Alveolar ridge augmentation using anodized implants coated with *Escherichia coli*–derived recombinant human bone morphogenetic protein 2

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Objective. The aim of this study was to examine the effect of *Escherichia coli*–derived recombinant human bone morphogenetic protein 2 (ErhBMP-2) coated onto anodized implant to stimulate local bone formation, including osseointegration and the vertical augmentation of the alveolar ridge.
Study design. Six young male adult beagle dogs were used. A crestal area was leveled on both sides of each test subject by removing minimal cortical bone using a round bur and without exposing cancellous bone. After a 2-month healing period, 3 anodized implants (length 8 mm, diameter 4 mm; Cowellmedi, Busan, Korea) were placed 5 mm into the mandibular alveolar ridge in either side. Each animal received 6 implants that were either coated with ErhBMP-2 (0.75 or 1.5 mg/mL concentration; Cowellmedi) or uncoated. This was performed using a randomized split-mouth design. A total of 36 implants were used for this study. Twelve noncoated implants were used as control, and 24 BMP-coated implants were used as our experimental group, which was further divided into 2 groups of 12 implants each with different BMP concentration of 0.75 and 1.5 mg/mL. Radiologic examinations were performed immediately after implant placement and 4 and 8 weeks after implant placement. The amount of bone augmentation was evaluated by measuring the distance from the uppermost point of the cover screw to the marginal bone. Implant stability quotient (ISQ) values were measured immediately after surgery and 8 weeks after implant placement. Statistical analysis was performed using one-way analysis of variance (SPSS version 17.0) and multiple-comparison tests. Statistical significance was established at the 95% confidence level.
Results. Implants coated with ErhBMP-2 at 0.75 mg/mL (BMP 0.75 group) and 1.5 mg/mL (BMP 1.5 group) exhibited significant vertical bone formation compared with the control group (mean ± SD): 0.88 ± 0.94 versus 0.60 ± 0.64 versus −0.52 ± 0.64 mm, respectively; $P < .05$. There was a significant difference between the 3 groups in bone level change ($P < .05$). The BMP 0.75 and BMP 1.5 groups exhibited significant changes in ISQ compared with the control group: 8.17 ± 8.31 versus 11.50 ± 9.02 versus 2.17 ± 7.61, respectively; $P < .05$.
Conclusion. Within the limits of this study, the ErhBMP-2 coating on an anodized implant may stimulate vertical bone augmentation, which significantly increases implant stability on completely healed alveolar ridges. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;xx:xxx)**

The current trend in the surface treatment of implants, which are developed from smooth machined surfaces, is to apply biologically active factors, such as bone morphogenetic protein (BMP), on implant surfaces to shorten the healing period and to promote osseointegration and bone augmentation. BMP, a protein from a subgroup of the transforming growth factor β superfamily,[1] promotes ossification by controlling the essential factors of the bone induction cascade to facilitate the biosynthesis of bone matrix and the proliferation of osteoblasts from the mesenchymal stem cells.[2-7] BMP can be classified into several subgroups. BMP-2, one of

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the subgroups, has been proven by preclinical and clinical studies for use for various medical treatments.[8] It has been reported that recombinant human BMP-2 (rhBMP-2), which is produced by a gene recombination technique, can be an effective carrier when coated on the anodized surfaces of implants.[9,10]

Recently, rhBMPs have been produced by BMP gene-transfected mammalian cell (Chinese hamster ovary [CHO]) cultures,[11,12] and rhBMP-2 and BMP-7 (rhBMP-7/osteogenic protein 1) are commercially available for the treatment of bony defects.[13,14] Problems associated with clinical application of CHO cell–derived rhBMP-2 (CrhBMP-2) are its low yield (ng/mL) and high cost due to the need for high doses. To develop a viable commercial product, the cost of acquisition must also be considered. One possible way of solving this problem is production of monomer rhBMPs in BMP gene–transformed *Escherichia coli*, which has a high efficiency of production and low cost. Besso et al.[15] examined the bone-inducing ability of an *E. coli*–derived rhBMP-2 (ErhBMP-2) variant with an N-terminal sequence and compared it with CrhBMP-2. Quantitative analysis has indicated that the activity of ErhBMP-2 is similar to that of CrhBMP-2.

