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Bone formation at rhBMP-2-coated titanium implants in the rat ectopic model

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Abstract

Background: The objective of this study was to evaluate local bone formation at titanium porous oxide (TPO) implant surfaces adsorbed with recombinant human bone morphogenetic protein-2 (rhBMP-2).

Methods: In vitro studies were used to estimate the kinetics of I^{125}-labeled rhBMP-2 released from TPO surfaces with narrow (N) or open (O) pores. Machined/turned titanium (MT) surfaces served as control. The rat ectopic model was used to assess local bone formation. Briefly, TPO-N, TPO-O, and MT disc implants adsorbed with 5, 10, or 20 μg rhBMP-2, respectively, were implanted subcutaneously into the ventral thoracic region in 5-week-old male Long Evans rats. The animals were euthanized at day 14 postsurgery when implants with surrounding tissues were removed, radiographed, and gross observations recorded. The specimens were processed for histologic evaluation using conventional cut-and-grind techniques. TPO implants without rhBMP-2 included in a preliminary evaluation revealed no evidence of bone formation, tissue encapsulation, or vascularity, thus such controls were not further used.

Results: TPO and MT implant surfaces adsorbed with 5 μg rhBMP-2 retained 2.3–5.4% rhBMP-2 following immersion and rinse in buffer, and 1.1–2.2% rhBMP-2 following repeated immersions and rinses over 27 days. TPO implants retained the most rhBMP-2 and MT implants retained the least. Explants revealed increased hard tissue formation, tissue encapsulation, and vascularity at TPO compared with MT implants. Radiographic observations were consistent with the explant observations. The histologic analysis showed greater amounts of bone formation, osteoblastic cells, osteoid, marrow, tissue encapsulation, vascularity, and bone voids for implants adsorbed with 10 and 20 μg rhBMP-2, and for TPO implants at the 5-μg rhBMP-2 dose. The histometric analysis revealed significantly greater bone formation at TPO-O than at MT implants at the 5-μg rhBMP-2 dose. All surfaces showed significant bone formation at the 10- and 20-μg dose.

Conclusions: rhBMP-2 adsorbed onto TPO implant surfaces executes an osteoinductive effect including bone contacting the implant surface. This effect is surface- and dose-dependent; the TPO-O surface yielding the most bone at the low discriminating rhBMP-2 dose.

Key words: bone; I^{125} binding and release; rat ectopic model; rhBMP-2; tissue engineering; titanium implants; titanium porous oxide

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Conflict of interest and source of funding statement

This study was conducted at Genetics Institute Inc. (Wyeth Research) Andover, MA, by the authors. Dr. Wikesjö was the Head/Principal Scientist, Preclinical Oral/Maxillofacial Research, Genetics Institute Inc., Andover, MA, at the time of study.

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Successful treatment using load-bearing implants relies on implant stability. The most important factor that determines the stability for non-cemented orthopedic and oral implants is likely the geometry of the implant, but surface properties also play an important role. Most manufacturers strive to enhance implant stability and load-bearing capacity for sub-optimal bone qualities by modifications of the implant surface, thus several surface variations have been developed using blasting, plasma spraying, and etching techniques. A different approach would be to modify the tissues adjoining the implant. It is likely that bone organization and rate of formation may be optimized introducing molecules such as extracellular matrix, growth, and differentiation factors to the

implant site. This report is the first from a series of studies aimed at developing a load-bearing implant, which presents such biologic factors to the implant site. This novel implant technology could have several applications in orthopedic and oral/maxillofacial rehabilitation, where a distinct advantage would be that the implant induces and/or promotes local bone formation without the use bone grafts, additional biomaterials or devices.

