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: 992–1000 doi: 10.1111/j.1600-051X.2008.01322.x

# Bone formation at recombinant human bone morphogenetic protein-2-coated titanium implants in the posterior maxilla (Type IV bone) in non-human primates

**Ulf M. E. Wikesjö[1], Yi-Hao Huang[1], Andreas V. Xiropaidis[1], Rachel G. Sorensen[2], Michael D. Rohrer[3], Hari S. Prasad[3], John M. Wozney[2] and Jan Hall[4]**

[1]Laboratory for Applied Periodontal and Craniofacial Regeneration, Medical College of Georgia School of Dentistry, Augusta, GA, USA; [2]Women's Health and Musculoskeletal Biology, Wyeth Research, Cambridge, MA, USA; [3]Division of Oral and Maxillofacial Pathology, University of Minnesota School of Dentistry, Minneapolis, MN, USA; [4]Research and Development, Nobel Biocare AB, Göteborg, Sweden

*Wikesjö UME, Huang Y-H, Xiropaidis AV, Sorensen RG, Rohrer MD, Prasad HS, Wozney JM, Hall J. Bone formation at recombinant human bone morphogenetic protein-2-coated titanium implants in the posterior maxilla (Type IV bone) in non-human primates. J Clin Periodontol 2008; 35: 992–1000. doi: 10.1111/j.1600-051X.2008.01322.x.*

***Abstract***

**Background:** Studies using ectopic rodent and orthotopic canine models (Type II bone) have shown that titanium porous oxide (TPO) surface implants adsorbed with recombinant human bone morphogenetic protein-2 (rhBMP-2) induce local bone formation including osseointegration. The objective of this study was to evaluate local bone formation and osseointegration at such implants placed into Type IV bone.

**Material and Methods:** rhBMP-2-coated implants were installed into the edentulated posterior maxilla in eight young adult Cynomolgus monkeys: four animals each received three TPO implants adsorbed with rhBMP-2 (2.0 mg/ml) and four animals each received three TPO implants adsorbed with rhBMP-2 (0.2 mg/ml). Contra-lateral jaw quadrants received three TPO implants without rhBMP-2 (control). Treatments were alternated between left and right jaw quadrants. Mucosal flaps were advanced and sutured to submerge the implants. The animals received fluorescent bone markers at weeks 2, 3, 4, and at week 16 when they were euthanized for histologic analysis.

**Results:** Clinical healing was uneventful. Extensive local bone formation was observed in animals receiving implants adsorbed with rhBMP-2 (2.0 mg/ml). The newly formed bone exhibited a specific pinpoint bone–implant contact pattern regardless of rhBMP-2 concentration resulting in significant osseointegration; rhBMP-2 (2.0 mg/ml): 43% and rhBMP-2 (0.2 mg/ml): 37%. Control implants exhibited a thin layer of bone covering a relatively larger portion of the implant threads. Thus, TPO control implants bone exhibited significantly greater bone–implant contact ($\sim$ 75%; $p < 0.05$). There were no statistically significant differences between rhBMP-2-coated and control implants relative to any other parameter including peri-implant and intra-thread bone density.

**Conclusion:** rhBMP-2-coated TPO implants enhanced/accelerated local bone formation in Type IV bone in a dose-dependent fashion in non-human primates resulting in significant osseointegration. rhBMP-2-induced de novo bone formation did not reach the level of osseointegration observed in native resident bone within the 16-week interval.

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

Accepted for publication 17 August 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 M. Wozney is an employee of Wyeth Research. Ulf M. E. Wikesjö was an employee of Wyeth Research, and serves as consultant to Wyeth Research and Nobel Biocare AB.

Since the discovery (Urist 1965), purification and molecular cloning (Wozney et al. 1988, Celeste et al. 1990, Özkaynak et al. 1990), and characterization (Wang et al. 1990, Sampath et al. 1992, Hötten et al. 1996) of bone morphogenetic proteins (BMPs), recombinant human BMPs have become available for preclinical and clinical evaluation including a variety of indications in the axial and appendicular skeleton; and for some indications already incorporated into clinical practice (Cook 1999, Boden 2001, Friedlaender 2001, Wikesjö et al. 2001, Valentin-Opran et al. 2002, Huang et al. 2008). It has been shown, primarily rodent models, that BMPs have an affinity to titanium and as such titanium implants have been considered as potential vehicles for BMPs (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, Hartwig et al. 2003). In a series of studies we have evaluated the possibility of developing a load-bearing implant, which could deliver BMPs primarily for oral/maxillofacial reconstruction (Hall & Lausmaa 2000, Hall et al. 2007, Wikesjö et al. 2008). In these studies, the lead implant technology features a titanium porous oxide (TPO) surface exhibiting a 95% increase in surface area compared with conventional turned titanium surfaces and could potentially provide a higher level and rate of osseointegration as well as serve as an effective vehicle for BMPs.

Large animal models have shown favourable results comparing the native TPO surface as a stand-alone technology with other surface technologies in various settings (Gottlow et al. 2000, Zechner et al. 2003, Huang et al. 2005, Xiropaidis et al. 2005). A first study evaluating the TPO surface coated with recombinant human BMP-2 (rhBMP-2) using the rat ectopic model showed that rhBMP-2/TPO implants induced local bone formation and osseointegration in a dose-dependent order (Hall et al. 2007). rhBMP-2/TPO implants were subsequently evaluated in a large animal orthotopic model (Wikesjö et al. 2008). Implants coated with rhBMP-2 exhibited accelerated bone formation compared with control; in some cases, bone formation appeared completed within 3–4 weeks upon implant installation in the edentulated posterior mandible (Type II bone; Lekholm & Zarb 1985) in the dog. However, implants placed into the posterior mandible in dogs could not discern how this novel technology would affect bone formation at sites with poor bone quality, a perennial challenge in implant dentistry. Thus, the objective of this study was to examine bone formation and osseointegration at rhBMP-2-coated TPO implants in Type IV bone (Lekholm & Zarb 1985) using a non-human primate model.

## Material and Methods

### Animals

Eight young adult male *Macaca fascicularis* monkeys were used. The animals had access to a standard laboratory diet and water until the beginning of the experimental segment of the study. Teeth were scaled and brushed with chlorhexidine (Chlorhexidine Gluconate 20%, Xttrium Laboratories Inc., Chicago, IL, USA; 20–30 ml of a 2% solution) 2 weeks before the surgical procedures. The study was approved by the Institutional Animal Care and Use Committee, Wyeth Research, Cambridge, MA, USA.

