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

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Abstract

Background: Pre-clinical studies have shown that recombinant human bone morphogenetic protein-2 (rhBMP-2) coated onto purpose-designed titanium porous-oxide surface implants induces clinically relevant bone formation and osseointegration. The objective of this study was to examine the potential of rhBMP-7, also known as recombinant human osteogenic protein-1 (rhOP-1), coated onto titanium porous-oxide surface implants to support vertical alveolar ridge augmentation and implant osseointegration.

Materials and Methods: Bilateral, critical-size, 5 mm, supraalveolar peri-implant defects were created in six young adult Hound Labrador mongrel dogs. The animals received implants coated with rhBMP-7 at 1.5 or 3.0 mg/ml randomized to contralateral jaw quadrants. The mucoperiosteal flaps were advanced, adapted, and sutured to submerge the implants for primary intention healing. The animals received fluorescent bone markers at 3, 4, 7, and 8 weeks post-surgery when they were euthanized for histological evaluation.

Results: Without striking differences between treatments, the implant sites exhibited a swelling that gradually regressed to become hard to palpation disguising the implant contours. The histological evaluation showed robust bone formation; the newly formed bone assuming characteristics of the contiguous resident bone, bone formation (height and area) averaging 4.1 ± 1.0 *versus* 3.6 ± 1.7 mm and 3.6 ± 1.9 *versus* 3.1 ± 1.8 mm^2; and bone density 56% *versus* 50% for implants coated with rhBMP-7 at 1.5 and 3.0 mg/ml, respectively. Both treatments exhibited clinically relevant osseointegration, the corresponding bone–implant contact values averaging 51% and 47%. Notable peri-implant resident bone remodelling was observed for implants coated with rhBMP-7 at 3.0 mg/ml.

Conclusions: rhBMP-7 coated onto titanium porous-oxide surface implants induces clinically relevant local bone formation including osseointegration and vertical augmentation of the alveolar ridge, the higher concentration/dose associated with some local side effects.

Key words: alveolar augmentation; bone morphogenetic protein; dental/oral implants; dogs; osseointegration; rhBMP-7; rhOP-1; seroma; tissue engineering; titanium; titanium porous oxide

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Conflict of interest and source of funding statement

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Dental implants today are often placed at sites compromised by trauma, periodontal disease, and crestal resorption to meet aesthetic and functional demands. Execution of these treatments requires bone augmentation procedures and autograft bone, bone derivatives or synthetic bone biomaterials, devices for guided bone regeneration, and implantable technologies based on growth and differentiation factors have been used in the support of local bone formation (Aghaloo & Moy 2007, Wikesjö et al. 2008c). Recent technological advances including direct coating of implant surfaces with bone morphogenetic proteins (BMPs) have been evaluated in pre-clinical models and show promise as a viable stand-alone alternative to current bone augmentation procedures (Hall & Lausmaa 2000, Hall et al. 2007, Leknes et al. 2008a, b, Wikesjö et al. 2008a, b, d, Polimeni et al. 2010).

Most studies evaluating growth, differentiation, and matrix factors as candidate agents in support of alveolar bone augmentation have focused on recombinant human BMP-2 (rhBMP-2) (Huang et al. 2008). Nevertheless, rhBMP-2 is not the only member of the BMP family of proteins approved for clinical use projecting a relevant potential to induce also alveolar bone formation. An equally intriguing member of the BMP family, BMP-7, also known as osteogenic protein-1 (OP-1), was first cloned in the late 1980s (Özkaynak et al. 1990). rhBMP-7 (Sampath et al. 1992) has been approved both in Europe and the United States for use as an alternative to autogenous bone grafts in the axial and appendicular skeleton. Clinical studies support the use of rhBMP-7 to treat tibial non-unions, tibial fractures, scaphoid non-unions, atrophic long bone non-unions, and spine fusion (Garrison et al. 2007, White et al. 2007).

The bone inductive potential of rhBMP-7 for craniofacial applications has been evaluated in a number of pre-clinical settings using large animal models (Cook et al. 1995, Ripamonti et al. 1996a, b, 2001a, b, c, Giannobile et al. 1998, Margolin et al. 1998, McAllister et al. 1998, Terheyden et al. 1999a, b, 2001a, b, 2004, Abu-Serriah et al. 2004, Roldán et al. 2004, Wang et al. 2004, Springer et al. 2005a, b) and in a limited clinical case series/trial evaluating subantral augmentation (Groeneveld et al. 1999a, b, van den Bergh et al. 2000) and clinical reports/series on heterotopically engineered vascular bone flaps for mandibular reconstruction (Warnke et al. 2004, Heliotis et al. 2006, Ayoub et al. 2007, Clokie & Sándor 2008). Recently, we have presented radiographic observations of the effect of rhBMP-7-coated implants on alveolar ridge augmentation using the critical-size supraalveolar peri-implant defect model in dogs (Leknes et al. 2008b). Robust radiographic bone formation could be observed at as early as 4 weeks with clear vertical augmentation of the alveolar ridge at 8 weeks. The objective of this study was to histologically assess the potential of rhBMP-7 coated onto titanium porous oxide surface-modified implants to stimulate local bone formation and osseointegration using the critical-size supraalveolar peri-implant defect model in dogs.

