The Bone Regenerative Effects of Chinese Hamster Ovary Cell-Expressed Recombinant Human Bone Morphogenetic Protein-2 using Demineralized Bone Matrix Carrier in Rabbit Cranial Defect

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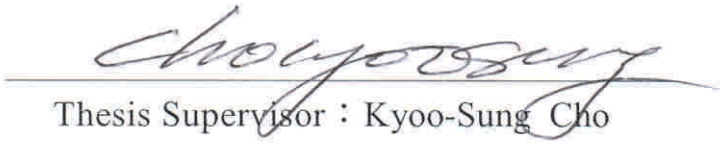
Directed by Professor : Kyoo-Sung Cho

A Dissertation
Submitted to the Department of Dental Science
and the Graduate School of Yonsei University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy of Dental Science

Min-Jung Kim

June 2012

This certifies that the dissertation
of Min-Jung Kim is approved.



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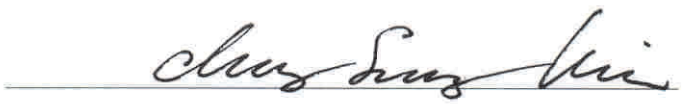

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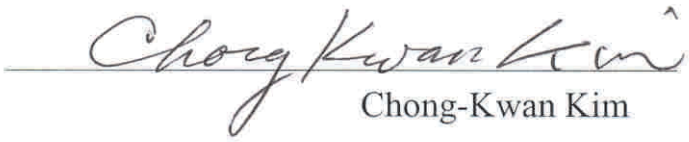

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감사의 글

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

연구 내내 많은 도움을 주신 치주과 교실원 여러분, 특히 김재신, 김유진 선생님께 고마움을 전합니다.

그리고, 늘 아낌없는 사랑과 헌신적인 도움으로 든든하고 따뜻한 버팀목이 되어준 사랑하는 나의 아내와 항상 한없는 따뜻함이 되어준 사랑하는 딸 연지에게 진정으로 사랑과 고마움의 마음을 전합니다.

마지막으로, 믿음과 사랑으로 이해해 주시고 항상 곁에서 든든하게 후원해 주신 부모님과 장인어른, 장모님께 감사의 마음을 담아 이 논문을 드립니다.

모든 분들께 진심으로 감사 드립니다

2012년 6월

저자 씀

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Abstract

The Bone Regenerative Effects of Chinese Hamster Ovary Cell-Expressed Recombinant Human Bone Morphogenetic Protein-2 using Demineralized Bone Matrix carrier in Rabbit Cranial Defect

Recombinant human bone morphogenetic protein-2 (rhBMP-2) has long been believed to have the potential to accelerate the healing process and augment tissue formation in challenging regenerative procedures. The objective of this study was to evaluate the bone regenerative effects of DBM coated by newly-developed Chinese hamster ovary (CHO) cell-expressed rhBMP-2 in rabbit cranial defect.

Two symmetric, 11-mm ø, critical-size osteotomy defects were created in 7 New Zealand white rabbit craniums received DBM and rhBMP-2 coated DBM, respectively. Implant sites were evaluated by microcomputed tomography and histologic and histometric parameters following an 8-week healing interval.

Surgical implantation of rhBMP-2 coated DBM resulted in enhanced local bone formation at 8 weeks. In defect closure, the rhBMP-2 coated DBM group was statistically significant greater than the DBM group ($P<0.05$). Total augmented area was not significantly different between the DBM group and the rhBMP-2 coated DBM group. However, New bone area of the rhBMP-2 coated DBM group was a

significantly greater than those of the DBM group ($P<0.05$).

In conclusion, DBM coated by newly-developed CHO-rhBMP-2 produced significant new bone and tissue formation in rabbit cranial defect. It is suggested that DBM may be an available carrier for rhBMP-2.

Key Words: bone regeneration, recombinant human bone morphogenetic protein-2, Chinese hamster ovary cell, demineralized bone matrix, rabbit cranial defect

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I. Introduction

Clinicians frequently encounter the challenge of the treating patients with significant bone loss resulting from periodontal disease, congenital abnormalities, traumatic injury, tumors, and resorption secondary to tooth loss. Although a variety of biologic and synthetic materials are currently used by clinicians to treat bone defects and enhance bone regeneration, conventional procedures often result in inadequate bone regeneration, leaving both the clinician and the patient dissatisfied with outcome. Growth factors have long been believed to have the potential to accelerate the healing

process and augment tissue formation in challenging regenerative procedures (Cochran et al., 1999).

