J Clin Periodontol 2010; 37: 582–590 doi: 10.1111/j.1600-051X.2010.01557.x

Journal of Clinical Periodontology

Evaluation of implants coated with rhBMP-2 using two different coating strategies: a critical-size supraalveolar peri-implant defect study in dogs

Jaebum Lee[1,2], John F. Decker[1,3], Giuseppe Polimeni[1,4,5], Carlo Alberto Cortella[1,5,6,7], Michael D. Rohrer[8], John M. Wozney[9], Jan Hall[10], Cristiano Susin[1,2] and Ulf M. E. Wikesjö[1,2]

[1]Laboratory for Applied Periodontal and Craniofacial Regeneration (LAPCR); [2]Departments of Periodontics and Oral Biology, Medical College of Georgia School of Dentistry, Augusta, GA, USA; [3]US Army, Fort Gordon, GA, USA; [4]Private Practice, Bologna, Rome, Italy; [5]Department of Periodontics, Medical College of Georgia School of Dentistry, Augusta, GA, USA; [6]Private Practice, Genoa, Italy; [7]Department of Periodontology, University of Milan San Rafaele, Milan, Italy; [8]Division of Oral and Maxillofacial Pathology, University of Minnesota School of Dentistry, Minneapolis, MN, USA; [9]Tissue Repair, Wyeth Research, Cambridge, MA, USA; [10]Research and Development, Nobel Biocare AB, Göteborg, Sweden;

Lee J, Decker JF, Polimeni G, Cortella CA, Rohrer MD, Wozney JM, Hall J, Susin C, Wikesjö UME. Evaluation of implants coated with rhBMP-2 using two different coating strategies: a critical-size supraalveolar peri-implant defect study in dogs. J Clin Periodontol 2010; 37: 582–590. doi: 10.1111/j.1600-051X.2010.01557.x.

Abstract

Background: Implants coated with recombinant human bone morphogenetic protein-2 (rhBMP-2) induce relevant bone formation but also resident bone remodelling.

Objectives: To compare the effect of implants fully or partially coated with rhBMP-2 on new bone formation and resident bone remodelling.

Materials and Methods: Twelve, male, adult, Hound Labrador mongrel dogs were used. Critical-size, supraalveolar, peri-implant defects received titanium porous oxide surface implants coated in their most coronal aspect with rhBMP-2 (coronal-load/six animals) or by immersion of the entire implant in an rhBMP-2 solution (soak-load/six animals) for a total of 30 μg rhBMP-2/implant. All implants were air-dried. The animals were euthanized at 8 weeks for histometric evaluation.

Results: Clinical healing was uneventful. Supraalveolar bone formation was not significantly affected by the rhBMP-2 application protocol. New bone height and area averaged ($\pm$ SE) 3.4 ± 0.2 *versus* 3.5 ± 0.4 mm and 2.6 ± 0.4 *versus* 2.5 ± 0.7 mm^2 for coronal-load and soak-load implants, respectively ($p > 0.05$). The corresponding bone density and bone–implant contact (BIC) recordings averaged $38.0 \pm 3.8\%$ *versus* $34.4 \pm 5.6\%$ and $25.0 \pm 3.8\%$ *versus* $31.2 \pm 3.3\%$ ($p > 0.05$). In contrast, resident bone remodelling was significantly influenced by the rhBMP-2 application protocol. Bone density outside the implants threads averaged $74.7 \pm 3.8\%$ and $50.8 \pm 4.1\%$ for coronal-load and soak-load implants, respectively ($p < 0.05$); bone density within the thread area averaged $51.8 \pm 1.2\%$ and $37.8 \pm 2.9\%$, and BIC $70.1 \pm 6.7\%$ and $43.3 \pm 3.9\%$ ($p < 0.05$).

Conclusion: Local application of rhBMP-2 appears to be a viable technology to support local bone formation and osseointegration. Coronal-load implants obviate resident bone remodelling without compromising new bone formation.

Key words: bone density; bone formation; bone remodelling; dental implant; osseointegration; rhBMP-2; tissue engineering

Accepted for publication 30 January 2010

Conflict of interest and source of funding statement

Jan Hall is an employee of Nobel Biocare AB and John M. Wozney is an employee of Wyeth Research. Ulf ME Wikesjö was previously employed by Wyeth Research and presently serves as consultant to Nobel Biocare AB.

This study was supported by a grant from Nobel Biocare AB, Göteborg, Sweden.

A paradigm shift in implant dentistry places restorative factors associated with aesthetics and function in front of implant site selection based on bone quantity and quality. Implant use has become the standard of care for the treatment of edentulous sites due in part to an increased need for prosthetic replacement of teeth (Carlsson & Omar 2006). Moreover, compared with traditional dental practice, implants provide a fixture for restorations without prosthetic involvement of adjoining teeth, minimizing the loss of tooth structure (Christensen 2008). Favourable success rates for endosseous implants have led to challenging new and innovative uses from their traditionally envisioned role.

Implants are nowadays placed at sites compromised by bone loss, trauma, and periodontal disease. Bone augmentation procedures including autograft bone, bone biomaterials (derivatives or substitutes), biologic agents, guided bone regeneration, or combinations thereof have been used in challenging sites to support implant placement (Wikesjö et al. 2008c). New technology involving direct coating of implant surfaces with bone morphogenetic proteins may add to the arsenal of techniques to overcome implant site inadequacies and complications (Hall & Lausmaa 2000, Hall et al. 2007, Leknes et al. 2008a, b, Wikesjö et al. 2008a, b, d, Polimeni et al. 2010).

Using the critical-size, supraalveolar, peri-implant defect model (Wikesjö et al. 2006), implants coated with recombinant human bone morphogenetic protein-2 (rhBMP-2) induce radiographic bone formation extending up to and above the implant platform within 4 weeks (Leknes et al. 2008a). Clinically relevant bone formation and osseointegration at all implants coated with rhBMP-2 compared with control have been demonstrated in histologic evaluations of the implant sites following an 8-week healing interval (Wikesjö et al. 2008b). Similar accelerated local bone formation has been observed for implants coated with rhBMP-2 placed into type II bone in dogs and type IV bone in non-human primates (Wikesjö et al. 2008a, d). Throughout these studies, a significant dose-dependent remodelling of the immediate peri-implant resident bone has been observed, sometimes including the entire buccal crestal plate, resulting in undesirable implant displacement at higher rhBMP-2 concentrations (Leknes et al. 2008a, Wikesjö et al. 2008b). Remodelling of the resident bone modifies immediate peri-implant bone density and likely primary implant stability. The implants in previous studies have been coated with rhBMP-2 using a laboratory bench soak-load method, i.e., each implant was immersed in an appropriate rhBMP-2 solution and then air-dried for several hours before use (Wikesjö et al. 2008a, b, d). We hypothesize that coating the implants with rhBMP-2 restricted to the implant collar, i.e., the most coronal aspect of the implant, would significantly decrease resident bone remodelling, whereas it would not significantly affect new bone formation. Thus, the objective of this study was to compare local bone formation and osseointegration at endosseous oral implants partially or fully coated with rhBMP-2.

