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Research Paper
Dental Implants

Effects of anodized implants coated with *Escherichia coli*-derived rhBMP-2 in beagle dogs

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Abstract. This study evaluated the effects of *Escherichia coli*-derived rhBMP-2 (ErhBMP-2) coated onto anodized implants to stimulate bone formation, osseointegration and vertical bone growth in a vertical bone defect model. Six young adult beagle dogs were used. After a 2-month bone healing period, anodized titanium implants (8 mm in length) were placed 5.5 mm into the mandibular alveolar ridge. Eighteen implants coated with ErhBMP-2 (BMP group) and another 18 uncoated implants (control group) were installed using a randomized split-mouth design. The implant stability quotient (ISQ) values were measured. Specimens were fabricated for histometric analysis to evaluate osseointegration and bone formation. The ISQ values at 8 weeks after implant placement were significantly higher in the BMP group than in the control group ($p < 0.05$). Histological observations showed that the changes in bucco-lingual alveolar bone levels were higher in the BMP group than in the control group ($p < 0.05$). The ErhBMP-2 coated anodized implants can stimulate bone formation and increase implant stability significantly on completely healed alveolar ridges in dogs. Further studies evaluating the effects of ErhBMP-2 on osseointegration in the bone–implant interface are warranted.

Key words: rhBMP-2; coating; anodized implant; osseointegration; bone growth.

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Urist et al.[1] introduced the 'bone induction principle' which states that protein extracts from bone induce ectopic and orthotopic cartilage and bone formation. Recently, further attempts have been made to apply bio-activating elements like recombinant human bone morphogenetic protein (rhBMP-2), a growth factor, onto the implant surface. Only a few studies evaluating the effect of the rhBMP-2 on osseointegration and alveolar bone growth have been completed.[2,3] Wikesjö et al.[4,5] examined the clinical effect of rhBMP-2 around the implant immediately after tooth extraction as well as after considerable bone absorption. This study attempted to determine whether rhBMP-2 coating on an anodized implant is effective in promoting alveolar bone growth on a completely healed alveolar ridge.

BMP, a protein derived from a subgroup of the transforming growth factor β family, accelerates ossification by controlling the essential factors of the bone induction cascade, resulting in the proliferation of osteoblasts from mesenchymal stem cells and the biosynthesis of bone matrix.[6–11] Since BMP-2 possesses high osteoinductive capacity,[12] it has been considered as a prime candidate among

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growth factors for coating titanium implants. BMP-2 has proved to be beneficial and can be used in various medical treatments.[13] It has been reported that rhBMP-2, which is produced by the gene recombination technique, can exert an osteoinductive effect when coated on the implant surface.[2,3,5] In particular, Hall et al.[2] concluded that an osteoinductive effect, including bone contact with the implant surface, is an advantage of titanium porous oxide surfaces (anodized surfaces) yielding the most bone at a low rhBMP-2 dose. Leknes et al.[3] radiologically evaluated the clinical outcomes of implants coated with rhBMP-2 in terms of local bone formation when the implants were placed 5 mm into the alveolar ridge following extraction of the premolar teeth and reduction of the alveolar ridge.

Recently, rhBMPs have been produced by BMP gene-transfected mammalian cell (Chinese hamster ovary (CHO)) cultures,[14,15] and rhBMP-2 and BMP-7 (rhBMP-7/osteogenic protein-1 (OP-1)) are now commercially available for the treatment of bony defects.[16,17] One of the problems associated with the clinical application of CHO-cell-derived rhBMP-2 (CrhBMP-2) is its high cost due to the need for use of high doses for effective treatment. One possible way of solving this problem is to produce monomer rhBMPs from BMP-gene-transfected *Escherichia coli* (*E. coli*), which has a high efficiency of production and low cost. Bessho et al.[18] 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. A quantitative analysis indicated that the activity of ErhBMP-2 was similar to that of CrhBMP-2. It is unclear whether the characteristics of ErhBMP-2 are appropriate for clinical application. In particular, the outcomes and effects of ErhBMP-2 on osseointegration have not yet been determined.

In a previous study by Wikesjö et al.,[19] bilateral, critical-size, supra-alveolar defects were created in the mandibular premolar region, which exposed a wide area of myeloid tissue resulting in high cell activity and easy flap manipulation. In a clinical situation, implants are installed at the completion of physiological healing after tooth extraction. There have been a few studies evaluating the effect of implants coated with ErhBMP-2 on bone augmentation in alveolar bones physiologically completely healed after tooth extraction.