### Titanium implants

Titanium implants with a TPO surface (TiUnite™, ∅3.75 × 8.5 mm; Nobel Biocare AB, Göteborg, Sweden) were used. The sterile implants were coated with rhBMP-2 (Wyeth Research) or left uncoated (control). Using aseptic technique, lyophilized rhBMP-2 was reconstituted with sterile water for injection to produce a 4.0 mg/ml solution. rhBMP-2 solutions at 0.2 and 2.0 mg/ml were prepared by diluting the reconstituted rhBMP-2 4.0 mg/ml solution in appropriate volumes of MFR 00169 buffer (5 mM glutamic acid, 5 mM NaCl, 2.5% glycine, 0.5% sucrose, 0.01% polysorbate 80, pH 4.5; Wyeth Research). Next, sterile implants with a TPO surface were removed from their packaging and exposed to ozone radiation for 10–15 min. before exposure to rhBMP-2. Each implant was placed into a sterile micro-Eppendorf tube containing 0.3 ml of the prepared rhBMP-2 solution (0.2 or 2.0 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.

### Surgical procedures

Food was withheld the night preceding surgery. Animals were pre-anesthetized with atropine [0.04 mg/kg intramuscularly (IM)/buprenorphine HCl (0.03 mg/kg) subcutaneously (SQ)], induced with ketamine (10 mg/kg IM), intubated and maintained on gas anesthesia (1–2% isoflurane/$O_2$ to effect). A sterile catheter was placed and the animals received a slow constant rate infusion of lactated Ringer's solution [10 ml/kg/h intravenously (IV)] to maintain hydration while anesthetized. A broad-spectrum antibiotic (cefazolin, 25 mg/kg IM) was administered for infection control.

In the maxilla, buccal and palatal sulcular incisions were made in the premolar/molar region reflecting buccal and palatal mucoperiosteal flaps and the premolar and molar teeth were extracted bilaterally. The flaps were re-apposed and sutured (GORE-TEX Suture CV5, W. L. Gore & Associates Inc., Flagstaff, AZ, USA) ensuring wound closure for primary intention healing. The extraction sites were allowed to heal for 12 weeks. The animals were placed on a soft-food diet for 2 weeks post-extraction.

For the implant installation, bilateral mid-crestal incisions were initiated immediately distal to the maxillary canine teeth and continued palatally approximately 10 mm and then distally to the second molar region where the incision was brought back mid-crestally. Following reflection of mucoperiosteal flaps, three rhBMP-2-coated implants (rhBMP-2 at 2.0 or 0.2 mg/ml) and three implants without rhBMP-2 (control) were installed into contra-lateral edentulated maxillary sites following routine protocols (Fig. 1). Four animals received implants coated with rhBMP-2 (2.0 mg/ml) and controls in contra-lateral jaw quadrants and four animals similarly received implants coated with rhBMP-2 (0.2 mg/ml). Each implant received a cover-screw and the mucoperiosteal flaps were readapted and sutured using vertical and horizontal mattress sutures (W. L. Gore & Associates Inc.) to en-

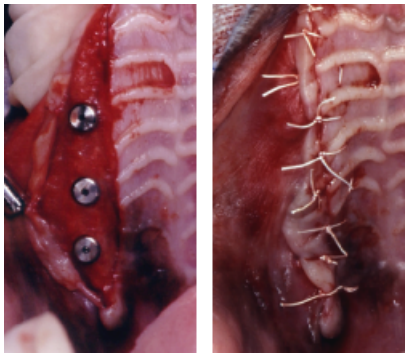

*Fig. 1.* Three titanium porous oxide surface implants were each placed into the left and right edentulated posterior maxilla. Wound closure for primary intention healing was achieved for all surgeries using a combination of mattress and interrupted sutures.

sure wound closure for primary intention healing. Treatments were alternated between left and right jaw quadrants in subsequent animals.

#### Post-surgery procedures

A long-acting opioid (buprenorphine HCl, 0.03 mg/kg IM, twice daily for 48 h) was administered for pain control. The broad-spectrum antibiotic (cefazolin, 25 mg/kg IM, BID for 7 days) was continued for infection control. Plaque control was maintained by daily topical rinse-application of chlorhexidine (Xttrium Laboratories; 20–30 ml of a 2% solution) until completion of the study. In addition, the animals' teeth were brushed with the chlorhexidine solution when they were sedated for suture removal and radiographic registrations. The animals were fed a soft-food diet for 2 weeks post-implantation and were then returned to the standard laboratory diet. Sutures were removed under sedation (ketamine, 10 mg/kg IM) at 10–14 days post-implantation.

#### Fluorescent bone labelling

Fluorescent markers were administered at 2, 3 and 4 weeks post-implantation and at 10 and 3 days pre-euthanasia to monitor bone formation (Li & Jee 2005) as follows: Alizarin Complexone (Fisher Scientific, Houston, TX, USA), 20 mg/kg IM at 2 weeks post-implantation; Oxytetracycline hydrochloride (Maxim-200, Phoenix Pharmaceuticals Inc., Belmont, CA, USA; 200 mg/ml), 20 mg/kg IM at 3 weeks post-implantation; Xylenol orange (Sigma-Aldrich Inc., St. Louis, MO, USA; 200 mg/ml), 25 mg/kg IM for 2 days at 4 weeks post-implantation; and Calcein green (Sigma-Aldrich Inc.; 25 mg/ml), 5 mg/kg IM at 10 and 3 days pre-euthanasia.

#### Clinical and radiographic recordings

Intra-surgery photographs were obtained before and immediately after placement of the implants, and following wound closure. Observations of experimental sites with regards to mucosal health, maintenance of suture line closure, edema and evidence of tissue necrosis or infection were made at suture removal, and while the animals were sedated for radiographic registrations, immediately post-implantation, and at weeks 2, 4, 8, 12 and 16 post-implantation.

#### Euthanasia

The animals were euthanized at week 16 post-implantation. Following sedation with ketamine (10 mg/kg IM), the animals were euthanized using an overdose of pentobarbital sodium (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 transferred to 10% neutral buffered formalin at a volume 10 × that of the block section.

#### Histological 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, Heraeus Kulzer, Verheim, Germany). The implants were cut mid-axially in a buccal–palatal plane into sections of 200 $\mu$m thickness using the cutting–grinding technique (EXAKT Apparatebau, Norderstedt, Germany) and were subsequently ground and polished to a final thickness of approximately 40 $\mu$m for fluorescence microscopy examination (Donath & Breuner 1982, Rohrer & Schubert 1992). Upon completion of the fluorescence microscopy examination, the sections were stained with Stevenel's blue and van Gieson's picro fuchsin. The most central section from each implant was used for the histologic and histometric analysis.

