Journal of Clinical Periodontology
Official Journal of The European Federation of Periodontology
Founded by the British, Dutch, French, German, Scandinavian, and Swiss Societies of Periodontology

J Clin Periodontol 2008; 35: 985–991 doi: 10.1111/j.1600-051X.2008.01318.x

Bone formation at recombinant human bone morphogenetic protein-2-coated titanium implants in the posterior mandible (Type II bone) in dogs

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Wikesjö UME, Xiropaidis AV, Qahash M, Lim WH, Sorensen RG, Rohrer MD, Wozney JM, Hall J. Bone formation at recombinant human bone morphogenetic protein-2-coated titanium implants in the posterior mandible (Type II Bone) in dogs. J Clin Periodontol 2008; 35: 985–991. doi: 10.1111/j.1600-051X.2008.01318.x.

Abstract

Background: Conventional oral/maxillofacial implants reach osseointegration over several months during which the titanium fixtures interact with alveolar bone. The objective of this study was to determine if adsorbing recombinant human bone morphogenetic protein-2 (rhBMP-2) onto a titanium porous oxide (TPO) implant surface might enhance or accelerate local bone formation and support osseointegration in a large animal oral/maxillofacial orthotopic model.

Material and Methods: Endosseous implants with a TPO surface were installed into the edentulated posterior mandible in eight adult Hound Labrador mongrel dogs. The implant surface had been adsorbed with rhBMP-2 at 0.2 or 4.0 mg/ml. TPO implants without rhBMP-2 served as control. Treatments were randomized between jaw quadrants. Mucosal flaps were advanced and sutured leaving the implants submerged. Clinical and radiographic evaluations were made immediately post-surgery, at day 10 (suture removal), and week 4 and 8 post-surgery. The animals received fluorescent bone markers at week 3, 4, and at week 8 post-surgery, when they were euthanized for histologic analysis.

Results: TPO implants coated with rhBMP-2 exhibited dose-dependent bone re-modelling including immediate resorption and formation of implant adjacent bone, and early establishment of clinically relevant osseointegration. The resulting bone–implant contact, although clinically respectable, appeared significantly lower for rhBMP-2-coated implants compared with the control [rhBMP-2 (0.2 mg/ml) 43.3 ± 10.8% *versus* 71.7 ± 7.8%, $p < 0.02$; rhBMP-2 (4.0 mg/ml) 35.4 ± 10.6% *versus* 68.2 ± 11.0%, $p < 0.03$].

Conclusions: rhBMP-2 adsorbed onto TPO implant surfaces initiates dose-dependent peri-implant bone re-modelling resulting in the formation of normal, physiologic bone and clinically relevant osseointegration within 8 weeks.

Key words: bone morphogenetic protein; dental/oral implants; dogs; osseointegration; porous titanium oxide; rhBMP-2; tissue engineering; titanium

Accepted for publication 31 July 2008

Conflict of interest and source of funding statement

This study was supported by a grant from Wyeth Research, Cambridge, MA, USA, and Nobel Biocare AB, Göteborg, Sweden. Jan Hall is an employee of Nobel Biocare AB. Rachel G. Sorensen was an employee of Wyeth Research at the time of study. John Wozney is an employee of Wyeth Research. Ulf M. E. Wikesjö was an employee of Wyeth Research, and presently serves as consultant to Wyeth Research and Nobel Biocare AB.

A concept of applying bone morphogenetic proteins (BMPs) onto titanium surfaces for enhanced local bone formation and osseointegration has evolved from observations in rodent ectopic and orthotopic models. Kawai et al. (1993) evaluated titanium sponges infused with