Materials and Methods

Animals

Twelve, male, adult (18–24 months) Hound Labrador mongrel dogs, approximate weight 25 kg, acquired from a USDA-licensed vendor were used following a protocol approved for this study by the Medical College of Georgia Institutional Animal Care and Use Committee. The animals were fed a canned soft dog food diet throughout the study. Oral prophylaxis was performed under sedation (telazol 5 mg/kg – xylazine 1 mg/kg i.v.) using an aseptic technique within 2 weeks before experimental surgeries.

rhBMP-2-coated implants

Titanium implants ($\varnothing 4.3 \times 10$ mm with 2–3 additional grooves at the 1.5 mm long collar; TiUnite™NobelReplace Tapered Groovy, RP, Nobel Biocare AB, Göteborg, Sweden) were used. The implants were coated with rhBMP-2 in their most coronal aspect (coronal-load) or by immersion of the entire implant in an rhBMP-2 solution (soak-load) and then air-dried. A total of 30 μg rhBMP-2/implant was applied. Coronal-load implants were prepared by the manufacturer using a proprietary protocol. The implants were then shipped to the surgical laboratory and maintained at 4°C until use.

Soak-load implants were prepared in the surgical laboratory. Briefly, using aseptic routines, lyophilized rhBMP-2 (Wyeth Research, Cambridge, MA, USA) was reconstituted with sterile water (sterile water for injection, USP; Abbot Laboratories, North Chicago, IL, USA) to produce a 4.0 mg/ml solution. MFR 00169 buffer (5 mM glutamic acid, 5 mM sodium chloride, 2.5% glycine, 0.5% sucrose, 0.01% polysorbate 80, pH 4.5; Wyeth Research) was added to the reconstituted 4.0 mg/ml rhBMP-2 solution to produce a 1.5 mg/ml rhBMP-2 stock solution stored at 4°C until use. Next, 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 of the 1.5 mg/ml rhBMP-2 stock solution to reach the implant platform. Implants were incubated in the rhBMP-2 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 Clean Room Model Vertical Flow Component System, Class 100 with three MAX8005 HEPA filtered modules (Tech Rite Sales and Manufacturing Inc., San Ramon, CA, USA) at room temperature.

Routine scanning electron microscopy evaluating a subset of coated and uncoated (control) implants (two implants/treatment category) was used to illustrate the rhBMP-2 coating on the titanium implants (Fig. 1).

Experimental surgery

Food was withheld the night preceding the surgery. The animals were pre-anaesthetized with atropine (0.02–0.04 mg/kg i.m.), buprenorphine HCl (0.01–0.03 mg/kg i.m.), and acepromazine (0.2–0.3 mg/kg i.m.). After tranquilization, a 20/23 G i.v. catheter was placed in the foreleg for induction with propofol (5–7 mg/kg i.v.). Animals were intubated with an appropriately sized (7–9 mm) endotracheal tube and then moved to the surgical theatre to be maintained on gas anaesthesia (1.5–2% isoflurane/O_2 to effect). The animals received a slow constant-rate infusion of lactated Ringer's solution (10–20 ml/kg/h i.v.) to maintain hydration during surgery. The depth of anaesthesia was monitored by evaluating the response to a toe pinch, corneal reflex, and by monitoring the depth of respiration, respiratory rate, and heart rate.

Bilateral, critical-size, supraalveolar peri-implant defects were created in the mandibular pre-molar region (Wikesjö et al. 2006; Figs 2 and 3). Briefly, following routine dental infiltration anaesthesia (lidocaine HCl 2%, epinephrine 1:100,000), buccal and lingual mucoperiosteal flaps were reflected and alveolar bone was 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 was amputated at the level of the reduced alveolar crest. Three titanium implants were placed into osteotomies prepared into the extraction sites of the distal root of the third and the mesial root and distal root of the fourth pre-molar in each jaw quadrant. Five millilitres of the implant was placed within the surgically reduced alveolar ridge, creating a 5 mm, supraalveolar,

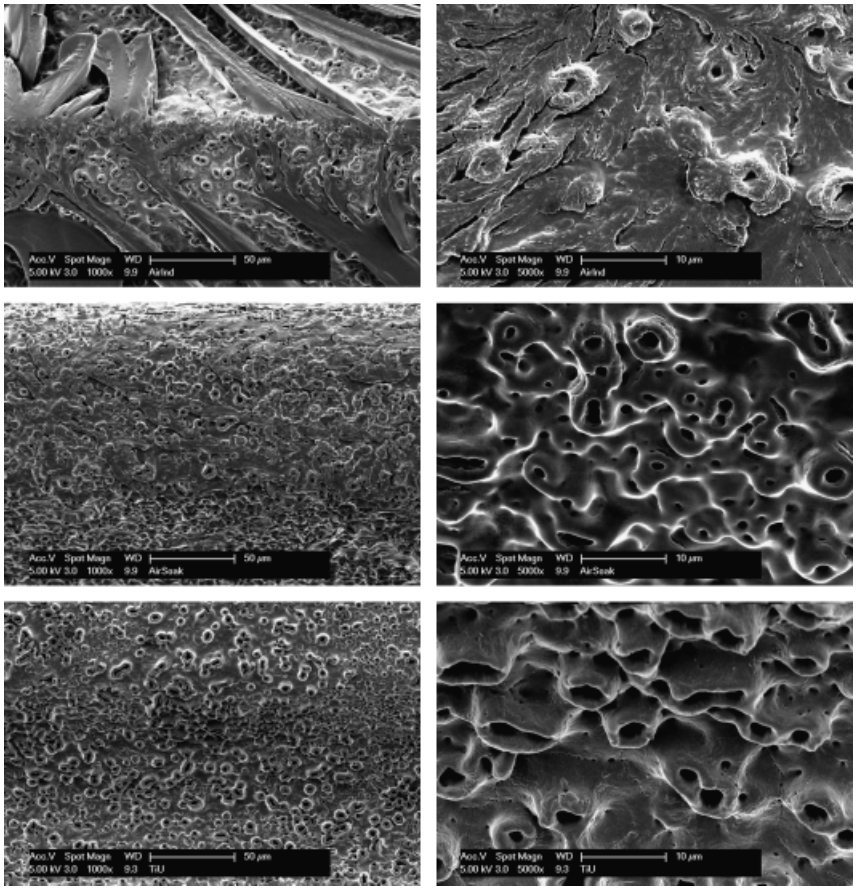

Fig. 1. Representative scanning electron microscopy photomicrographs (× 1000 left and × 5000 right panels) of the coronal aspect of titanium porous oxide implants coated with recombinant human bone morphogenetic protein-2 (30 μg/implant). Top panels show the coronal-load, centre panels show the soak-load, and bottom panels show the uncoated titanium porous oxide implants.