#### Data analysis

Two experienced masked examiners (U. M. E. W., Y.-H. H.) using a magnifier/masking device (Viewscope 2 ×, Flow X-Ray Corp., Hempstead, NY, USA) evaluated the radiographs from immediately post-implantation and from week 16 post-implantation for peri-implant bone formation, crestal bone resorption, and potential implant penetration into the subantral space.

The histopathologic evaluation by the two masked examiners (U. M. E. W., Y.-H. H.) using incandescent and polarized light microscopy (BX 60, Olympus America Inc., Melville, NY, USA) included observations of peri-implant bone formation, bone resorption, woven and lamellar bone, cortex formation, seroma formation, fibrovascular tissue and marrow, inflammatory responses, vascularity and implant penetration into the subantral space.

One masked examiner (U. M. E. W.) using fluorescent light microscopy (Olympus America Inc.) analysed the fluorochrome bone histodynamic markers. Active bone formation was observed relative to the presence or absence, intensity and width of the fluorochrome markers at the 2-, 3- and 4-week and pre-sacrifice observations (evidenced by red, yellow, orange, or green markers, respectively).

One calibrated masked examiner (Y.-H. H.) performed the histometric analysis using incandescent and polarized light microscopy (Olympus America Inc.), a microscope digital camera system (DP10, Olympus America Inc.) and a PC-based image analysis system (Image-Pro Plus™, Media Cybernetic, Silver Spring, MD, USA). The following measurements were recorded for the buccal and palatal surfaces of the most central section of each implant:

- *Crestal bone height*: distance between the most coronal extent of peri-implant crestal bone and the implant top surface;
- *Coronal bone contact*: distance between the most coronal bone contact and the implant top surface;
- *Peri-implant bone density*: density of peri-implant alveolar bone immediately outside the implant threads in an area extending 500 $\mu$m from the implant surface along the five most coronal threads;
- *Intra-thread bone density*: density of peri-implant alveolar bone within the five most coronal threads;
- *Bone–implant contact*: percentage bone–implant contact along the five most coronal threads of the implant surface below the alveolar crest.

### Statistical analysis

Intra-examiner reproducibility was evaluated by duplicate histometric registrations using a subset of the implant sections 1 week apart. Repeat measurements of crestal bone height, coronal bone contact, bone density within and immediately outside the implant threads and percentage of bone–implant contact showed high reproducibility (correlation coefficient: 1.0; $p < 0.0001$).

Summary statistics (means ± SD) based on animal means for the experimental conditions were calculated using the selected sections. Paired *t*-tests were performed to evaluate differences between treatment conditions for each group ($N = 4$) (StatView 4.5, Abacus Concepts, Berkeley, CA, USA). Significance was accepted at a probability level of $p \leqslant 0.05$.

## Results

### Clinical and radiographic observations

At implant installation, 12 weeks following tooth extractions, the maxillary alveolar ridges all showed sufficient width for implant placement. The implants were placed into Type IV bone in every animal. Some of the implants dropped into the osteotomy passing through the cortical bone. Seven implants did not obtain primary stability. Three implants were perceived perforating into the subantral space; however, only one could be confirmed radiographically immediately post-implantation. Primary wound closure was achieved in all surgical sites. Healing following implant placement was generally uneventful.

Seven implants became exposed during the healing interval. The cover screws were exposed in part or completely without clinical signs of inflammation of the adjoining mucosal tissues. All the implants were maintained clinically stable until euthanasia. Radiographs obtained at sacrifice showed no significant differences between experimental and control groups. Some crestal bone loss was observed at the exposed implants. Radiographic evaluation of the gross specimens suggested that implant sinus penetration had occurred at 10 implant sites (Fig. 2).

### Light microscopy observations

Five rhBMP-2-coated implants [four rhBMP-2 (2.0 mg/ml)] and one control showed exposed cover screws. There was no appreciable inflammatory reaction associated with the exposures. Notably, the crestal bone level was reduced to the first thread at the exposed implants regardless of treatment. In contrast, the bone level reached up to the implant shoulder to even cover the entire implant at implants that remained submerged (Fig. 3).

Five implants showed penetration into the subantral space; one of which coated with rhBMP-2 (0.2 mg/ml). Three implants exhibited limited sinus penetration maintaining the integrity of the sinus mucosa. Two implants apparently penetrated the Schneiderian membrane without an appreciable inflammatory reaction. There was no bone formation associated with the implant intrusions (Fig. 3).

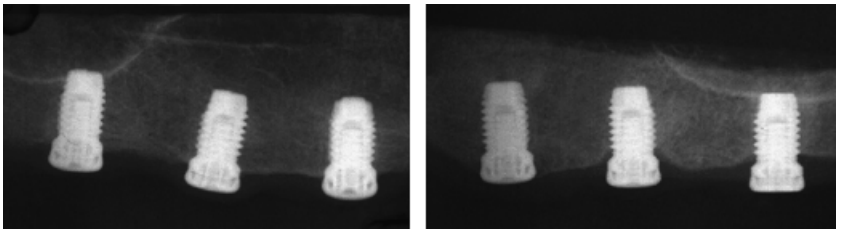

*Fig. 2.* Radiographs from the left and right maxilla obtained at week 16 post-implantation. The titanium porous oxide surface implants in the right panel were coated with recombinant human bone morphogenetic protein-2 (rhBMP-2; 2.0 mg/ml). No appreciable differences may be noticed between jaw quadrants receiving rhBMP-2-coated or uncoated control implants. The distal implants appear to have penetrated the floor of the maxillary sinus.

*Fig. 3.* Photomicrographs of recombinant human bone morphogenetic protein-2 (rhBMP-2; 2.0 mg/ml)-coated and uncoated control titanium porous oxide surface implants placed into the non-human primate posterior maxilla (Type IV bone) also shown in Fig. 2. The top row shows (posterior → anterior) control implants. The second row shows (anterior → posterior) rhBMP-2 (2.0 mg/ml)-coated implants. The posterior control implant partially intrudes into the sinus. The rhBMP-2 (2.0 mg/ml)-coated implants exhibit a pinpoint pattern of bone–implant contact. The controls show thin layers of bone covering the implant threads. The distal rhBMP-2 (2.0 mg/ml)-coated implants are exposed, the alveolar bone reaching the top thread only (healing interval 16 weeks; stain: Stevenel's blue and van Gieson's picro fuchsin).