bovine BMP in an ectopic mouse model. New bone formation was observed within 3 weeks using soft X-ray analysis. Chondrocytes and new bone formation occurred in contact with the titanium surface. X-ray microanalysis demonstrated new bone formation inside the pores of the titanium sponges. Herr et al. (1996) observed, macroscopically and histologically, local bone formation at disc-shaped titanium alloy implants treated by corundum blasting, coated with hydroxyapatite or pure titanium plasma spraying, coated with porcine BMP-3 and implanted into abdominal wall muscle pouches in rats. Uncoated controls remained inactive at 25 days post-implantation. In other studies, Cole et al. (1997) evaluated recombinant human BMP-2 (rhBMP-2) conditioned porous, plasma-sprayed or hydroxyapatite-coated titanium implants in a rat quadriceps muscle pouch model. The rhBMP-2-conditioned implants formed significantly more bone than controls independent of the dose or presence of hydroxyapatite. Endochondral ossification began within 7 days. Bone and marrow largely replaced induced cartilage by 21 days. In still other studies, the osteoinductive potential of a porous titanium fibre mesh with or without a calcium phosphate coating loaded with rhBMP-2 or a rhBMP-2/bovine BMP combination was evaluated in a rat ectopic model (Vehof et al. 2001). All BMP combinations induced cartilage and bone formation within 5 days. At 20 days, bone formation was characterized by trabecular bone and marrow-like tissue. At 40 days, lamellar bone and haemopoietic marrow-like tissue were observed. Others observed accelerated osseointegration to BMP-3-coated, hydroxyapatite-coated or corundum-blasted titanium alloy implants. BMP-3-coated and non-coated implants were placed into the femoral part of the patello-femoral joint in giant rabbits (Esenwein et al. 2001). BMP-3-coated titanium implants exhibited improved osseointegration compared with non-coated controls. Light microscopy demonstrated osseointegration without a connective tissue membrane at 2, 5 and 8 weeks. In all, the evidence suggests that a variety of titanium implant surfaces may serve as vehicle for BMPs while maintaining its osteoinductive potential. The utility of such technologies remains to be evaluated for clinical application in large animal orthotopic models.

The current report is the second from a series of studies aimed at developing a load-bearing implant, which could present BMPs to the implant site. This novel implant technology could have applications in a multiple of orthopaedic and oral/maxillofacial settings, where an undeniable advantage would be a prosthetic device that induces or promotes local bone formation and osseointegration without the additional use of bone grafts, bone biomaterials or devices for guided bone re-generation. In a previous publication, we reported bone induction and osseointegration at novel surface-modified titanium implants coated with rhBMP-2 in the rat ectopic model (Hall et al. 2007). The novel implant surfaces featured a titanium porous oxide (TPO) dioxide layer of approximately 5 μm thickness with narrow or open pores (Hall & Lausmaa 2000); the control was the standard machined/turned ''Brånemark'' surface. The TPO surfaces allowed a comparably slower release of rhBMP-2 relative to the control in vitro. The in vivo observations suggested that the rhBMP-2-coated titanium implants induced lamellar bone formation and osseointegration in a dose- and surface-dependent order. The TPO surface with the open pore structure appeared the most effective surface at the low discriminating dose. Abundant bone formation was observed for all surface variations at higher doses without significant dose dependency. The objective of this study was to determine if adsorbing rhBMP-2 onto a TPO implant surface might enhance or accelerate local bone formation and support osseointegration in a large animal oral/maxillofacial orthotopic model.

Material and Methods

Animals

Eight, male, 18-month-old, Hound Labrador mongrel dogs, approximate weight 25 kg, were used. Animal selection and management, and surgical protocol followed routines approved by the local Institutional Animal Care and Use Committee. The animals were fed a canned soft dog-food diet and had free access to water. Their teeth were cleaned immediately pre-surgery.

Implants

Titanium implants (TiUnite™, ∅ 3.75 mm × 8.5 mm; Nobel Biocare AB, Göteborg, Sweden) exhibiting an open pore TPO surface were used. The sterile implants were coated with rhBMP-2 (Wyeth Research, Cambridge, MA, USA), or left uncoated (control). Using aseptic techniques, lyophilized rhBMP-2 was re-constituted with sterile water for injection to produce a 4.0 mg/ml solution. An rhBMP-2 (0.2 mg/ml) solution was produced by diluting the reconstituted rhBMP-2 (4.0 mg/ml) solution in MFR 00842 buffer (5 mM L-glutamic acid, 2.5% glycine, 0.5% sucrose, 0.01 % polysorbate 80; pH 4.5; Wyeth Research). Next, sterile TPO implants were removed from their packaging. Each implant was placed into a sterile micro-Eppendorf tube containing 0.3 ml of the prepared rhBMP-2 solution (0.2 or 4.0 mg/ml). The implant was incubated in the rhBMP-2 solution for 30 min. at room temperature and then removed to air-dry for approximately 12 h before surgical implantation. Adsorbed rhBMP-2 dose estimated from results of in vitro binding and release studies, was 4.1 ± 0.6 and 43.7 ± 9.4 μg rhBMP-2/implant, respectively.

Surgical 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)/acepromazine (0.1 mg/kg IM), induced with xylazine (1 mg/kg IV), and maintained on gas anaesthesia (1–2% isoflurane/O_2 to effect). A sterile catheter was placed and animals received a slow constant rate infusion of lactated Ringer's solution (10–20 ml/kg/h IV) to maintain hydration while anaesthetized. Prophylactic antibiotics (enrofloxacin, 2.5 mg/kg IM) were administered pre-surgery and re-dosed post-surgery.

