# The BMPs expression and histomorphometric study of β-TCP / rhBMP-2 Grafting on the rabbit cranial bone defects

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## Abstract

**Objective:** The Purpose of the study was to investigate the bone morphogenic protein expression of rhBMP-2(recombinant human bone morphogenic protein-2) as singnaling molecule and β-TCP(Tricalcium phosphate) as a bone substitute and carrier medium of rhBMP-2.

**Materials and Methods:** 16 rabbits divided into 2 group of each 8 rabbit. Two standardized bone defect, round bilateral defect was made in the cranium of the 8 rabbit of first group, and was grafted with 150~500㎛ diameter β-TCP 0.25g in one side, which was soaked with rhBMP-2, and autogenous bone was grafted on another side as a positive control. Second group of 8 rabbit, only β-TCP was grafted with same size and same manner. After 2, 4, 8, and 12 weeks, specimen was taken for microscopic immunohiostochemical and histomorphometric analysis.

**Result:** Grafting β-TCP with rhBMP show the early formation of the bone regenerative factor (BMP-4) and more quantity of new bone formation than only use of β-TCP (8,12 week), even show less new bone formation than autogenous bone.

**Conclusion :** The experimental study result that β-TCP graft combination with rhBMP-2 as a delivery system is an effective with osteoinductive capacity and biodegradable properties, so that provide clinical availibility of composite use in reconstruction of bony defect.

**Key words**
Beta-Tricalcium phosphate, bone graft, Bone Morphogenic Protein, bone substitute, histomorphometry, Immunohistochemistry

## INTRODUCTION

Oral and maxillofacial bone defect can result from many causes such as trauma, tumor, resective surgery, and atrophy with aging. The goal of bone graft is to stimulate or facilitate the growth of new bone into the defect. The gold standard for recovering these defects to use the autogenous bone. Although highly effective, it has some disadvantages such as limitation of quantity, donor site morbidity, fear of patient, need for additional surgery and various complication (such as pain, bleeding, infection and paresthesia). Therefore graft of synthetic biomaterials come into common use with unlimited availability and without the risk of transmitting infection.

The ideal bone substitute should maintains biologic supports during the healing period and is then gradually replaced by the newly formed bone. Several alloplastic materials have been examined as a substitute for autogenous bone, such as allografts[1], ceramics , and various osteoinductive agents. Synthetic ceramics, such as hydroxyapatite, tricalcium phosphate (TCP)[2], and biphasic calcium phosphate are commonly used as bone substitute[3].

However, alloplastic materials have insufficient activity in osteogenic or osteoinductive activity, so it takes long period in bone remodeling, it is needed therefore add the signaling agent of cell growth in order to reinforce the new bone formation.

The bone morphogenic protein (BMP) is known as

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endogenous growth factor, binding to cell surface receptor that is likely to play a crucial role in osteoblast stimmulation and resulting in bone formation.

Urist[4] reported that there exists a substance within bone that is capable of inducing new bone formation and observed that bone was produced in the muscle of a rat by this substance. Since this group of protein from demineralized bone of rabbit in 1979[5], many clinical research continued in oral and maxillofacial surgery, and the use of BMP alone or in conjunction with other graft modalities shows great experimental result in the reconstruction of defects and deficiencies in recents[6,7]. According to Wozney[8], BMP combination with a graft material effects as biologic catalyst that proliferate the cell and promote chondrogenesis and osteogensis. BMPs are growth factor proteins secreted by cells and serve as signaling agent. They work as part of a large cellular communication network that influences the cell functions of division, matrix synthesis, and tissue differentiation[9].

The ideal material of BMP delivery system should have a function as prevent invasion by the surrounding soft tissue until induction of new bone, and are then gradually absorbed with new bone formation, and many carrier have already been examined, such as TCP, Hydroxyapatite, collagen, Polylactic acid-polyglycolic acid copolymer, and titanium[10-14]. $\beta$-TCP is a synthetic porous ceramic which has shown osteoconduction and very favorable substitution rate in standardized bone defects[15], and has good tissue tolerance with no immunological or toxic reactions, good biocompatibility, characteristics of absorption, and is effectively replaced by natural bone in an appropriated period of time[16-19].

Since there are little study of grafting $\beta$-TCP in conjunction with rhBMP-2 with incremental new bone formation by period, we evaluate immunologic and histomorphometric changes of bone grafting material and new bone formation in sequence of weeks in this study. In addition, we investigate the osteoinductive activity of $\beta$-TCP/rhBMP-2 through immunohistochemical expression of the BMP-2, -4. -7.

## MATERIALS AND METHODS

The protocol of animal study was approved by Hanyang University Hospital Animal Laboratory and the study was performed in 16 Newzealand White rabbits.