As load-bearing implants usually are manufactured from metals, limitations of the implant surface to carry pharmacologically relevant amounts of an osteoinductive or osteoconductive factor presents a challenge. Moreover, a candidate biologic factor must be sufficiently potent to induce or enhance local bone formation, and it must be released allowing adequate concentration gradients and residence time in the adjoining tissues that clinically relevant bone formation may occur. In this first report we investigate possible differences in bone inductive capacity for three implant surfaces adsorbed with recombinant human bone morphogenetic protein-2 (rhBMP-2) using a rat ectopic model (Urist 1965, Wang et al. 1990). Rat ectopic models have also been used in previous studies to observe bone formation when bone morphogenetic proteins (BMPs) have been added to titanium implants. For example, bone induction has been shown using BMP-3 at titanium and calcium phosphate-coated implants (Herr et al. 1996), rhBMP-2 at plasma-sprayed titanium and calcium phosphate-coated implants (Cole et al. 1997), and at titanium fibre mesh loaded with rhBMP-2 and native bovine BMP (Vehof et al. 2001). Bone induction using BMP-3 has also been shown at corundum-blasted titanium and hydroxyapatite-coated implants in an orthotopic rabbit model (Esenwein et al. 2001), and bone induction by BMP-2 has been shown at poly(D, L-lactide)-coated titanium wires in a rat tibia model (Schmidmaier et al. 2002). Both the chemical nature and the implant surface area appear to influence BMP-induced bone formation (Herr et al. 1996, Keller 1998). Porous hydroxyapatite coatings with specific pore sizes (300–400 μm), shape, and chemical nature appear suitable carriers for BMPs (Gao et al. 1996, Tsuruga et al. 1997, Kuboki et al. 1998). Increased bone formation and osseointegration have also been observed following surgical implantation of rhBMP-2 in conjunction with titanium implants in various alveolar settings (Hanisch et al. 1997, Sigurdsson et al. 1997, Wikesjö et al. 2002, 2003, 2004).

The hypothesis for our studies was that it must be possible to develop an implant that induces clinically relevant quantities of bone tailoring the implant surface for ample adsorption and release of rhBMP-2 to ultimately develop and manufacture oral and orthopedic implants featuring enhanced and/or accelerated osseointegration and clinical utility. We herein report two novel titanium porous oxide (TPO)-modified implant surfaces adsorbed with rhBMP-2. The two TPO surfaces were expected to feature different rhBMP-2 release kinetics and tissue stability at the implant surface. A standard machined/turned titanium (MT) surface served as control.

Material and Methods

In vitro rhBMP-2 binding and release

In vitro binding and release studies using I^{125}-labelled rhBMP-2 (Wyeth Research, Cambridge, MA, USA) were performed to estimate the amount of protein retained on implant surfaces. Two experimental TPO surfaces were manufactured in an anodic oxidation process, which resulted in porous structures with pore sizes in the low micorometer range (Fig. 1; Nobel Biocare AB, Göteborg, Sweden). The TPO surfaces exhibited a titanium dioxide layer of approximately 5 μm thickness with either narrow (TPO-N) or open (TPO-O) pores. The narrow and open pore structures were used as they would allow for variations in release kinetics;