Hall et al.[9] have reported that heterotopic ossification is faster on the anodized porous surface of an implant than on a smooth surface when applied to the breast of a rat. Leknes et al.[16] have demonstrated that when implants coated with rhBMP-2 are placed in the 5-mm-deep vertical defects processed right after extracting teeth in adult dogs, a higher bone augmentation can be obtained compared with control dogs which were given implants without coating. In the study by Leknes et al.,[16] most of the vertical defects were formed by removing alveolar ridges after extracting the teeth, which exposed a wide area of myeloid tissue, resulting in high cell activity and easy flap manipulation. However, in a clinical setting implants are placed when physiologic healing, and corticalization has been achieved after tooth extraction.

There has been no study on the effect of implants coated with ErhBMP-2 on bone augmentation in alveolar bones physiologically completely healed after tooth extractions. Therefore, the present study aimed to evaluate the effect of anodized implants coated with ErhBMP-2 on the augmentation of the alveolar bone in vertical bone defects, which are formed in physiologically completely healed alveolar bones after tooth extraction.

MATERIALS AND METHODS

Animals

Six healthy male adult beagle dogs, aged 2 -3 years and weighing 10-15 kg, were used for this study. None of the dogs had general dental problems. Animal selection management and the surgical protocol were approved by the Ethics Committee on Animal Experimentation of Chonnam National University (CNU IACUC-YB-R-2010-10). The dogs were caged in single-unit housing at a temperature of 23 ± 3°C and a relative humidity of 40 ± 10% and fed with a soft diet (Science Diet, Hill's Co., Topeka, KS, USA) twice daily, with water provided ad libitum.

Preparation of implants

Thirty-six implants (length 8 mm, diameter 4 mm; Cowellmidi Co., Busan, Korea) were fabricated. All thread-type implants were made from pure titanium and were designed to have microthreads on the upper part and wider threads on the lower part. A total of 36 implants were used for this study. Twelve noncoated implants were used as control, and 24 BMP coated implants were used as our experimental group, which was further divided into 2 groups of 12 implants each with different BMP concentration. The surfaces of each implant were properly anodized (watery phosphoric acid and sulfuric acid solution used at low voltage in a DC field) and coated with ErhBMP-2 (Cowellmedi) at concentrations of 0.75 mg/mL (BMP 0.75 group) and 1.5 mg/mL (BMP 1.5 group). To coat with ErhBMP-2, they were immersed 3 times in protein solution up to the microthreads of the implants and were lyophilized (freeze drying at −40°C, followed by vacuum drying at a maximum 20°C). The amounts of protein coated on were 20 μg in the BMP 1.5 group and 10 μg in the BMP 0.75 group.

Primary operation

Food was withheld the night before surgery. The animals were administered atropine sulfate (.05 mg/kg SC; Dai Han Pharm Co., Seoul, Korea) and tiletamine/zolazepam (5 mg/kg IV; Zoletil 50; Virbac, Carros, France). During the operation, anesthesia was maintained with isoflurane (Choongwae Co., Seoul, Korea) and oxygen. Lactated Ringer solution was administration at a rate of 5 mL/kg/h until the completion of the surgical procedure. Ampicillin sodium (Penbrook, 20 mg/kg IV; Chong Kun Dang Co., Seoul, Korea) and meloxicam (Metacam, 0.2 mg/kg IV; Boehringer Ingelheim Co., Ridgefield, CT, USA) were administration before the operation. After performing infiltration anesthesia in the area of the experiment by using lidocaine (Yu-Han Co., Gunpo, Korea) that contained 1:100,000 epinephrine, the maxillary first, second, third, and forth premolar teeth were surgically extracted. In the mandible, all premolars and the first molars were surgically extracted. A tooth furcation area was cut using a fissure bur and the mesiodistal root was carefully extracted while attempting not to damage the extraction socket. The residual root was checked with radiological examination of the root apex, and suture