1) Surgical procedure

The surgery was performed the Animal Experimental Regulation Animal Laboratory, Hanyang University Medical Center, Seoul, Korea. Before Surgery, intramuscular injection of ketamine 20mg/kg body weight (Ketamine®HCl, Youhan Yanghang) and Xylazine hydrochloride 2mg/kg body weight (Rompun®, Bayer Korea) and general anesthesia was induced. Cranial area was shaved and local anesthesia was induced with 2% lidocaine, 1:100000 epinephrine (1.8ml). Disinfection with povidone iodine solution and drap was done. Through the central incision, the cranial bone was sufficiently exposed and bilateral round and standardized cranial defect was designed with ready-made round template (15mm diameter) and preperated throught saline irrigation with low speed round bur (106 Komet®, Germeny) to depth of meninges. Lateral aspect of subperiosteal incision extend to periorbital area, and symmetrcial round defect does not invade cranial suture line. The harveseted corticocancellous bone blocks were crushed and ground to a particulate size about 1~1.5mm using a bone mill and filled in left side of cranial bone defect. And in first group of 8 rabbits, in Right side, distilled water diluted 0.01% rhBMP-2(Fig. 1) solution was soaked to 0.25g of $\beta$-TCP(Fig. 2) and filled in another right side of cranial defect. In second group of 8 rabbits, only $\beta$-TCP was grafted in the defect on one side, and autogenous bone graft was done in the other side with same method. Periosteal approximation was done with 4-0 vicryl(Ethicon, Norderstedt, Germany) and dermal suture by 3-0 silk(Fig. 3-5). During the first 3 day, antibiotic (Gentamycin®. Dong Hwa. Korea 30mg/kg body weight) was injected throught intramuscular for prevention of infection andanimals were checked daily.

2) Healing period

The 16 animals were divied into 2 groups of 8 animals following periods of grafting operation : 2, 4, 8, 12 weeks

At the end of each periods, the animals were sacrificed and cranial bone defect specimen contained to round grafted defect with 2mm margin were obtained by drill.

3) rhBMP-2 and $\beta$-TCP

rhBMP-2 (CHO cell derived, RND system, Mineapolice. USA : 10$\mu$g/ml >90% purity) was diluted by 0.1ml distilled water into 0.01% volume concentration (10$\mu$g/ml), and soaked to 150~500$\mu$m of $\beta$-TCP (Cerasorb®, Curasan AG, Kleinostheim, Germany ; Particle size 150~500$\mu$m) 0.25g for 1 hour(Fig. 6).


**Fig. 1.** rhBMP-2(CHO cell derived, RND system. mineapolice).

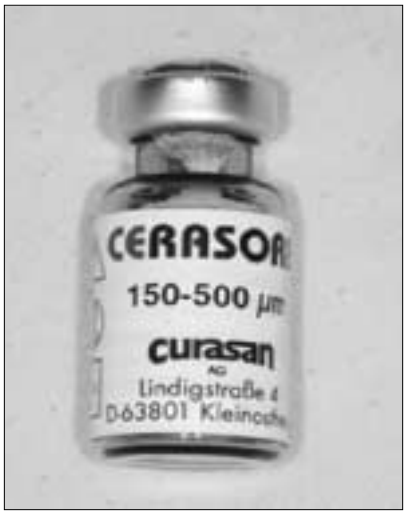


**Fig. 2.** β-TCP (Cerasorb®, Curasan AG, Kleinostheim, Germany ; Particle size 150~500μm).

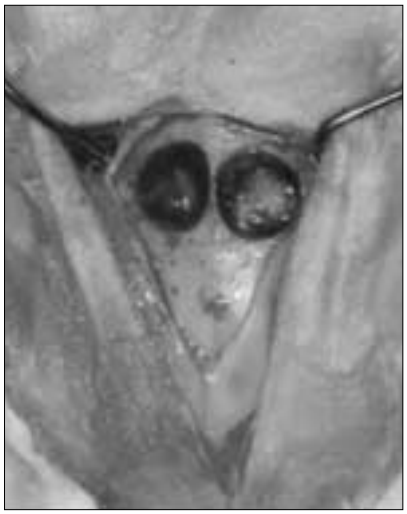

**Fig. 3.** Bilateral round and standardized cranial defect (15mm diameter).

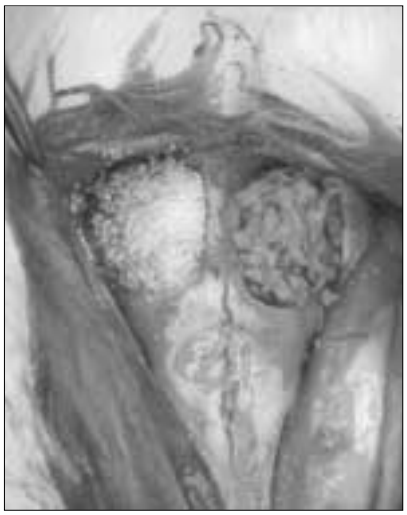

**Fig. 4.** Grafted in rabbit cranial defects(β-TCP(or rhBMP-2 with β-TCP) in Rt. side,Autogenous particulated bone in Lt. side).