The maxillary first, second, and third premolar teeth were surgically extracted, and the fourth premolars were reduced in height to the level of gingival margin and the exposed pulpal tissues were sealed (Cavit®, ESPE, Seefeld/Oberbayern, Germany). This was performed to alleviate potential trauma from the maxillary teeth to the experimental mandibular sites post-surgery. In the mandible, all premolars and first molars were surgically extracted. Care was taken to preserve the buccal, lingual, and lateral walls of the alveolar sockets. The extraction sites were allowed to heal for 12 weeks.

Titanium implants were installed into the edentulated mandibular alveolar ridge 12 weeks following surgical extractions (Fig. 1). Briefly, the animals were pre-medicated and anaesthetized as described above. A mid-crestal incision from the region of the first premolar to the second molar was made reflecting buccal and lingual mucoperiosteal flaps. TPO implants with or without rhBMP-2 were installed using routines for the Brånemark system. Four animals received two TPO implants coated with rhBMP-2 (0.2 mg/ml) in one jaw quadrant *versus* control implants without rhBMP-2 in the contra-lateral jaw quadrant, and four animals received two TPO implants coated with rhBMP-2 (4.0 mg/ml) in one jaw quadrant *versus* control implants without rhBMP-2 in the contra-lateral jaw quadrant. Coated and uncoated implants were alternated between left and right jaw quadrants in subsequent animals. Each jaw quadrant received two additional implants exhibiting an alternative surface technology (Xiropaidis et al. 2005) similarly coated with rhBMP-2 or left uncoated. Data from these implants will be reported elsewhere. Each implant received a cover-screw and the mucoperiosteal flaps were advanced, adapted, and sutured (GORE-TEX™ Suture CV5, W. L. Gore & Associates Inc., Flagstaff, AZ, USA) to submerge the implants.

Post-surgical procedures

A long-acting opioid (buprenorphine HCl, 0.015 mg/kg IM bid for 48 h) was administered for post-surgery pain control. The broad-spectrum antibiotic (enrofloxacin, 2.5 mg/kg IM bid) was used for continued infection control for 7 days following extractions and implant installations. The animal's temperature was monitored and recorded for 10 days. Plaque control was maintained by daily flushing of the oral cavity with chlorhexidine (Chlorhexidine Gluconate 20%, Xttrium Laboratories Inc., Chicago, IL, USA; 20–30 ml of a 2% solution) until suture removal and daily thereafter (Monday through Friday) until completion of the study. Sutures were removed under sedation (propofol, 4 mg/kg IV bolus; 0.2–0.6 mg/kg/min. IV) at approximately 10 days post-surgery.

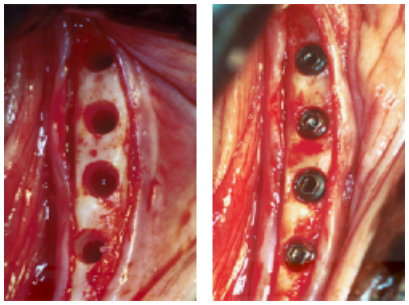

Fig. 1. Surgical installation of the titanium implants.

Oxytetracycline hydrochloride (Maxim-200, Phoenix Pharmaceuticals, St. Joseph, MO, USA; 20 mg/kg SQ) was administered at week 3 and xylenol orange (Sigma-Aldrich Inc., St. Louis, MO, USA; 200 mg/ml; 90 mg/kg SQ, twice 1 day apart) at week 4 post-implantation, and calcein green (Sigma-Aldrich Inc.; 25 mg/ml; 5 mg/kg SQ) at day 10 and 3 pre-euthanasia to monitor bone formation (Li & Jee 2005).

Test material traceability was recorded in the animal's chart. The pre-surgery condition of the oral tissues was noted. Intra-surgery photographs were taken before and after placement of the TPO implants, and following wound closure. Observations of experimental sites with regards to mucosal health, maintenance of suture line closure, oedema, and evidence of tissue necrosis or infection were made daily until suture removal, and at least twice weekly thereafter. Radiographs were obtained immediately post-surgery, at suture removal (approximately 10 days post-surgery), and at week 4 and 8 post-surgery.

The animals were euthanized at week 8 post-surgery. Following sedation with atropine (0.02–0.04 mg/kg)/buprenorphine (0.01–0.03 mg/kg)/acepromazine (0.1 mg/kg) SQ, the animals were euthanized using an overdose of pentobarbital (100 mg/kg IV). Block sections including titanium implants, alveolar bone, and surrounding mucosa were collected and radiographed. The specimens were rinsed in sterile saline, and the tissue blocks transferred to 10% neutral buffered formalin at a volume 10 times that of the block section.