One of the most extensively studied growth factors is bone morphogenetic proteins (BMPs). BMPs are multifunctional polypeptides that belong to the transforming growth factor-β superfamily of proteins (Wozney et al., 1988). The most remarkable feature of BMPs is the ability to induce ectopic bone formation (Urist, 1965). Bone morphogenetic protein-2, isolated and named by Celeste et al and Wozney et al (Celeste et al., 1990; Wozney et al., 1988), stimulates bone growth via a mechanism recapitulating that of in utero intramembranous and endochondral ossification (Sampath, 1992).

Demineralized bone matrix (DBM) has been shown to allow for complete bony healing of critical sized defects. Lindholm and associates demonstrated this in rabbits where they placed 30 mgs of rabbit DBM into sized cranial vault defects and found 50 to 75% new bone fill at 12 weeks (Lindholm et al., 1993). In 1986, Oklund et al. reported that calvarial defects in dogs grafted with fresh autogenous bone demonstrated 99% bone fill whereas those filled with DBM resulted in 77% bone fill (Oklund et al., 1986). A number of other studies have supported these findings as well and suggest therefore that DBM may be a reasonable alternative to autogenous bone.

Critical-size osseous defects are defects that must not spontaneously regenerate following reconstructive surgery without adjunctive measures (Schmitz et al., 1986). The rabbit critical-size defect model has been used to study the efficacy of a variety

of bone substitute materials and adjunctive techniques to promote defect healing (Moghadam et al, 2004). In previous studies, we found that it is possible to prepare two symmetric 11-mm defects in most rabbit craniums without involving sagittal suturing, which could be an alternative to creating one 15-mm defect, especially for evaluating the mid-phase (8weeks) or late-healing period (12 weeks). If several materials need to be compared only for early-phase healing (2-4 weeks), four 8-mm defects could be created in a single rabbit cranium. (Sohn et al., 2010).

The purpose of this study was to evaluate the bone regenerative effects of DBM coated by newly-developed Chinese hamster ovary (CHO) cell-expressed rhBMP-2 in rabbit cranial defect.

II. Materials & methods

A. Materials

1. Animals

A total of 7 male New Zealand white rabbits (weight 2.8-3.2 kg) were used. The animals were maintained in standard laboratory, fed a standard diet, in separate cages. Animal selection and management, surgical protocol, and preparation followed routines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea.

2. Graft materials

In this study, DBM[£] and rhBMP-2[¢] coated DBM[£] were used. DBM which is the control group was produced by the following methods. After cleaning the cortical bones with distilled water and grinding them into 0.5-1.0mm particle size, the lipid and fat were removed in 70% ethanol and 3% H_2O_2 for two hours. The bone specimens were lyophilized at -70℃ vacuum condition after being decalcified in 0.6N hydrochloride for 72 hours and stored at room temperature. And rhBMP-2 coated DBM which is the test group was produced by the following methods. 1cc

[£]DBM Powder, Korea Bone Bank Co. Ltd., Seoul, Korea

[¢]RhBMP-2, Korea Bone Bank Co. Ltd., Seoul, Korea

CHO cell-expressed rhBMP-2[¢] of 0.05mg/cc was dispersed on 1cc DBM and it was stored at -70℃ for overnight. And then it was freeze dried at -70℃ vacuum condition. The freeze dried powder was stored at 4℃ until animal implantation.

It might be argued that it would be desirable to include a sham-surgery control group (no material applied) in the study. This study was part of a larger study including a robust control group (Sohn et al., 2010). Adding an internal control represents unnecessary duplication in violation of the seminal principles Refinement–Reduction–Replacement, key strategies to provide a systematic framework to achieve the goal of humane experimental techniques (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011).

3. Surgical procedures

The animals were anesthetized with and intramuscular injection, using a mixture of Ketamin hydrochloride[§] and Xylazine[‖]. Surgical sites were shaved and draped with alcohol and povidone iodine. Surgical sites were locally anesthetized with 2% lidocaine[¶]. An incision was made along the sagittal midline from the frontal bone to the occipital bone. A full thickness flap was elevated, exposing the calvarial bone. Two standardized, circular defects were made using a trephine bur (outer diameter: 11 mm) under saline irrigation. After removal of the trephined calvarial disk, the experimental and control treatments were applied to the calvarial defects (Figure 1).

The flap was sutured layer by layer with a resorbable suture material[#]. Animals were sacrificed eight weeks after surgery.