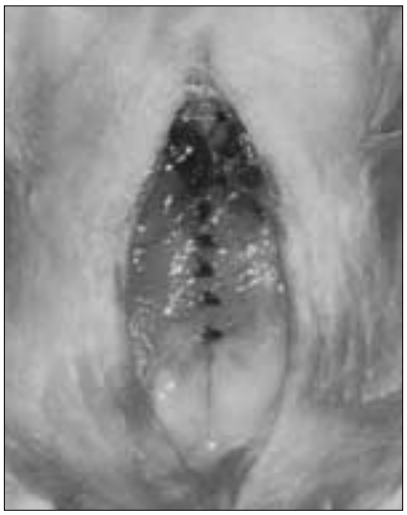

**Fig. 5.** Perosteal approximation.

**Fig. 6.** rhBMP-2 was diluted by 0.1ml .0 distilled water with 0.01% volume concentration (10μg/ml), and soaked to 150~500μm of β-TCP.

4) Histologic analysis

The specimen was fixed by 4% neutral buffered paraformaldehyde solution for 4 weeks and were washed with 0.1M phosphoric buffer solution(PBS) for 30 minutes, and it was demineralized with EDTA 10% with 0.1M PBS for 80 days and embedded in methyl methacrylate. Specimen was stained with H&E, analyzed by microscope (U-MOD1083, Olympus Japan ; ×100 magnification)

5) Histomorphometric analysis

20μm sections of specimen stained with H&E were analyzed by OPTIMUS (6.5 system, Nethelands), and remaining bone, new bone, soft tissue area was detected and divided by total defect area and expressed as percentage.

6) Immunohistochemistry

Streptavidin-biotin method was used for immunohistochemical stain and analyzing specimen. Paraffin of slide

was removed and soaking with alcohol. It was washed three times by distilled water and in 0.01M sodium citrate buffer (pH6.0) microwave was applied, twice for eeach 5 min as pre-treatment for prevention of internal peroxide activation, 3% Hydro-peroxide was applied for 10 min, and washed by distilled water and PBS.

DAKO LSAB kit (DAKO, U.S.A) blocking agent was adjusted for 20 minutes and for pervention of unspecific IgG crossaction. In room temperature, BMP-2, -4, -7 primary antibody was applied for 1 hour and washed three times by distilled water. Polyclonal goat anti-BMP-2, -4, -7 (SantaCruz, U.S.A) was used for primary antibody with 1:50 solution. Later, biotin marked secondary antibody was incubated for 15 min and washed by PBS. Peroxidase marked streptavidin was incubated for 15 minutes and washed by PBS. Specimen was dyed by brown DAB(3,3-diaminobenzidine) for 5 minutes and washed by distilled water, and counter-colored by Meyer's hematoxyline, and permount was examined by light microscopic. Each specimen was examined, and amount of color was divided into 4 steps for dyed area percantage of total, 0-5%(+/-), 6-25%(+), 26-50%(++), more than 50%(+++). Two pathologic specialists were studied and total score was integrated.

## RESULT

Sixteen Newzealand rabbits have no vital problems and signs of infection in experimental period, and showed no other complications. For histomorphometry, total size were examined by two examiners twice for each specimens by microscopy (magnification ×20), and mean volume percent were noted with standard deviation.

For immunohistochemistry, ×200 magnification was applied, and two pathologic specielists choose the specific area and analyze.

To compare new bone formations, One Way ANOVA method was utilized (SPSS. version 11.0).

### 1. Histologic analysis

1) Autogenous bone graft(Fig. 7-10)

The more week went by, the more new bone formation was finding around grafted bone. At 4 week, osteoblast, activated fibroblast and proliferation of endothelial vessel cell were found and after 8 week were not founded. At 8 week, reduction of grafted bone was seen.

2) β-TCP graft + rhBMP-2(Fig. 11-14)

The more week went by, the more new bone formation was finding around β-TCP and some of them were linked each other and expanded. At 4 week, slightly resorption of β-TCP granule was found. At 8 week, activated osteoblast and connective tissue with vessel were found around the new bone.

3) β-TCP only(Fig. 15-18)

At 4 week, slightly resorption of β-TCP granule was found and new bone was found around β-TCP, but the connection of new bone was not found. At 8 week, new bone was found around resorbed β-TCP and some of them were linked each other at some area.

### 2. Histomorphometric Comparison of New Bone Formation(Table 1)

(1) 2 week

New bone formation in autogenous bone was 12.88% and it was more than other β-TCP graft group(6.7%) and β-TCP graft with rhBMP-2 grafted group(8.24%) with significance ($p>0.05$). But there was no difference between β-TCP graft group and β-TCP graft with rhBMP-2 grafted group.

