniofac Surg*, 24(4): 1383-1387.

Hu J, Qi MC, ZouSJ, Li JH, Luo E (2007). Callus formation enhanced by BMP-7 ex vivo gene therapy during distraction osteogenesis in rats. *J Orthop Res*, 25(2): 241-251.

Ilizarov GA (1989a). The tension-stress effect on the genesis and growth of tissues. Part I. The influence of stability of fixation and soft-tissue preservation. *Clin Orthop Relat Res*(238): 249-281.

Ilizarov GA(1989b). The tension-stress effect on the genesis and growth of tissues: Part II. The influence of the rate and frequency of distraction. *Clin Orthop Relat Res*(239): 263-285.

Jensen OT, Cockrell R, Kuhike L, Reed C (2002). Anterior maxillary alveolar distraction osteogenesis: a prospective 5-year clinical study. *Int J Oral Maxillofac Implants*, 17(1): 52-68.

Kempen DH, Lu L, Hefferan TE, Creemers LB, Maran A, Classic KL, Dhert WJ, Yaszemski MJ (2008). Retention of in vitro and in vivo BMP-2

bioactivities in sustained delivery vehicles for bone tissue engineering. *Biomaterials*, 29(22): 3245-3252.

Konas E, Emin Mavili M, Korkusuz P, Demir D, Oner F, Canter HI (2009). Acceleration of distraction osteogenesis with drug-releasing distractor. *J Craniofac Surg*, 20(6): 2041-2048.

Li G, Bouxsein ML, Luppen C, Li XJ, Wood M, Seeherman HJ, Wozney JM, Simpson H (2002). Bone consolidation is enhanced by rhBMP-2 in a rabbit model of distraction osteogenesis. *J Orthop Res*, 20(4): 779-788.

Liou EJ, Chen PK, Huang CS, Chen YR (2000). Interdental distraction osteogenesis and rapid orthodontic tooth movement: a novel approach to approximate a wide alveolar cleft or bony defect. *Plast Reconstr Surg*, 105(4): 1262-1272.

Mehrara BJ, Rowe NM, Steinbrech DS, Dudziak ME, Saadeh PB, McCarthy JG, Gittes GK, Longaker MT (1999). Rat mandibular distraction osteogenesis: II. Molecular analysis of transforming growth factor beta-1 and osteocalcin gene expression. *Plast Reconstr Surg*, 103(2): 536-547.

Meling TR, Hogevold HE, Due Tonnessen BJ, Skjelbred P (2011). Midface distraction osteogenesis: internal vs. external devices. *Int J Oral Maxillofac Surg*, 40(2): 139-145.

Mitsukawa N, Satoh K, Morishita T(2010). Le Fort I distraction using internal devices for maxillary hypoplasia in patients with cleft lip, palate, and alveolus: complications and their prevention and management. *J Craniofac Surg*, 21(5): 1428-1430.

Moore C, Campbell PM, Dechow PC, Ellis ML, Buschang PH(2011). Effects of latency on the quality and quantity of bone produced by dentoalveolar distraction osteogenesis. *Am J Orthod Dentofacial Orthop*, 140(4): 470-478.

Moore DC, Ehrlich MG, McAllister SC, Machan JT, Hart CE, Voigt C, Lesieur Brooks AM, Weber EW (2009). Recombinant human platelet-derived growth factor-BB augmentation of new-bone formation in a rat model of distraction osteogenesis. *J Bone Joint Surg Am*, 91(8): 1973-1984.

Ozdemir F, Tozlu M, Germec Cakan D (2014). Quantitative evaluation of alveolar cortical bone density in adults with different vertical facial types using cone-beam computed tomography. *Korean J Orthod*, 44(1): 36-43.

Paley D, Herzenberg JE, Paremain G, Bhave A (1997). Femoral lengthening over an intramedullary nail. A matched-case comparison with Ilizarov femoral lengthening. *J Bone Joint Surg Am*, 79(10): 1464-1480.

Perry M, Hodges N, Hallmon DW, Rees T, Opperman LA (2005). Distraction osteogenesis versus autogenous onlay grafting. Part I: outcome of implant integration. *Int J Oral Maxillofac Implants*, 20(5): 695-702.

Picard A, Diner PA, Galliani E, Tomat C, Vazquez M, Carls FP (2011). Five years experience with a new intraoral maxillary distraction device (RID). *Br J Oral Maxillofac Surg*, 49(7): 546-551.

Plate JF, Brown PJ, Walters J, Clark JA, Smith TL, Freehill MT, Tuohy CJ, Stitzel JD, Mannava S (2014). Advanced age diminishes tendontobone healing in a rat model of rotator cuff repair. *Am J Sports Med*, 42(4): 859-868.

Rihn JA, Makda J, Hong J, Patel R, Hilibrand AS, Anderson DG, Vaccaro AR, Albert TJ (2009). The use of rhBMP-2 in singlelevel transforaminal lumbar interbody fusion: a clinical and radiographic analysis. *Eur Spine J*, 18(11): 1629-1636.

Sailhan F, Gleyzolle B, Parot R, Guerini H, Viguier E (2010). Rh-BMP-2 in distraction osteogenesis: dose effect and premature consolidation. *Injury*, 41(7): 680-686.

Saulacic N, Schaller B, Bosshardt DD, Buser D, Jaun P, Haeniwa H, Iizuka T (2012). Periosteal distraction osteogenesis and barrier membrane application: an experimental study in the rat calvaria. *J Periodontol*, 83(6): 757-765.