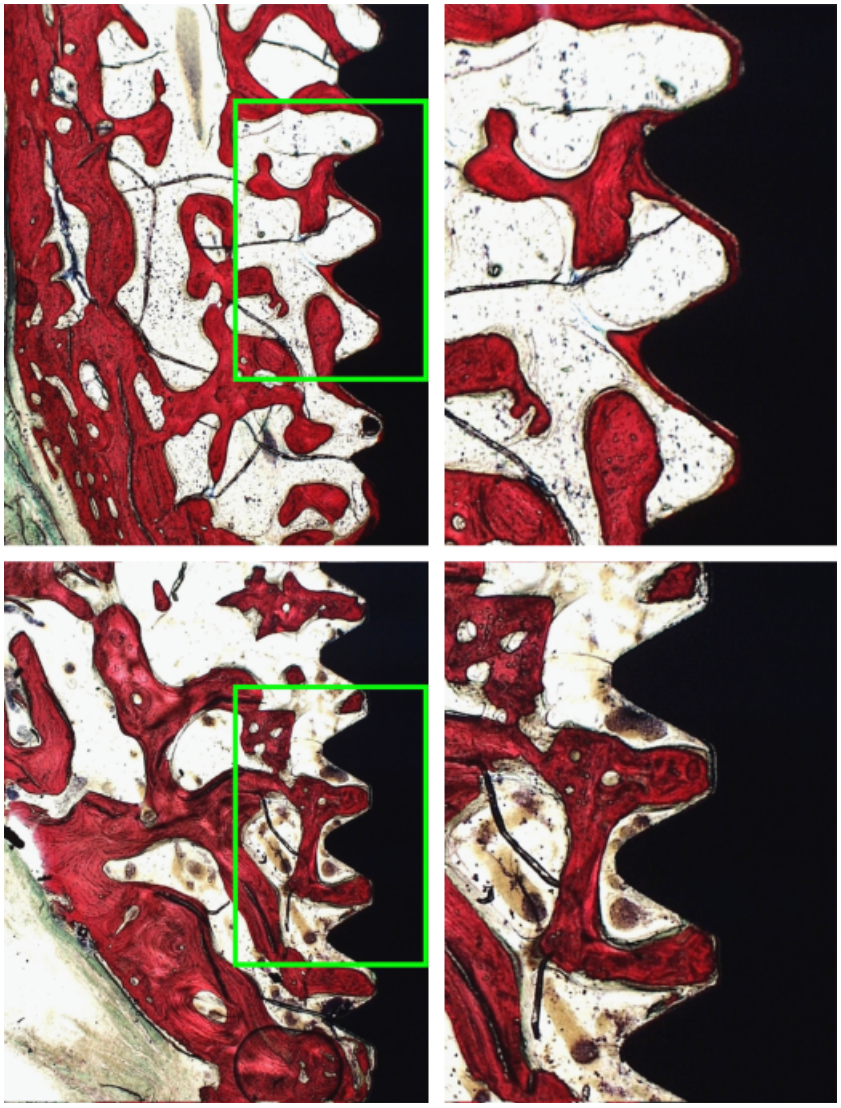

*Fig. 4*. Photomicrographs showing characteristic bone–implant contact patterns at control (top) and recombinant human bone morphogenetic protein-2 (rhBMP-2)-coated implants. The rhBMP-2-coated titanium porous oxide surface implant exhibits a commonly observed pinpoint bone–implant contact pattern. In contrast, a typical thin layer of bone along the threads in contact with the implant surface may be observed in the control (healing interval 16 weeks; stain: Stevenel's blue and van Gieson's picro fuchsin).

Type IV bone was observed for all jaw quadrants (Lekholm & Zarb 1985, Hanisch et al. 1997a). Two animals exhibited relatively denser peri-implant bone formation at implants coated with rhBMP-2 (2.0 mg/ml). There were no other striking differences between experimental and control sites with the exception for the character of bone–implant contact patterns (Fig. 3).

The rhBMP-2-coated implants exhibited a characteristic bone–implant contact pattern without major differences between rhBMP-2 concentrations. The surrounding bone projected into the root of the threads forming pinpoint bone–implant contacts. Areas of broader bone–implant contact were also observed. In contrast, the predominant observation for the control was a thin layer of bone covering most of the implant threads. This characteristic appeared to produce a high-level bone–implant contact (Fig. 4).

#### Fluorescence microscopy observations

Sites receiving rhBMP-2 (2.0 mg/ml)-coated implants exhibited new peri-implant bone formation extending up to 2 mm from the implant surface (Fig. 5). The zone of newly formed bone usually did not exhibit residual native bone. Diffuse orange and yellow bands suggested the new bone being formed within 4 weeks. This observation was consistent for each of the four animals receiving rhBMP-2 (2.0 mg/ml)-coated implants, however appeared more pronounced in two animals. Contralateral control sites exhibited residual native bone in close proximity to the implant surface (Fig. 5). Linear yellow, orange and green bands indicative normal bone turnover was observed. A few sites receiving rhBMP-2 (2.0 mg/ml)-coated implants did not show appreciable differences from the control.

Sites receiving rhBMP-2 (0.2 mg/ml)-coated implants exhibited limited new bone formation (data not shown). Residual native bone exhibiting linear yellow, orange and green bands indicative of normal bone turnover was observed in the immediate peri-implant area. The contralateral control exhibited similar appearance without major differences among animals (data not shown).

#### Histometric analysis

For animals receiving rhBMP-2 (2.0 mg/ml)-coated implants (Table 1), crestal bone height and coronal bone contact averaged $0.4 \pm 0.3$ and $0.8 \pm 0.4$ mm *versus* $0.2 \pm 0.2$ and $0.4 \pm 0.3$ mm for the control ($p > 0.05$). Peri-implant and intra-thread bone density averaged $39.8 \pm 14.8\%$ and $45.7 \pm 14.1\%$ for the rhBMP-2-coated implants *versus* $37.9 \pm 10.4\%$ and $32.8 \pm 13.1\%$ for the control ($p > 0.05$). However, there was a statistically significant difference in bone–implant contact between rhBMP-2-coated and control implants ($43.0 \pm 6.5\%$ *versus* $74.4 \pm 13.8\%$; $p < 0.01$).