peri-implant defect. Six animals received coronal-load rhBMP-2-coated implants and six animals received soak-load rhBMP-2-coated implants. Treatments were alternated between left and right jaw quadrants in subsequent animals. Contra-lateral jaw quadrants received treatments reported elsewhere. Cover screws were placed onto the implants and the periostea of the mucogingival flaps were fenestrated at the base of the flaps to allow tension-free flap apposition and wound closure. The flaps were advanced 3–4 mm coronal to the implants and the flap margins were adapted and sutured (GORE-TEX™ Suture CV5, W. L. Gore & Associates Inc., Flagstaff, AZ, USA) to cover the implants. Photographs were taken following implant placement and wound closure.

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

Postsurgery procedures

A long-acting opioid (buprenorphine HCl, 0.01–0.03 mg/kg, i.m., b.i.d./3 days) was administered for pain control. A broad-spectrum antibiotic (enrofloxacin; 5 mg/kg, i.m., s.i.d./7 days) was administered for infection control. Plaque control was maintained by twice-daily flushing of the oral cavity with a chlorhexidine gluconate solution (Xttrium Laboratories Inc., Chicago, IL, USA; 20–30 ml of a 2% solution) until completion of the study. Sutures were removed under sedation (telazol 5 mg/kg – xylazine 1 mg/kg i.v.) at approximately 10 days. The experimental areas were monitored daily until suture removal and thereafter at least weekly for swelling/dehiscencies/infection.

Radiographic registrations

Radiographic registrations were obtained under sedation immediately postsurgery, and at 4 and 8 weeks postsurgery (telazol 5 mg/kg – xylazine 1 mg/kg i.v.) using a mobile X-ray unit (Irix 70/CCX Digital, Trophy Radiologie SA, Marne la Vallé, France) and a standardized protocol at 70 kVp, 7 mA, and 30 impulses. An ANSI size #4 Kodak Ultra-speed film (Eastman Kodak Company, Rochester, NY, USA) was used. The mandibles of the animals were placed flat on the films, and the distance from the focal spot to the films approximated 6 in. The projection angle was 65° from the operating table. Radiographs were processed using an automatic dental film processor (A/T 2000, Air Techniques, Hicksville, NY, USA).

Fluorescent bone labelling

The animals were administered fluorescent bone labels (Li & Jee 2005) for a qualitative evaluation of bone formation. Oxytetracycline hydrochloride (Maxim-200, Phoenix Pharmaceuticals, St. Joseph, MO, USA; 25 mg/kg, s.q.) was administered at 3 weeks, xylenol orange (Sigma-Aldrich Inc., St. Louis, MO, USA; 200 mg/ml; 90 mg/kg, s.q., twice 1 day apart) at 4 weeks, and calcein (Sigma-Aldrich Inc., St. Louis, MO, USA; 25 mg/ml; 5 mg/kg, s.q.) at 10 and 3 days pre-euthanasia.

Euthanasia

The animals were anaesthetized as above and euthanized at week 8 postsurgery using an intravenous injection of concentrated sodium pentobarbital (Euthasol®, Delmarva Laboratories Inc., Midlothian, VA, USA; 150 mg/kg). Following euthanasia, block sections including titanium implants, alveolar bone, and surrounding mucosa were collected and radiographed. The specimens were rinsed in sterile saline and transferred to 10% neutral-buffered formalin at a volume 10 times that of the block section.

Histotechnical processing

The tissue blocks were fixed in 10% buffered formalin for 3–5 days, dehydrated in alcohol, and embedded in methylmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). The implants were cut mid-axially in a buccal–lingual plane into