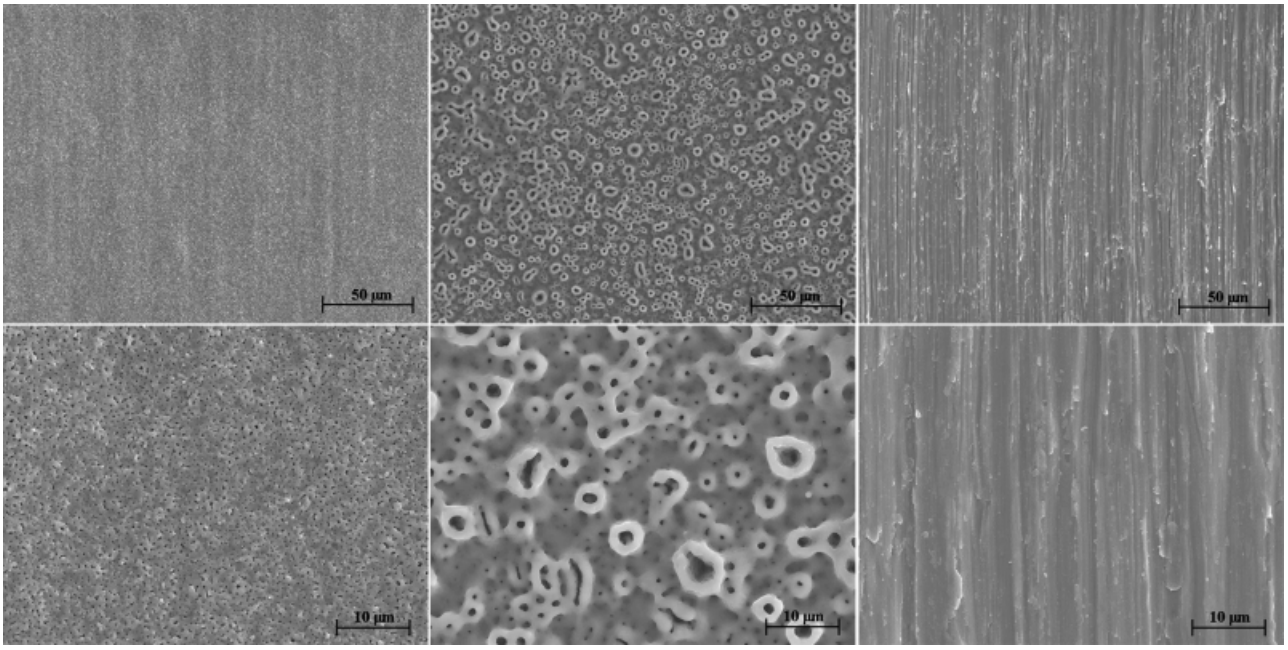

Fig. 1. Scanning electron photomicrographs of the TPO-N (left), TPO-O (centre) and MT surfaces. MT, machined/turned titanium; TPO, titanium porous oxide; N, narrow pore; O, open pore.

the open pores expected to exhibit a somewhat faster release of rhBMP-2. Near instant (bolus) release was expected for the MT control surface. The TPO-O surface has been introduced under the label TiUnite™ for clinical use without rhBMP-2 and has been detailed elsewhere (Hall & Lausmaa 2000).

Five γ-irradiated (25 kGy), ozone-cleaned, titanium disc implants ($\varnothing 7 \times 1.5$ mm) exhibiting either a TPO-N, TPO-O, or MT surface were used (Nobel Biocare). The MT implants were cleaned using a validated commercial process (Nobel Biocare) before sterilization and ozone treatment. The TPO implants were manufactured by an electrochemical process in a mixture of acids and thereafter thoroughly rinsed in ion-free sterile water before sterilization and ozone treatment. The manufacture and cleaning processes effectively removed all impurities. Each implant was adsorbed with 10 μl of 42.3 μg unlabelled and 2.1 μCi of I^{125}-labelled rhBMP-2, and air dried for 12 h, at which time initial counts were obtained. Each implant was immersed in 1 ml aliquots of MFR 00842 buffer (0.5% sucrose, 2.5% glycine, 5 mM L-glutamic acid, 5 mM NaCl, 0.01% polysorbate 80, pH 4.5; Wyeth Research) for approximately 1 min., thereafter rinsed in fresh 1.0 ml buffer for approximately 5 s, counted and then placed into fresh buffer and gently agitated at 37°C between time points (15 and 30 min., 1, 3, and 6 h, and 1, 3, 5, 7, 10, 14, and 27 days). Both buffer and implants were counted at each time point. Following each count, implants were placed into fresh 1-ml buffer aliquots.

Rat ectopic implant study

rhBMP-2/titanium implant preparation

Using aseptic procedures, lyophilized rhBMP-2 in MFR 00842 buffer was reconstituted to produce a 4.45 mg/ml stock solution diluted to a final concentration of 0.33 mg/ml. γ-irradiated (25 kGy) TPO-N, TPO-O, and MT disc implants were placed in a sterile field and exposed to ozone for 10 min. before rhBMP-2 coating. Using aseptic procedures, 15 μl of the prepared rhBMP-2 solution was uniformly dispensed/pipetted onto each implant 1, 2, or 4 times for a final rhBMP-2 dose of 5, 10, and 20 μg, respectively. Implants were air dried for 100 min. between dispenses.

Implant procedure

Twenty-seven 5-week-old male Long Evans rats, divided into groups of nine, were used following a protocol approved by the Institutional Animal Care and Use Committee, Wyeth Research. For each group, three animals received implants, TPO-N, TPO-O, or MT, adsorbed with rhBMP-2 at a dose of 5, 10, or 20 μg, respectively. Briefly, the animals were anaesthetized with isoflurane, weighed, and ear tagged. The upper abdominal wall and thorax were shaved and scrubbed with isopropyl alcohol. Two incisions were made in the ventral thoracic region ($\sim$ 5 mm caudal to the ziphoid process and $\sim$ 10 mm to the right and left of the midline). Using blunt scissors, the skin was retracted away from the underlying tissue, creating a subcutaneous pocket. One rhBMP-2-coated implant was inserted within each subcutaneous space with the coated side toward the muscle bed. A reference notch located on the uncoated side of the implant differentiated opposing sides. Incisions were closed with wound clips and the animals placed in lateral recumbency until ambulatory. The animals were fed ad libitum and were assessed daily for general health and for signs of infection (abnormal swelling, incision line discharge, wound opening, and inappetence).

TPO implants without rhBMP-2 were included in a preliminary evaluation. Gross observations revealed no evidence of bone formation, tissue encapsulation, or vascularity at these implants; thus, such controls were not further used.