For animals receiving rhBMP-2 (0.2 mg/ml)-coated implants (Table 2), crestal bone height and coronal bone contact averaged $0.1 \pm 0.2$ and $0.3 \pm 0.4$ mm *versus* $0.1 \pm 0.1$ and $0.3 \pm 0.4$ mm for the control ($p > 0.05$). Peri-implant and intra-thread bone density averaged $31.4 \pm 11.5\%$ and $35.5 \pm 6.4\%$ for the rhBMP-2-coated implants *versus* $38.1 \pm 5.4\%$ and $32.0 \pm 6.4\%$ for the control ($p > 0.05$). Nevertheless, there was a statistically significant difference in bone–implant contact between rhBMP-2-coated and control implants ($36.8 \pm 9.8\%$ *versus* $74.6 \pm 15.7\%$; $p < 0.05$).

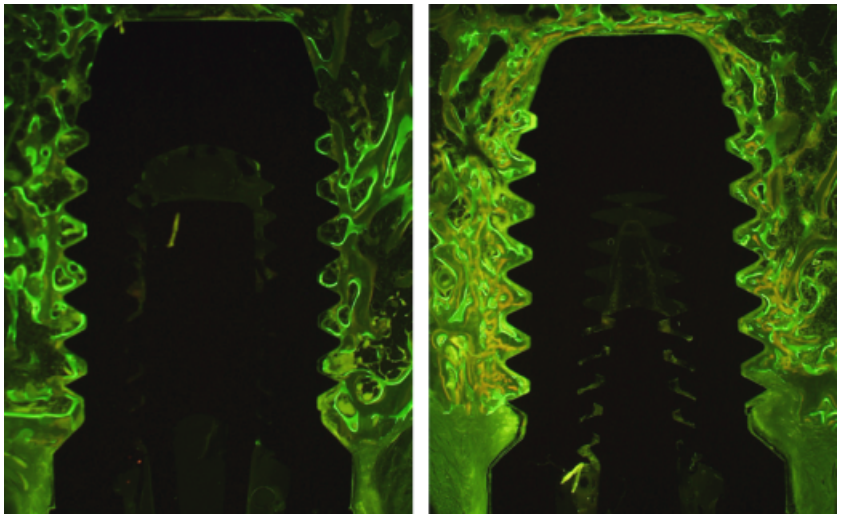

*Fig. 5*. Fluorescence microscopy photomicrographs of the central control and recombinant human bone morphogenetic protein-2 (rhBMP-2; 2.0 mg/ml)-coated titanium porous oxide surface implants also shown in Figs 2 and 3. The rhBMP-2-coated implant (right) exhibits extensive early peri-implant bone formation (wide diffuse yellow and orange bands). The control implant shows limited new bone formation (narrow fluorescent markers indicate normal bone turnover). The native alveolar bone (dark green) remains in close contact with the control but not the rhBMP-2-coated implant (healing interval 16 weeks).

*Table 1*. Comparisons between jaw quadrants receiving rhBMP-2 (2.0 mg/ml)-coated implants *versus* uncoated controls (means ± SD; $N = 4$)

| | Crestal bone height (mm) | Coronal bone contact (mm) | Peri-implant bone density (%) | Intra-thread bone density (%) | Bone–implant contact (%) |
|---|---|---|---|---|---|
| rhBMP-2 | 0.4 ± 0.3 | 0.8 ± 0.4 | 39.8 ± 14.8 | 45.7 ± 14.1 | 43.0 ± 6.5 |
| Control | 0.2 ± 0.2 | 0.4 ± 0.3 | 37.9 ± 10.4 | 32.8 ± 13.1 | 74.4 ± 13.8 |
| *p*-value | 0.0714 | 0.0707 | 0.8232 | 0.2811 | 0.0079 |

*Table 2*. Comparisons between jaw quadrants receiving rhBMP-2 (0.2 mg/ml)-coated implants *versus* uncoated controls (means ± SD; $N = 4$)

| | Crestal bone height (mm) | Coronal bone contact (mm) | Peri-implant bone density (%) | Intra-thread bone density (%) | Bone–implant contact (%) |
|---|---|---|---|---|---|
| rhBMP-2 | 0.1 ± 0.2 | 0.3 ± 0.4 | 31.4 ± 11.5 | 35.5 ± 6.4 | 36.8 ± 9.8 |
| Control | 0.1 ± 0.1 | 0.3 ± 0.3 | 38.1 ± 5.4 | 32.0 ± 6.4 | 74.6 ± 15.7 |
| *p*-value | 0.4959 | 0.6990 | 0.4844 | 0.4718 | 0.0348 |

## Discussion

This study was designed to evaluate local bone formation and osseointegration at rhBMP-2-coated TPO surface implants using a Type IV bone non-human primate model; the edentulated posterior maxilla. TPO implants without rhBMP-2 implanted in contralateral jaw quadrants served as control. The animals were euthanized for histologic analysis following a 16-week healing interval. Sites receiving implants coated with rhBMP-2 (2.0 mg/ml) exhibited extensive (within 4 weeks) local bone formation compared with sites receiving implants coated with rhBMP-2 (0.2 mg/ml) or control. The newly formed bone exhibited a specific pinpoint bone–implant contact pattern resulting in significant, clinically relevant osseointegration regardless of rhBMP-2 concentration. The controls, on the other hand, exhibited a thin layer of bone covering a relatively larger percentage of the implant threads. Thus, the controls exhibited statistically significantly greater osseointegration than the rhBMP-2-coated implants.

The survival or success rate of endosseous implants placed into Type IV bone, in particular in the posterior maxilla, appears dramatically decreased compared with that in other bone qualities (Adell et al. 1981, Albrektsson et al. 1988, Jaffin & Berman 1991, Lekholm et al. 1994, Jemt et al. 1996, Buser et al. 1997). This was one motivation to use the edentulated posterior maxilla in non-human primates to study bone formation and osseointegration following installation of the rhBMP-2 conditioned TPO implants. Several previous studies evaluating BMP technologies have preferred non-human primate models (Boyne 1996, Hanisch et al. 1997a, b, Boyne et al. 1998). The non-human primate posterior maxilla has been shown to consistently present Type IV bone (Hanisch et al. 1997a). Moreover, the appositional bone formation rate in the Cynomolgus monkey closely parallels that in humans (Simmons 1976, Schenk 1991). Thus, observations in this non-human primate model may directly translate to clinical applications.