**Table 1.** Histomorphometric analysis

| | Autogenous bone graft | | | β-TCP graft + rhBMP-2 | | | β-TCP only | | |
|---|---|---|---|---|---|---|---|---|---|
| | Graft Bone | New Bone | Soft Tissue | Graft Bone | New Bone | Soft Tissue | Graft Bone | New Bone | Soft Tissue |
| 2 W | 67.06±7.95 | 12.88±3.11 | 20.06±4.84 | 46.30±3.97 | 8.24±4.70 | 51.20±8.66 | 84.52±1.95 | 6.79±0.92 | 8.69±1.04 |
| 4 W | 66.91±8.74 | 20.39±5.37 | 12.70±3.38 | 50.20±14.06 | 15.05±7.73 | 34.74±20.03 | 78.91±0.98 | 10.28±4.34 | 10.81±3.36 |
| 8 W | 47.65±11.36 | 47.83±10.37 | 4.51±0.99 | 19.42±3.75 | 35.50±6.77 | 44.08±4.40 | 49.30±3.36 | 14.00±2.24 | 36.68±4.52 |
| 12 W | 29.09±9.19 | 67.31±8.82 | 2.78±0.36 | 14.79±18.03 | 37.54±10.92 | 47.67±10.50 | 51.30±9.68 | 21.91±9.42 | 26.77±6.44 |

(Area Mean Volume Percent ± Standard deviation)