Clinical observations and recordings

At 14 days post-implantation, the animals were euthanized by CO_2 inhalation and the skin excised to observe the implants in situ. The consistency, shape, presence of fluids, and vascularity was recorded for each implant. The implants were then carefully separated from the subcutaneous tissues, weighed, photographed, and radiographed.

Histotechnical protocol

The implants were placed into labelled cassettes, fixed with 70% ethanol for 1 week, dehydrated through a gradient of alcohols, cleared in xylene, and infiltrated and embedded undecalcified in methylmethacrylate. Implants were cross-sectioned (1–2 mm thickness) using an Isomet low-speed saw (Buehler™, Lake Bluff, IL, USA), and ground and polished to a final thickness of 30–60 μm (Buehler™ Petrothin) according to Donath & Breuner (1982). Sections were mounted on white plastic, clear glass or plastic, stained with Sanderson's rapid bone stain, and lightly counterstained with fast green.

Histologic and histometric analysis

Qualitative microscopic observations were recorded for each implant. The most central section for each implant was used for the histometric analysis. The following parameters were recorded for the rhBMP-2-coated surface of each section:

- bone area (mineralized bone and marrow);
- bone density (% mineralized bone); and
- bone–implant contact (% mineralized bone contact to the implant surface).

Bone area measurements were obtained using the point counting method with a standard 10×10 mm grid at $\times$ 10 magnification. Bone density and bone–implant contact assessments were acquired using computer-based image analysis software (Image-Pro Plus™, Media Cybernetics, Silver Springs, MD, USA).

Statistical analysis

Summary statistics included means ($\pm$ SD) for each recorded parameter for the six implants adsorbed with 5, 10, or 20 μg of rhBMP-2, respectively. A two-factor analysis of variance was used to test the effects of rhBMP-2 dose (independent of surface variation), surface variation (independent of rhBMP-2 dose), and the combined effects of rhBMP-2 dose and surface variation. Statistical significance was determined at $p < 0.05$.

Results

In vitro rhBMP-2 binding and release

Results of the binding and release assay are summarized in Fig. 2. Defining the initial count as 100%, the amount of rhBMP-2 bound to the implants following the initial MFR 00842 rinse decreased to 5.4%, 3.9%, and 2.3% for

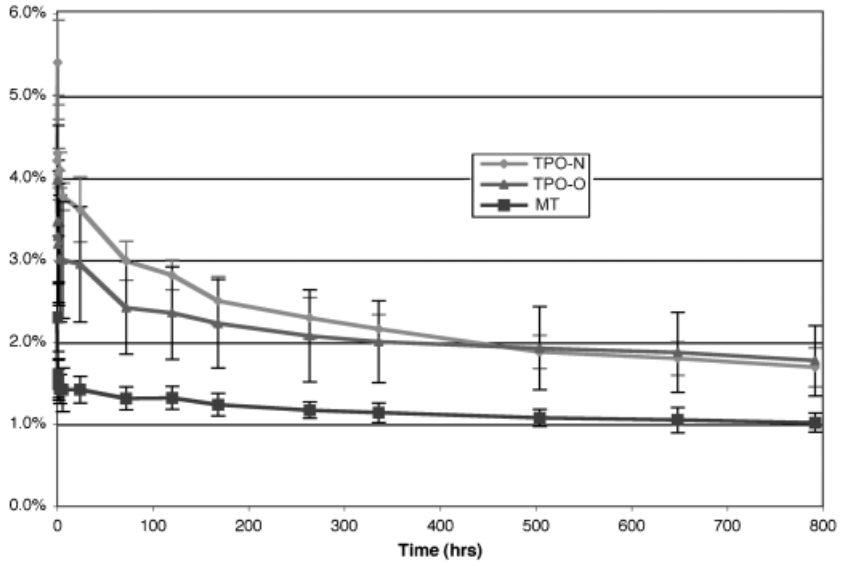

Fig. 2. Mean (± SD) percentage retention I^{125}-labelled rhBMP-2 at TPO and MT implants ($N = 5$). MT, machined/turned titanium; TPO, titanium porous oxide.