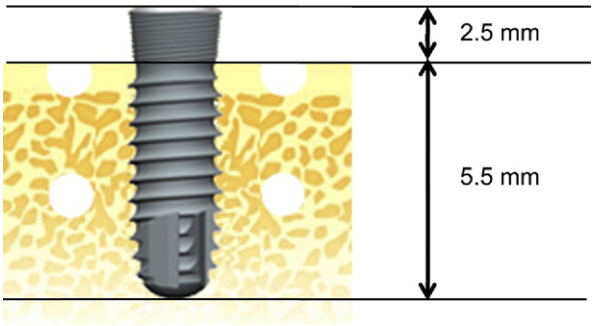

Fig. 1. Schematic diagram of the implant position. In each group, 12 implants were inserted, and the position of implant placement per group was randomized by the split-mouth design. While maintaining cortical bone, the implants were placed so that 2.5 mm of the upper part was exposed out of the alveolar bone with the microthread as the guide. To reduce bleeding, cancellous bone between the implants was exposed by punching out cortical bone (buccal, crestal and lingual aspects) using a 1-mm round bar.

with 4-0 nylon (Mersilk, Ethicon Co., Livingston, UK) was carried out to promote healing. After all surgical procedures, the dog was placed on meloxicam (Metacam, 0.1 mg/kg PO; Boehringer Ingelheim Co., Ridgefield, CT, USA) for pain and received amoxicillin (amoxicillin, 20 mg/kg PO; Choongwae Co.) every 12 hours for 6 days.

Secondary operation

Approximately 2 months after the primary operation and after confirming that the alveolar bone was sufficiently healed, general and local anesthesia were applied with the same method as during the primary operation. Three implants were placed on either side of the lower jaw. The position of implant placement per group was randomized by the split-mouth design.

After crestal incision and separation of a full-thickness flap, the crestal area was leveled by removing minimal cortical bone using a round bur. The guide drill in the implant surgical set (Cowellmedi) was used to drill cortical bone, and a 2-mm first drill, a 3-mm pilot drill, and a 3.2-mm final drill were used sequentially before countersinking. Then 6 implants were placed in the edentulous mandible on both sides. Because ErhBMP-2 released from ErhBMP-2–coated implants could modify the surrounding environment, the experimental implants were placed at one side of the jaw and the control implants on the other side. The implants protruded from the top of the alveolar bone by 2.5 mm with the microthread of the implant as the guide (Fig. 1). After confirming the arrangement of the implant and the crestal area by radiologic examination of the root apex, a cover screw was applied, incision was performed inside the periosteum to form a tension-free flap, and suture was applied with 5-0 Gore-Tex (W.L. Gore and Associates, Flagstaff, AZ, USA).

Control after operation

The experimental animals were treated with penicillin G procaine and penicillin G benzathine by intramuscular injection (1 mL/5 kg) immediately following and 48 hours after the operation. Two-percent chlorhexidine was sprayed into their mouths once or twice a day for plaque control by using a syringe. The dogs were fed a soft diet.

Radiographic examination

Radiologic studies were conducted immediately and 4 and 8 weeks after the operation using a portable X-ray system (Port-X II, Genoray, Co, SungNam, Korea). The intraoral paralleling technique was used while filming, where the film and implant were parallel and perpendicular to the cone. The marginal bone level was estimated with the PACS software (Digi-X verson 2.7.5.1; Hanjin Digi-X Co., Seoul, Korea). The actual distance between the macrothreads was measured and compared with the distance (0.8 mm) between the macrothreads on each image, and then corrections were made to minimize errors on the radiographic images.

Implant stability measurement

The stability of implants was measured using the Osstell Mentor (Integration Diagnostic, Göteborg, Sweden) immediately after and 8 weeks after implant placement. The values were recorded 5 times for each implant, and 3 values excluding the minimum and maximum values were used for calculating the mean and standard deviation for the evaluation of changes in implant stability.

Statistical analysis

Statistical analysis was performed using 1-way analysis of variance and the multiple comparison test. Statistical significance was established at the 95% confidence level. SPSS for Windows (version 17.0; SPSS, Chicago, IL, USA) was used for data analysis.

RESULTS

Surgical findings

Eight weeks after flap elevation, the 2 experimental groups showed more bone formation in both vertical and horizontal dimensions than the control group (Fig. 2). However, no difference was noted between the 2 experimental groups.

