

Effects of rhBMP-2 Coating Tricalcium Phosphate on Socket Preservation in Dog Extraction Socket

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Abstract : Recently, research about bone morphogenetic protein(BMP) has become a field in which advances are consistently and constantly being made due to possibility in substitution for bone graft. The purpose of present study was to evaluate the efficacy of BMP-2 for bone regeneration during socket healing after teeth extraction through the volumetric analysis of extraction sockets using cone beam computed tomography(CBCT). In six dogs, the both side 3rd and 4th mandibular pre-molars were hemi-sected and the distal roots were removed. The distal extraction socket of left side of the pre-molars were filled with BMP-2 coating TCP. The socket of the contra-lateral pre-molars were left without bone graft. The three dogs were sacrificed after 4 weeks of healing, the other three dogs were sacrificed after 12 weeks. Then, the tooth socket was scanned with CBCT and data were uploaded in consecutive 1 mm-thick two-dimensional slices. The total volume of the delineated socket was computed. The results were statistically analyzed using ANOVA and Multiple comparison test. There were no differences in the width of socket between 2 groups after four and twelve weeks healing. However, BMP-2 coated TCP have efficacy to preserve vertical dimension of bone as a bone graft material in socket after 4 weeks of healing($p < 0.05$). Within the limit of the results of this study, BMP-2 coating TCP can preserve the socket height in early stage in dog mandible.

Key words: rhBMP-2, extraction socket, socket preservation, CBCT

1. Introduction

An extraction causes instant damage and loss in alveolus and soft tissues. Hence, complicated biochemical and histological wound healing process follows, which eventually results in a structural change of alveolus and soft tissues. The changing pattern of alveolus after extraction usually shows decrease in bone mass, and such bone resorption causes difficulties in restoration of the extraction site, especially in the implant restoration. Hence, studies regarding the procedure that minimizes the bone resorption after extraction and the preservation and formation of the bone tissues are currently under progress.

A number of papers examining the alveolar resorption after

extraction with animal experiments or clinical experiments have been published so far. In 1967, Carlsson¹ reported that 40% of the alveolus can be reduced after tooth extraction. The decrease of size of edentulous site after tooth loss has been discussed since early 1960s by several researchers, such as Atwood² and Johnson.

In order for bone tissue to be formed in defect sites that were created by extraction or damage, osteogenic cells that induce bone formation, scaffold that can accommodate the proliferation and differentiations of those cells at appropriate location, and the osteoinductive growth factors that stimulate the differentiation induction of the bone tissue formation are necessary. Among these, the osteoinductive growth factors include bone morphogenetic proteins(BMPs)⁴, transforming growth factors(TGF- β), insulin-like growth factor(IGF), etc., among which BMPs is known as the most effective bone formation factor.^{5,6} In 1965, Urist⁷ transplanted BMPs that were

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identified from bone matrix intomuscular tissue of rats and found out that the BMPs induced bone formation in soft tissue. In 1992, Reddi⁸ and Ripamonti⁹ reported, in an experiment about osteoinductivity with a combination of BMP-2 and BMP-7, that relatively large bone loss was recovered when collagen, demineralization bone matrix, biocompatible polymer, etc. were used together.

Tricalcium phosphate(TCP), one of the alloplastic grafting materials, is a carrier that has osteoconductivity. In 1986, Jarcho¹⁰ verified the effectiveness of TCP on the reconstruction of the site without pathologic condition, and afterwards TCP has been known as an ideal bone graft material for the sockets without inflammation.

Cone beam computer topography(CBCT) can obtain information by a single rotation that takes about 9~40 seconds using cone-shaped radioactive beam and 2-D detector. As a result, CBCT provides very high spatial resolution. CBCT is especially useful in obtaining image of hard tissues in the maxillofacial area as well as in a morphological assessment of the bone frame.

The purpose of this study was to examine the effects of BMP-2 on the bone maintenance and formation in socket. The difference was analyzed using CBCT between test group where TCP coated with BMP-2 was applied to the socket and control group where no grafting material was applied.