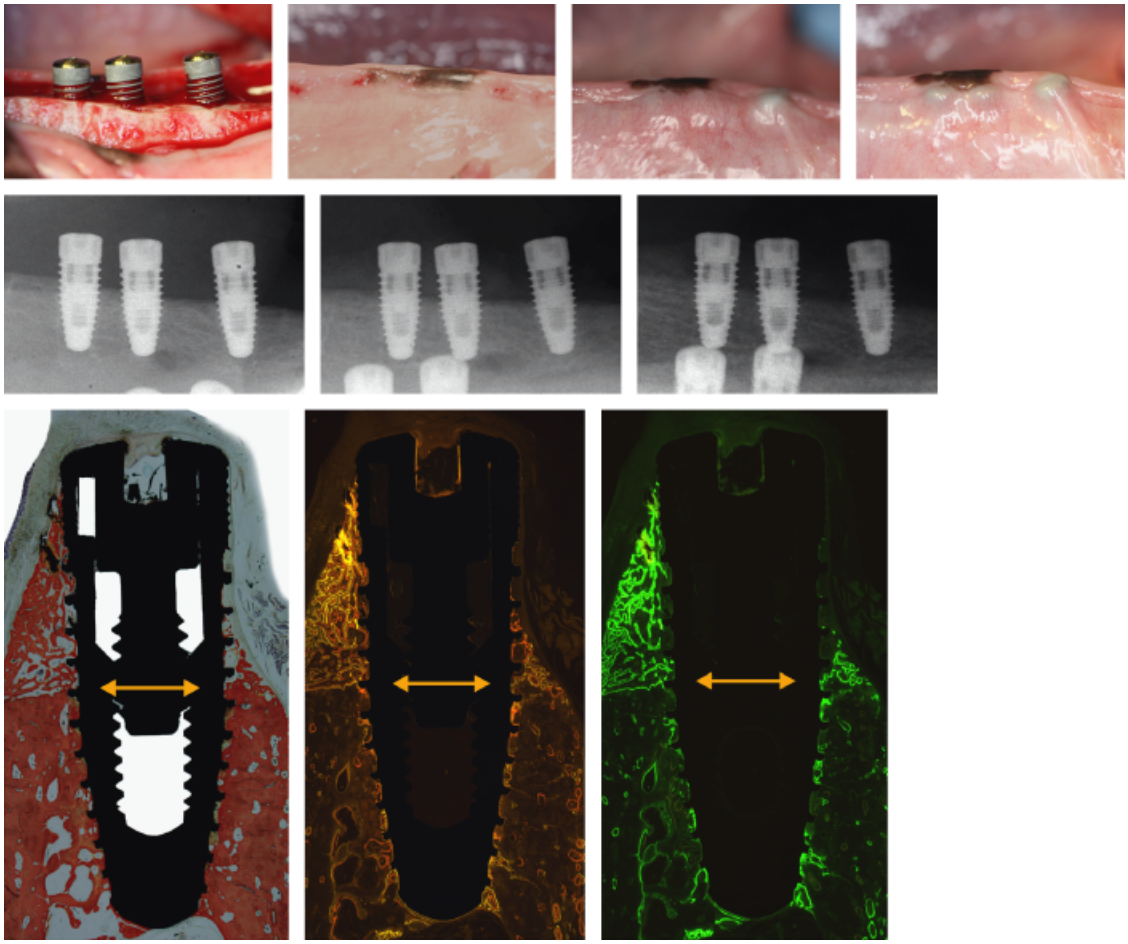

Fig. 2. Clinical photographs showing 10 mm titanium porous oxide implants coated in their most coronal aspect with recombinant human bone morphogenetic protein-2 (rhBMP-2) (30 μg/implant) following placement (left), suture removal (centre left), and healing at week 4 (centre right) and 8 (right). Note that swelling at suture removal resolved at week 4. Radiographic recordings from immediately postsurgery (right), and weeks 4 (centre) and 8 (left) show increased peri-implant radiopacity from week 4 to 8. Light (left) and fluorescence (centre/right representing week 3/4 and 7/8, respectively) microscopy photomicrographs show new bone formation approaching the implant platform. Buccal bone formation appears to be more limited. Note the limited remodelling in resident bone (centre and left panels). Orange arrows delineate the border between rhBMP-2-induced and resident alveolar bone.

200 μm thick sections using the cutting-grinding technique (EXAKT Apparatebau, Norderstedt, Germany), and subsequently ground and polished to a final thickness of approximately 40 μm (Donath & Breuner 1982, Rohrer & Schubert 1992). Unstained central sections were used for fluorescent light microscopy analysis and imaging. The same selected sections were stained with Stevenel's blue and van Gieson's picrofuchsin for histopathologic and histometric analysis using incandescent and polarized light microscopy.

Radiographic analysis

The radiographs were converted to digital images using a film scanner (Epson Perfection® 4990 Photo, Epson America Inc., Long Beach, CA, USA) at 600 dpi. Two masked experienced examiners (J. L. and J. F. D.) evaluated computer-enhanced radiographic images obtained immediately postsurgery, at week 4 and 8 weeks in a dark room. The following evaluations were made:

- An implant was scored positive for peri-implant bone remodelling when a radiolucent zone was observed around the implant in resident bone at 4 and/or 8 weeks compared with immediately postsurgery.
- An implant was scored positive for dislocation when the implant had tipped, drifted, extruded, or rotated at 4 and/or 8 weeks compared with its position immediately postsurgery.
- Unscrewed or missing cover screws at weeks 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.

Histopathologic analysis

Two masked experienced examiners (J. L., U. M. E. W.) performed the histopathologic evaluation including observations of bone formation and resorption, cortex formation, seroma formation, fibrovascular tissue and marrow, and inflammatory reactions using computer-enhanced images projected on high-resolution screens, and routine incandescent, polarized, and fluorescent light microscopy at a wide range of magnifications (BX 51, Olympus America Inc., Melville, NY, USA).