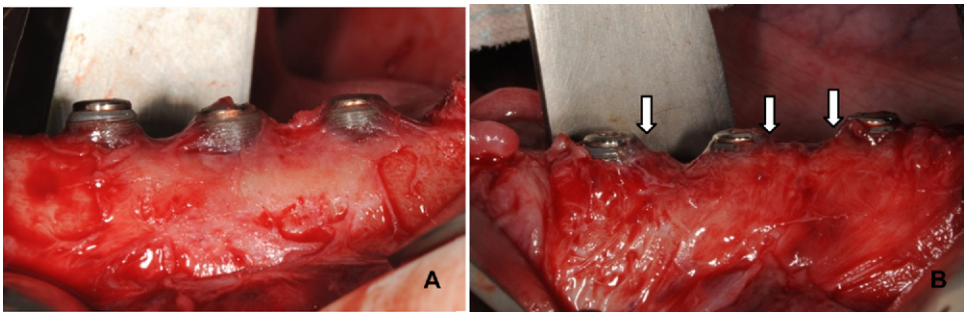

Fig. 2. Surgical findings before killing the animals. In the control group, there was no evidence of bony overgrowth (**A**), but in the 0.75 group (**B**; 0.75 mg/mL concentration), there was newly overgrowthed compact bone (*arrows*). However, there was no significant difference between the 2 experimental groups.

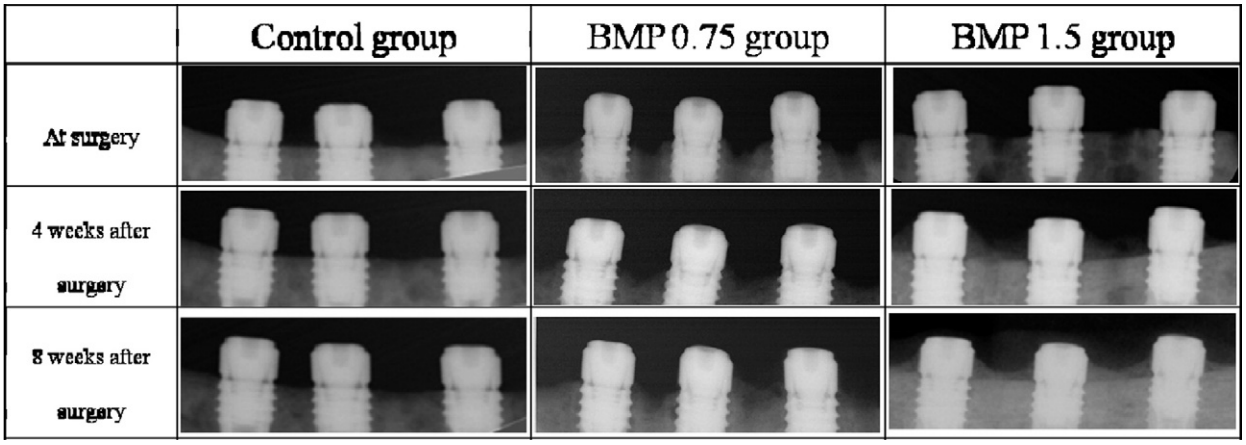

Fig. 3. Radiographs of the 2 experimental groups and the control group at different time points. The 2 experimental groups showed vertical bone gain at 4 and 8 weeks, but the control group did not show any significant changes.

Radiographic findings

The radiograph showed marginal bone augmentation along the fixture in the 2 experimental groups, whereas no significant change in the marginal bone level was observed in the control group (Fig. 3). The average mean (±SD) of bone augmentation was 0.52 (±0.48) and 0.60 (±0.64) at 4 and 8 weeks, respectively, in the BMP 0.75 group and 0.89 (±0.79) and 0.88 (±0.94) at 4 and 8 weeks, respectively, in the BMP 1.5 group. In contrast, the control group showed bone loss with an average loss rate of −0.22 (±0.56) at 4 weeks and −0.52 (±0.64) at 8 weeks. There was a significant difference between the 2 experimental groups and the control group ($P < .05$). However, there was no significant difference between the 2 experimental groups ($P > .05$; Figs. 4 and 5; Table I).

Changes in implant stability

Comparing ISQ values immediately after surgery and at 8 weeks, ErhBMP-2–coated implants in the 2 experimental groups showed increased values ($P < .05$). The mean change in ISQ value (±SD) was 2.12 (±7.61) in the control group, 8.16 (±8.31) in the BMP 0.75 group, and 11.50 (±9.02) in the BMP 1.5 group. Although the BMP 1.5 group showed the greatest change, the difference between BMP 0.75 and BMP 1.5 group was not statistically significant.