Histological processings

After fixation, the tissue blocks were dehydrated in alcohol and embedded in methylmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). The embedded specimen blocks were mounted and oriented to allow mid-axial sections in a mesial–distal plane. Sections were cut to a thickness of 200 μm using the cutting–grinding technique (EXAKT Apparatebau, Norderstedt, Germany), and were subsequently ground and polished to a final thickness of approximately 40 μm for fluorescence microscopy examination (Donath & Breuner 1982, Rohrer & Schubert 1992). Upon completion of the fluorescence microscopy examination, the sections were stained with Sanderson's Rapid Bone Stain (Surgipath, Richmond, IL, USA) and counterstained with Fast Green (Wyeth Research).

Analysis

Radiographs were evaluated for crestal and immediate peri-implant bone formation by one masked examiner (U. M. E. W.). Fluorochrome bone histodynamic markers were analysed under fluorescent light by two masked examiners (U. M. E. W., X. J. L.). The entire peri-implant area in one to two sections per implant was evaluated for bone formation rate and location as delineated by the fluorescent markers, residual resident bone, fibrovascular tissue and marrow.

One masked examiner (R. G. S.) performed the histometric analysis using a PC-based image analysis system (Image-Pro Plus™, Media Cybernetic, Silver Spring, MD, USA) under light microscopy. The following measurements were recorded for mesial and distal surfaces for each implant:

- *Peri-implant bone density*: Ratio of mineralized bone to fibrovascular tissue and marrow within an area extending 600 μm immediately outside the five most coronal threads of the implant surface below the alveolar crest.
- *Intra-thread bone density*: Ratio of mineralized bone to fibrovascular tissue and marrow within the five most coronal threads of the implant surface below the alveolar crest.
- *Coronal bone contact*: Distance from the implant platform to the most coronal bone–implant contact.
- *Bone–implant contact*: Percent of bone–implant contact along the five most coronal threads of the implant surface below the alveolar crest.

Group means and standard deviations were calculated for each parameter. Differences between experimental conditions were analysed using appropriate Student's

t-test (StatView 4.5, Abacus Concepts, Berkeley, CA, USA). A *p*-value of <0.05 was required for statistical significance.

Results

Clinical observations

Localized swelling was observed at sites receiving implants coated with rhBMP-2 at suture removal; swelling being pronounced at sites receiving implants coated with rhBMP-2 (4.0 mg/ml) compared with that at implants coated with rhBMP-2 (0.2 mg/ml) or controls without rhBMP-2. There were minimal differences in swelling between sites receiving implants coated with rhBMP-2 (0.2 mg/ml) and control. Localized tissue reactions generally resolved within 14 days. All implant sites were covered by keratinized alveolar mucosa until euthanasia. As part of the biopsy protocol, surgical re-entry at euthanasia revealed that implants and cover-screws were covered with bone for all implants coated with rhBMP-2 (4.0 mg/ml). Implants coated with rhBMP-2 (0.2 mg/ml) presented with cover-screws covered with bone in two animals and partially covered in two animals. Cover-screws typically remained exposed without bone coverage in control sites.

Radiographic observations

At suture removal, radiolucent halos were observed at the coronal aspect of implants coated with rhBMP-2 (4.0 mg/ml) in all animals (Fig. 2). Implants coated with rhBMP-2 (0.2 mg/ml) exhibited radiolucent halos around the coronal aspect of the implants in one animal. Peri-implant radiolucencies generally resolved within 4 weeks. Bone formation did not only resolve the peri-implant radiolucent area but also covered the cover-screw of the implants at these sites.

Light microscopy observations

Limited bone re-modelling within and immediately adjacent to the implant surface was the predominant observation for TPO control implants. In contrast, TPO implants coated with rhBMP-2 (0.2 mg/ml) exhibited a picture of exuberant bone metabolic activity (Figs 3 and 4). With apparent variations between animals, bone re-modelling extended 0.5–2 mm from the implant surface. The newly formed trabecular bone established bone–implant contact. Islands and peninsulas of resident bone remained within the newly formed bone and approached the implant surface at variable distances.