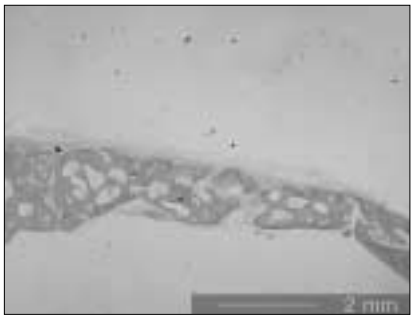

**Fig. 7.** Microscopic exam. shows the autogenous bone graft, 2 weeks (× 20, H&E).

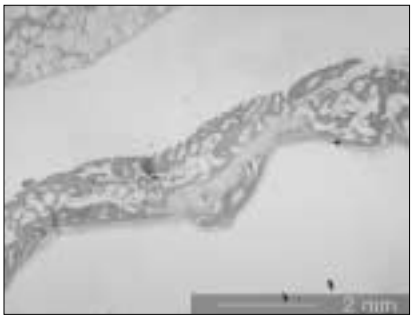

**Fig. 8.** Microscopic exam. shows the autogenous bone graft, 4 weeks (× 20, H&E).

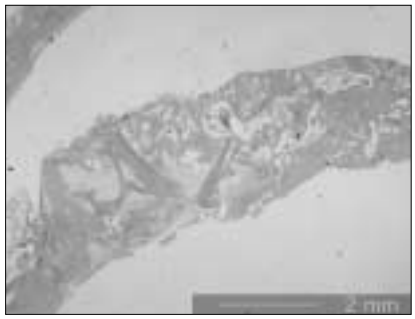

**Fig. 9.** Microscopic exam. shows the autogenous bone graft, 8 weeks (× 20, H&E).

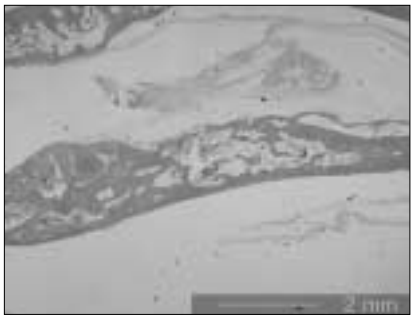

**Fig. 10.** Microscopic exam. shows the autogenous bone graft, 12 weeks (× 20, H&E).

**Fig. 11.** Microscopic exam. shows the β-TCP with rhBMP-2 graft, 2 weeks (× 20, H&E).

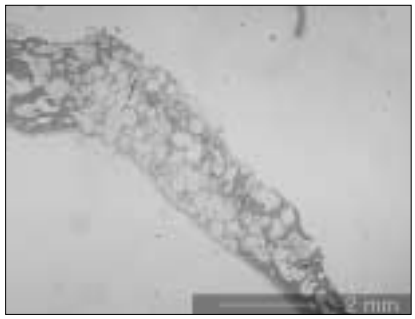

**Fig. 12.** Microscopic exam. shows the β-TCP with rhBMP-2 graft, 4 weeks (× 20, H&E).

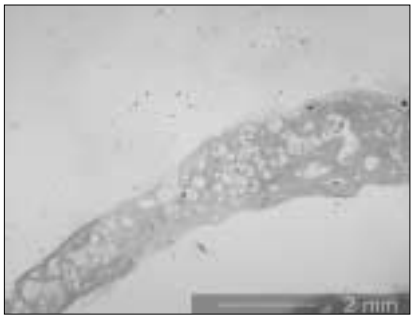

**Fig. 13.** Microscopic exam. shows the β-TCP with rhBMP-2 graft, 8 weeks (× 20, H&E).

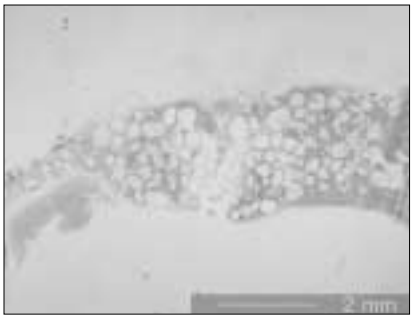

**Fig. 14.** Microscopic exam. shows the β-TCP with rhBMP-2 graft, 12 weeks (× 20, H&E).

**Fig. 15.** Microscopic exam. shows the β-TCP graft, 2 weeks (× 20, H&E).

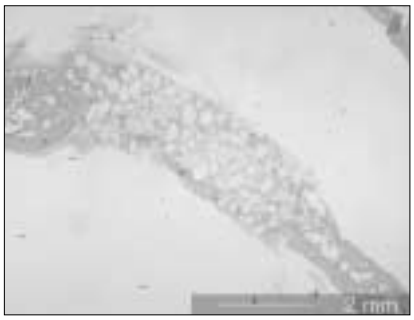

**Fig. 16.** Microscopic exam. shows the β-TCP graft, 4 weeks (× 20, H&E).

**Fig. 17.** Microscopic exam. shows the β-TCP graft, 8 weeks (× 20, H&E).

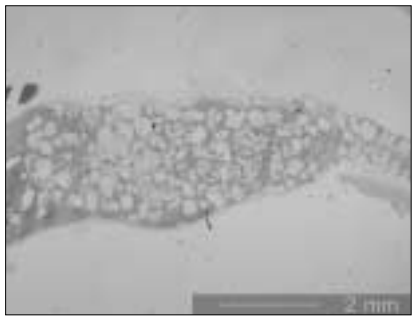

**Fig. 18.** Microscopic exam. shows the β-TCP graft, 12 weeks (× 20, H&E).

**Fig. 19.** Immunohistochemical exam. shows BMP-2 expression on the autogenous bone graft, 2 weeks (× 200).

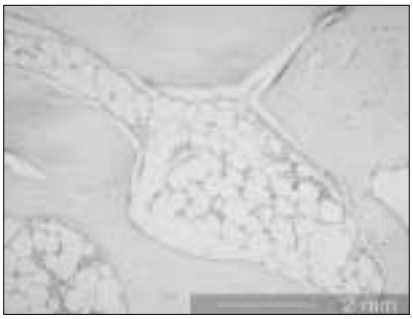

**Fig. 20.** Immunohistochemical exam. shows BMP-2 expression on the autogenous bone graft, 8 weeks (× 200).

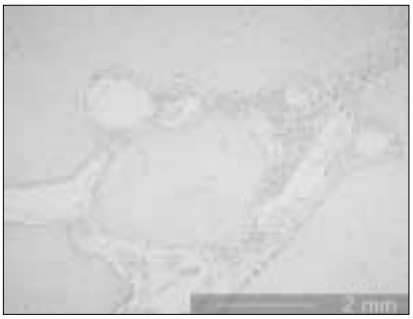

**Fig. 21.** Immunohistochemical exam. shows BMP-2 expression on the autogenous bone graft, 12 weeks (× 200).

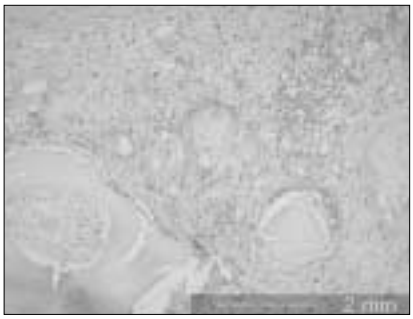

**Fig. 22.** Immunohistochemical exam. shows BMP-4 expression on the autogenous bone graft, 2 weeks (× 200).

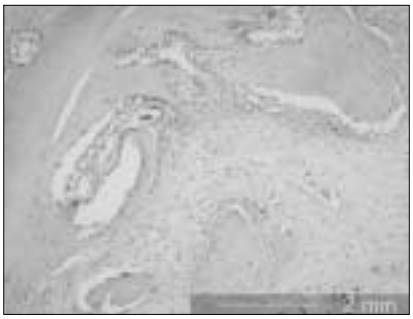

**Fig. 23.** Immunohistochemical exam. shows BMP-4 expression on the autogenous bone graft, 4 weeks (× 200).

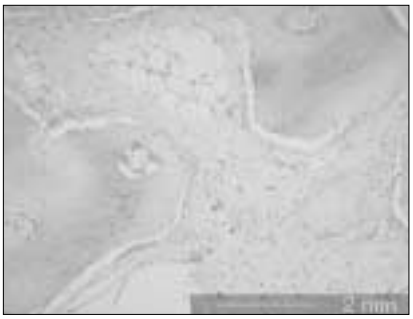

**Fig. 24.** Immunohistochemical exam. shows BMP-4 expression on the autogenous bone graft, 8 weeks (× 200).

**Fig. 25.** Immunohistochemical exam. shows BMP-4 expression on the autogenous bone graft, 12 weeks (× 200).

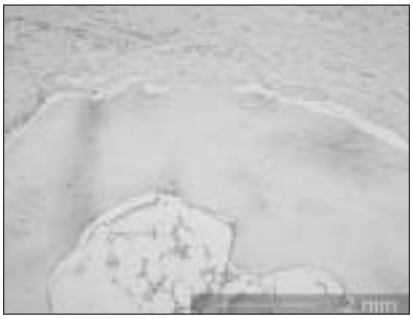

**Fig. 26.** Immunohistochemical exam. shows BMP-7 expression on the autogenous bone graft, 8 weeks (× 200).

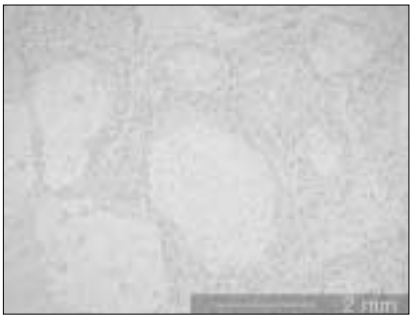

