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Alveolar ridge augmentation using implants coated with recombinant human bone morphogenetic protein-7 (rhBMP-7/rhOP-1): radiographic observations

Knut N. Leknes[1], Jie Yang[2], Mohammed Qahash[3], Giuseppe Polimeni[3], Cristiano Susin[3] and Ulf M. E. Wikesjö[3]

[1]Department of Clinical Dentistry – Periodontics, Faculty of Medicine and Dentistry, University of Bergen, Bergen, Norway; [2]Department of Oral and Maxillofacial Pathology, Medicine and Surgery, Division of Oral and Maxillofacial Radiology, Temple University School of Dentistry, Philadelphia, PA, USA; [3]Laboratory for Applied Periodontal and Craniofacial Regeneration, Departments of Periodontics and Oral Biology and Maxillofacial Pathology, Medical College of Georgia School of Dentistry, Augusta, GA, USA

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Abstract

Aim: The objective of this study was to radiographically evaluate the potential of a purpose-designed titanium porous-oxide implant surface coated with recombinant human bone morphogenetic protein-7 (rhBMP-7), also known as recombinant human osteogenic protein-1 (rhOP-1), to stimulate alveolar ridge augmentation.

Material and Methods: Six young-adult Hound Labrador mongrel dogs were used. Three 10 mm titanium oral implants per jaw quadrant were placed 5 mm into the alveolar ridge in the posterior mandible following surgical extraction of the pre-molar teeth and reduction of the alveolar ridge leaving 5 mm of the implants in a supra-alveolar position. The implants had been coated with rhBMP-7 at 1.5 or 3.0 mg/ml and were randomized to contralateral jaw quadrants using a split-mouth design. The mucoperiosteal flaps were advanced, adapted, and sutured to submerge the implants. Radiographic registrations were made immediately post-surgery (baseline), and at weeks 4 and 8 (end of study).

Results: rhBMP-7-coated implants exhibited robust radiographic bone formation. At 8 weeks, bone formation averaged 4.4 and 4.2 mm for implants coated with rhBMP-7 at 1.5 and 3.0 mg/ml, respectively. There were no significant differences between the rhBMP-7 concentrations at any observation interval. A majority of the implant sites showed voids within the newly formed bone at week 4 that generally resolved by week 8. The newly formed bone assumed characteristics of the resident bone.

Conclusions: The titanium porous-oxide implant surface serves as an effective carrier for rhBMP-7 showing a clinically significant potential to stimulate local bone formation.

Key words: BMP-7; bone; bone morphogenentic protein; dog; OP-1; oral/dental implant; radiology; seroma; tissue engineering; titanium

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Conflict of interest and source of funding statement

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Bone is a living structure, a framework of proteins reinforced by calcium phosphate, and capable of regeneration. The process of bone formation, resorption, and remodelling occurs through the dynamic integration of biochemical, cellular, and hormonal events (Reddi 1992, Kalfas 2001). Three parameters appear essential for successful tissue engineering of bone: (1) soluble osteoinductive signals; (2) cells capable of differentiating into bone-forming cells; and (3) a suitable extracellular matrix (Ripamonti & Duneas 1996, Reddi 2000, Bianco & Robey 2001). One overall goal for tissue engineering of bone has been to mimic

native processes by designing biomaterials capable of initiating predictable bone formation.

Bone matrix is known to contain growth and differentiation factors including basic and acidic fibroblast growth factor, insulin-like growth factor I and II, transforming growth factor-β, platelet-derived growth factor, and bone morphogenetic proteins (BMPs). The BMPs, a group of related proteins originally identified by their presence in extracts of demineralized bone (Urist 1965), can be divided into three subfamilies based on the primary amino acid sequence in the mature region of the molecule (Wozney 1992). A primary action of several BMPs in post-foetal life includes differentiation of mesenchymal precursor cells into cartilage- and bone-forming cells (Thies et al. 1992, Yamaguchi et al. 1992).