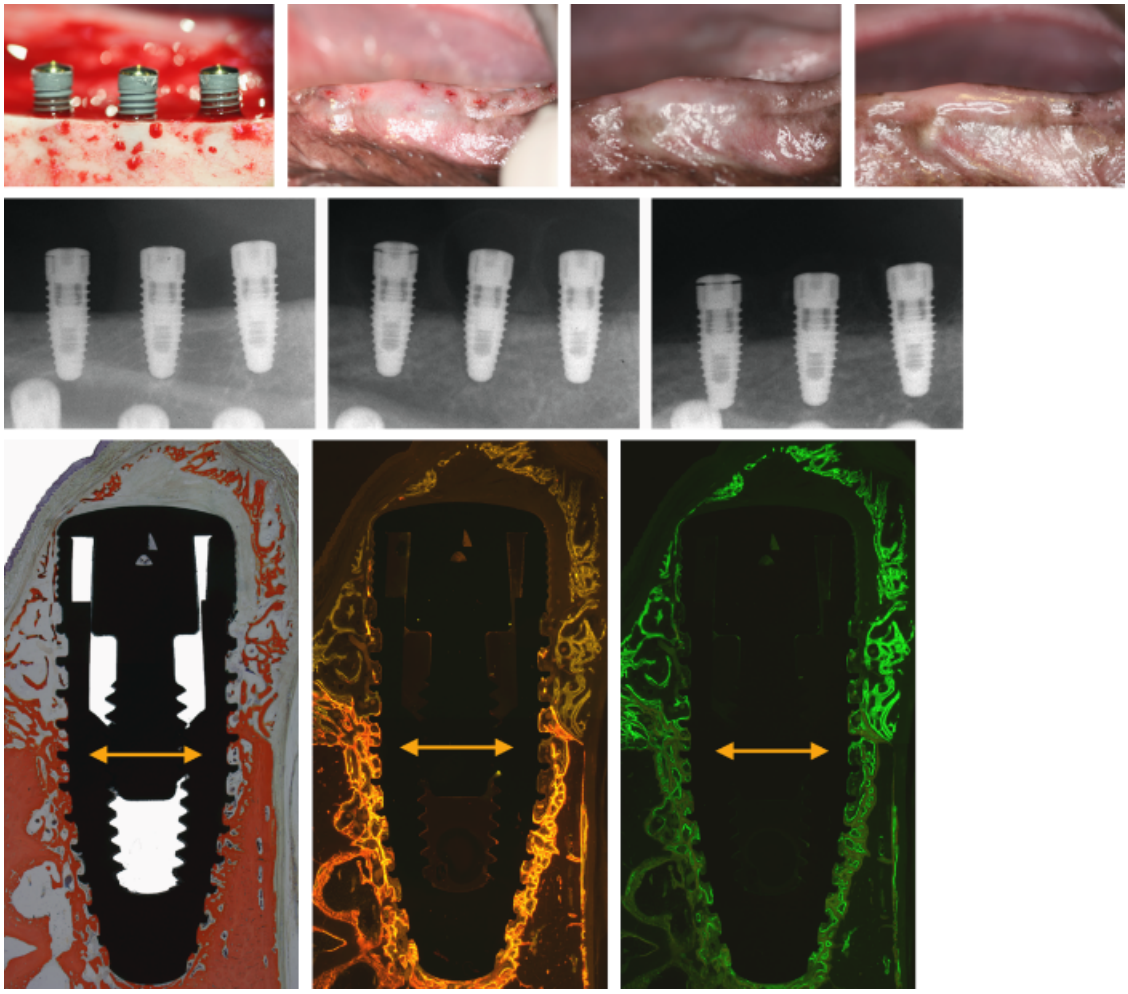

Fig. 3. Clinical photographs showing 10-mm titanium porous oxide implants soak-loaded with recombinant human bone morphogenetic protein-2 (rhBMP-2) (30 μg/implant) following placement (left), suture removal (centre left), and healing at weeks 4 (centre right) and 8 (right). Note that swelling at suture removal resolved at week 4. Radiographic recordings from immediately postsurgery (right), and weeks 4 (centre) and 8 (left) show radiolucent halos around implants at week 4 suggestive of seroma formation in part resolved (filled with bone) at week 8. Light (left) and fluorescence (centre/right representing weeks 3/4 and 7/8, respectively) microscopy photomicrographs show new bone formation exceeding the implant platform and a residual seroma embracing the implant collar area. Note extensive peri-implant remodelling in resident bone within 3 weeks (centre panel). Orange arrows delineate the border between rhBMP-2-induced and resident alveolar bone.

Histometric analysis

One masked, calibrated examiner (J. F. D.) performed the histometric analysis using incandescent and polarized light microscopy (BX 51, Olympus America Inc.), a microscope digital camera system (Retiga 4000R QImaging, Burnaby, BC, Canada), and a PC-based image analysis system (Image-Pro Plus™, Media Cybernetic, Silver Spring, MD, USA) with a custom macro for the critical-size, supraalveolar, peri-implant defect model. The most central section from each implant was used for the histometric analysis. Histologic slides and digitized photomicrographs were examined using a wide range of magnifications as above. The following measurements were recorded for the buccal and lingual surfaces of each implant (Fig. 4):

- *Bone Regeneration (height)*: distance between the 5 mm thread and the vertical extension of newly formed bone along the implant. Bone formation exceeding the implant platform was not included.
- *Bone Regeneration (area)*: area of newly formed bone along the implant above the 5 mm thread. Bone formation exceeding the implant platform was not included.
- *Bone Density (new bone)*: ratio bone/marrow spaces in newly formed bone.
- *Osseointegration (new bone)*: percent bone–implant contact (BIC) measured between the 5 mm thread and the vertical extension of newly formed bone along the implant.
- *Bone Density Outside the Implant Threads*/BD_{OT} *(resident bone)*: ratio bone/marrow spaces in a 400 × 4000 μm area (width × height) immediately outside the implant threads in resident bone.
- *Bone Density Within the Implant Threads*/BD_{WT} *(resident bone)*: ratio bone/marrow spaces within the implant threads in resident bone.
- *Osseointegration (resident bone)*: percent BIC within resident bone measured from the 5 mm thread to the apex of the implant.

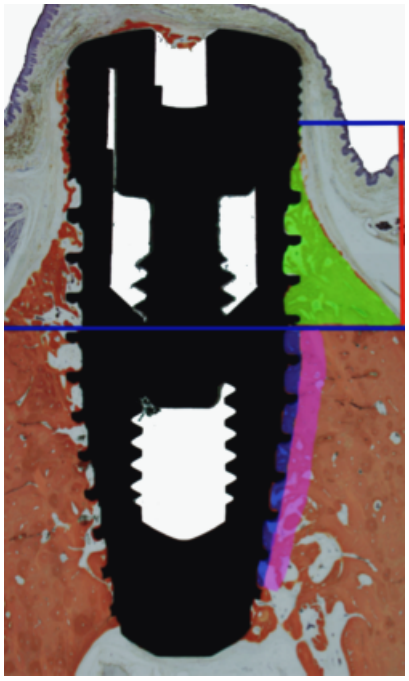

Fig. 4. Landmarks and parameters used in the histometric analysis: border between recombinant human bone morphogenetic protein-2-induced bone and resident alveolar bone (lower blue line); bone regeneration height (red line); bone regeneration area (green outline); bone density within the implant threads (BD_{WT}/blue outline); and bone density outside the implant threads (BD_{OT}/purple outline).

Statistical analysis

The statistical analysis was performed using a statistical software (Stata 9.2 for Windows, Stata Corporation, College Station, TX, USA). Linear models were used to compare the experimental groups. Appropriate variance estimators were used to account for the clustering of observations within animals. Significance was set at 5% and *p*-values were adjusted for multiple comparisons. Means ($\pm$ SE) are presented. Examiner reliability for the histometric evaluation was assessed using the Concordance Correlation Coefficient (Barnhart et al. 2007). Within the context of the present study, this coefficient ranges between 0 and 1 and values close to 1 indicate high reliability. The concordance correlation coefficient was $\geqslant 0.99$ for all parameters evaluated, demonstrating high reliability for all parameters assessed.

Results

Clinical observations

Healing was uneventful. All defect sites exhibited some swelling without apparent differences among rhBMP-2 application techniques (Figs 2 and 3). No implant exposures or losses occurred during the 8-week observation period. Starting at week 4 and remaining throughout the 8-week observation period, sites surrounding the implants were firm, smooth, and hard to palpation, suggestive of new tissue formation consistent with bone. No distinguishable clinical differences were observed between the two groups.

Radiographic observations

Radiographic evidence of bone formation was observed from week 4 and increased in radiopacity by week 8. None of the radiographs, whether representing implants processed using a coronal- or a soak-load rhBMP-2 application, revealed remarkable peri-implant bone remodelling. A single 10° implant displacement in the soak-load group was observed starting at suture removal and remained unaltered in this position throughout the study.

Seroma formation appeared to be a frequent sequel of healing without an obvious preference for the application method. The radiolucent round or ovoid shapes circumscribing the implants at week 4 were not apparent or had significantly regressed at week 8 in all but two animals. These animals and their corresponding four coronal-load implants maintained observable radiographic evidence of seroma formation despite apparent bone fill.

Cover screw unwinding occurred in only two soak-load implants, which became partially unscrewed beginning at suture removal and did not progress further during the observation interval. An additional two implants in the soak-load group also demonstrated gaps between the implant and the cover screw. However, immediate postsurgery radiographs revealed that these observations could be attributed to intra-surgery manipulations.