The present study used TPO surface implants (Hall & Lausmaa 2000) as a vehicle for rhBMP-2. The TPO surface may not only increase the contact area to bone but also incorporate biologic factors that enhance bone formation. Potentially, a BMP-coated implant may improve the success rate of implant treatment in the posterior maxilla and other sites with poor bone quality or where aberrations of the alveolar ridge disallows desired immersion of the implant in resident bone. A previous study evaluated various implant surfaces to serve as vehicles for rhBMP-2 (Hall et al. 2007). TPO implants with narrow or open pores and implants with a turned titanium surface coated with rhBMP-2 were implanted into the ventral thoracic region in rats. The histologic evaluation showed significant bone formation and osseointegration following a 14-day healing interval at implant surfaces conditioned with rhBMP-2. The TPO surface with open pores, used in the present study, appeared the most effective rhBMP-2 vehicle. Subsequently, rhBMP-2-coated TPO surface modified implants with open pores were evaluated in Type II bone using the edentulated posterior mandible in dogs (Wikesjö et al. 2008). Two concentrations of rhBMP-2 (0.2 and 4.0 mg/ml) were evaluated. Peri-implant

bone formation and implant osseointegration was recorded following an 8-week healing interval. Similar to the present study, implants coated with rhBMP-2 exhibited dose-dependent accelerated local bone formation. In the present study, the fluorescence microscopy evaluation provided evidence of new bone formation at rhBMP-2 (2.0 mg/ml)-coated TPO implants within 4 weeks. Implants coated with rhBMP-2 at 0.2 mg/ml did not show appreciable new bone formation other than the specific bone–implant contact pattern. Taken together, the observations in the present and previous studies using relevant ectopic and orthotopic models without doubt demonstrate that rhBMP-2 can successfully be delivered to induce local bone formation and osseointegration using the TPO surface as a discrete vehicle.

Distinct bone–implant contact patterns were observed for rhBMP-2-coated and TPO control implants. Bone density within the thread area was not significantly different between experimental and control implants, that is between rhBMP-2-induced de novo bone formation and remodelling resident alveolar bone in the control sites. However, bone distribution appeared unique comparing rhBMP-2-coated implants with the control. In most sites, including rhBMP-2-coated implants, bone formed in the centre of the space among the threads. Thus, the root area among the threads exhibited a characteristic pinpoint bone–implant contact. On the other hand, sites receiving TPO control implants typically exhibited a thin layer of bone covering the implant thread surface producing a higher bone–implant contact rate. The actual mechanism(s) affecting the uniquely different bone healing patterns is unknown other than that they are associated with distinctly different processes, that is, de novo bone formation *versus* bone remodelling. It is unknown, however likely, that bone–implant contact patterns at rhBMP-2-coated implants eventually may assume characteristics of the TPO control. Nevertheless, the TPO surface exhibited convincing osteoconductive properties supporting significant osseointegration both in the presence of rhBMP-2-induced de novo bone formation and native Type IV bone.

Peri-implant bone density and bone–implant contact approximated 38% and 75%, respectively for the controls in this study. Hanisch et al. (1997a) first demonstrated rhBMP-2-induced bone augmentation combined with implant treatment in the posterior maxilla. rhBMP-2 in a collagen sponge was implanted in a subantral non-human primate model. Turned titanium implants were placed into rhBMP-2-induced bone following a 12-week healing interval and allowed to osseointegrate for 12 weeks. Peri-implant bone density approximated 14% in rhBMP-2-induced and native resident bone. Bone–implant contact approximated 40% for rhBMP-2-induced and native resident bone. In other reports, Hürzeler et al. (1997a, b) and Quinones et al. (1997a, b) evaluated bone–implant contact at pure titanium, titanium plasma sprayed and hydroxyapatite-coated implants inserted into the edentulated posterior maxilla in non-human primates following a 16-week healing interval. While bone–implant contact at pure titanium and titanium plasma sprayed implants ranged between 52% and 68%, the corresponding observation for the hydroxyapatite-coated implants was 98%. Bone density for the native resident bone was not reported. Although strict comparisons between studies cannot be made due to differences in design, implant technology and analysis, the observations in the present study demonstrate that the native TPO implant establishes substantial bone–implant contact within a short time interval. These observations are consistent with a previous report of this surface technology in Type II bone in the canine mandible (Xiropaidis et al. 2005).

Radiographic registrations were used to guide the clinical management at implant installation and to monitor healing events. The diagnostic reliability of conventional radiographs has been questioned (Benn 1990, 1992). The present study corroborates, in part, the diagnostic reliability of conventional radiographs when used to evaluate implants. Several implant specimens conditioned with rhBMP-2 exhibited increased peri-implant bone density under light microscopy and early peri-implant bone formation under fluorescence microscopy. Nevertheless, no appreciable differences could be discerned from radiographs comparing these specimens with controls. Moreover, the histopathologic evaluation identified five implants penetrating into the subantral space. During implant surgery, three implants were perceived perforating into the maxillary sinus, only one could immediately be confirmed radiographically. Radiographic evaluation of the gross specimens at sacrifice suggested that implant sinus penetration had occurred at 10 implant sites. In contrast, crestal bone resorption reaching the first thread of the exposed implants was always confirmed by the radiographic registrations. These observations emphasize difficulties using conventional radiographs as a diagnostic tool for implant evaluations.

## Conclusions

The rhBMP-2-coated TPO surface implants enhanced/accelerated local bone formation in Type IV bone in a dose-dependent fashion in non-human primates resulting in significant osseointegration. Nevertheless, rhBMP-2-induced de novo bone formation did not reach the level of osseointegration observed in native bone within the 16-week interval.

## Acknowledgements

This study was supported by a grant from Wyeth Research, Cambridge, MA, USA, and Nobel Biocare AB, Göteborg, Sweden.

## References

Adell, R., Lekholm, U., Rockler, B. & Brånemark, P. I. (1981) A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. *International Journal of Oral Surgery* **10**, 387–416.

Albrektsson, T., Dahl, E., Enbom, L., Engevall, S., Engquist, B., Eriksson, A. R., Feldmann, G., Freiberg, N., Glantz, P. O., Kjellmann, O., Kristersson, L., Kvint, S., Köndell, P. Å., Palmquist, J., Wenndahl, L. & Åstrand, P. (1988) Osseointegrated oral implants. A Swedish multicenter study of 8139 consecutively inserted Nobelpharma implants. *Journal of Periodontology* **59**, 287–296.

Benn, D. K. (1990) A review of the reliability of radiographic measurements in estimating alveolar bone changes. *Journal of Clinical Periodontology* **17**, 14–21.