A more pronounced reaction was realized following placement of TPO implants coated with rhBMP-2 (4.0 mg/ml)

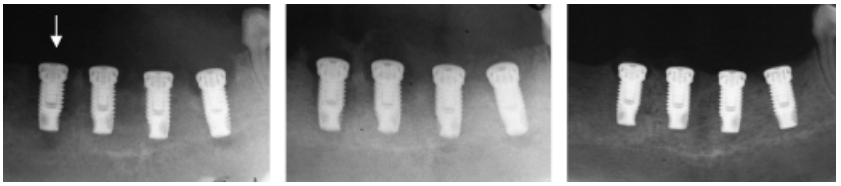

Fig. 2. Representative radiographs of tissue reactions at implants coated with recombinant human bone morphogenetic protein-2 (rhBMP-2) (4.0 mg/ml) from day 12 (left), week 4 (centre) and week 8 post-implantation. Note radiolucent halos around the coronal aspect of the implants at day 12 that gradually resolved over the 8-week osseointegration interval. The implant indicated by the arrow is also shown in Figs 3 and 4.

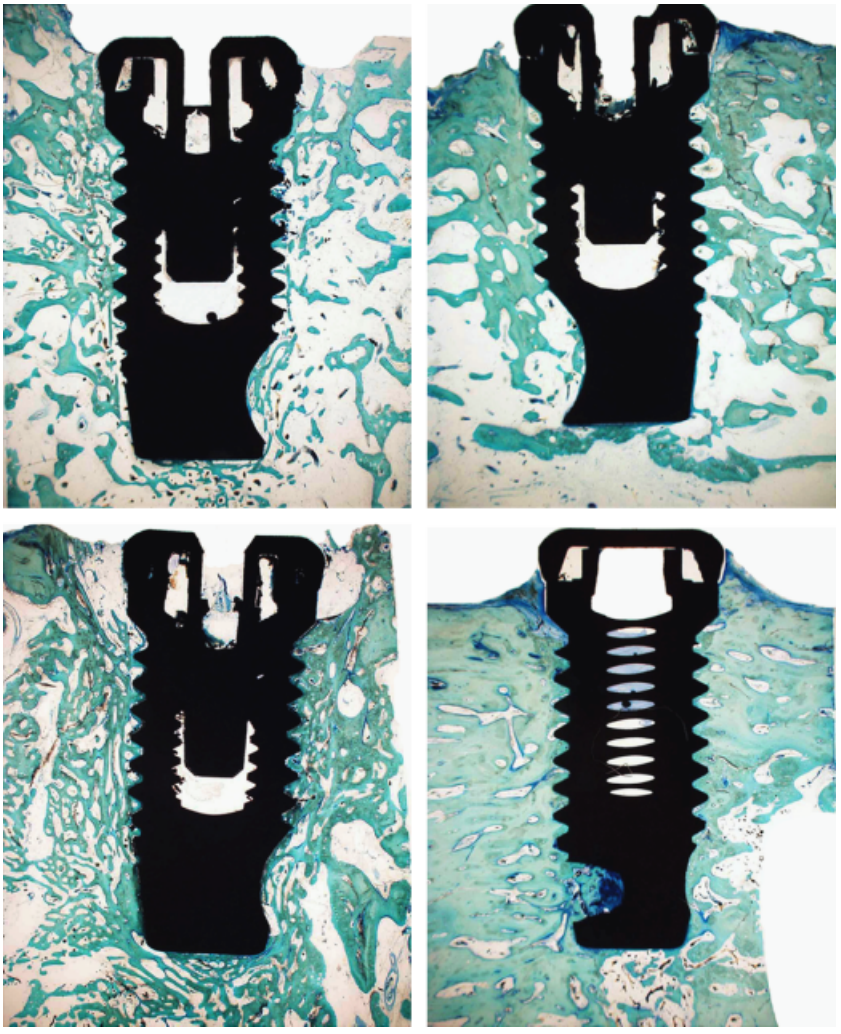

Fig. 3. Representative incandescent light microscopy photomicrographs of titanium porous oxide surface-modified implants coated with recombinant human bone morphogenetic protein-2 (rhBMP-2) at 0.2 and 4.0 mg/ml and uncoated controls at 8 weeks post-surgery. The top row shows rhBMP-2 (0.2 mg/ml) coated (left) and corresponding control (right) implants from the same animal. The bottom row shows rhBMP-2 (4.0 mg/ml) coated implant (left; also shown in Fig. 2) and the corresponding control (right) from a second animal. Note pronounced peri-implant bone re-modelling at implants coated with rhBMP-2 in particular rhBMP-2 (4.0 mg/ml).