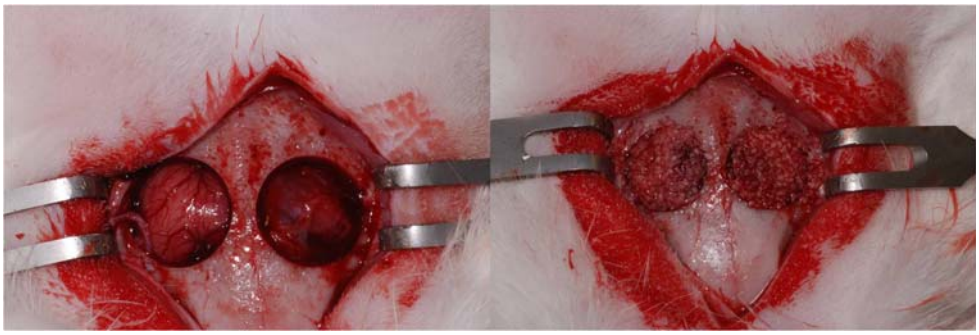

Figure 1. A series of photographs illustrating the surgical procedure used in the cranial defect.

[§] Ketalar®, Yuhan Co., Seoul, Korea

[||] Rumpun®, Bayer Korea, Seoul, Korea

[¶] 2% lidocaine, 1:100,000 epinephrine, Kwangmyung Pharm., Seoul, Korea

[#] Vicryl®, Ethicon, Johnson & Johnson Int., Edinburgh, UK

4. Microcomputed tomography

The specimens were fixed in 10% buffered formalin for 10 days and scanned using microcomputed tomography[£] (micro-CT) at a resolution of 35μm (100 kV and 100μA). The area of interest was reconstructed using Ondemand 3D software[¥].

5. Histologic processing and histometric measurements

Block sections were fixed in 10% neutral buffered formalin, decalcified in 5% formic acid, and embedded in paraffin. Serial sections of 5 μm thickness were cut along the center of the calvarial defects. The two most central sections were chosen and stained with Hematoxylin and Eosin (HE) and Masson's trichrome stain (TRC). Masson's trichrome staining was used for histomorphometric measurement. Each section was examined by a binocular microscope[††] equipped with a camera[**].

After the conventional microscopic examination, an automated image analysis system[‡‡] was used. The following parameters were measured: defect closure, augmented area, new bone area. Defect closure was determined by measuring the distance between the defect margin and the new bone margin and was expressed as a percentage of the total defect width. Augmented area (mm^2) was considered to be all tissue within the boundaries of newly formed bone. New bone area (mm^2) was measured as newly formed mineralized bone, excluding residual DBM, marrow and fibrovascular tissue (Figure 2).

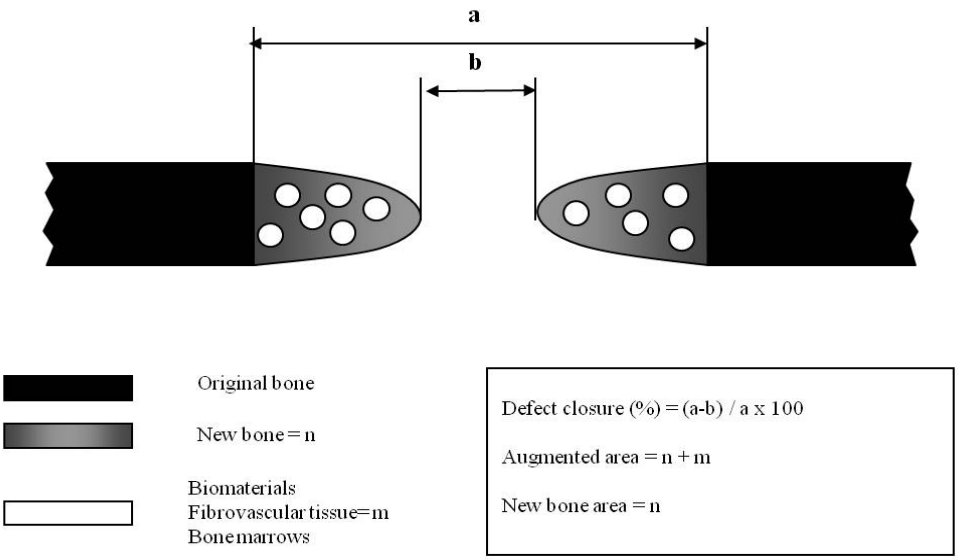

Figure 2. Schematic drawings of calvarial osteotomy defect showing histometric analysis.

6. Statistical analysis

Mann-Whitney U test method was used to evaluate the statistical significance between the two groups.

[£] Skyscan 1072, Skyscan, Aartselaar, Belgium

[¥] Cybermed, Seoul, Korea

[††] Leica DM LB, Leica Microsystems Ltd., Wetzlar, Germany

[**] Leica DC300F, Leica Microsystems Ltd., Heerburgg, Switzerland

[‡‡] Image-Pro Plus®, Media Cybernetics, Silver Spring, MD, U.S.