Complications of operation

Three implants were exposed from the buccal side of the fixture owing to an open flap. Of the 3 implants that were exposed, 2 implants were from the BMP 1.5 group and 1 from the control group. However, they were not related to the implant that failed in osseointegration. Because they did not show any significant difference in the bone level with the submerged implant between the 2 experimental groups and the control group, the data were included in the evaluation.

DISCUSSION

BMPs were originally identified as osteoinductive proteins >40 years ago.[1] Numerous studies and clinical

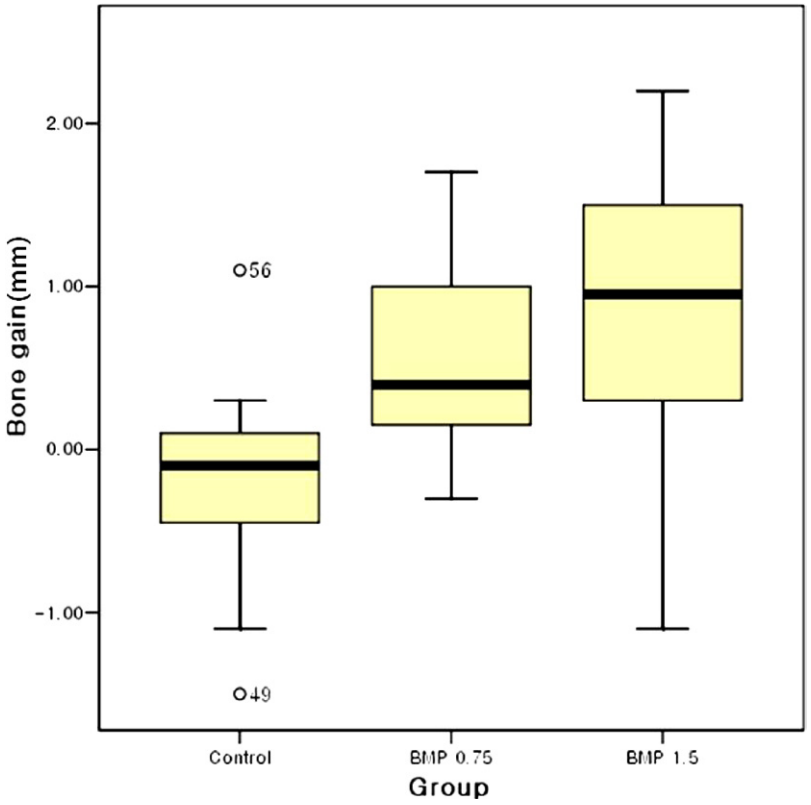

Fig. 4. Box-plot chart of bone gain at 4 weeks. The 2 experimental groups showed increased vertical bone volume at 4 weeks. There was no statistically significance difference between the 2 experimental groups, whereas the 2 experimental groups showed a significantly different increase in vertical bone volume compared with the control group.

applications of BMP have been performed since BMP genes were first cloned. Earlier, most rhBMPs were produced in mammalian cells, such as CHO cells.[12] Problems associated with the general clinical use of BMPs are the low yield (ng/mL) and their high cost of production.[17] Many attempts have been made to produce and evaluate biologically active rhBMPs in *E. coli* as an alternative to mammalian cells.[15,17] Sebald's team devised a novel method to produce rhBMP-2–derived from *E. coli* and to convert BMP monomers to biologically active dimers (ErhBMP-2).[18,19] ErhBMP-2 has an osteoinductive activity that is similar to that of CHO-derived rhBMP-2 both in vivo and in vitro.[20]

A sufficient volume of alveolar bone covering the whole implant is required for the placement and long-term success of dental implants.[21] In some clinical cases in which physiologic bone resorption has developed in the edentulous area, the vertical height of alveolar bone may be insufficient to accommodate implants of an ordinary length. The present study was carried out to test whether coating implants with ErhBMP-2 could induce bone augmentation under such conditions.

There were several limitations in this study. First, we used a supra-alveolar defect model in the beagle dog mandible. Possibly the most difficult aspect in this model was to prevent flap dehiscence after surgery. Despite doing our best to achieve tension-free flap closure, 3 implants were exposed. Second, bone quality in the mandible of the beagle dog may have been harder than the human mandible at the sites of implant placement. Therefore, it is uncertain whether ErhBMP-2 can produce the same outcome in human bone. Third, the sample size of each group was relatively small to yield substantial results, and thus the study is likely to be underpowered.