**Fig. 27.** Immunohistochemical exam. shows BMP-2 expression on the $\beta$-TCP with rhBMP-2 graft, 8 weeks (× 200).

**Fig. 28.** Immunohistochemical exam. shows BMP-2 expression on the $\beta$-TCP with rhBMP-2 graft, 12 weeks (× 200).

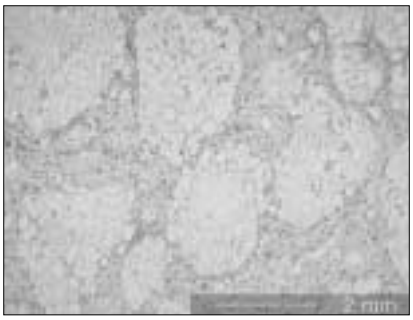

**Fig. 29.** Immunohistochemical exam. shows BMP-4 expression on the $\beta$-TCP with rhBMP-2 graft, 2 weeks (× 200).

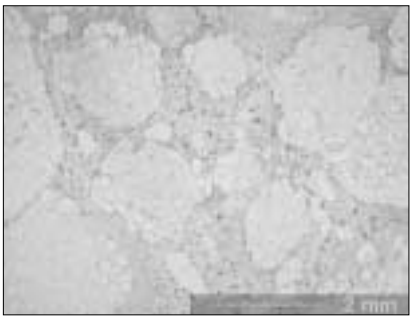

**Fig. 30.** Immunohistochemical exam. shows BMP-4 expression on the $\beta$-TCP with rhBMP-2 graft, 4 weeks (× 200).

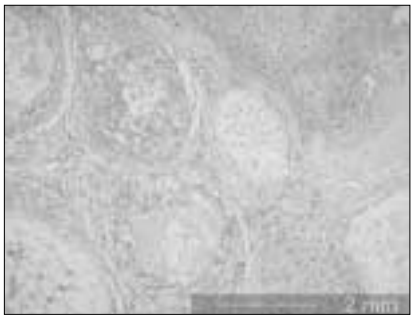

**Fig. 31.** Immunohistochemical exam. shows BMP-4 expression on the β-TCP with rhBMP-2 graft, 8 weeks (× 200).

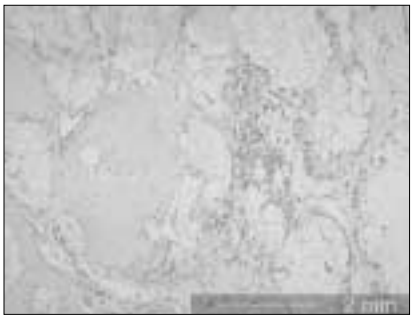

**Fig. 32.** Immunohistochemical exam. shows BMP-4 expression on the β-TCP with rhBMP-2 graft, 12 weeks (× 200).

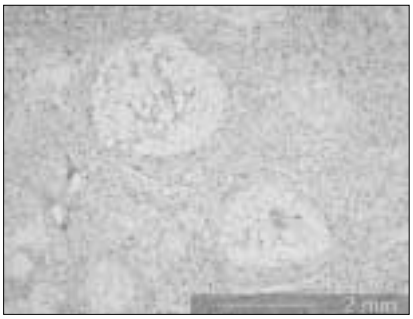

**Fig. 33.** Immunohistochemical exam. shows BMP-7 expression on the β-TCP with rhBMP-2 graft, 8 weeks (× 200).

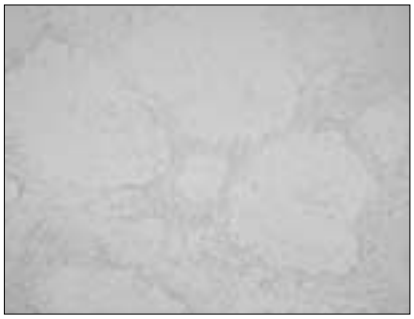

**Fig. 34.** Immunohistochemical exam. shows BMP-2 expression on the β-TCP graft, 4 weeks (× 200).

**Fig. 35.** Immunohistochemical exam. shows BMP-4 expression on the β-TCP graft, 4 weeks (× 200).

**Fig. 36.** Immunohistochemical exam. shows BMP-4 expression on the β-TCP graft, 12 weeks (× 200).

**Table 2.** Immunohistochemical study on BMP-2 expression

| | Autogenous bone graft | | | | β-TCP graft + rhBMP-2 | | | | β-TCP only | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 W | 4 W | 8 W | 12W | 2 W | 4 W | 8 W | 12W | 2 W | 4 W | 6 W | 8W |
| Sample 1 | +/- | - | + | + | - | - | - | + | - | - | + | +/- |
| Sample 2 | +++/- | - | - | ++ | - | +/- | - | ++ | - | - | +/- | + |

Positive was graded on a scale, with - representing no staining, +/- indistinct, + weak stainig, ++ moderate staining, and +++ strong staining.

**Table 3.** Immunohistochemical study on BMP-4 expression

| | Autogenous bone graft | | | | β-TCP graft + rhBMP-2 | | | | β-TCP only | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 W | 4 W | 8 W | 12W | 2 W | 4 W | 8 W | 12W | 2 W | 4 W | 6 W | 8W |
| Sample 1 | ++ | + | + | + | + | ++ | +++ | ++ | - | + | + | + |
| Sample 2 | + | ++ | + | ++ | +/- | ++ | +++ | + | +/- | +/- | +/- | + |

Positive was graded on a scale, with - representing no staining, +/- indistinct, + weak stainig, ++ moderate staining, and +++ strong staining.

(2) 4 week