III. Results

A. Clinical observations

Wound healing was generally uneventful and inflammatory reaction was not appeared in all groups.

B. Micro-CT observations

The defect coverage was minimal in the control group. In the test group, the radiopacity is conspicuous than the control group (Figure 3).

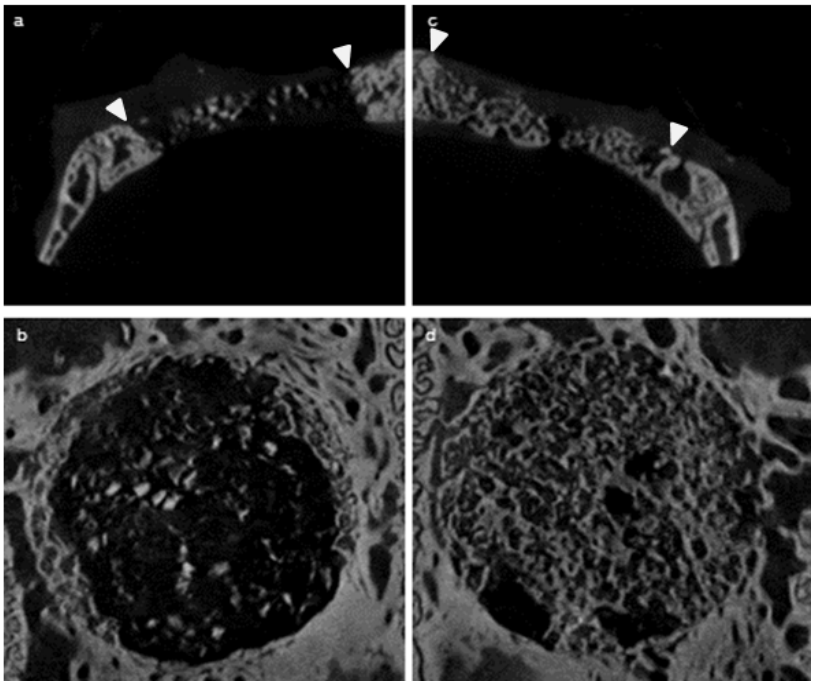

Figure 3. Representative micro-CT images of the control and test groups (arrow head = defect margin, **a**, **b**, control group; **c**, **d**, test group).

C. Histologic observations

Control group: In the control group, defects filled with loose fibrous connective tissue with a minimal amount of new bone formation originating from the defect margins were observed the defects. The DBM particles remained within the connective tissue (Figure 4).

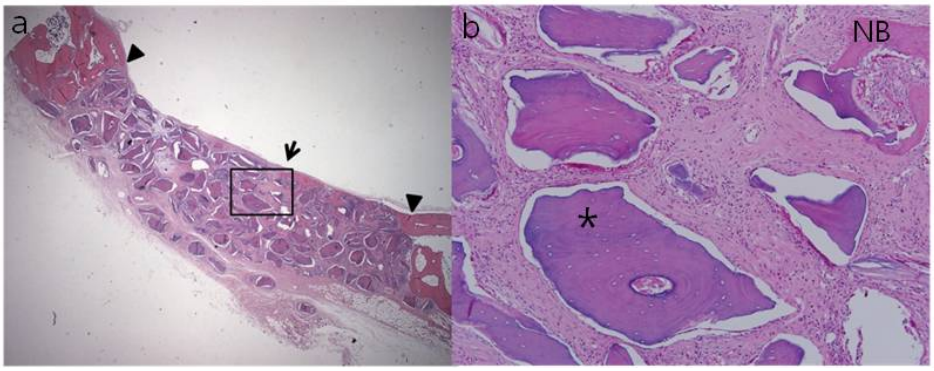

Figure 4. Representative photomicrographs of the control group. DBM particles were embedded within the connective tissue with a minimal amount of new bone. The defect was almost filled with loose connective tissue and DBM particles
(arrow = new bone margin or closed defect margin, arrow head = defect margin, *: DBM, NB: new bone; H-E stain; original magnification a ×12.5; b ×100).

Test group: Defects were almost filled with the newly formed bone. Loose connective tissue was also observed in the defect. New bone formation was observed at the margin of defect. Compared to the control group, less residual DBM particles observed within the new bone and connective tissue (Figure 5).