Carrier technologies are required to transfer osteogenic or osteoinductive factors, to maintain them at the site of implantation, and to optimize their release for bone formation (Babensee et al. 2000, Uludag et al. 2001). A concept of applying BMPs onto titanium surfaces has been evaluated in rodent ectopic and orthotopic models (Kawai et al. 1993, Herr et al. 1996, Cole et al. 1997, Esenwein et al. 2001, 2003, Vehof et al. 2001, Schmidmaier et al. 2002). The bone inductive potential of a purpose-designed titanium porous-oxide implant surface (Hall & Lausmaa 2000) coated with recombinant human BMP-2 (rhBMP-2) has been evaluated in a rat ectopic model, and in canine and nonhuman primate orthotopic models (Hall et al. 2007, Leknes et al. 2008, Wikesjö et al. 2008a, b, c). Using a standardized canine supra-alveolar peri-implant defect model (Wikesjö et al. 2006), it was shown that rhBMP-2 coated onto a titanium porous-oxide implant surface induced robust radiographic bone formation extending to and above the implant platform as evaluated at weeks 4 and 8 post-surgery (Leknes et al. 2008). The histologic and histometric evaluation showed that this novel implant technology supported clinically relevant bone formation and osseointegration (Wikesjö et al. 2008c). It was also shown that higher doses of rhBMP-2 were associated with some undesirable side effects. Whether this purpose-designed titanium porous-oxide implant surface combined with other members of the BMP family possesses similar bone-inductive potential remains unknown. The objective of this study was to radiographically evaluate the bone-inductive potential of the titanium porous-oxide implant surface coated with rhBMP-7, also known as recombinant human osteogenic protein-1 (rhOP-1), using the supra-alveolar peri-implant defect model in the dog.

Material and Methods

Animals

Six male Hound Labrador mongrel dogs, age 10–12 months, weight 20–25 kg, obtained from a USDA-approved dealer were used. Animal selection and management, surgery protocol, and alveolar defect preparation followed routines approved by the local Institutional Animal Care and Use. The animals were fed a canned soft dog food diet throughout the study.

Titanium implants

Titanium porous-oxide surface-modified oral implants (TiUnite™, $\varnothing 4.0 \times 10$ mm; Nobel Biocare AB, Göteborg, Sweden) were used. The titanium implants, custom made for the supra-alveolar peri-implant defect model, were manufactured with a reference notch 5 mm from the implant platform. The reference notch was designed to facilitate the surgical placement leaving 5 mm of the implant in a supra-alveolar position and to serve as a reference point in the radiographic and histologic analysis. The sterile implants were coated with rhBMP-7 at 1.5 or 3.0 mg/ml. rhBMP-7 at 3.0 mg/ml in a 5% lactose vehicle (Stryker Biotech, Hopkinton, MA, USA) and 5% lactose vehicle alone (Stryker Biotech) were shipped overnight on dry ice to the surgical laboratory and stored at -80°C until use. Using aseptic technique, rhBMP-7 solutions at 1.5 mg/ml were prepared by diluting the 3.0 mg/ml rhBMP-7 solution with 5% lactose. Sterile implants were placed into sterile 0.5 ml wells (96 MicroWell™ Plates – Round Well Polypropylene, Nunc™ A/S, Roskilde, Denmark) and the wells were filled with 0.4 ml freshly prepared 1.5 or 3.0 mg/ml rhBMP-7 solution to reach the implant platform. Implants were incubated in the rhBMP-7 solution for 30 min. and were then moved to air-dry for a minimum of 6 h or overnight before implantation. All preparations were performed in a Biogard, Class II, type A, laminar flow hood (Baker Company, Sanford, ME, USA) at room temperature.