2. Material and Methods

2.1 Management of Animals

Six adult beagle dogs older than 12 months with weights about 12 kg were used in this study. The dogs were kept in standard laboratory conditions of a light-dark schedule and relative humidity, were fed a standard diet and were isolated in separate cages.

2.2 Preparation of TCP Coated with rhBMP-2

TCP coated with BMP-2(Cowellmedi Co, Busan, Korea) was used as a bone grafting material. As for rhBMP-2, 0.75 mg/cc concentrations were used. The rhBMP-2 was absorbed on

TCP by incubating TCP for 24 hrs.

2.3 Surgical Procedures

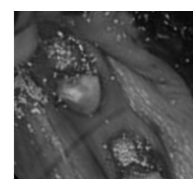
The experimental animals were fasted a day before the surgery. Atropine(0.04 mg/kg, subcutaneously) was injected as preoperative medication and propofol(5 mg/kg, IV) was used to induce anesthesia. Endotracheal tube was located and isoflurane gas was used for anesthesia in 100% oxygen supply(2~3%). After the infiltration anesthesia into membrane with lidocaine with 1:100000 epinephrine, mesial roots of the third and fourth premolar in submaxilla were access-opened. Apex was measured using portable x-ray(Xenoray2[®], Korea) after cleaning with NaOCl, and the condensation was completed using the lateral condensation method with Gutta-percha(Dentsply, Germany). After cutting the furcation area using fissure bur and carefully extracting distal root not to damage the socket, we put TCP coated with BMP-2(Cowellmedi, Inc., Korea) in the socket and confirmed that it was uniformly inserted down to the apical site by taking autoradiograph. As for control group, the opposite side received the same endodontic treatment, while an interrupted suture was performed using 4-0 Vicryl after extraction without inserting anything in the socket(Fig 1). All experimental animals received intramuscular injection of Amikacin(Dong-A Co, Korea) 4 mg/kg after the surgery with a purpose of preventing infection, along with tooth brushes three times a week with a careful plague care.

2.4 Radiological Evaluation

Three dogs were sacrificed after 4 weeks and another three were sacrificed after 12 weeks with an overdose of Pentothal.



Control group(no graft)



Test group(BMP-2 coating TCPgroup)

Figure 1.

Table 1. Summary of Experimental Design.

	Control group	BMP-2 coating TCP group
No. of sacrificed animal at 4 weeks	3	3
No. of sacrificed animal at 12 weeks	3	3
Number	6	6

BMP: bone morphogenetic protein, TCP: tricalcium phosphate

After injecting fixing agent (glutaraldehyde 5% and formaldehyde 4%) through carotid, the mandible was dissected and preserved in fixing solution.

An apical shooting was performed orthogonal to long axis of tooth to observe the change of height of alveolar crest in furcation area and the height of the distal root socket. The width and length of the alveolar ridge were measured by DCTpro[®] (Vatech, Seoul, Korea), which uses CBCT. When measuring the bone width and bone level based on CBCT, the bone width was measured at the top of the socket, while the bone level was measured as a distance between furcation area of the neighboring root and the center of the socket (Figs 2 and 3).

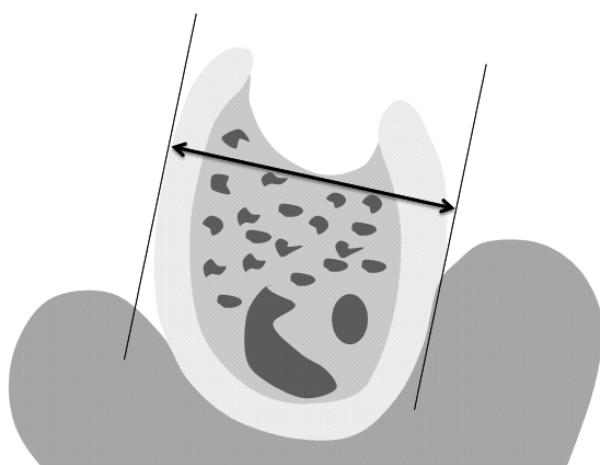


Figure 2. Bone width was measured at the top of the socket.