Benn, D. K. (1992) Estimating the validity of radiographic measurements of marginal bone height changes around osseointegrated implants. *Implant Dentistry* **1**, 79–83.

Boden, S. D. (2001) Clinical application of the BMPs. *The Journal of Bone and Joint Surgery (AM)* **83**, S161.

Boyne, P. J. (1996) Animal studies of the application of rhBMP-2 in maxillofacial reconstruction. *Bone* **19**, 83S–92S.

Boyne, P. J., Nath, R. & Nakamura, A. (1998) Human recombinant BMP-2 in osseous reconstruction of simulated cleft palate defects. *The British Journal of Oral and Maxillofacial Surgery* **36**, 84–90.

Buser, D., Mericske-Stern, R., Bernard, J. P., Behneke, A., Behneke, N., Hirt, H. P., Belser, U. C. & Lang, N. P. (1997) Long-term evaluation of non-submerged ITI implants. Part I: an 8-year life table analysis of a prospective multi center study with 2359 implants. *Clinical Oral Implants Research* **8**, 161–172.

Celeste, A. J., Iannazzi, J. A., Taylor, R. C., Hewick, R. M., Rosen, V., Wang, E. A. & Wozney, J. M. (1990) Identification of transforming growth factor ß family members present in bone-inductive protein purified from bovine bone. *Proceedings of the National Academy of Sciences of the United States of America* **87**, 9843–9847.

Cole, B. J., Bostrom, M. P., Pritchard, T. L., Sumner, D. R., Tomin, E., Lane, J. M. & Weiland, A. J. (1997) Use of bone morphogenetic protein 2 on ectopic porous coated implants in the rat. *Clinical Orthopaedics and Related Research* **345**, 219–228.

Cook, S. D. (1999) Preclinical and clinical evaluation of osteogenic protein-1 (BMP-7) in bony sites. *Orthopedics* **22**, 669–671.

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.

Esenwein, S. A., Esenwein, S., Herr, G., Muhr, G., Küsswetter, W. & Hartwig, C. H. (2001) Osteogenetic activity of BMP-3-coated titanium specimens of different surface texture at the orthotopic implant bed of giant rabbits. *Der Chirurg* **72**, 1360–1368, (in German).

Esenwein, S. A., Esenwein, S., Herr, G., Muhr, G., Küsswetter, W. & Hartwig, C. H. (2003) Histologic and histomorphometric follow-up observations of osseointegration of corundum-blasted BMP-3 coated titanium test implants (Ti6Al4V) at orthotopic site in the giant rabbit. *Biomedizinische Technik (Berlin)* **48**, 217–224, (in German).

Friedlaender, G. E. (2001) OP-1 clinical studies. *The Journal of Bone and Joint Surgery (AM)* **83**, S160–S161.

Gottlow, J., Henry, P. J., Tan, A. E. S., Allan, B. P., Johansson, C. & Hall, J. (2000) Biomechanical and histologic evaluation of the TiUnite and Osseotite implant surfaces in dogs. *Applied Osseointegration Research* **1**, 28–30.

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.

Hanisch, O., Tatakis, D. N., Boskovic, M. M., Rohrer, M. D. & Wikesjö, U. M. E. (1997b) Bone formation and reosseointegration in peri-implantitis defects following surgical implantation of rhBMP-2. *The International Journal of Oral and Maxillofacial Implants* **12**, 604–610.

Hanisch, O., Tatakis, D. N., Rohrer, M. D., Wöhrle, P. S., Wozney, J. M. & Wikesjö, U. M. E. (1997a) Bone formation and osseointegration stimulated by rhBMP-2 following subantral augmentation procedures in non-human primates. *The International Journal of Oral and Maxillofacial Implants* **12**, 785–792.

Hartwig, C.-H., Esenwein, S. A., Pfund, A., Küsswetter, W. & Herr, G. (2003) Improved osseointegration of titanium implants of different surface characteristics by the use of bone morphogenetic protein (BMP-3): an animal study performed at the metaphyseal bone bed in dogs. *Zeitschrift für Orthopadie und Ihre Grenzgebiete* **141**, 705–711.

Herr, G., Hartwig, C. H., Boll, C. & Küsswetter, W. (1996) Ectopic bone formation by composites of BMP and metal implants in rats. *Acta Orthopaedica Scandinavica* **67**, 606–610.

Hötten, G. C., Matsumoto, T., Kimura, M., Bechtold, R. F., Kron, R., Ohara, T., Tanaka, H., Satoh, Y., Okazaki, M., Shrai, T., Pan, H., Kawai, S., Pohl, J. S. & Kudo, A. (1996) Recombinant human growth/differentiation factor 5 stimulates mesenchyme aggregation and chondrogenesis responsible for the skeletal development of limbs. *Growth Factors* **13**, 65–74.

Huang, Y.-H., Polimeni, G., Qahash, M. & Wikesjö, U. M. E. (2008) Bone morphogenetic proteins and osseointegration. Current knowledge – future possibilities. *Periodontology 2000* **47**, 206–223.

Huang, Y.-H., Xiropaidis, A. V., Sorensen, R. G., Albandar, J. M., Hall, J. & Wikesjö, U. M. E. (2005) Bone formation at TiUnite™ implants in type IV bone. *Clinical Oral Implants Research* **16**, 105–111.

Hürzeler, M. B., Quinones, C. R., Kirsch, A., Gloker, C., Schüpbach, P., Strub, J. R. & Caffesse, R. G. (1997b) Maxillary sinus augmentation using different grafting materials and dental implants in monkeys. Part I. Evaluation of anorganic bovine-derived bone matrix. *Clinical Oral Implants Research* **8**, 476–486.

Hürzeler, M. B., Quinones, C. R., Kirsch, A., Schüpbach, P., Krausse, A., Strub, J. R. & Caffesse, R. G. (1997a) Maxillary sinus augmentation using different grafting materials and dental implants in monkeys. Part III. Evaluation of autogenous bone combined with porous hydroxyapatite. *Clinical Oral Implants Research* **8**, 401–411.

Jaffin, R. A. & Berman, C. L. (1991) The excessive loss of Brånemark fixtures in type IV bone: a 5-year analysis. *Journal of Periodontology* **62**, 2–4.

Jemt, T., Chai, J., Harnett, J., Heath, M. R., Hutton, J. E., Johns, R. B., McKenna, S., McNamara, D. C., van Steenberghe, D., Taylor, R., Watson, R. M. & Herrmann, I. (1996) A 5-year prospective multicenter follow-up report on overdentures supported by osseointegrated implants. *The International Journal of Oral and Maxillofacial Implants* **11**, 291–298.