Autogenous bone was found with 20.39% and it was more than other β-TCP graft group (10.28%) and β-TCP graft with rhBMP-2 grafted group (15.05%) but there was no difference of significance ($p>0.05$).

(3) 8 week

New bone formation of autogenous bone graft (47.83%) and β-TCP graft with rhBMP-2 grafted group (35.50%) were more than other β-TCP graft group (14.00%) with significance ($p>0.05$) but there is no difference between Autogenous bone graft and β-TCP graft with rhBMP-2 grafted group ($p>0.05$).

(4) 12 week

New bone formation of autogenous bone was 67.31% and it was more than other β-TCP graft group (21.91%) and β-TCP graft with rhBMP-2 grafted group (37.54%) but there was no difference of significance ($p>0.05$).

**3. Immunohistochemical study on BMP expression (Table 2, 3)**

1) Autogenous bone graft(Fig. 19-26)

BMP-2 (except at 4week), BMP-4 were founded around grated bone but BMP-7 was not founded except at 4week.

2) β-TCP graft + rhBMP-2(Fig. 27-33)

BMP-4 was founded in osteoblast round grafted bone, and no BMP-2 and BMP-7 were not founded at 2,4,8 week. At 12 week, BMP-2 and BMP-4 were founded but BMP-4 was decreased than 8 week.

3) β-TCP only(Fig. 34-36)

Less than +/- of BMP-2, -4 were founded around osteoblast with grafted bone. At 12 week, BMP-2, -4 were slightly dercrease than 8 week.

## DISCUSSION

Calcium phosphate is on the major substances comprising hard tisseue, tooth and bone. It is non-toxic, osteoconductive, and biocompatible to bone and soft tissue.

Molecular structure of TCP is similar to bone and TCP has a good boicompitibility, and optimal resorption period, so that there is various clinical use and contemporary experimental research of β-TCP, because it show high solubility than HA.

Usually porous β-TCP allows new bone to grow to pores of TCP and replace them, and it was used as the filler of new bone formation in bone defect.

Although calcium phosphate biomaterials have developed to similar composition of bone matrix, which is porous, resorbable, dense and bioactive, TCP is osteoconductive material and only provide a scaffold for new bone formation, Therefore, its clinical sue is restricted to relatively small bone defect[20], and the combination of bone substitute materials with osteoinductivity would be favorable to reduce the period to form new bone.

Osteoinduction is defined as the mechanism by which a mesenchymal tissue is induced to change its cellular structure to become osteogenic[21], and BMP have an osteoinductive carriers in various experimental study and the value of BMP treatment also has been reported in human study[22].