Histological observations

Histological observations using incandescent, polarized, and fluorescent light microscopy are summarized in Table 1 and shown Figs 2 and 3. Induced supraalveolar bone formation was similar for all implants. In general, induced bone was limited, thin, trabecular woven bone with restricted BIC.

Seromas were evenly distributed among the soak- and coronal-load groups without predilection to the processing technique (Table 1). Bone associated with seromas was sparsely trabecular, usually located at or over the implant platform, and exhibited limited BIC. Lamellar bone formation was observed in a few sites at implants subject to a localized rhBMP-2 coating, whereas it was not observed in sites exhibiting soak-load implants.

While the application method had no apparent influence on rhBMP-2-induced bone formation and osseointegration, a distinguishing difference between the coating protocols, coronal- or soak-load, was observed in the resident bone. All soak-load implants displayed a characteristic resident bone peri-implant remodelling zone. In these implants, a darkly stained peri-implant and fluorescent zone comprised of woven bone spanned the entire length of the implant in resident bone. Coronal-loaded implants showed limited resident bone remodelling.

Histometric observations

The results of the histometric analysis are shown in Tables 2–4. With respect to supraalveolar bone formation, no statistically significant difference was observed between the coronal- and the soak-load application (Table 2). The mean bone height ranged between 3.4 ± 0.2 mm for coronal-load and 3.5 ± 0.4 mm for soak-load implants ($p > 0.05$). The mean bone area amounted to $2.6 \pm 0.4\,mm^2$ for coronal-load *versus* $2.5 \pm 0.7\,mm^2$ for soak-load implants ($p > 0.05$). Bone density averaged $38.0 \pm 3.8\%$ and $34.4 \pm 5.6\%$ for coronal- and soak-load implants, respectively ($p > 0.05$). The corresponding BIC-values averaged $25.0 \pm 3.8\%$ and $31.2 \pm 3.3\%$ ($p > 0.05$).

Peri-implant remodelling in resident bone was significantly influenced by the

Table 1. Light and fluorescence microscopy observations: frequency of implant sites (animals) exhibiting seroma formation, lamellar and woven bone formation, and resident bone remodelling

	Seroma formation	Lamellar bone formation	Woven bone formation	Resident bone remodelling
Coronal-load	3/18 (2/6)	3/18 (2/6)	18/18 (6/6)	0/18 (0/6)
Soak-load	5/18 (2/6)	0/18 (0/6)	18/18 (6/6)	18/18 (6/6)

Table 2. Histometric results (means ± SE) for rhBMP-2-induced bone formation

	Bone height (mm)	Bone area (mm^2)	BD (%)	BIC (%)
Coronal-load	3.40 ± 0.23^{A}	2.63 ± 0.35^{A}	38.03 ± 3.83^{A}	25.02 ± 3.78^{A}
Soak-load	3.47 ± 0.38^{A}	2.47 ± 0.70^{A}	34.36 ± 5.59^{A}	31.21 ± 3.26^{A}

Means followed by the same capital letters do not differ statistically ($p > 0.05$).
BD, bone density; BIC, bone–implant contact; rhBMP-2, recombinant human bone morphogenetic protein-2.

Table 3. Histometric results (means ± SE) for rhBMP-2-induced resident bone remodelling

	BD_{OT} (%)	BD_{WT} (%)	BIC (%)
Coronal-load	74.66 ± 3.84^{A}	51.79 ± 1.22^{A}	70.05 ± 6.73^{A}
Soak-load	50.81 ± 4.05^{C}	37.79 ± 2.93^{B}	43.29 ± 3.91^{B}

Means followed by the same capital letters do not differ statistically ($p > 0.05$).
BD_{OT}, bone density immediately outside the threads; BD_{WT}, bone density within the threads; BIC, bone–implant contact; rhBMP-2, recombinant human bone morphogenetic protein-2.

Table 4. Histometric results (means ± SE) for rhBMP-2-induced bone formation according to buccal and lingual sites

	Bone height (mm)	Bone area (mm^2)	BD (%)	BIC (%)
Coronal-load				
Buccal sites	2.98 ± 0.52^{A}	2.22 ± 0.76^{A}	24.64 ± 5.72^{A}	21.67 ± 3.57^{A}
Lingual sites	3.81 ± 0.24^{A}	3.05 ± 0.46^{A}	51.43 ± 3.69^{B}	28.37 ± 6.22^{A}
Soak-load				
Buccal sites	3.42 ± 0.51^{a}	2.44 ± 0.97^{a}	34.42 ± 5.22^{a}	30.46 ± 4.27^{a}
Lingual sites	3.52 ± 0.32^{a}	2.48 ± 0.62^{a}	34.30 ± 7.22^{a}	31.97 ± 2.60^{a}

Coronal-load comparisons: means followed by the same capital letters do not differ statistically ($p > 0.05$).
Soak-load comparisons: means followed by the same lowercase letters do not differ statistically ($p > 0.05$).
BD, bone density; BIC, bone–implant contact; rhBMP-2, recombinant human bone morphogenetic protein-2.

rhBMP-2 coating protocol (Table 3). Coronal-load and soak-load implants showed statistically significant differences in BD_{OT}, BD_{WT}, and BIC. The mean BD_{OT} amounted to $74.7 \pm 3.8\%$ for coronal-load *versus* $50.8 \pm 4.1\%$ for soak-load implants ($p < 0.05$). The mean BD_{WT} amounted to $51.8 \pm 1.2\%$ for coronal-load *versus* $37.8 \pm 2.9\%$ for soak-load implants ($p < 0.05$). Similar differences between coronal-load and soak-load implants were recorded for BIC averaging $70.1 \pm 6.7\%$ *versus* $43.3 \pm 3.9\%$ ($p < 0.05$).

New bone density was significantly increased for lingual compared with buccal sites for coronal-load implants. No other significant difference was observed when comparing buccal and lingual sites within experimental groups (Table 4).

Discussion

The objective of this study was to compare local bone formation, remodelling and osseointegration at endosseous oral implants fully or partially coated with rhBMP-2. Two application methods were investigated: local application of rhBMP-2 onto the most coronal aspect of the implants and soak-load of the entire implant in an rhBMP-2 solution. Our experimental hypothesis was that coating the implants with rhBMP-2 restricted to the coronal aspect of the implant would reduce resident bone remodelling without compromising new bone formation. No statistically significant differences were observed between groups for new bone height, area, density, and BIC. Coronal-load implants showed statistically significantly greater resident bone density and BIC than soak-load implants, indicating a clinically relevant decrease in bone remodelling.