Surgery and experimental procedures

Food was withheld the night preceding surgery. The animals were pre-anaesthetized with atropine (0.02–0.04 mg/kg; IM), buprenorphine HCl (0.01–0.03 mg/kg; IM), and acepromazine (0.2–0.3 mg/kg; IM). After tranquilization, an intravenous catheter was placed in the foreleg for induction with propofol (5–7 mg/kg; IV). Animals were moved to the operating room and maintained on gas inhalation anaesthesia (1–2% isoflurane/O_2 to effect). Conventional dental infiltration anaesthesia (lidocaine 2% epinephrine 1:100,000) was used at the surgical sites. The animals received a slow constant rate infusion of lactated Ringer's solution (10–20 ml/kg/h IV) to maintain hydration during surgery.

One experienced surgeon (U. M. E. W.) performed all experimental surgical procedures. In brief, bilateral, critical-size, supra-alveolar peri-implant defects (Wikesjö et al. 2006) were created in the mandibular pre-molar region (Fig. 1). Buccal and lingual mucoperiosteal flaps were reflected and alveolar bone removed around the circumference of the pre-molar teeth to a level approximately 6 mm from the cemento-enamel junction using water-cooled rotating burs. The pre-molar teeth were then extracted and the first molar amputated at the level of the reduced alveolar crest. Three titanium implants were placed into osteotomies prepared into the extraction sites of the third and fourth pre-molar teeth in each jaw quadrant. A

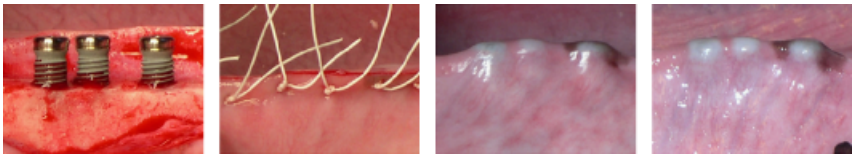

Fig. 1. Critical size, supra-alveolar peri-implant defect in the mandibular pre-molar region (left) following wound closure advancing the mucogingival flap to cover the implants (left centre), and following 4 (right centre) and 8 (right) weeks of healing.

few implants were placed into osteotomy defects prepared in the reduced alveolar process when placement into extraction sites was impossible. Five mm of the implant was placed within the surgically reduced alveolar ridge to the level of the reference notch, creating 5 mm, supra-alveolar, peri-implant defects.

The animals received implants coated with rhBMP-7 at a concentration of 1.5 or 3.0 mg/ml in contralateral jaw quadrants. Treatments were randomized between left and right jaw quadrants in consecutive animals (Table 1). The periostea were fenestrated at the base of the mucogingival flaps to allow tension-free apposition and wound closure. The flaps were advanced 3–4 mm coronal to the implants and the margins adapted and sutured (GORE-TEX™ Suture CV5, W.L. Gore & Associates Inc., Flagstaff, AZ, USA). Photographs were taken following implant placement and wound closure. It might be argued that it would be desirable to include uncoated control implants in the study.

The maxillary first, second, and third pre-molar teeth were surgically extracted and the maxillary fourth premolars reduced in height and exposed pulpal tissues sealed (Cavit®, ESPE, Seefeld/Oberbayern, Germany) in order to alleviate potential trauma from the maxillary teeth to the experimental mandibular sites.

This study was part of a larger study including a robust control group (Wikesjö et al. 2006). 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 (Russell & Burch 1959, Institute of Laboratory Animal Resources 1996).

Post-surgery procedures

A long-acting opioid, buprenorphine HCl (0.01–0.03 mg/kg IM) was administered immediately post-surgery and re-dosed BID for 3 days. A broad-spectrum antibiotic (enrofloxacin; 2.5 mg/kg IM) was administered immediately post-surgery and re-dosed BID for 7 days. Sutures were removed under sedation (propofol; 5–7 mg/kg IV) at approximately 10 days. Plaque control was maintained by daily flushing the oral cavity with chlorhexidine gluconate (Xttrium Laboratories Inc., Chicago, IL, USA; 20–30 ml of a 2% solution) until completion of study. Observations of experimental sites with regards to gingival health, maintenance of suture line closure, oedema, and evidence of tissue necrosis or infection were recorded daily.