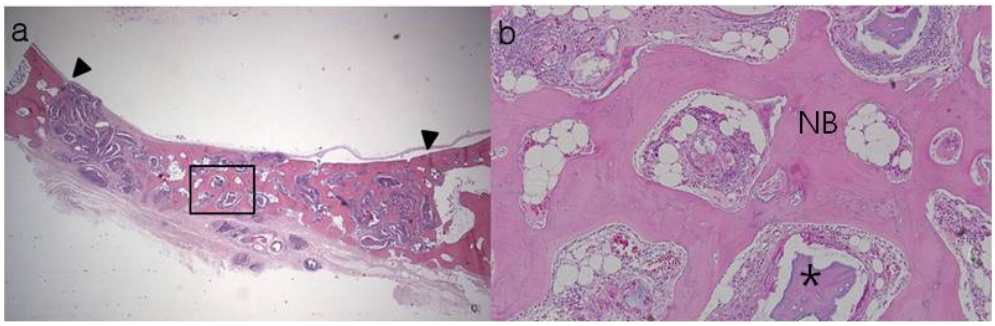

Figure 5. Representative photomicrographs of the test group. Some DBM particles were embedded within the new bone, and there was some adipose tissue. The defect was almost filled with the new bone
(arrow head = defect margin, *: DBM, NB: new bone; H-E stain; original magnification a ×12.5; b ×100).

D. Histometric analysis

Tables 1-3 show the results of the histometric analysis. In defect closure, the test group was statistically significant greater than the control group ($P<0.05$). Total augmented area was not significantly different between these two groups. But, New bone area of the test group was significantly greater than the control group ($P<0.05$).

Table 1. Defect Closure (group means ± SD; n=7, %)

	8 weeks
DBM	40.03 ± 26.5
DBM + rhBMP-2	90.64 ± 11.9*

*: Statistically significant difference

Table 2. Augmented area (group means ± SD; n=7, mm^2)

	8 weeks
DBM	28.2 ± 10.7
DBM + rhBMP-2	35.85 ± 8.6

No Statistically significant difference

Table 3. New Bone area (group means ± SD; n=7, mm^2)

	8 weeks
DBM	4.7 ± 2.3
DBM + rhBMP-2	15.0 ± 6.9*

`*: Statistically significant difference

IV. Discussion

The objective of this study was to evaluate the bone regenerative effects of DBM coated by newly-developed Chinese hamster ovary (CHO) cell-expressed rhBMP-2 in rabbit cranial defect. Micro-CT imaging showed that the rhBMP-2 coated DBM group revealed extensive bone formation compared with the DBM group. In histometric analysis, there were significant differences in defect closure and new bone area between the DBM group and the rhBMP-2 coated DBM group (Table 1 and 3), which may be explained by the effect of rhBMP-2 on bone regeneration. In the DBM alone group, significantly less bone formed. Although this study did not include sham-operated control, our previous study evaluated the spontaneous healing capacity of surgically produced 11mm cranial defect in rabbits with an 8 weeks healing period (Sohn et al., 2010). The defect closure and new bone area in the DBM group were similar to those in the previous control study, although statistical analysis was not performed (50.03 ± 25.92 % of defect closure, 3.88 ± 0.96 mm^2 of new bone area).

Demineralized bone matrix (DBM) is a well known osteoinductive substance (Nichter et al., 1992). Nevertheless, the scientific literature remains controversial about whether DBM is clinically effective in bone-regenerative procedures. For instance, some studies reported poor osteoinductivity of DBM in animals (Becker et al., 1995; Schwartz et al., 1996) and humans (Becker et al., 1996; Becker et al., 1998). On the contrary, other studies reported the use of DBM to be effective in bone repair

(Pacaccio et al., 2005; Oztürk et al., 2006) especially in cases involving periodontal defects (Cortellini et al., 1995; Kim et al., 1998). Even meta-analyses of previous clinical trials seem to be controversial; one reported a beneficial effect of DBM (Reynolds et al., 2003) whereas another stated that its use may not be beneficial (Laurell et al., 1998).

Reddi and associates reported that significantly less bone formation occurs when DBM powder is placed alone in the defect (Reddi et al., 1987). The limited osteoinductivity of DBM seen in the present investigation confirms that DBM powder may not be effective in bone regeneration of critical-size defect. This is presumably due to the lack of control at the time of placement as well as the inability to modulate BMP release from the DBM (Carnes DL Jr et al., 1999).

The proliferation and differentiation of sufficient progenitor cells are critical for bone regeneration, and these processes are regulated by growth factors such as TGF-β and BMPs (Sykaras et al., 2003). Two of these proteins, BMP-2 (INFUSE® Bone Graft, Medtronic, USA) and BMP-7 (Osigraft®, Stryker GmbH & Co. KG, Duisburg Germany), have been developed commercially for use as bone inductive materials (Boyan et al., 2006). Both proteins initiate endochondral bone formation when implanted heterotopically (Schreiber et al., 2005) and stimulate bone formation clinically (Wozney et al., 2002). RhBMPs were produced in mammalian cells, like Chinese hamster ovary cells (CHO-rhBMP-2) (Wozney et al., 1988) or in prokaryotic cells, such as *Eschericha coli* (ErhBMP-2) (Kübler et al., 1998). The efficacy of

CHO-rhBMP-2 was estimated to be about one order of magnitude higher than the efficacy of ErhBMP-2 (Kimura et al., 2000).