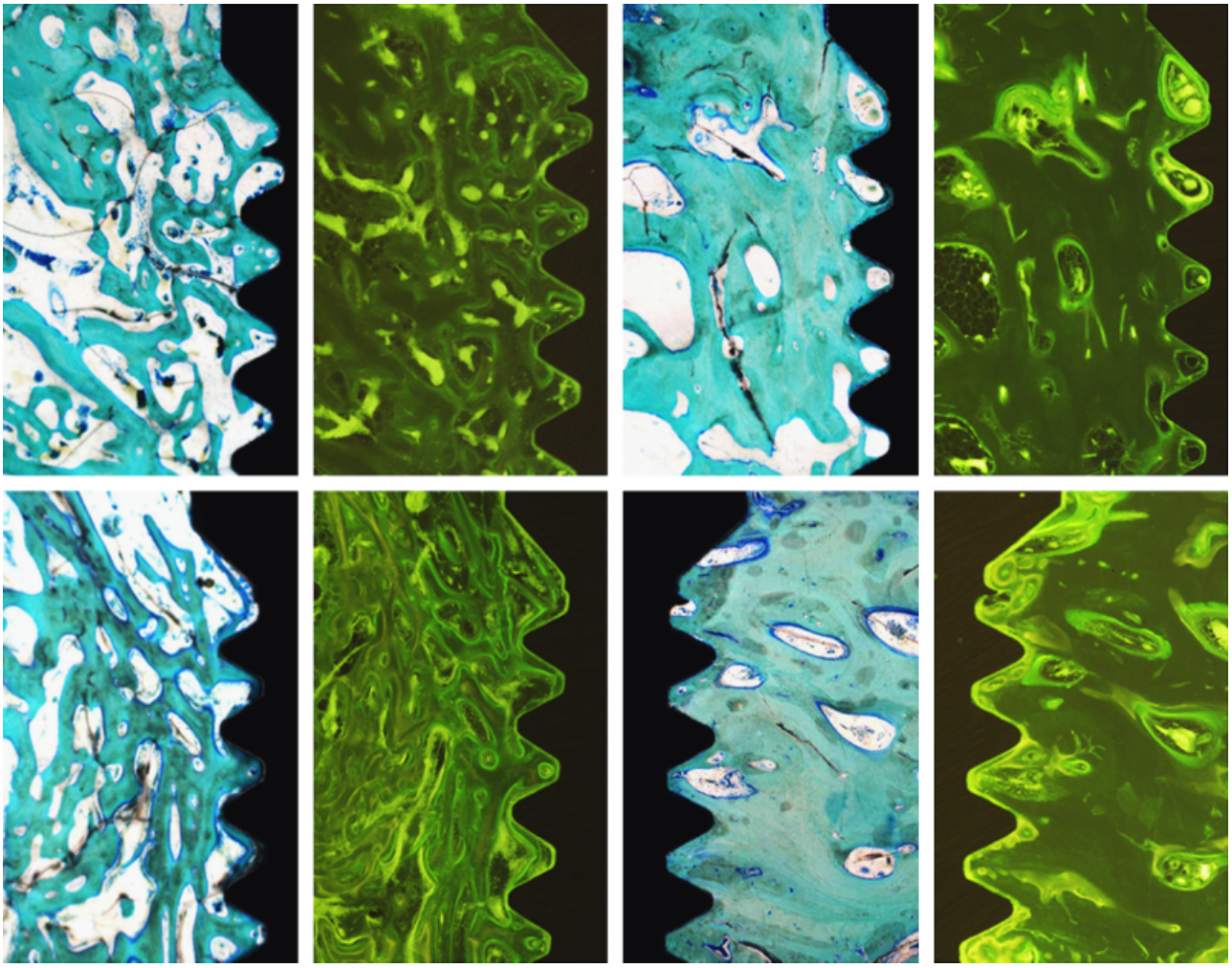

Fig. 4. Close-up incandescent and fluorescence light microscopy photomicrographs of the titanium porous oxide surface-modified implants coated with recombinant human bone morphogenetic protein-2 (rhBMP-2) at 0.2 and 4.0 mg/ml and the corresponding uncoated controls shown in Fig. 3. The top row shows the rhBMP-2 (0.2 mg/ml) coated (left) and control (right) implants. The bottom row shows the rhBMP-2 (4.0 mg/ml) coated (left; also shown in Fig. 2) and corresponding control (right) implants. Note peri-implant bone re-modelling at implants coated with rhBMP-2, in particular rhBMP-2 (4.0 mg/ml).

(Figs 3 and 4). Bone re-modelling encompassed a 2-mm radius from the implant surface, the area filled with newly formed fine trabecular bone also establishing bone–implant contact. As observed for implants coated with rhBMP-2 (0.2 mg/ml), the extent of bone–implant contact appeared lower than that observed for the TPO control implants. Variations in peri-implant bone formation appeared related to variations between animals.

Fluorescence microscopy observations

Unsupported native bone undergoing re-modelling was observed within and immediately outside the thread area for TPO control implants. Fluorescent bone markers indicated limited bone re-modelling appreciated by narrow oxytetracycline (week 3), xylenol orange (week 4), and calcein green markers (week 8), and limited bone formation between the markers (Figs 3 and 4).