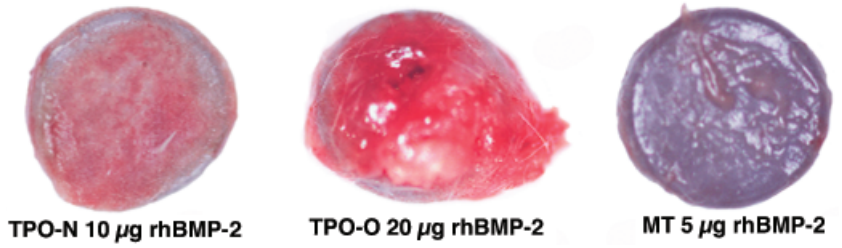

Fig. 3. Clinical representation of TPO and MT implants adsorbed with rhBMP-2 upon explant. MT, machined/turned titanium; TPO, titanium porous oxide.

the TPO-N, TPO-O, and MT implants, respectively. At week 2 post-adsorption, the amount retained decreased to 2.2%, 2.0%, and 1.1% for the TPO-N, TPO-O, and MT implants, respectively.

Explant observations

Healing was uneventful for all animals. There were no signs of infection or rejection. The amount of hard tissue, vascularity, and tissue encapsulation varied with surface variation and rhBMP-2 dose (Fig. 3). Hard tissue generally circumscribed the implants rather than being limited to the surface adsorbed with rhBMP-2.

At 5 μg rhBMP-2, TPO-O implants amassed the most hard tissue, vascularity, and tissue encapsulation. TPO-N implants exhibited a moderate amount of hard tissue, vascularity, and tissue encapsulation. Hard tissue formation, vascularity, and tissue encapsulation was not apparent on MT implants.

Only small differences in bone formation between surface variations were observed for the 10- and 20-μg doses. At 10 μg rhBMP-2, hard tissue formation was observed on the majority of the implants. TPO-O implants appeared to amass the most hard tissue formation. There were minimal differences between TPO-N and MT implants. A moderate amount of vascularity and tissue encapsulation was observed for the majority of the implants. At 20 μg rhBMP-2, a moderate to large amount of hard tissue formation was observed on TPO-N and TPO-O implants. Minimal differences were noted in vascularity and tissue encapsulation between these implants. Only a small amount of hard tissue formation was observed on MT implants; a moderate amount of vascularity and tissue encapsulation was however apparent.

There were no significant differences in pre-implantation weights between the implants. Both surface variation and rhBMP-2 dose significantly affected the post-implantation wet weight. Independent of dose, the MT implants weighed significantly less than the TPO implants. Independent of surface variation, implants adsorbed with 5 μg rhBMP-2 weighed significantly less than implants adsorbed with 10 or 20 μg rhBMP-2. There was no significant difference between implants adsorbed with 10 and 20 μg rhBMP-2; surface variation and dose together, did not have a significant effect on post-implantation wet weight (data not shown).

Radiographic observations

Radiopaque areas were seen on all implants observed to have at least a moderate amount of hard tissue. Implants observed to have no or minimal hard tissue showed no radiographic evidence of bone formation (data not shown).

Histologic and histometric observations

Implants were observed under incandescent light microscopy for marrow and fibrovascular elements, mineralized bone, osteoblastic and osteoclastic cells, osteoid, osteocytes, and bone voids (acellular cavities within bone). The extent of bone–implant contact was also determined. A separation between the induced bone and the implant surface was apparent in a majority of the histologic sections. However, the border of induced bone tissue mirrored that of the implant surface. The separation was therefore considered a sectioning artefact. Bone formation was observed circumscribing the implants and thus was not limited to the rhBMP-2-coated surface.

At 5 μg rhBMP-2 (Figs 4 and 5), a small number of osteoblasts and a moderate amount of marrow elements and fibrovascular tissue were observed for all implants. A small amount of osteoid was observed for all but the MT implants. Osteoclasts were not observed. Small bone voids were most prevalent at the TPO-O implants. In general, MT implants exhibited minimal bone formation and consequently less defined elements.

At 10 μg rhBMP-2 (Figs 4 and 5), a small to medium number of osteoblasts, and a small to medium amount of marrow and fibrovascular tissue were observed for all implants. Osteoblasts were most prominent at the TPO-N and

TPO-O implants. Osteoid was only observed at the TPO-N implants (2/6). Osteoclasts were not observed. A moderate number of bone voids were observed for all but the MT implants, exhibiting few bone voids.

At 20 μg rhBMP-2 (Figs 4 and 5), osteoblasts were present in greater numbers at TPO-N implants. Compared to the 5- and 10-μg rhBMP-2 dose, the osteoblasts appeared more prevalent and greater in size for all implants. The largest amount of marrow and fibrovascular tissue was observed at TPO-N implants. Osteoid was most commonly observed at TPO-O followed by TPO-N and MT implants. A limited number of osteoclasts were observed for TPO-N and TPO-O implants. It appeared that bone voids were increased in number and size for all implants at this dose. Overall, extensive bone formation was observed at 10 and 20 μg rhBMP-2 and the newly formed bone appeared adapted to the implant surface.