Kawai, T., Mieki, A., Ohno, Y., Umemura, M., Kataoka, H., Kurita, S., Koie, M., Jinde, T., Hasegawa, J. & Urist, M. R. (1993) Osteoinductive activity of composites of bone morphogenetic protein and pure titanium. *Clinical Orthopaedics and Related Research* **290**, 296–305.

Lekholm, U., van Steenberghe, D., Herrmann, I., Bolender, C., Folmer, T., Gunne, J., Henry, P., Higuchi, K., Laney, W. & Lindén, U. (1994) Osseointegrated implants in the treatment of partially edentulous patients: a prospective 5-year multicenter study. *The International Journal of Oral and Maxillofacial Implants* **9**, 627–635.

Lekholm, U. & Zarb, G. A. (1985) Patient selection and preparation. In: Brånemark, P. I., Zarb, G. A. & Albrektsson, T. (eds). *Tissue Integrated Prostheses: Osseointegration in Clinical Dentistry*, pp. 199–209. Chicago: Quintessence.

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

Özkaynak, E., Rueger, D. C., Drier, E. A., Corbett, C., Ridge, R. J., Sampath, T. K. & Oppermann, H. (1990) OP-1 cDNA encodes an osteogenic protein in the TGF-β family. *The EMBO Journal* **9**, 2085–2093.

Quinones, C. R., Hürzeler, M. B., Schüpbach, P., Arnold, D. R., Strub, J. R. & Caffesse, R. G. (1997b) Maxillary sinus augmentation using different grafting materials and dental implants in monkeys. Part IV. Evaluation of hydroxyapatite-coated implants. *Clinical Oral Implants Research* **8**, 497–505.

Quinones, C. R., Hürzeler, M. B., Schüpbach, P., Kirsch, A., Blum, P., Caffesse, R. G. & Strub, J. R. (1997a) Maxillary sinus augmentation using different grafting materials and osseointegrated dental implants in monkeys. Part II. Evaluation of porous hydroxyapatite as a grafting material. *Clinical Oral Implants Research* **8**, 487–496.

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, Oral Pathology* **74**, 73–78.

Sampath, T. K., Maliakal, J. C., Hauschka, P. V., Jones, W. K., Sasak, H., Tucker, R. F., White, K. H., Coughlin, J. E., Tucker, M. M., Pang, R. H., Corbett, C., Özkaynak, E., Oppermann, H. & Rueger, D. (1992) Recombinant human osteogenic protein-1 (hOP-1) induces new bone formation in vivo with a specific activity comparable with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation in vitro. *The Journal of Biological Chemistry* **267**, 20352–20362.

Schenk, R. K. (1991) Zur Problematik der Knochenersatzstoffe: Histophysiologie des Knochenumbaus und der Substitution von Knochenerstoffen. In: Huggler, A. H. & Kuner, E. H. (eds). *Aktueller Stand beim Knochenersatz. Hefte der Unfallheilkunde*, pp. 23–35. Berlin: Springer.

Schmidmaier, G., Wildemann, B., Cromme, F., Kandziora, F., Haas, N. P. & Raschke, M. (2002) Bone morphogenetic protein-2 coating of titanium implants increases biomechanical strength and accelerates bone remodeling in fracture treatment: a biomechanical and histological study in rats. *Bone* **30**, 816–822.

Simmons, D. J. (1976) Comparative physiology of bone. In: Bourne, G. H. (ed). *The Biochemistry and Physiology of Bone*, pp. 445–516. London: Academic Press.

Urist, M. R. (1965) Bone: formation by autoinduction. *Science* **150**, 893–899.

Valentin-Opran, A., Wozney, J., Csimma, C., Lilly, L. & Riedel, G. E. (2002) Clinical evaluation of recombinant human bone morphogenetic protein-2. *Clinical Orthopaedics and Related Research* **395**, 110–120.

Vehof, J. W., Mahmood, J., Takita, H., van't Hof, M. A., Kuboki, Y., Spauwen, P. H. & Jansen, J. A. (2001) Ectopic bone formation in titanium mesh loaded with bone morphogenetic protein and coated with calcium phosphate. *Plastic and Reconstructive Surgery* **108**, 434–443.

Wang, E. A., Rosen, V., D'Alessandro, J. S., Bauduy, M., Cordes, P., Harada, T., Israel, D. I., Hewick, R. M., Kerns, K. M., LaPan, P., Luxenburg, D. P., McQuaid, D., Moutsatsos, I. K., Nove, J. & Wozney, J. M. (1990) Recombinant human bone morphogenetic protein induces bone formation. *Proceedings of the National Academy of Sciences of the United States of America* **87**, 2220–2224.

Wikesjö, U. M. E., Sorensen, R. G. & Wozney, J. M. (2001) Augmentation of alveolar bone and dental implant osseointegration: clinical implications of studies with rhBMP-2. A comprehensive review. *The Journal of Bone and Joint Surgery (AM)* **83**, S136–S145.

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

Wozney, J. M., Rosen, V., Celeste, A. J., Mitsock, L. M., Whitters, M. J., Kriz, R. W., Hewick, R. M. & Wang, E. A. (1988) Novel regulators of bone formation: molecular clones and activities. *Science* **242**, 1528–1534.

Xiropaidis, A. V., Qahash, M., Lim, W. H., Shanaman, R. H., Rohrer, M. D., Wikesjö, U. M. E. & Hall, J. (2005) Bone–implant contact at calcium phosphate-coated and porous titanium oxide (TiUnite)-modified oral implants. *Clinical Oral Implants Research* **16**, 532–539.

Zechner, W., Tangl, S., Fürst, G., Tepper, G., Thams, U., Mailath, G. & Watzek, G. (2003) Osseous healing characteristics of three different implant types. *Clinical Oral Implants Research* **14**, 150–157.

Address:
*Dr. Ulf M. E. Wikesjö*
*Laboratory for Applied Periodontal and Craniofacial Regeneration*
*Departments of Periodontics, Oral Biology and Maxillofacial Pathology*
*Medical College of Georgia School of Dentistry #1430*
*1120 Fifteenth Street*
*Augusta, GA 30912*
*USA*
E-mail: uwikesjo@mcg.edu

**Clinical Relevance**

*Scientific rationale for the study:* This study is a third 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 use of bone grafting, biomaterials, or devices for guided bone regeneration.

*Principal findings and practical implications:* Using an orthotopic non-human primate Type IV 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.