Histologic observations of induced bone formation were similar for both rhBMP-2 applications. Bone morphology was characterized as being sparsely trabecular, immature woven bone with limited bone formation on the buccal aspect of the implants. Similar characteristics of rhBMP-2-induced bone have been reported in previous studies utilizing comparable concentrations of rhBMP-2 coated onto implants (Wikesjö et al. 2008b) or delivered using an absorbable collagen sponge carrier (Tatakis et al. 2002, Wikesjö et al. 2003, 2004, Qahash et al. 2007). Higher concentrations of rhBMP-2 notably delayed bone maturation compared with lower concentrations, resulting in immature bone formation such as in the present study, whereas lower concentrations resulted in more mature bone including cortex formation (Wikesjö et al. 2008b). Seroma formation appears to be a normal dose-dependent sequel of rhBMP-2-induced bone formation (Hunt et al. 2001, Sigurdsson et al. 2001, Jovanovic et al. 2003, 2007,Leknes et al. 2008a, Wikesjö et al. 2008a, b, d). In the present study, a similar occurrence of seroma formation was observed in both groups, its resolution consistent with that of previous studies.

Implants exposed to rhBMP-2 soak-load applications exhibited a characteristic remodelling zone along the entire length of the implant in resident bone. This remodelling zone included not only bone formation within the threads but also the adjoining bone as can be seen from the histometric, and the incandescent, polarized, and fluorescent microscopy evaluation. In contrast, implants receiving the coronal-load rhBMP-2 application demonstrated limited peri-implant resident bone remodelling. Bone remodelling at these implants was limited to the implant surface within the supraalveolar defect. This difference in remodelling can only be attributed to the presence or absence of rhBMP-2. Remodelling zones were also observed in previous studies utilizing various rhBMP-2 concentrations and a soak-load protocol (Wikesjö et al. 2008a, b, d). The absence of a wide remodelling zone at the uncoated aspect of coronal-load implants in the present study is consistent with that observed at uncoated control implants in previous studies (Wikesjö et al. 2006, 2008a, b, d). Clinically, excess bone remodelling may reduce primary implant stability and delay implant loading protocols; thus, local application of rhBMP-2 onto the most coronal aspect of the implant appears to be the preferred technology for rhBMP-2-coated implants intended to support alveolar

augmentation. On the other hand, soak-load or similar full-implant rhBMP-2 applications may support osseointegration in type IV or compromised bone.

The animals in the present study displayed lesser new bone formation than that in a previous study using essentially the same protocol including soak-load laboratory bench-produced rhBMP-2-coated implants (Wikesjö et al. 2008b). New bone formation ranged between 4.2 and 4.4 mm in height for implants coated with rhBMP-2 at 0.75, 1.5, and 3.0 mg/ml, whereas in the present study, bone height approximated 3.4 mm for both coronal- and soak-load implants. Similarly, new bone area ranged between 5.0 and 7.4 mm^2 for our previous study, whereas in the current study, new bone area approximated 3.5 mm^2. Differences between studies are difficult to discern at least for implants belonging to the rhBMP-2 (1.5 mg/ml) soak-load protocol. The same surgical protocol was followed using essentially the same age animals, surgical protocol, and team. Possibly, differences in the release kinetics resulting from using slightly different titanium porous oxide implants may explain, at least in part, the discrepancies in bone formation.

The defect sites in the present study were not exposed to equivalent doses of rhBMP-2 for sites receiving soak-load and coronal-load implants. The bone-anchoring titanium porous oxide implant surface was coated in its entirety with rhBMP-2 for the soak-load implants, whereas the same 30 μg dose was applied exclusively to the collar region of the coronal-load implants. Because 5 mm of the implants were placed into the resident alveolar bone away from the defect area, the amount of rhBMP-2 available in the defect area was in all likelihood lower for soak-load than for coronal-load implants. In spite of this apparent difference in rhBMP-2 exposure, no significant differences in new bone formation or maturation were apparent between the groups. In a previous study, higher concentrations of rhBMP-2 (3.0 mg/ml) applied to titanium porous oxide implants using the soak-load application did not enhance bone formation compared with lower concentrations (0.75 and 1.5 mg/ml) at 8 weeks (Wikesjö et al. 2008b). Thus, it appears that lower doses/concentrations of rhBMP-2 may not necessarily decrease the bone-inductive potential; in fact, it may, as discussed above, contribute to accelerated bone maturation.

In conclusion, rhBMP-2-coated titanium porous oxide implants induce significant bone formation, and application techniques do not appear to have a significant effect on induced bone formation and osseointegration, but do affect resident bone remodelling. Local application of rhBMP-2 onto the most coronal aspect of implants appears to be preferred for continued study in clinical settings.

Acknowledgements

The authors thank Dr. Nancy A. Rodriguez for veterinary support; Cedrick Bouey and Jamie Frank for animal technical care and husbandry; Hari S Prasad, BS, MDT for expert histotechnical preparations; and Kimberly Curtis for administrative support. This study was supported by a grant from Nobel Biocare AB, Göteborg, Sweden.

References

Barnhart, H. X., Haber, M. J. & Lin, L. I. (2007) An overview on assessing agreement with continuous measurements. *Journal of Biopharmaceutical Statistics* **17**, 529–567.

Carlsson, G. E. & Omar, R. (2006) Trends in prosthodontics. *Medical Principles and Practice* **15**, 167–179.

Christensen, G. J. (2008) Three-unit fixed prostheses versus implant-supported single crowns. *Journal of the American Dental Association* **139**, 191–194.

Donath, K. & Breuner, G. (1982) A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage-Schliff (sawing and grinding) technique. *Journal of Oral Pathology* **11**, 318–326.

Hall, J. & Lausmaa, J. (2000) Properties of a new porous oxide surface on titanium implants. *Applied Osseointegration Research* **1**, 5–8.

Hall, J., Sorensen, R. G., Wozney, J. M. & Wikesjö, U. M. E. (2007) Bone formation at rhBMP-2 coated titanium implants in the rat ectopic model. *Journal of Clinical Periodontology* **34**, 444–451.

Hunt, D. R., Jovanovic, S. A., Wikesjö, U. M. E., Wozney, J. M. & Bernard, G. W. (2001) Hyaluronan supports recombinant human bone morphogenetic protein-2 induced bone reconstruction of advanced alveolar ridge defects in dogs. A pilot study. *Journal of Periodontology* **72**, 651–658.

Jovanovic, S. A., Hunt, D. R., Bernard, G. W., Spiekermann, H., Nishimura, R., Wozney, J. M. & Wikesjö, U. M. E. (2003) Long-term functional loading of dental implants in rhBMP-2 induced bone. A histologic study in the canine ridge augmentation model. *Clinical Oral Implants Research* **14**, 793–803.