Results of the histometric analysis for the 5-μg rhBMP-2 dose are shown in Table 1 and Fig. 6. The effect of rhBMP-2 dose and surface variation on bone area was significant. The combined effect of surface variation and rhBMP-2 dose on bone density was not significant, although there were individual significant effects of surface variation and rhBMP-2 dose. There was no significant effect of rhBMP-2 dose and surface variation on bone–implant contact, when analysed individually or in combination.

At 5 μg rhBMP-2, there were overall significant differences in bone area ($p = 0.019$). TPO-O implants induced greater bone area than MT implants ($p = 0.001$). There was also increased bone area at TPO-N compared with MT implants (although not statistically significant). There was greater bone density at TPO-N, and TPO-O implants than at MT implants (not all significantly different). Bone–implant contact was significantly different between implants ($p = 0.047$). Bone–implant contact was significantly greater at TPO-N than at MT implants.

Differences between surface variations were less apparent at the 10 and 20-μg dose. At 10 μg, there were no significant differences in bone area ($p = 0.822$) or bone–implant contact ($p = 0.402$). Bone density was only significantly greater at TPO-N implants ($p = 0.014$). At 20 μg, there were no significant differences in bone density ($p = 0.396$) or bone–implant contact ($p = 0.219$).

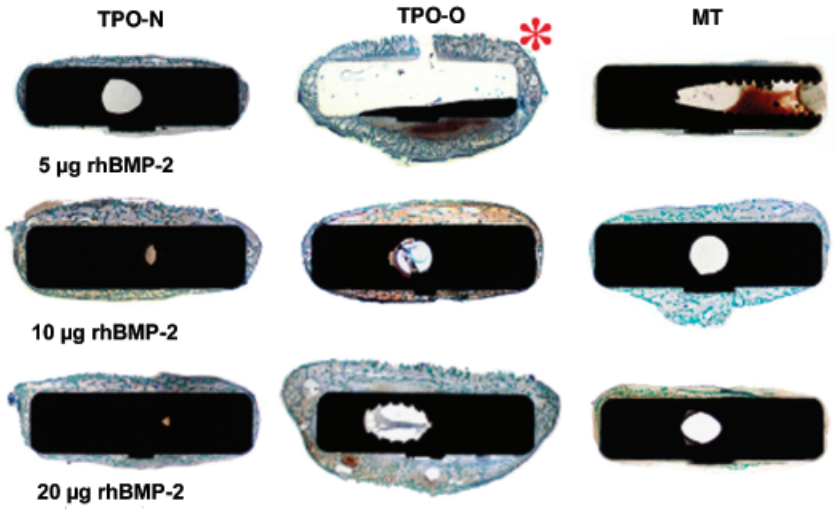

Fig. 4. Photomicrographs of TPO and MT implants adsorbed with rhBMP-2. The TPO-O* implant showed the greatest bone induction at the discriminating 5-μg dose (Sanderson's Rapid Bone Stain). MT, machined/turned titanium; rhBMP, recombinant human bone morphogenetic protein-2; TPO, titanium porous oxide; O, open pore.

Discussion

The objective of this study was to evaluate the potential of TPO surface implants adsorbed with rhBMP-2 to induce local bone formation. Implants with MT surfaces served as control. In vitro binding and release studies showed that implants adsorbed with 5 μg rhBMP-2 retained 2–5% rhBMP-2 after an immediate rinse in buffer, and 1–2% rhBMP-2 at 2 weeks post-adsorption. MT surfaces retained the least rhBMP-2. Thus, in vitro rhBMP-2 release appeared very rapid. Evaluations using the rat ectopic model showed that TPO-O surfaces exhibited greater bone formation than TPO-N and MT surfaces. Bone–implant contact was similar for TPO-O and TPO-N surfaces, and lower for MT surfaces at the discriminating 5-μg rhBMP-2 dose. Using 10 or 20 μg rhBMP-2, all surfaces exhibited significantly greater, but surface independent,