The discovery by Urist[16] in 1965 of the osteoinductive properties of demineralized , guanidine-extracted bone, eventually led to the isolation of bone morphogenic proteins in 1980, and the beginning of osseous tissue engeneering.

The BMPs belong to an expanding TGF-β (Transforming growth factor) superfamily and approximately 40 isoforms have been identified and they are the only growth factors that can stimulate differntiation of the MSCs into a chondroblastic and osteoblastic direction[23,24]. And these isoforms differ in their osteogenic potential, and their effects may be mitogenic, chemotactic, morphogenic, or apoptotic depending on the cell type to which the ligand is exposed, and the growth factor concentration[24].

After injury to the bone matrix, BMPs are released. They are responsible for various mechanisms that contribute to bone, such as angiogenesis, and chemotaxis and diffrentiation of mesenchymal cells.

BMPs have pleomorphic functions that range from non-skeletal and skeletal organogenesis to bone generation and regeneration[25], and BMPs have been tested in clinical trials. Initial data from these trials have suggested that BMP-induced bone formation is at least equvalent to autogenous bone-grafting when used to treat tibial nonunions and to promote spinal fusions[26-28].

Our study focus on rhBMP-2 because BMP-2 are essential for osteoblast and osteoclast formation, and have

been shown to be the most strongly expressed BMPs in rodent models of mandibular fracture healing, as well as in new bone formation during distraction osteogenesis of the mandible[23].

The bone substitute used as a carrier for BMP-2 controls the release kinetics of BMP-2. Unlike the gelatine sponge or direct injection, BMP-2 soaking within β-TCP continuosly released with resorption mechanism of TCP granule.

Uludag et al.[29], showed that the release kinetics depend on the protein itself and on the carrier. They found differences in BMP-2 release from collagen, glycolic acid, demineralized bone matrix, and bovine bone mineral with the highest recovery rate found in collagen and a recovery rate of about 30% in TCP. Nevertheless, in their model, rhBMPs was added via soaking process, it may be possible that the BMP adhere only to the outer surface of the carrier, resulting in an earlier burst release. Blood invades from the defect into the TCP, it might result in early wash-out of the BMP, which served as a cell recruitment signal, attracting the BMP-responding cells into the TCP.

Many carriers have been tried for BMP-2, and the most important requirments for BMP carriers are bioactive, osteoconductivity, and biomechanical stability. The substance should be resorbable, be replaced by new bone during normal remodeling, and should be moldable, easy to handling with surgeon, and filled completely with close contact between bone and implant[30].

Calcium phosphate ceramics are well known as carriers for BMP[31,32], because of their micro-architecture. The micro-architecture of biomaterials also interferes with the tissue response at the site of bone graft and at the same time, the porosity can act as housing of BMP to continuously release for a long time. Studies on the porosity of carriers have revealed that hardness of surface can impair the ingrowth of graft material. Because bone tissue would be physical barrier, it can limit the proliferation of blood vessels essential for bone repair. Pore smaller than 50μm are too small to allow the ingrowth, so macroporosity (>100μm)[33] plays an important role in the osteoconductivity. And the microporosity (<5μm)[34] is important for the bioresorbability of the material.

However, most tested calcium phosphate-BMP-composites are supplied in form of preformed cubes, wedges, or even granules, which have only little biomechanical stability, leading to a poor contour adaptation between bone and implant. This reduces the contact area, resulting in less osseous ingrowth, with the risk of instability and pseudoarthrosis, however, calcium phosphate can be injected or molded during the operation and fill defects are any size or shape, resulting in an excellent bone-implant contact[35], so that BMP could have more chance to surface of bone defects than other biomaterials.

The use of TCP as a carrier for BMP combines the advantage of the optimized bone-implant contact and this osteoconductive effect with enhanced new bone formation induced by BMP. This should lead to a faster bone healing with improved biomechanical stability[3].

The combination of bioactive and osteoconductive calcium phosphate ceramics, or similar materials, and the osteoinductive BMPs seems to be synergistic on the healing of bone because of the bioactive properties of calcium phosphate ceramics, which also scaffold for cell ingrowth and differentiation, and whose osteoconductive properties stimulate bone healing as well.

## CONCLUSION

In animal study of various graft materials, such as autogenous bone, β-TCP and β-TCP with rhBMP-2 to cranial bone defect of rabbit, we investigate new bone formation through histologic, histomorphometric, and immunohistochemistric analysis.

In immunomorphometric analysis, grafting β-TCP with rhBMP show the early formation (2, 4 and 8 week) of the bone regenerative factor (BMP-4) than β-TCP graft group (4 and 8 week), and in histomorphometric analysis more quantity of new bone formation than only use of β-TCP (8 and 12 week $p>0.05$), even show less new bone formation than autogenous bone(2,4,8 and 12 week).

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