Radiographs were taken under sedation (propofol; 5–7 mg/kg IV bolus) immediately post-surgery (baseline), and at weeks 4 and 8 using a mobile X-ray unit (Philips Oralix 70, Monza, Italy) and a standardized protocol at 70 kVp, 7 mA, 30 impulses, and ANSI size #4 Kodak ultra-speed film (Eastman Kodak Company, Rochester, NY, USA). To standardize the radiographic procedure, the mandibles of the dogs were placed flat on the films and the distance from the focal spot to the films was approximately 6 in. The projection angle was 65° from the operating table. The radiographs were processed using an automatic dental film processor (A/T 2000, Air Techniques, Hicksville, NY, USA).

The animals were anaesthetized and euthanized at week 8 post-surgery by an intravenous injection of concentrated sodium pentobarbital (Euthasol®, Delmarva Laboratories Inc., Midlothian, VA, USA). Following euthanasia, block sections including titanium implants, alveolar bone, and surrounding mucosa were collected and radiographed.

Radiographic analysis

Two masked experienced examiners (K. N. L., J. Y.) evaluated computer enhanced radiographic images obtained immediately post-surgery (baseline), and at weeks 4 and 8 in a dark room. The radiographs had been converted to digital images using a film scanner (Epson Perfection® 2400 Photo, Epson America Inc., Long Beach, CA, USA) at 600 dpi. The following evaluations were made; a positive score to be acknowledged by both examiners.

- An implant was scored positive for bone resorption when a radiolucent zone was observed around the implant in resident bone at week 4 and/or 8 compared with immediately post-surgery (baseline).
- An implant was scored positive for dislocation when the implant had tipped, drifted, extruded, or rotated at week 4 and/or 8 compared with its position immediately post-surgery (baseline).
- Unscrewed or missing cover screws at week 4 and/or 8 were noted.
- Presence of a circular/ovoid peri-implant radiolucent zone in induced bone at week 4 and/or 8 was scored as seroma formation.

One masked, calibrated examiner (K. N. L.) evaluated vertical bone augmentation from computer-enhanced radiographic images obtained immediately post-surgery (baseline), and at weeks 4 and 8 in a dark room using a PC-based image analysis system (Image-Pro Plus™, Media Cybernetic, Silver Spring, MD, USA). Vertical augmentation of the alveolar ridge (bone height) along the mesial and distal aspect of each implant was measured from the reference notch. Bone formation above the implant platform was not included.

Statistical analysis

Intra-examiner reliability was assessed by measuring radiographic bone gain along mesial and distal aspect of 10 implants, twice, 5 days apart. The concordance correlation coefficient, ranging between 0 and 1, the higher the coefficient the greater the reliability, was used to test the reliability (Lin 1989, 2000). The coefficient for repeated radiographic bone height measurements was 0.98.

The animal was used as the unit of analysis. The χ^2 test was applied to evaluate a potential relationship between rhBMP-7 dose and response. All measurements at site level were averaged for each jaw quadrant. Radiographic analysis baseline measurements were subtracted from measurements obtained at weeks 4 and 8 yielding a radiographic delta bone gain over time. A general linear model including a population-averaged panel-data methodology to account for the split-mouth

Table 1. Study design

No. of animals		6
Test item	rhBMP-7	1.5 *versus* 3.0 mg/ml
Implants per jaw quadrant		3
Healing interval		8 weeks

design was used. A robust variance estimation was used. Analysis of differences between doses was performed using Wald's tests adjusted for multiple comparisons. The level of significance was set at 5%.

Results

Clinical observations

Healing was generally uneventful. Nevertheless, jaw quadrants receiving implants coated with rhBMP-7 exhibited significant swelling. No implant was lost.