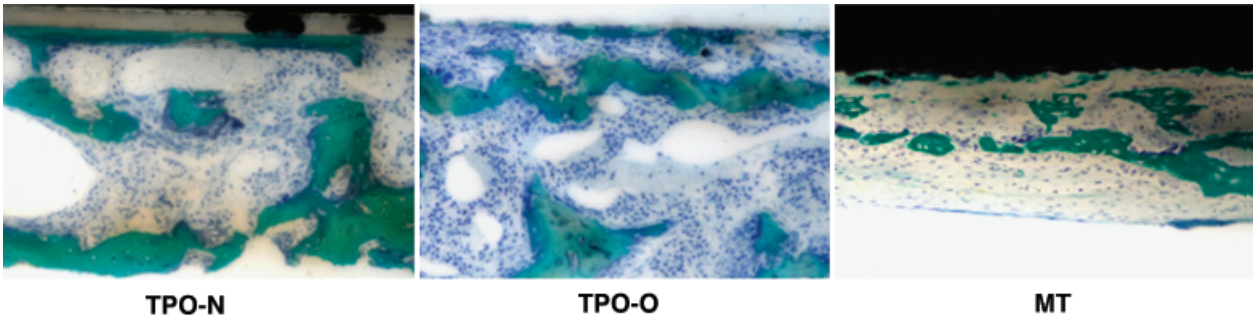

Fig. 5. Representative photomicrographs of bone induction at TPO-N, TPO-O and MT implants adsorbed with 5 μg rhBMP-2 (Sanderson's Rapid Bone Stain, × 20 magnification). MT, machined/turned titanium; N, narrow pore; O, open pore; rhBMP, recombinant human bone morphogenetic protein-2; TPO, titanium porous oxide.

Table 1. Results of the two-factor ANOVA for peri-implant bone area and density, and bone–implant contact (BIC)

	Bone area	Bone density	BIC
Surface modification	NS	0.004	NS
rhBMP-2 dose	0.004	0.004	NS
Surface modification × rhBMP-2 dose	0.023	NS	NS

NS, not significant; rhBMP, recombinant human bone morphogenetic protein-2.

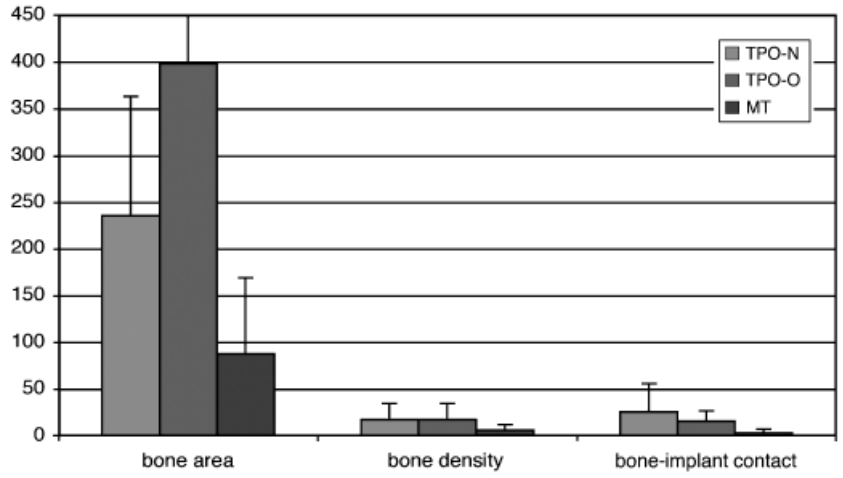

Fig. 6. Mean (± SD) bone area (arbitrary units), bone density (%), and bone–implant contact (%) at TPO and MT implants adsorbed with 5 μg rhBMP-2 ($N = 6$). MT, machined/turned titanium; rhBMP, recombinant human bone morphogenetic protein-2; TPO, titanium porous oxide.

bone formation and bone–implant contact. In summary, rhBMP-2 adsorbed onto TPO surfaces executed a bone inductive effect including bone–implant contact. This effect was surface- and dose dependent, the TPO-O surface appearing the most effective surface.

In vitro, less than 6% rhBMP-2 was retained on the implant surfaces following an initial rinse in buffer. While the TPO surfaces retained more rhBMP-2 than the control MT surface, the amount was small, 0.2–1 μg after the initial rinse. Approximately 1–2% rhBMP-2 was retained at 2 weeks post-adsorption. Nevertheless, the TPO surfaces retained a factor 2 more rhBMP-2 than the MT surface, indicating somewhat improved sustained release kinetics, which may be desirable in vivo.