This study aimed to evaluate the effects of *E. coli*-derived rhBMP-2 (ErhBMP-2) coated onto anodized implants to stimulate bone formation, osseointegration and vertical bone growth in a model of vertical bone defects, which are formed in physiologically completely healed alveolar bones after tooth extraction.

Materials and methods

36 implants (8.0 mm in length, 4.0 mm in diameter; Cowellmedi Co, Busan, Korea) were fabricated. All treated implants were made of pure titanium and were designed with microthreads on the upper part and broader threads on the lower part. The implant surface was treated by the anodizing method (Cowellmedi Co), and half of the implants were processed with the ErhBMP-2 coating agent (Cowellmedi Co.). To coat with ErhBMP-2, each implant was immersed 3 times in a protein solution (1.5 mg/ml concentration) up to the microthreads and freeze dried under sterile conditions (freeze drying at −40 °C, followed by vacuum drying at maximum 20 °C). The amount of ErhBMP-2 coated on the surface was about 20 μg.

The surface morphologies of anodized implants without ErhBMP-2 (control group) and anodized implants with ErhBMP-2 (BMP group) were investigated by a scanning electron microscope (SEM, S2300, Hitachi, Japan). The substrates were coated with gold using a sputter coater (Eiko IB, Japan). The SEM was operated at 15 kV.

In vitro ErhBMP-2 release study

Only the anodized surface was used for this in vivo study, but the authors wanted to determine the merits of anodized surfaces regarding release mechanics. The anodized surface coated with ErhBMP-2 was compared with a pristine titanium surface coated with ErhBMP-2 in a release study.

To evaluate the amount of ErhBMP-2 released from the pristine titanium surface coated with ErhBMP-2 and the anodized titanium surface coated with ErhBMP-2, each sample was immersed in 50 ml conical tube containing 1 ml of PBS buffer (pH 7.4) and gently shaken at 100 rpm at 37 °C. At predetermined time intervals of 1 h, 3 h, 6 h, 10 h, and 1, 3, 5, and 7 days, the supernatants were collected and replaced with fresh PBS. All samples were was stored at −20 °C until analysis. The amount of ErhBMP-2 released from the surface was evaluated with an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions using a microplate reader (Bio-Rad, Hercules, CA, USA) at 450 nm. Cumulative release of ErhBMP-2 was determined as a percentage of initial loading concentration.

Experimental animals and surgery

The animal selection management and the surgical protocol were approved by the Ethics Committee on the Animal Experimentation of Chonnam National University (IACUC-YB-R-2010-10). Six 3-year-old beagle dogs, approximately 10–15 kg in weight, were used for the study and were allowed to acclimatize for 2 weeks. The animals were fed a soft diet and had free access to water.

During the first surgery, the premolars and molars of the upper and lower jaws were extracted. The dogs were premedicated with atropine sulfate (0.05 mg/kg intramuscular injection; Dai Han Pharm Co., Seoul, Korea) and anaesthesia was maintained using isoflurane (Choongwae Co., Seoul, Korea). 1 ml of a mixture of lidocaine (Yu-Han Co., Gunpo, Korea) and 1:100,000 epinephrine was infiltrated into the mucosa at the surgical sites. Each premolar and molar was separated into two pieces, the mesial and distal roots. Care was taken to preserve the buccal, lingual, and lateral walls of the alveolar socket. The two pieces were carefully extracted without causing any damage to the extraction site. The extraction site was sutured using 4-0 nylon (Mersilk, Ethicon Co., Livingston, UK) to enhance healing. The extraction sites were allowed to heal for 2 months.

The second surgery was performed 2 months after complete healing of the extraction socket. Local infiltration and general anaesthesia were performed in the same way as in the first surgery. Experimental implants were installed into the edentulous mandibular alveolar ridges 2 months after surgical extraction. All 36 anodized implants with ErhBMP-2 (BMP group) or without ErhBMP-2 (control group) were installed using the Cowellmedi (Busan, Korea) implant system. Three experimental implants were installed in the right and left edentulous mandibular alveolar ridge areas each using a split-mouth design. Treatments were randomized between the left and right jaw quadrants in all dogs. Then, 5.5 mm implants were placed within the reduced alveolar ridges to the depth of the reference notch, creating 2.5 mm, supra-alveolar, peri-implant defects (Fig. 1a). In order to ensure symmetrical placement of implants on both sides, the planned implant placement sites along the exposed