Sciadini MF, Johnson KD(2000). Evaluation of recombinant human bone morphogenetic protein-2 as a bone-graft substitute in a canine segmental defect model. *J Orthop Res*, 18(2): 289-302.

Song HR, Kim HG, Yun YP, Bae JH, Kim HJ, Hae RS, Sung EK, HyoGK, YoungPY, JiHB, Hak JK (2011). *Tissue Engineering and Regenerative Medicine,* 8:158-163.

Song HR, Oh CW, Kyung HS, Park IH, Kim PT, Baek SH, Kim SJ, Lee ST (2004). Injected calcium sulfate for consolidation of distraction osteogenesis in rabbit tibia. *J Pediatr Orthop B*, 13(3): 170-175.

Terbish M, Choi HY, Park YC, Yi CK, Cha JY (2014). Premaxillary Distraction Osteogenesis Using an Intraoral Appliance for Unilateral Cleft Lip and Palate: Case Report. *Cleft Palate Craniofac J.*

Tong AC, Yan BS, Chan TC (2003). Use of interdental distraction osteogenesis for orthodontic tooth alignment and correction of maxillary hypoplasia: a case report. *Br J Oral Maxillofac Surg*, 41(3): 185-187.

Uckan S, Veziroglu F, Dayangac E (2008). Alveolar distraction osteogenesis versus autogenous onlay bone grafting for alveolar ridge augmentation: Technique, complications, and implant survival rates. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 106(4): 511-515.

Uludag H, D'Augusta D, Palmer R, Timony G, Wozney J (1999). Characterization of rhBMP-2 pharmacokinetics implanted with biomaterial carriers in the rat ectopic model. *J Biomed Mater Res*, 46(2): 193-202.

Verseijden F, Posthumusvan Sluijs SJ, Pavljasevic P, Hofer SO, van Osch GJ, Farrell E(2010). Adult human bone marrow- and adipose tissue-derived

stromal cells support the formation of prevascular-like structures from endothelial cells in vitro. *Tissue Eng Part A*, 16(1): 101-114.

Wolvius EB, Scholtemeijer M, Weijland M, Hop WC, Van der Wal KG(2007). Complications and relapse in alveolar distraction osteogenesis in partially dentulous patients. *Int J Oral Maxillofac Surg*, 36(8): 700-705.

Yasko AW, Lane JM, Fellinger EJ, Rosen V, Wozney JM, Wang EA (1992). The healing of segmental bone defects, induced by recombinant human bone morphogenetic protein (rhBMP-2). A radiographic, histological, and biomechanical study in rats. *J Bone Joint Surg Am*, 74(5): 659-670.

Yen SL (1997). Distraction osteogenesis: application to dentofacial orthopedics. *Semin Orthod*, 3(4): 275-283.

Zhang S, Doschak MR, Uludag H (2009). Pharmacokinetics and bone formation by BMP-2 entrapped in polyethylenimine-coated albumin nanoparticles. *Biomaterials*, 30(28): 5143-5155.

Zheng LW, Cheung LK (2006). Effect of recombinant human bone morphogenetic protein-2 on mandibular distraction at different rates in a rabbit model. *Tissue Eng*, 12(11): 3181-3188.

Zheng LW, Wong MC, Rabie AB, Cheung LK (2006). Evaluation of recombinant human bone morphogenetic protein-2 in mandibular distraction osteogenesis in rabbits: Effect of dosage and number of doses on formation of bone. *Br J Oral Maxillofac Surg*, 44(6): 487-494.

국 문 요 약

치조골 골신장 부위의 재조합 인간 골형성 단백질-2 주입 후 골형성 촉진

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본 연구는 신장된 치조골에 rhBMP-2 를 주입했을 때, rhBMP-2 가 재생된 뼈의 골질과 골량에 미치는 효과를 평가하기 위한 것이다.

열여섯 마리의 성체 beagle dog 을 대조군과 rhBMP-2 그룹으로 나누었다. 치조골 신장술 후, 실험군에서 신장된 치조골의 근심, 중앙, 원심 측 치조정에 330 μg 의 rhBMP-2 가 포함된 0.33 ml 의 용액을 천천히 주입하였다. 2 주, 6 주의 골 경화기 후에 재생된 골에서 조직학적 분석과 마이크로 CT 분석을 시행 하였다.

6 주의 골 경화기 후, 재생된 골의 수직적 결손이 대조군 (3.4 mm) 에 비해 rhBMP-2 군 (2.2 mm) 에서 유의하게 낮았다 (P<0.05). 또한, 재생골의 폭은 대조군 (2.8 mm) 보다 rhBMP-2 군 (4.3 mm) 에서 유의하게 높았다 (P<0.05). 6 주의 골경화기 후에 rhBMP-2 군은 대조군에 비해 재생된 골의 골밀도가 더 높았고, 골량은 더 컸다 (P <0.001).

골 신장술 후 재생된 골에 rhBMP-2 를 주입함으로 골경화기 6 주째 신장된 부분의 골량이 유의하게 증가하였으며, 치조제의 폭경과 높이가 향상되었고, 골질도 증가되었다.

핵심되는 말: rhBMP- 2, 골 재생, 뼈 조직, 골 신장술, 치조골 신장술, 골 형성.