Significant surface-dependent bone formation was observed in the rat ectopic model. The TPO surfaces showed enhanced bone formation at the rhBMP-2 5-μg dose compared to the MT surface apparently dependent on the concentration of rhBMP-2 in the near and immediate vicinity of the implant surface. It is unlikely that the large difference in bone formation between TPO and MT surfaces using the rhBMP-2 5-μg dose may be explained by the relatively small differences in release kinetics in vitro. However, it seems reasonable to assume that the difference in concentration of rhBMP-2 near and in the immediate vicinity of the implant surface would be larger in vivo than in vitro. The clotting blood may retain higher concentrations of rhBMP-2 within the structures of the corrugated TPO surfaces due to limited transport of rhBMP-2 from the porous structures and limited mobility of the clotting blood at the implant–tissue interface. This may explain the higher degree of bone–implant contact for the TPO surfaces as compared to the relatively smooth MT surface for which faster release of rhBMP-2 and higher implant–tissue mobility can be anticipated. One may further speculate that the most corrugated TPO surface (TPO-O) in addition to enhancing the stability of the blood clot within its structure also in part stabilizes the clot extending from the surface. Enhanced stability of the extended clot may explain the larger bone volume induced at TPO-O surfaces compared with that at TPO-N and MT surfaces using the 5-μg rhBMP-2 dose. However, the stability of the extended clot may have had limited effect on the density of the induced bone, which is determined by rhBMP-2 concentration within the clot. The less corrugated TPO-N surface may have caused sufficient retention of rhBMP-2 and stability of the blood clot within its surface structure to render favorable conditions for bone–implant contact; however, the surface corrugation being less pronounced than at the TPO-O surface limiting retention of the blood clot may explain the smaller volume but equal density of induced bone at this surface. Following the same reasoning, limited bone formation and bone–implant contact observed at the MT surface may be due to excessive mobility at the tissue–implant interface and limited retention of rhBMP-2 resulting in unfavorable perturbation of the bone forming process.

No remarkable differences in bone induction between the TPO surfaces and the MT control were observed for the rhBMP-2 10- and 20-μg dose; similar amounts of bone formation and bone–implant contact were observed for all surface variations with the exception for the MT surface where the bone quantity was somewhat lower. The relatively crude rat ectopic model apparently did not allow quantitative discrimination between the surface variations using higher rhBMP-2 doses. The higher doses seemingly compensated for the obvious ineffective release kinetics of the MT surface; thus, the evaluation in this model system became independent of the surface variation at rhBMP-2 doses exceeding 5 μg. This lack of discrimination may in part be due to compartmental restrictions by surrounding tissues limiting peri-implant bone formation.

Histologic observations consistent with histometric results showed that the osteoinductive activity was greatest for the rhBMP-2 10- and 20-μg doses as demonstrated by the number of osteoblastic cells, significant presence of osteoid, and associated bone voids and fibrovascular tissue at these doses; no obvious correlations between surface variation and osteoinductive capacity were seen. These elements were also present for TPO-N and TPO-O implants adsorbed with 5 μg rhBMP-2. Osteoclast differentiation was not prominent in this model; osteoclasts were only observed for the highest rhBMP-2 dose. In

perspective, an unexpected observation was that bone formation circumscribed the entire implant and was thus not confined to the implant surface adsorbed with rhBMP-2. This observation may be explained by the diffusion of released rhBMP-2 into the fibrin clot surrounding the implant. The fibrin compartment was created by the surgical incisions and blunt dissection. Post-implantation, the spatial extension of the fibrin compartment was likely influenced by tissue motion by the animal allowing an immediate or extended osteoinductive effect around the entire periphery of the implant.

In the present study using a rat ectopic model and in subsequent studies using orthotopic canine and non-human primate models, we have used rhBMP-2 at doses smaller than 100 μg per implant to support significant local bone formation. These are considerably lower doses than those used with the regulatory-approved rhBMP-2/absorbable collagen sponge implants (InductOs™, Wyeth Research; InFuse® Bone Graft, Medtronic, Memphis, TN, USA) for which doses in the milligram range typically are used for indications in the axial and appendicular skeleton. However, the concentration and residence time for low doses of rhBMP-2 generated in the near vicinity of the TPO surface may be sufficient for indications including oral implants.

Conclusions

The results from this study show that rhBMP-2 adsorbed onto TPO implant surfaces executes an osteoinductive effect including bone contacting the implant surface. This effect is surface and dose dependent; the TPO-O surface yielding the greatest effect at the low discriminating rhBMP-2 dose. In consequence, the TPO-O surface was selected for study and development of a bone inductive load bearing implant.

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Clinical Relevance

Scientific rationale for the study: Severe alveolar resorption and other ridge deformities make optimal oral implant placement difficult without bone augmentation procedures. This study is a first in a series describing the development of an implant surface that combined with bone morphogenetic protein induces significant 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 the ectopic rat model, we show that bone formation is surface and dose dependent and that a TPO surface with open pores appears the most effective candidate surface in the development of this bone inductive implant.