The clinical application of native bone growth factors without any carrier is mainly limited by their short half-life and the problem of delivery (Fischer et al., 2011). Carriers have an important role in maintaining the BMP concentration at the target site for sufficient time to promote chemotaxis, migration of bone-forming cells to the target site as well as the proliferation and differentiation of these cells (Seeherman et al., 2002). In general, a suitable carrier must consist of the following properties: it should provide controlled release of the protein, adequate exposure to inducible cells, be biocompatible with host tissue and have an architecture conducive to the rapid ingrowth of newly formed bone (Sandhu et al., 2001).

Considerable research has been focused on the examination of delivery systems to evaluate their efficacy and biocompatibility as carriers for BMPs. The candidate carrier materials include particulate and putty formulations of inorganic biomaterials from natural or synthetic sources based on hydroxyapatite (Sigurdsson et al., 1996; Boyne et al., 2001), β-tricalcium phosphate (Pang et al., 2004), calcium sulphates/plaster of Paris (Yamazaki et al., 1988), calcium phosphates (Sorensen et al., 2004), calcium carbonates (Boyne et al., 2001), bioglass technologies (Barboza et al., 2004) and organic polymers, including allogeneic/xenogeneic collagen preparations (Sigurdsson et al., 1996; Hunt et al., 2001; Barboza et al., 2004), hyaluronan (Hunt et

al., 2001), poly-α-hydroxy acids (Sigurdsson et al., 1996; Boyne et al., 2001), and methylmethacrylate (Boyne et al., 2001).

In the present study, we evaluated newly developed CHO-rhBMP-2-induced bone formation and maturation using the DBM carrier system in rabbit cranial defect. DBM has been used with success as a carrier for allogenic BMP and rhBMP-2 (Ripamonti et al., 1993). Both quantity and quality of rhBMP-2-induced bone has been shown to be high in periodontal defects where DBM is used as a carrier (Sigurdsson et al., 1996). It was shown that almost a complete closure of defect and a significantly larger new bone area at 8 weeks in this study. Although comparisons between studies should be made judiciously, the studies collectively suggest that rhBMP-2–DBM is an effective device for bone regeneration.

In conclusion, DBM coated by newly-developed CHO-rhBMP-2 produced significant new bone and tissue formation in rabbit cranial defect. It is suggested that DBM may be an available carrier for rhBMP-2.

V. Conclusion

The objective of this study was to evaluate the bone regenerative effects of DBM coated by newly-developed Chinese hamster ovary (CHO) cell-expressed rhBMP-2 in rabbit cranial defect.

Two symmetric, 11-mm ø, critical-size osteotomy defects were created in 7 New Zealand white rabbit craniums received DBM and rhBMP-2 coated DBM, respectively. Implant sites were evaluated by microcomputed tomography and histologic and histometric parameters following an 8-week healing interval.

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References

Barboza E, Caúla AL, Caúla F, et al. Effect of recombinant human bone morphogenetic protein-2 in an absorbable collagen sponge with space-providing biomaterials on the augmentation of chronic alveolar ridge defects. *J Periodontol* 75:702-708, 2004.

Becker W, Clokie C, Sennerby L, Urist MR, Becker BE. Histologic findings after implantation and evaluation of different grafting materials and titanium micro screws into extraction sockets: Case reports. *J Periodontol* 69:414-421, 1998.

Becker W, Urist M, Becker BE, et al. Clinical and histologic observations of sites implanted with intraoral autologous bone grafts or allografts. 15 human case reports. *J Periodontol* 67:1025-1033, 1996.

Becker W, Urist MR, Tucker LM, Becker BE, Ochsenbein C. Human demineralized freeze-dried bone: Inadequate induced bone formation in athymic mice. A preliminary report. *J Periodontol* 66: 822-828, 1995.

Boyan DB, Ranly DM, Schwartz Z. Use of growth factors to modify osteoinductivity of demineralized bone allografts: Lessons for tissue engineering of bone. *Dent Clin N Am* 50:217-228, 2006.

Boyne PJ, Shabahang S. An evaluation of bone induction delivery materials in conjunction with root-form implant placement. *Int J Periodontics Restorative Dent* 21:333-343, 2001.

Carnes DL Jr, De La Fontaine J, Cochran DL et al. Evaluation of 2 novel approaches for assessing the ability of demineralized freezed-dried bone allograft to induce new bone formation. *J Periodontol* 70:353-363, 1999.