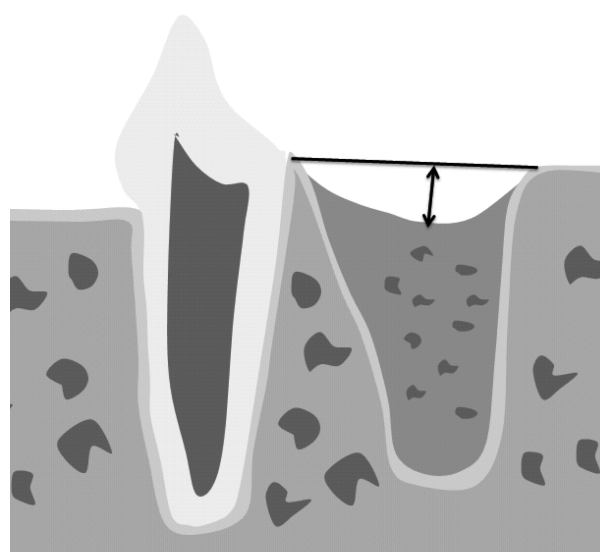


Figure 3. Bone level was measured as a distance between furcation area of the neighboring root and the center of the socket.

2.5 Statistical Analysis

Mean and standard deviation of the values obtained from the experiment were computed and the statistical analysis was performed using ANOVA. Multiple comparison analysis was performed to test the difference between control group and test group. All statistics were verified at 95% significance level. Statistical significance was established at $p < 0.05$.

3. Results

3.1 Clinical Observation

All animals remained healthy during the observation period and all sites of implantation healed uneventfully. There were no signs of infection, edema or the extrusion of implant materials in test group.

3.2 Radiologic Findings

3.2.1 Periapical Radiographic Findings

Four weeks after the surgery, both of test group (a group that bone grafting material was applied) and control group (a group that no bone grafting material was applied) showed that most of the sockets were filled with newly formed bone, revealing pattern of socket being partially remodeled. While test group showed clear border between middle third of the socket and coronal third, as well as an active bone remodeling pattern in apical third, only the border of coronal third was clear in control group, which indicates that bone remodeling also takes place in middle third. Moreover, the particles of the bone grafting material were still clearly observed in coronal third in Group 2.

Both of the test group and control group showed remodeling of most of the socket wall 12 weeks after the surgery. The bone density inside the socket was higher in test group than in group, and especially, the particle of bone grafting material was clearly observed in coronal third. The bone level of the socket was also higher in test group.

3.2.2 CBCT finding

The radiopaque inside the socket was clear in test group 4 weeks after the surgery, in general. Even though the buccolingual width did not differ between two groups, the lingual bone wall was preserved better in test group.

The overall radiopaque inside the socket was similar 12 weeks after the surgery. No difference was observed between two groups in the measurement of the buccolingual width, and the height of the lingual bone wall was similar to the buccal one

in both of the two groups. However, the overall bone level was higher in test group compared to control group.

There was no difference between test group and control group in bone width either after 4 weeks or after 12 weeks. However, the bone level showed less change in test group compared to control group. A statistically significant difference between control group and test group was observed only in the height of the alveolar ridge among the animals sacrificed after 4 weeks (Table 2 and Figs 4 and 5).

4. Discussion

A morphological change that accompanies extraction can be partially limited by using alveolar ridge preservation surgical approach that is currently used. The resorption of the alveolar ridge after extraction can be minimized by performing extraction using periotome or similar device that minimizes traumatism along with an appropriate use of some of the hard

tissue grafting material, filter film, soft tissue transplant, etc. However, a partial loss of the width and height of the alveolus after extraction is inevitable. The findings that buccal bone is thinner than lingual bone and hence is more susceptible to surgical damage resulting in more resorption than lingual in the same area were reported by many researchers, such as Wilderman *et al.* (1960, 1963), Wood *et al.* (1972), and Araújo *et al.* (2005)¹¹. Especially, according to Schroeder ('tooth-related' tissue 1986), the fact that bundle bone has large proportion in buccal alveolar ridge compared to lingual one implies that the buccal alveolar ridge height decreases even more after the extraction. Bone regeneration in the dogs occurs at a rate of 1.5 times faster than in humans.