Variable re-modelling leaving native resident bone close to the implant surface followed by rapid bone formation (week 3 and 4) into the thread area (diffuse/wide yellow and orange markers) was characteristic for TPO implants coated with rhBMP-2 (0.2 mg/ml) (Figs 3 and 4). The newly formed bone exhibited high density.

The immediate implant area was characterized by re-modelling extending 1–2 mm from the implant surface leaving little, if any, resident bone within and immediately outside the thread area for TPO implants coated with rhBMP-2 (4.0 mg/ml) (Figs 3 and 4). Extensive bone formation within and immediately outside the thread area was observed from week 2 through 4 (diffuse/wide oxytetracycline yellow and xylenol orange markers with bone formation preceding the yellow label) with little bone formation at week 8 (limited separation between narrow calcein green markers). The newly formed bone exhibited high density.

Histometric evaluation

The results from the histometric evaluation of TPO implants coated with rhBMP-2 (0.2 mg/ml) and contra-lateral TPO controls without rhBMP-2 are shown in Table 1. Bone density within a 600-μm radius from the implant threads was significantly lower for implants coated with rhBMP-2 compared with control ($46.4 \pm 6.9\%$ *versus* $64.4 \pm 12.5\%$; $p < 0.03$). However,

Table 1. Titanium porous oxide (TPO) implants coated with recombinant human bone morphogenetic protein-2 (rhBMP-2) (0.2 mg/ml) *versus* uncoated controls (means ± SD, $N = 4$)

	Bone density outside threads (%)	Bone density within threads (%)	Coronal bone contact (mm)	Bone–implant contact (%)
rhBMP-2	46.4 ± 6.9	44.6 ± 9.2	0.4 ± 0.2	43.3 ± 10.8
Control	64.4 ± 12.5	52.6 ± 16.5	0.3 ± 0.1	71.7 ± 7.8
p-value	0.0298	0.3431	0.0366	0.0157

Table 2. Titanium porous oxide (TPO) implants coated with recombinant human bone morphogenetic protein-2 (rhBMP-2) (4.0 mg/ml) *versus* uncoated controls (means ± SD, $N = 4$)

	Bone density outside threads (%)	Bone density within threads (%)	Coronal bone contact (mm)	Bone–implant contact (%)
rhBMP-2	45.3 ± 7.1	35.2 ± 11.4	0.5 ± 0.2	35.4 ± 10.6
Control	57.4 ± 18.4	53.8 ± 17.6	0.8 ± 0.5	68.2 ± 11.0
p-value	0.1329	0.1621	0.1347	0.0249

there were no significant differences in bone density within the thread area. There were statistically significant differences in coronal bone contact (0.4 ± 0.2 *versus* 0.3 ± 0.1 mm; $p < 0.04$) and bone–implant contact (43.3 ± 10.8% *versus* 71.7 ± 7.8%; $p < 0.02$) between rhBMP-2-coated and TPO control implants.

The results from the histometric evaluation of TPO implants coated with rhBMP-2 (4.0 mg/ml) and contra-lateral TPO controls without rhBMP-2 are shown in Table 2. There were no significant differences in bone density within a 600-μm radius outside the thread area, bone density within the thread area, and coronal bone contact. However, there were statistically significant differences in bone–implant contact between rhBMP-2-coated and TPO control implants (35.4 ± 10.6% *versus* 68.2 ± 11.0%; $p < 0.03$).

Discussion

The objective of this study was to determine if coating implants with rhBMP-2 might enhance or accelerate local bone formation and support osseointegration in a large animal oral/maxillofacial orthotopic model. TPO surface modified implants coated with rhBMP-2 at 0.2 or 4.0 mg/ml and TPO controls without rhBMP-2 were installed into the edentulated posterior mandible (Type II bone) in eight Hound Labrador mongrel dogs using split mouth designs. The animals were euthanized at 8 weeks post-surgery when block biopsies were collected for histologic and histometric analysis. Radiographic and incandescent/fluorescent light microscopy evaluations suggest that rhBMP-2 induces immediate peri-implant bone re-modelling including the formation of normal physiologic bone. The rhBMP-2-induced bone exhibits significant bone density and bone–implant contact. The extent of bone re-modelling appears correlated to the rhBMP-2 dose.