Celeste AJ, Iannazzi JA, Taylor RC, et al. Identification of transforming growth factor beta family members present in bone-inductive protein purified from bovine bone. *Proc Natl Acad Sci U S A* 87:9843-9847, 1990.

Cochran DL, Wozney JM. Biological mediators for periodontal regeneration. *J Periodontol 2000* 19:40-58, 1999.

Cortellini P, Bowers GM. Periodontal regeneration of intrabony defects: An evidence-based treatment approach. *Int J Periodontics Restorative Dent* 15:128-145, 1995.

Fischer J, Kolk A, Wolfart S, Pautke C, Warnke PH, Plank C, Smeets R: Future of local bone regeneration - Protein versus gene therapy. *J Craniomaxillofac Surg* 39:54-64, 2011.

Hunt DR, Jovanovic SA, Wikesjö UME, et al. Hyaluronan supports recombinant

human bone morphogenetic protein-2 induced bone reconstruction of advanced alveolar ridge defects in dogs: a pilot study. *J Periodontol* 2001;72:651-8.

Kim CK, Cho KS, Choi SH, Prewett A, Wikesjö UM. Periodontal repair in dogs: effect of allogenic freeze-dried demineralized bone matrix implants on alveolar bone and cementum regeneration. *J Periodontol* 69(1):26-33, 1998.

Kimura M, Zhao M, Zellin G, Linde A. Bone-inductive efficacy of recombinant human bone morphogenetic protein-2 expressed in *Escherichia coli*: an experimental study in rat mandibular defects. *Scand J Plast Reconstr Surg Hand Surg* 34(4): 289-299, 2000.

Kübler NR, Reuther JF, Faller G, Kirchner T, Ruppert R, Sebald W. Inductive properties of recombinant human BMP-2 produced in a bacterial expression system. *Int J Oral Maxillofac Surg* 27(4):305-309, 1998.

Laurell L, Gottlow J, Zybutz M, Persson R. Treatment of intrabony defects by different surgical procedures. A literature review. *J Periodontol* 69:303-313, 1998.

Lindholm TC, Gao TJ, Lindholm TS. Granular hydroxyapatite and allogenic demineralized bone matrix in rabbit skull defect augmentation. *Ann Chirurg*

Gynaecol 82:91-98, 1993.

Moghadam HG, Sándor GK, Holmes HH, Clokie CM. Histomorphometric evaluation of bone regeneration using allogeneic and alloplastic bone substitutes. *J Oral Maxillofac Surg* 62:202-213, 2004.

National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th edition. Washington (DC): National Academy Press (US), 2011.

Nichter LS, Yazdi M, Kosari K, et al. Demineralized bone matrix/polydioxanone composite as a substitute for bone graft: a comparative study in rats. *J Craniofac Surg* 3:63-69, 1992.

Oklund SA, Prolo DJ, Gutierres RV, et al. Quantitative comparison of healing in cranial fresh autografts, frozen autografts, processed autografts and allografts in canine skull defects. *Clin Orthop* 205:269-291, 1986.

Oztürk A, Yetkin H, Memis L, Cila E, Bolukbasi S, Gemalmaz C. Demineralized bone matrix and hydroxyapatite / tri-calcium phosphate mixture for bone healing in rats. *Int Orthop* 30:147-152, 2006.

Pacaccio DJ, Stern SF. Demineralized bone matrix: Basic science and clinical applications. *Clin Podiatr Med Surg* 22:599-606, 2005.

Pang EK, Im SU, Kim CS, Chi SH, Chai JK, Kim CK, Han SB, Cho KS. Effect of recombinant human bone morphogenetic protein-4 dose on bone formation in a rat calvarial defect model. *J Periodontol* 75:1364-1370, 2004.

Reddi AH, Wientroub S, Mathukumaran N. Biological principles of bone induction. *Orthop Clin North Am* 18:207-212, 1987.

Reynolds MA, Aichelmann-Reidy ME, Branch-Mays GL, Gunsolley JC. The efficacy of bone replacement grafts in the treatment of periodontal osseous defects. A systematic review. *Ann Periodontol* 8:227-265, 2003.

Ripamonti U, Ma SS, Cunningham NS, Yeates L, Reddi AH. Reconstruction of the bone-bone marrow organ by osteogenin, a bone morphogenetic protein and demineralized bone matrix in calvarial defects of adult primates. *Plast Reconstr Surg* 91:27-36, 1993.

Sampath TK, Maliakal JC, Hauschka PV et al. Recombinant human osteogenic protein-1 (hOP-1) induces new bone formation in vivo with a specific activity comparable with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation in vitro. J Biol Chem 267(28):20352-20362, 1992.