In this study, according to the radiological observation in 4 weeks, even if overall healing began in extraction socket in a control group where suture was performed after extraction without any insertion of grafting material, the border of the socket was clearly observed. After 12 weeks, the border of the socket disappeared and it was observed that old bone was mostly

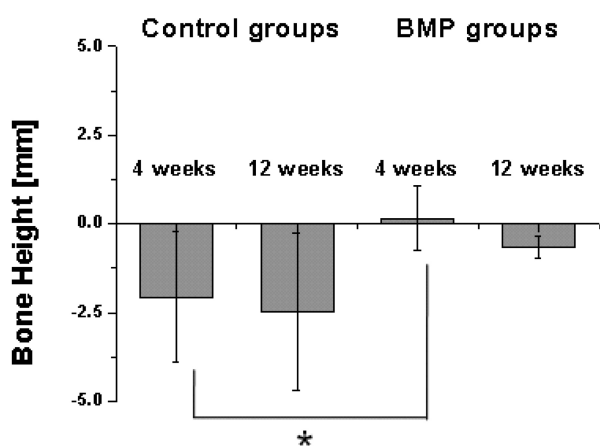


Figure 4. A statistically significant difference between control group and test group was observed in the height of the alveolar ridge sacrificed after 4 weeks.

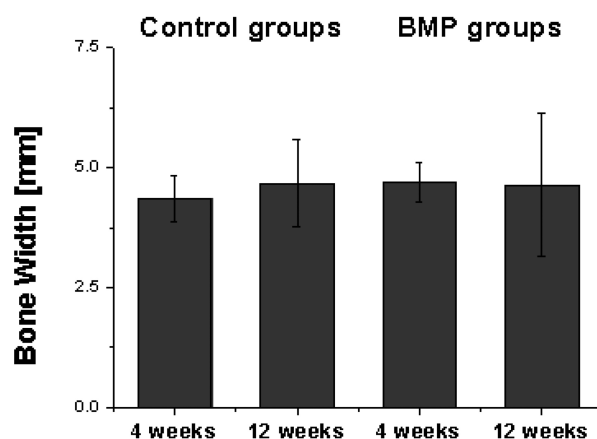


Figure 5. Nosignificant difference between control group and test group was observed in the height.

Table 2. Measurement results of bone level and bone width.

		Dog					
		1	2	3	4	5	6
Bone Level	Control	1.53	0.74	-0.6	-0.93	-0.95	-0.32
	Group	0.27	0	-0.89	-0.4	-0.46	-0.92
	BMP	-1.31	-1.89	-1.07	-1.8	-0.85	-3.3
	graft	-0.29	-5.59	-2.19	-3.56	-1.59	-0.73
Bone width	Control	4.58	5.49	4.68	4.82	6.59	4.53
	Group	4.58	4.43	4.37	4.15	5.57	2.15
	BMP	4.41	4.02	4.03	4.98	5.24	3.64
	graft	4.25	5.28	4.05	4.81	5.86	3.52

replaced by mature bone. This result was similar to Cardaropoli *et al.*¹²(2003), which reports that 88% of the socket were replaced with mineralized bone 30 days after extraction and bone remodeling occurred at the border of the socket after 90 days, where mature bone of bone marrow constituted 61%. However, the border of the socket was unclear in test group after 4 weeks, indicating that bone remodeling occurred due to the repair of socket that was stimulated by the application of BMP-2.