This study followed a modification of the supraalveolar defect study model presented by Lekness et al.[16] There are some differences between their model and our modification. First, implants were coated with CHO cell–derived BMP by a dipping method in their study, whereas *E. coli*–derived BMP-2 was lyophilized for easy storage and handling in this study. Second, this study was carried out in a 2.5-mm vertical defect model, and implants were placed within cortical bone as in a clinical situation, whereas their model used

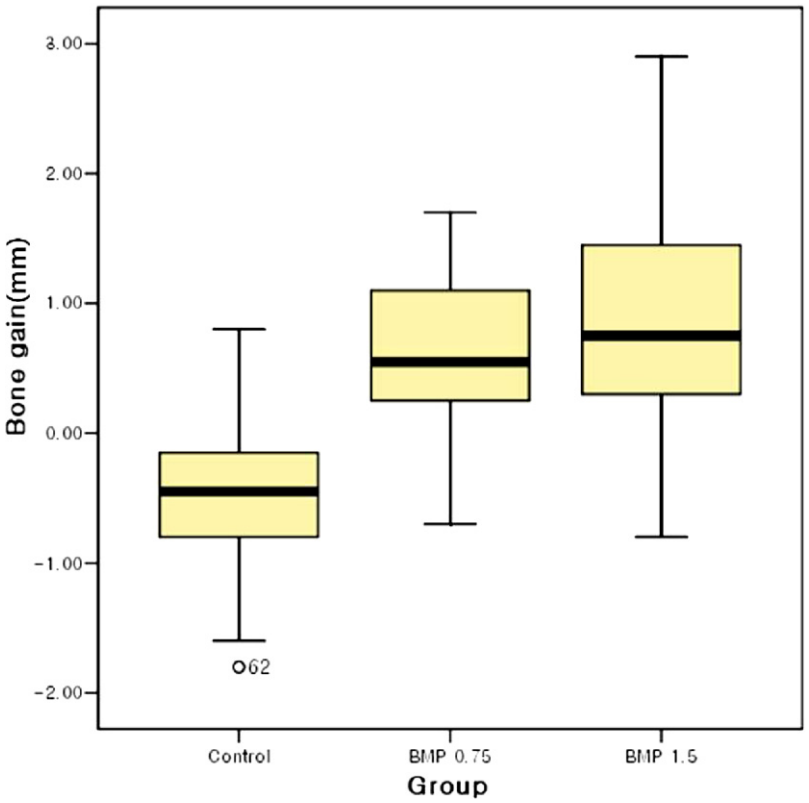

Fig. 5. Box-plot chart of bone gain at 8 weeks. In the 2 experimental groups, there was no significance difference between bone gains at 4 and 8 weeks. A statistically significant increase in vertical bone volume was found between the 2 experimental groups and the control group.

Table I. Mean (±SD) radiographic bone gain (mm) at different time points

	Week 4	*Week 8*
Control group	−.22 (±.56)*	−.52 (±.64)*
BMP 0.75 group	.52 (±.48)	.60 (±.64)
BMP 1.5 group	.89 (±.79)	.88 (±.94)

*$P < .05$.

5-mm vertical defects within cancellous bone. Despite the limitations in the present study with beagle dogs, the experimental groups showed a greater vertical bone gain. Radiography showed bone augmentation of 0.6 mm at 8 weeks in the BMP 0.75 group and 0.88 mm at 8 weeks in the BMP 1.5 group, which filled 25% and 30%, respectively, of the total 2.5-mm defect. The difference in ultimately created marginal bone between the control and BMP 1.5 groups was approximately 1.4 mm, because the control group showed a bone loss of approximately .52 mm at 8 weeks. This result differs from that of Leknes et al.,[16] which showed a bone augmentation of 4.4 mm in the 5-mm defect, and a bone augmentation of 0.9 mm even in the control group. This difference probably resulted from the difference in the cell activities of cortical bone and bone marrow. This discrepancy might also have been caused by the difference in the bone formation abilities of the BMPs derived from *E. coli* and CHO cells. Bessho et al.[15] have reported that the *E. coli*–derived group develops a lower bone density and forms a fattier marrow after performing experiments with the 2 kinds of rhBMP-2. Further studies are necessary to verify this point.