Jovanovic, S. A., Hunt, D. R., Bernard, G. W., Spiekermann, H., Wozney, J. M. & Wikesjö, U. M. E. (2007) Bone reconstruction following implantation of rhBMP-2 and guided bone regeneration in canine alveolar ridge defects. *Clinical Oral Implants Research* **18**, 224–230.

Leknes, K. N., Yang, J., Qahash, M., Polimeni, G., Susin, C. & Wikesjö, U. M. E. (2008a) Alveolar ridge augmentation using implants coated with recombinant human bone morphogenetic protein-2: radiographic observations. *Clinical Oral Implants Research* **19**, 1027–1033.

Leknes, K. N., Yang, J., Qahash, M., Polimeni, G., Susin, C. & Wikesjö, U. M. E. (2008b) Alveolar ridge augmentation using implants coated with recombinant human bone morphogenetic protein-7 (rhBMP-7/rhOP-1). Radiographic observations. *Journal of Clinical Periodontology* **35**, 914–919.

Li, X. J. & Jee, W. S. S. (2005) Integrated bone tissue anatomy and physiology. In: Deng, H-W., Liu, Y.-Z., Guo, C.-Y. & Chen, D. (eds). *Current Topics in Bone Biology*, pp. 11–56. Singapore: World Scientific Publishing Co.

Polimeni, G., Wikesjö, U. M. E., Susin, C., Qahash, M., Shanaman, R. H., Rohrer, M. D. & Hall, J. (2010) Alveolar ridge augmentation using implants coated with recombinant human growth/differentiation factor-5 (rhGDF-5). Histologic observations. *Journal of Clinical Periodontology*, doi: 10.1111/j.1600-051X.2010.01554.x.

Qahash, M., Hardwick, W. R., Rohrer, M. D., Wozney, J. M. & Wikesjö, U. M. E. (2007) Surface-etching enhances titanium implant osseointegration in newly formed (rhBMP-2-induced) and native bone. *International Journal of Oral & Maxillofacial Implants* **22**, 472–477.

Rohrer, M. D. & Schubert, C. C. (1992) The cutting-grinding technique for histologic preparation of undecalcified bone and bone-anchored implants. Improvements in instrumentation and procedures. *Oral Surgery, Oral Medicine, and Oral Pathology* **74**, 73–78.

Sigurdsson, T. J., Nguyen, S. & Wikesjö, U. M. E. (2001) Alveolar ridge augmentation with rhBMP-2 and bone-to-implant contact in induced bone. *International Journal of Periodontics and Restorative Dentistry* **21**, 461–473.

Tatakis, D. N., Koh, A., Jin, L., Wozney, J. M., Rohrer, M. D. & Wikesjö, U. M. E. (2002) Peri-implant bone regeneration using recombinant human bone morphogenetic protein-2 in a canine model: a dose-response study. *Journal of Periodontal Research* **37**, 93–100.

Wikesjö, U. M. E., Huang, Y-H., Xiropaidis, A. V., Sorensen, R. G., Rohrer, M. D., Prasad, H. S., Wozney, J. M. & Hall, J. (2008a) Bone formation at human bone morphogenetic protein-2 coated titanium implants in the posterior maxilla (Type IV bone) in nonhuman primates. *Journal of Clinical Periodontology* **35**, 992–1000.

Wikesjö, U. M. E., Qahash, M., Polimeni, G., Susin, C., Shanaman, R. H., Rohrer, M. D., Wozney, J. M. & Hall, J. (2008b) Alveolar ridge augmentation using implants coated with recombinant human bone morphogenetic protein-2 (rhBMP-2). Histologic observations. *Journal of Clinical Periodontology* **35**, 1001–1010.

Wikesjö, U. M. E., Qahash, M., Thomson, R. C., Cook, A. D., Rohrer, M. D., Wozney, J. M. & Hardwick, W. R. (2003) Space-providing expanded polytetrafluoroethylene devices define alveolar augmentation at dental implants induced by recombinant human bone morphogenetic protein 2 in an absorbable collagen sponge carrier. *Clinical Implant Dentistry and Related Research* **5**, 112–123.

Wikesjö, U. M. E., Qahash, M., Thomson, R. C., Cook, A. D., Rohrer, M. D., Wozney, J. M. & Hardwick, W. R. (2004) rhBMP-2 significantly enhances guided bone regeneration. *Clinical Oral Implants Research* **15**, 194–204.

Wikesjö, U. M. E., Simion, M. & Reddy, M. S. (2008c) Biomaterials and Substances for Site Optimizing. Alveolar augmentation: past, present, and future. In: Jokstad, A. (ed). *Osseointegration and Dental Implants [Proceedings from The Toronto Osseointegration Conference Revisited, May 9–10, 2008]*, pp. 183–196. Oxford: Wiley-Blackwell.

Wikesjö, U. M. E., Susin, C., Qahash, M., Polimeni, G., Leknes, K. N., Shanaman, R. H., Prasad, H. S., Rohrer, M. D. & Hall, J. (2006) The critical-size supraalveolar peri-implant defect model: characteristics and use. *Journal of Clinical Periodontology* **33**, 846–854.

Wikesjö, U. M. E., Xiropaidis, A. V., Qahash, M., Lim, W. H., Sorensen, R. G., Rohrer, M. D., Wozney, J. M. & Hall, J. (2008d) Bone formation at recombinant human bone morphogenetic protein-2 coated titanium implants in the posterior mandible (Type II bone) in dogs. *Journal of Clinical Periodontology* **35**, 985–991.

Address:
Jaebum Lee
Laboratory for Applied Periodontal and Craniofacial Regeneration (LAPCR)
Departments of Periodontics and Oral Biology
Medical College of Georgia School of Dentistry
1120 15th Street AD-1432
Augusta, GA 30912, USA
E-mail: jaelee@mcg.edu

Clinical Relevance

Scientific rationale: This study is one in a series describing the preclinical development of a titanium implant surface that, combined with rhBMP-2, induces significant local bone formation for optimal implant placement and osseointegration without bone grafting, biomaterials, or bone regeneration devices. We have observed that coating the entire implant with rhBMP-2 yields significant new bone formation; however, significant resident bone remodelling potentially decreasing immediate implant stability and causing implant displacement was also observed.

Principal findings: Using the canine, critical-size, supraalveolar peri-implant defect model, we show that application of rhBMP-2 restricted to the coronal aspect of the implant supports local bone formation and osseointegration obviating resident bone remodelling.

Practical implications: Localized rhBMP-2 application appears to be a viable strategy in the development of an rhBMP-2-coated implant for clinical use.