Misch¹³(1993) argued that TCP is appropriate for mixed use for enhancing maneuverability of the osteogenesis such as BMPs or grafting materials with osteoinductivity due to its high stability and abrasion resistance, despite its porosity. Cho *et al.*¹⁴ concluded anorganic bovine bone can be a good carrier for peptide. In a comparison analysis among HA, collagen, and TCP as a mediator of BMP-2 conducted by Wesley(2004), no significant different among the groups was observed, while the groups with added BMP-2 showed difference from the group without added BMP-2. Meanwhile, BMP-2's induction effect of bone formation can differ not only by the type of mediator, but also by the transplant site. Murata¹⁵(1999) proved the osteoinductivity of BMP-2 in submaxilla of dogs and parietal bone of rats, respectively. Nevertheless, the socket is different from the bone loss area used in their studies in its vascular supply and neighboring bone condition. Moreover, the change of bone shows very different pattern after extraction from the change of bone in other areas. However, there are currently not enough studies regarding the effects of BMP-2 application in the socket.

The introduction of cone-beam CT opened a new era of clinical dentistry. Ziegler¹⁶ argued that CBCT provided more information with shorter scanning time and less exposure to radiation, compared to previously used CT. Hence, CBCT was especially suitable for maxillofacial area. With CT, a fan-shaped radioactive beam and continuous detector provide sliced image. These continued cross-sectional images are reprocessed based on many projection data obtained from rapid rotation to be eventually displayed in 3-D. Consequently, the vertical spatial resolution tends to decrease, which often results in failure to obtain accurate image of the anatomical structure in maxillofacial area. On the other hand, CBCT can obtain information from a single rotation that takes about 9~40 seconds, using a cone-shaped radioactive beam and 2-D detector, which provides very high vertical spatial resolution. Hashimoto¹⁷ argued that the accuracy of the image of CBCT is no less than that of CT and that CBCT is less affected by the site of patients' head during the image obtaining procedure than CT.

Moreover, Yajima *et al.*¹⁸ reported that using CBCT provides measurement accuracy of up to 0.1 mm of the distance of anatomical structure, such as mandibular canal or thickness of compact bone. According to Mah¹⁹, the amount of exposure to radiation of CBCT is only about 20% of that of CT and was similar to the full mouth periapical radiograph. In this study, CBCT was used in order to minimize the error of study results by using the digitalized measurement method.

When measuring the bone level after 4 weeks, it can be even higher than the furcation area due to the grafting material of the socket. In this case, the level was expressed in positive rather than in negative. It is conjectured that such difference caused larger difference of bone levels in measurement after 4 weeks than that after 12 weeks. The measurement of bone level after 12 weeks revealed higher value in test group, even though it was not statistically significant.

According to the results of the radiological observation of the effect of BMP-2 TCP that was transplanted to the socket of adult dogs on the alveolar ridge preservation of the socket, the radiopaque inside the socket 4 weeks after the surgery was clearer in test group, which used grafting materials. These new strategy may be applied in treatment of bony defects of periodontal or implant therapy. Even though the width of buccolingual of the alveolar ridge did not show much change, it turned out that the lingual bone wall is maintained better in case of BMP-2 transplantation. Animal and human studies showed that extraction sockets with completely intact bony walls are capable of socket defect bone regeneration on their own.²⁰⁻²² Despite preservation of the alveolar ridge and socket dimensions through the use of a variety of bone graft materials, the dynamics of the extraction socket healing processes reportedly were altered.²³

Both of the control group and test group showed level of the lingual bone wall similar to buccal one in 12 weeks after the surgery. Meanwhile, the overall bone level was higher in case of BMP-2 transplantation.

According to the comparison results of bone width and level using CBCT, the bone width did not differ between test group and control group both after 4 weeks and after 12 weeks, while the bone level showed less change in test group compared to control group. Only the values related to the alveolar ridge level among the animals that were sacrificed after 4 weeks showed statistically significant difference between control group and test group.