Radiographic observations

Radiographic observations for animals receiving implants coated with rhBMP-7 at 1.5 mg/ml *versus* rhBMP-7 at 3.0 mg/ml in contralateral jaw quadrants are shown in Tables 2 and 3. All animals exhibited robust new bone formation commonly exceeding the implant platform (Fig. 2). Almost all animals exhibited radiolucent voids at week 4 that with one exception resolved by week 8 (Fig. 3). One implant showed peri-implant bone resorption and implant extrusion at week 4 (Fig. 4). Both these implants had been coated with rhBMP-7 at 3.0 mg/ml. Several animals exhibited partially unscrewed or missing cover screws (Fig. 4). In all, there was no significant dose–response relationship of rhBMP-7 for bone resorption, implant dislocation, cover unscrewed, or void formation at week 4 or 8 ($p > 0.05$).

Quantitative analysis

A significant increase in bone height from baseline to week 4 was observed for the implants coated with rhBMP-7 at 1.5 and 3.0 mg/ml amounting to 4.6 and 4.4 mm, respectively (Table 4). At 8 weeks, bone formation averaged 4.4 and 4.2 mm for implants coated with rhBMP-7 at 1.5 and 3.0 mg/ml, respectively. In other words, new bone formation approximated the entire height of the supra-alveolar peri-implant defect. No significant differences were observed between the 1.5 and 3.0 mg/ml rhBMP-7 preparations at any interval (Table 4).

Discussion

The objective of this study was to radiographically evaluate the potential of a purpose-designed titanium porous-oxide implant surface combined with rhBMP-7 to stimulate alveolar ridge augmentation. Implants coated with rhBMP-7 exhibited robust radiographic bone formation extending to and above the implant platform. There were no significant differences among sites receiving rhBMP-7 at 1.5 mg/ml or rhBMP-7 at 3.0 mg/ml. The newly formed bone assumed characteristics of the resident bone. While several implants exhibited seroma-like formations at week 4, these were generally resolved by week 8. Only one implant exhibited peri-implant bone resorption. Partially unscrewed cover screws, not exposed to the oral environment, were observed in several animals indicating that forces associated with the bone-inductive process may have contributed to this observation. Mean radiographic bone gain for implants coated with rhBMP-7 at 1.5 and 3.0 mg/ml averaged 4.4 and 4.2 mm, respectively, at week 8. These observations are comparable to that observed in a parallel study for implants coated with rhBMP-2 at 0.75, 1.5, or 3.0 mg/ml showing radiographic bone gain averaging 4.4, 4.4, and 4.2 mm, respectively (Leknes et al. 2008). In contrast, uncoated sham-surgery control implants exhibited only limited mean radiographic bone gain (0.5 mm).

Delivery of morphogens for osteoinduction appears a critical issue in bone tissue engineering. Ideally, biomaterials used as carrier technologies for BMPs are designed to support local osteoinduction by a controlled presentation to target cells. One strategy used to enhance the efficacy of growth or differentiation factors intended for tissue engineering is to facilitate sustained release of the bioactive molecules over an extended period of time also known as sustained release kinetics. In a biodegradable system, the growth factor will be released to induce tissue regeneration as the scaffold biodegrades. The proteins can also be released by erosion mechanisms or in combination with diffusion (Babensee et al. 2000). A biomaterial might also potentiate the

Table 2. Radiographic observations at weeks 4 and 8 for sites (%) receiving implants coated with rhBMP-7 at 1.5 mg/ml or rhBMP-7 at 3.0 mg/ml

	Bone resorption		Implant dislocated		Cover unscrewed		Void formation	
	week 4	week 8	week 4	week 8	week 4	week 8	week 4	week 8
1.5 mg/ml	0/18	0/18	0/18	0/18	2/18 (11)	2/18 (11)	12/18 (67)	0/18
3.0 mg/ml	1/18 (6)	0/18	1/18 (6)	1/18 (6)	6/18 (33)	7/18 (39)	11/18 (61)	1/18 (6)

Table 3. Radiographic observations at weeks 4 and 8 for animals (%) receiving implants coated with rhBMP-7 at 1.5 mg/ml or rhBMP-7 at 3.0 mg/ml