The ISQ values of the 2 experimental groups, which showed insignificant difference at the time of operation compared with that of the control group, became significantly higher at 8 weeks. Comparing ISQ values immediately after surgery and at 8 weeks, ErhBMP-2–coated implants in the 2 experimental groups showed increased values ($P < .05$). Figure 6 shows the significant difference between changes in ISQ values. A small difference in ISQ value was observed in the control group from the time of surgery to 8 weeks after surgery, whereas the experimental groups showed a significant increase in ISQ value compared with control group. It is significant that vertical bone gain has a

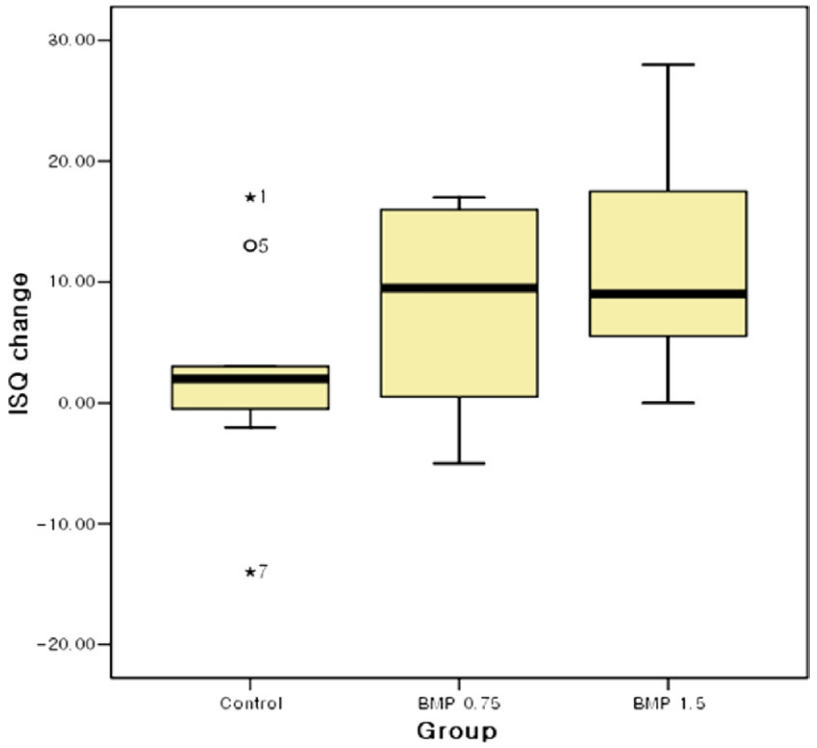

Fig. 6. Box-plot chart of changes in ISQ value at different time points. The ISQ value of the control group was less than that of the 2 experimental groups ($P > .05$), and there was no significant difference in ISQ value between the 2 experimental groups. The 2 experimental groups showed a significant increase in the stability of the implant. Change in ISQ = value at 8 weeks − value immediately after surgery.

possible effect on implant stability, showing a marked increase in ISQ value. Despite the possibility of increase in ISQ value due to the improvement in osseointegration affected BMP, it could not be confirmed in this study, because histometric analysis was not included. Further studies should be carried out to verify the correlation between BMP and osseointegration.

Wikesjö et al.[10] have reported that radiographically transparent areas appear at the early stage of placement of implants coated with 3.0 mg/mL rhBMP-2 and then disappears gradually. The results of that study conflicts with this result. Wikesjö et al. used implants coated with a higher concentration (3 mg/mL) than those used in the present study, and this concentration may have caused a temporary appearance of the radiographically transparent area while the bone around the implant may have completely regenerated. The rhBMP-2 concentration of 0.75 and 1.5 mg/mL has been shown to be safe by Wikesjö et al.[10] No adverse effects were found in our study where only 2 different concentrations (0.75 and 1.5 mg/mL) were used. However, it is uncertain whether this ErhBMP-2 concentration is ideal. Further studies should be performed using various concentrations to confirm these results.

CONCLUSIONS

From the results of this study, it is suggested that anodized implants coated with ErhBMP-2 can induce vertical bone augmentation when placed in the vertical defect of the alveolar bone that has been completely healed after tooth extraction. This provides the possibility of improving the stability of implants and recovery of vertical bone defects without additional bone transplantation.

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