Marked bone re-modelling was observed at TPO implants coated with rhBMP-2. This effect was dose-dependent, implants coated with rhBMP-2 (4.0 mg/ml) exhibiting markedly increased bone metabolic activity compared with that observed for implants coated with rhBMP-2 (0.2 mg/ml) and controls. Briefly, implants coated with rhBMP-2 (4.0 mg/ml) exhibited early soft tissue swelling, radiographic evidence of peri-implant bone resorption, histologic evidence of bone re-modelling reaching a 2 mm radius from the implant surface including accelerated bone formation within 2–3 weeks, and radiographic and clinical evidence of bone formation exceeding the implant cover-screw. Similar, however less noticeable, reactions were observed for implants coated with rhBMP-2 (0.2 mg/ml). The histologic observations provided evidence of bone re-modelling, however, leaving peninsulas and islands of resident bone in the immediate vicinity of the implant surface. Bone re-modelling appeared limited and, importantly, delayed at TPO control implants. These observations are the first to demonstrate an osteoinductive titanium root-form implant that supports bone formation and accelerated osseointegration in a large animal oral/maxillofacial orthotopic model. This novel implant technology may have significance for craniofacial rehabilitation, but also repair and re-construction elsewhere in the axial and appendicular skeleton.

Initial peri-implant bone re-modelling has been observed in a previous study when rhBMP-2 in an absorbable collagen sponge carrier placed into the hollow apex of a titanium implant was introduced into the edentulated posterior mandible in dogs (Sykaras et al. 2001). Comparable observations have also been made when rhBMP-2 constructs have been placed into trabecular bone sites such as the femoral neck (Rodeo et al. 1999). *De novo* bone formation followed initial resorption. It is apparent that rhBMP-2 constructs when placed within trabecular bone induce local re-modelling including *de novo* bone formation in a dose-dependent fashion, in the present study within 2–3 weeks. These observations corroborate our previous evaluation of novel implant surface technologies as candidates for rhBMP-2 delivery (Hall et al. 2007). Titanium implants with TPO-modified surfaces coated with rhBMP-2 at various doses and implanted into the rat ectopic model induced de novo bone formation in a dose-dependent fashion. The newly formed bone established contact to the implant surfaces. Collectively, these studies, corroborating the present study, suggest that localized re-modelling, bone resorption followed by rapid bone formation and osseointegration, may be expected with the use of rhBMP-2 technologies in orthotopic sites and that this may become particularly evident in a high-density bone environment such as the posterior mandible (Type II bone). Perhaps the posterior maxilla, generally exhibiting considerably lower bone density (Type IV bone) and thus presenting a greater challenge to the clinician, may be a more favourable environment to evaluate this novel osteoinductive implant technology.

Bone–implant contact approximated 70% following an 8-week healing interval for the TPO control implants also published elsewhere (Xiropaidis et al. 2005). This should be compared with that at similar turned/machined threaded ''Brånemark'' implants placed into rhBMP-2-induced or native resident bone also in the posterior mandible in dogs (Jovanovic et al. 2003). These implants were exposed for prosthetic re-construction following 4 months of submerged osseointegration and were subsequently subject to functional loading over 12 months. Bone–implant contact for these implants ranged from

40% to 50% following 4 months of osseointegration, and from 50% to 75% following 12 months of functional loading. Bone–implant contact following 8 weeks of osseointegration in the posterior mandible in the present study compares favourably to these observations suggesting that a relevant de novo bone–implant contact (rhBMP-2-coated TPO implants) and resident bone–implant contact (TPO control implants) can be obtained within 2 months. Nevertheless, these observations do not preclude that surface-dependent variations may exist at other sites with lesser bone density such as the posterior maxilla.

Conclusions

rhBMP-2 adsorbed onto TPO implant surfaces initiates dose-dependent peri-implant bone re-modelling resulting in the formation of normal, physiologic bone and clinically relevant osseointegration within 8 weeks.

Acknowledgements

Drs. Michael Danesh-Meyer and Richard H. Shanaman are acknowledged for support of the clinical protocol, and Hari S. Prasad, University of Minnesota, Mary Stevens and Janet Golden, Wyeth Research, for histotechnical preparations, and Dr. X. Jian Li for thoughtful review of the fluorescence microscopy. This study was supported by a grant from Wyeth Research and Nobel Biocare AB.

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Clinical Relevance

Scientific rationale for the study: This study is the second in a series describing the development of a titanium implant surface that combined with rhBMP-2 induces significant local bone formation for optimal implant placement and osseointegration without the use of bone grafting, biomaterials or devices for guided bone re-generation.

Principal findings and practical implications: Using an orthotopic canine Type II bone model, we show that a TPO surface implant coated with rhBMP-2 promotes immediate peri-implant bone formation including clinically relevant osseointegration in a dose-dependent fashion.