	Bone resorption		Implant dislocated		Cover unscrewed		Void formation	
	week 4	week 8	week 4	week 8	week 4	week 8	week 4	week 8
1.5 mg/ml	0/6	0/6	0/6	0/6	1/6 (17)	1/6 (17)	4/6 (67)	0/6
3.0 mg/ml	1/6 (17)	0/6	1/6 (17)	1/6 (17)	4/6 (67)	4/6 (67)	4/6 (67)	1/6 (17)

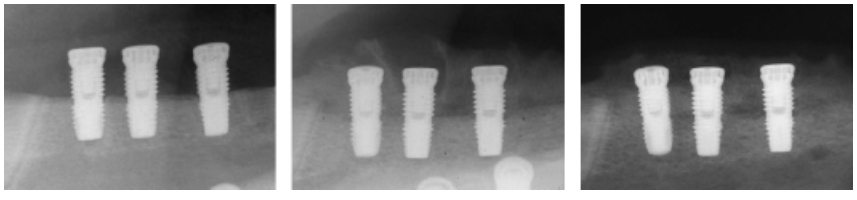

Fig. 2. Post-surgery (left), week 4 (centre), and week 8 (right) radiographs from sites receiving implants coated with recombinant human bone morphogenetic protein-7 (rhBMP-7) at 1.5 mg/ml showing bone formation exceeding the implant platform and peri-implant radiolucent zones at week 4 resolved by week 8.

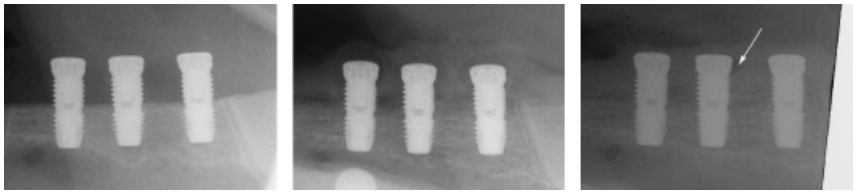

Fig. 3. Post-surgery (left), week 4 (centre), and week 8 (right) radiographs from sites receiving implants coated with recombinant human bone morphogenetic protein-7 (rhBMP-7) at 3.0 mg/ml showing bone formation exceeding the implant platform and peri-implant radiolucent zones at week 4 partly resolved by week 8 (arrow).

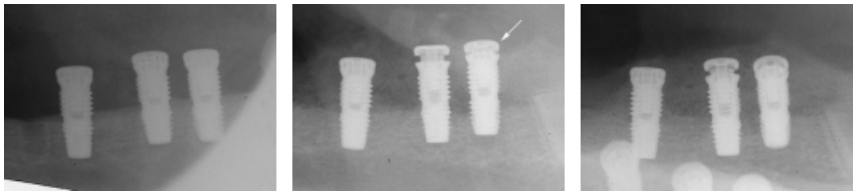

Fig. 4. Post-surgery (left), week 4 (centre), and week 8 (right) radiographs from sites receiving implants coated with recombinant human bone morphogenetic protein-7 (rhBMP-7) at 3.0 mg/ml showing one implant with peri-implant radiolucency within resident bone and apparent implant extrusion at week 4 (arrow). Two cover screws became partially unscrewed in spite of that the implants remained clinically submerged.

Table 4. Mean (±SD) radiographic bone gain by group and observation interval for animals receiving implants coated with rhBMP-7 at 1.5 mg/ml or rhBMP-7 at 3.0 mg/ml

Group	Δ Week 4	Δ Weeks 8
rhBMP-7 (1.5 mg/ml)	4.6 ± 0.5	4.4 ± 0.9
rhBMP-7 (3.0 mg/ml)	4.4 ± 0.8	4.2 ± 1.1

Δ Weeks 4 = difference between baseline and week 4.
Δ Weeks 8 = difference between baseline and week 8.