Sandhu HS, Khan SN, Suh DY, Boden SD. Demineralized bone matrix, bone

morphogenetic proteins, and animal models of spine fusion: an overview. *Eur Spine J* 10 Suppl 2:S122-131, 2001.

Schmitz JP, Hollinger JO. The critical size defect as an experimental model for craniomandibulofacial nonunions. *Clin Orthop Relat Res* 205:299-308, 1986.

Schreiber RE, Blease K, Ambrosio A, et al. Bone induction by AdBMP-2/collagen implants. *J Bone Joint Surg Am* 87:1059-1068, 2005.

Schwartz Z, Mellonig JT, Carnes DL Jr., et al. Ability of commercial demineralized freeze-dried bone allograft to induce new bone formation. *J Periodontol* 67:918-926, 1996.

Seeherman H, Wozney J, Li R: Bone morphogenetic protein delivery systems. *Spine* 27:S16-S23, 2002.

Sigurdsson TJ, Nygaard L, Tatakis DN, et al. Periodontal repair in dogs: evaluation of rhBMP-2 carriers. *Int J Periodontics Restorative Dent* 16:524-537, 1996.

Sohn JY, Park JC, Um YJ, Jung UW, Kim CS, Cho KS, Choi SH. Spontaneous healing capacity of rabbit cranial defects of various sizes. *J Periodontal Implant Sci* 40:180-187, 2010.

Sorensen RG, Wikesjö UME, Kinoshita A, et al. Periodontal repair in dogs: evaluation of a bioresorbable calcium phosphate cement (Ceredex®) as a carrier for

rhBMP-2. *J Clin Periodontol* 31:796-804, 2004.

Sykaras N, Opperman LA. Bone morphogenetic proteins (BMPs): how do they function and what can they offer the clinician? *J Oral Sci* 45(2):57-73, 2003.

Urist MR. Bone: formation by autoinduction. *Science* 150:893-899, 1965.

Wozney JM, Rosen V, Celeste AJ, et al. Novel regulators of bone formation: molecular clones and activities. *Science* 242:1528-1534, 1988.

Wozney JM. Overview of bone morphogenetic proteins. *Spine* 27(16 Suppl. 1):S2-8, 2002.

Yamazaki Y, Oida S, Akimoto Y, et al. Response of the mouse femoral muscle to an implant of a composite of bone morphogenetic protein and plaster of Paris. *Clin Orthop Relat Res* 234:240-249, 1988.

국문요약

백토 두개골 결손부에서 탈회골기질 전달체를 이용한 중국 햄스터 난소 세포 유례 rhBMP-2의 골재생 효과

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재조합 인간 골형성 유도 단백질-2 (Recombinant human bone morphogenetic protein-2, rhBMP-2) 는 골 재생 술식에서 치유와 조직 형성을 촉진하는 것으로 알려져 왔다. 본 연구에서는 백토 두개골 결손부에서 새롭게 개발된 중국 햄스터 유례 rhBMP-2의 골형성 유도 효과를 평가하고자 한다.

7마리의 뉴질랜드 백토에 대칭으로 2개의 지름 11 mm 임계 크기 두개골 결손부를 형성하였다. 대칭으로 형성된 결손부에 DBM과 rhBMP-2 coated DBM을 각각 이식하였다. 술 후 8주에 실험 동물을 희생하고, micro CT 이미지 촬영과 조직학적 및 조직계측학적 분석으로 비교 관찰하였다.

Micro CT 이미지와 조직학적 관찰 결과, rhBMP-2 coated DBM군에서 뚜렷한 골형성 유도 효과가 나타났다.

조직계측학적 관찰 결과, 결손부 폐쇄 (defect closure)는 rhBMP-2 coated DBM군에서 DBM군에 비해 유의성 있게 크게 나타났다($P<0.05$). 총조직형성량 (augmented area)은 DBM군과 rhBMP-2 coated DBM군에서 차이가 없었으나, 신생골형성량 (new bone area)은 rhBMP-2 coated DBM군에서 DBM군에 비해 유의성 있게 크게 나타났다($P<0.05$).

이상의 결과에서 볼 때, 백토 두개골 결손부에서 새롭게 개발된 중국 햄스터 난소 세포 유래 rhBMP-2를 피막시킨 DBM은 유의성 있는 골 재생과 조직 재생 효과를 보였으며, DBM은 rhBMP-2의 유효성 있는 전달체로 사료된다.

핵심되는 말: 골 재생, 재조합 인간 골형성 유도 단백질-2, 농도, 중국 햄스터 난소 세포, 탈회골기질(DBM), 백토 두개골 결손부