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References

1. GE Carlsson, N Ragnarson, P Astrand, Changes in height of the alveolar process in edentulous segments. A longitudinal clinical and radiographic study of full upper denture cases with residual lower anteriors, *Odontol Tidskr*, **75(3)**, 193 (1967).
2. DA Atwood, Some clinical factors related to rate of resorption of residual ridges, *J Prosthet Dent*, **86(2)**, 119 (2001).
3. K Johnson, A study of the dimensional changes occurring in the maxilla following tooth extraction, *Aust Dent J*, **14(4)**, 241 (1969).
4. JM Wozney, Novel regulators of bone formation: molecular clones and activities, *Science*, **242**, 1528 (1988).
5. P Knaus, W Sebald, Cooperativity of binding epitopes and receptor chains in the BMP/TGFbeta superfamily, *Biol Chem*, **382**, 1189 (2001).
6. D Chen, M Zhao, GR Mundy, Bone morphogenetic proteins, *Growth Factors*, **22**, 233 (2004).
7. MR Urist, Bone formation by autoinduction, *Science*, **150**, 893 (1965).
8. AH Reddi, Role of morphogenetic protein in skeletal tissue engineering and regeneration, *Nat Biotechnol*, **16**, 247 (1992).
9. U Ripamonti, The critical role of geometry of porous hydroxyapatite delivery system in induction of bone by osteogenin, a bone morphogenetic protein, *Matrix*, **12**, 202 (1992).
10. G Khang, MS Kim, SH Cho, *et al.*, Natural scaffolds biomaterials for tissue regeneration, *Tissue Eng Regen Med*, **1(1)**, 9 (2004).
11. MG Araujo, J Lindhe, Dimensional ridge alterations following tooth extraction. An experimental study in the dog, *J Clin Periodont*, **32**, 212 (2005).
12. G Cardaropoli, M Araujo, J Lindhe, Dynamics of bone tissue formation in tooth extraction sites. An experimental study in dogs, *J Clin Periodont*, **30**, 809 (2003).
13. IH Chung, YH Kang, HJ Ryu, *et al.*, Bioengineering of the tooth via organ culture system, *Tissue Eng Regen Med*, **1(1)**, 47 (2004).
14. YJ Cho, SI Yeo, JW Park, *et al.*, The effects of synthetic peptide derived from hBMP-2 on bone formation in rabbit calvarial defect, *Tissue Eng Regen Med*, **5(3)**, 488 (2008).
15. M Murata, BZ Huang, T Shibata, *et al.*, Bone augmentation by recombinant human BMP-2 and collagen on adult rat parietal bone, *Int J OralMaxillofac Surg*, **28(3)**, 232 (1999).
16. CM Ziegler, R Woertche, J Brief, *et al.*, Clinical indications for digital volume tomography in oral and maxillofacial surgery, *Dentomaxillofacial Radiology*, **31**, 126 (2002).
17. K Hashimoto, S Kawashima, M Araki, *et al.*, Comparison of image performance between conebeam computed tomography for dental use and four-rowmultidetectorhelical CT, *J Oral Scien*, **48**, 27 (2006).
18. A Yajima, M Otonari-Yamamoto, T Sano, *et al.*, Cone-beam CT(CB Throne) applied to dentomaxillofacial region, *Bulletin of Tokyo Dental College*, **47**, 133 (2006).
19. JK Mah, RA Danforth, A Bumann, *et al.*, Radiation absorbed in maxillofacial imaging with a new dental computed tomography device, *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, **96**, 508 (2003).
20. MH Amler, Histological and histochemical investigation of human alveolar socket healing in undisturbed extraction wounds, *J Am Dent Assoc*, **61**, 32 (1960).
21. IH Chung, PH Choung, YY Jo, Bioengineering of the tooth and jaw, *Tissue Eng Regen Med*, **2(2)**, 86 (2005).
22. Y Ohta, Comparative changes in microvasculature and bone during healing of implant and extraction sites, *J Oral Implantol*, **19**, 184 (1993).
23. W Becker, BE Becker, R Caffesse, A comparison of demineralized freeze-dried bone and autologous bone to induce bone formation in human extraction sockets, *J Periodontol*, **65**, 1128 (1994).