activity of BMPs by binding and presenting the proteins directly to cell receptors (Uludag et al. 2001). Candidate oral implant surfaces to serve as a carrier for rhBMP-2 have been evaluated in a rat ectopic model (Hall et al. 2007). The titanium porous-oxide surface with open pores used in this study appeared more effective than a turned titanium surface. In addition to be porous to allow cell infiltration, the carrier should be biocompatible to minimize inflammatory reactions, and, if biodegradable, not interfere with long-term properties of the newly formed tissues (Seeherman 2001). In this study, each animal received titanium implants with the titanium porous-oxide surface coated with rhBMP-7 at 1.5 or 3.0 mg/ml placed in contralateral jaw quadrants. Robust bone gain indicates that rhBMP-7 was successfully delivered to targeted cells and that in pharmacologically active concentrations. Moreover, the observations may suggest that even lower rhBMP-7 concentrations could provide a clinically relevant effect similar to that observed for rhBMP-2 possibly also reducing void formation observed at 4 weeks (Leknes et al. 2008, Wikesjö et al. 2008c).

The existence of several BMPs with osteogenic activity poses important questions about the biological significance of this redundancy and suggests multiple interactions during bone induction and periodontal regeneration. For example, Ripamonti et al. (2001) evaluated tissue induction and morphogenesis using hBMP-7 and hBMP-2 applied singly or in combination in surgically created furcation defects in the baboon. The results indicate that tissue morphogenesis induced by hBMP-7 and hBMP-2 is qualitatively different when the morphogens are applied singly. While hBMP-7 induced substantial cementogenesis, hBMP-2-treated defects showed limited cementum formation but substantial bone regeneration and remodelling. Combining hBMP-7 and hBMP-2 did not synergistically enhance periodontal regeneration. In another study, Wikesjö et al. (2004) showed significant bone formation following implantation of rhBMP-2 in supra-alveolar periodontal defects whereas implantation of rhBMP-12 was associated with modest bone formation. In contrast, sites implanted with rhBMP-12 exhibited formation of a functionally oriented periodontal ligament in a dose-dependent order whereas sites implanted with rhBMP-2 exhibited limited, if any, regeneration of the periodontal ligament. Threshold doses of BMPs apparently are required to initiate sequential but not necessarily related cellular responses (Reddi 1994). Chemotaxis is initiated and optimized at low threshold doses of BMPs while higher doses support mitosis and cell differentiation (Cunningham et al. 1992). Doses in the microgram range elicit bone differentiation. It is likely that the induction of specific cell phenotypes by BMPs also is regulated by the extracellular matrix microenvironment (Ripamonti & Duneas 1996). In the present study, implants coated with rhBMP-7 at 1.5 mg/ml or rhBMP-7 at 3.0 mg/ml showed substantial radiographic bone formation extending to and above the implant platform at 8 weeks. Bone gain was comparable to that observed for implants coated with rhBMP-2 (Leknes et al. 2008). Higher concentrations (3.0 mg/ml) of rhBMP-2 were, however, associated with extensive seroma formation in some cases resulting in remarkable implant dislocation indicating that rhBMP-2 may be a more potent bone inductive molecule than rhBMP-7 at least in the present setting.

In conclusion, the titanium porous-oxide implant surface serves as an effective carrier for rhBMP-7 showing a clinically significant potential to stimulate local bone formation, i.e. vertical augmentation of the alveolar ridge.

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Address:
Dr. Knut N. Leknes
Department of Clinical Dentistry – Periodontics
Faculty of Medicine and Dentistry
University of Bergen
Aarstadveien 17, N-5009 Bergen
Norway
E-mail: knut.leknes@odont.uib.no

Clinical Relevance

Scientific rationale for the study: One overall goal for tissue engineering of bone is to mimic the native processes by designing biomaterials capable of initiating predictable bone formation. Implants coated with rhBMP may have the potential to stimulate alveolar ridge augmentation.

Principal findings: Radiographic bone formation extended to and above the implant platform. The titanium porous-oxide surface served as an effective carrier for rhBMP-7.

Practical implications: The present findings indicate that the BMP technology holds promise as an alternative to other devices in support of local bone formation.