Materials and Methods

Animals

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

Titanium implants

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

Surgery and experimental procedures

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

One experienced surgeon (U. M. E. W.) performed all surgical procedures in this series of studies. Bilateral, critical size, supraalveolar peri-implant defects were created in the mandibular premolar region (Fig. 1; Wikesjö et al. 2006). Briefly, buccal and lingual mucoperiosteal flaps were reflected and the alveolar bone was removed around the circumference of the pre-molar teeth to a level approximately 6 mm apical to the cemento-enamel junction using water-cooled rotating burs. The pre-molar teeth were extracted and the first molar amputated at the level of the reduced alveolar crest. Three implants were placed in osteotomies prepared into the extraction sites of the third and fourth premolar teeth in each jaw quadrant. A few

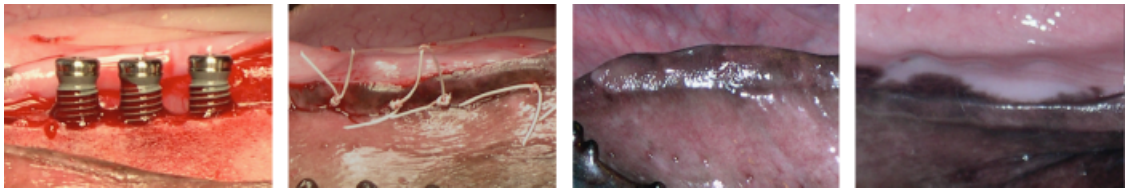

Fig. 1. Clinical protocol including implant placement, wound closure, and healing at 4 weeks (right centre; lateral view) and 8 weeks (right; coronal view).

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

The animals received implants coated with rhBMP-7 at 1.5 or 3.0 mg/ml randomized between left and right jaw quadrants using a split-mouth design. The periostea of the mucogingival flaps were fenestrated at the base of the flaps to allow tension-free flap apposition and wound closure. The flaps were advanced 3–4 mm coronal to the implants and the flap margins were adapted and sutured (GORE-TEX™ Suture CV5, W.L. Gore & Associates Inc., Flagstaff, AZ, USA). Photographs were obtained following implant placement and wound closure.

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

Post-surgery procedures

A long-acting opioid, buprenorphine HCl (0.01–0.03 mg/kg; IM) was administered immediately post-surgery and re-dosed b.i.d. for 3 days. A broad-spectrum antibiotic (enrofloxacin, 2.5 mg/kg; IM) was administered immediately post-surgery and re-dosed b.i.d. for 7 days. Sutures were removed under sedation (propofol, 5–7 mg/kg; IV) at approximately 10 days. Radiographs were obtained under sedation (propofol, 5–7 mg/kg; IV bolus) immediately post-surgery (baseline), and at weeks 4 and 8 post-surgery. Plaque control was maintained by daily flushing of the oral cavity with chlorhexidine gluconate (Xttrium Laboratories Inc., Chicago, IL, USA; 20–30 ml of a 2% solution) until completion of the study. Observations of experimental sites with regard to gingival health, maintenance of suture line closure, oedema, and evidence of tissue necrosis or infection were recorded daily.

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

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

Histotechnical procedures

The block sections were fixed in 10% buffered formalin for 3–5 days, dehydrated in alcohol, and embedded in methylmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). The implants were cut mid-axially in a buccal–lingual plane into 200-μm-thick sections using the cutting–grinding technique (EXAKT Apparatebau, Norderstedt, Germany), and were subsequently ground and polished to a final thickness of approximately 40 μm for fluorescent light microscopy (Donath & Breuner 1982, Rohrer & Schubert 1992). Upon completion of the fluorescent light examination, the sections were stained with Stevenel's blue and van Gieson's picro fuchsin for histopathological and histometric analysis using incandescent, polarized, and fluorescent light microscopy.

Clinical analysis

One examiner (G. P.) recapped the clinical observations from laboratory notebook entries and clinical photographs with a focus on 4- and 8-week observations including whether implants were visible and/or palpable through the mucosa; the cover screw or the body of the implant was exposed to the oral cavity; and whether signs of seroma formation including a reddish-bluish fluctuating swelling that could not be related to an infectious process were noticeable.

Histopathological analysis

Two masked experienced examiners (M. Q., U. M. E. W.) performed the histopathological evaluation including observations of bone formation and resorption, cortex formation, seroma formation, fibrovascular tissue and marrow, and inflammatory reactions using computer-enhanced images, fluorescent, incandescent, and polarized light microscopy (BX 60, Olympus America Inc., Melville, NY, USA).

Histometric analysis

One masked, calibrated examiner (M. Q.) performed the histometric analysis using incandescent and polarized light microscopy (BX 60, 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 most central section for each implant was used for the histometric analysis of the buccal and lingual surfaces of each implant including:

- *Defect height*: distance between the reference notch and the implant platform.
- *Bone regeneration (height)*: the distance between the reference notch and the vertical extension of newly formed bone along the implant;

excluding bone formation exceeding the implant platform.
- *Bone regeneration (area)*: the area of newly formed bone along the implant above the reference notch; excluding bone formation exceeding the implant platform.
- *Bone density (new bone)*: the fraction of mineralized bone within newly formed bone.
- *Osseointegration (new bone)*: the percent of bone–implant contact (BIC) as measured between the reference notch and the vertical extension of newly formed bone along the implant.
- *Bone density outside the implant threads (resident bone)*: the fraction of mineralized bone within a $300 \times 1800\,\mu m$ area (width $\times$ height) immediately outside the implant threads in resident bone.
- *Bone density within the implant threads (resident bone)*: the fraction of mineralized bone within the implant threads in resident bone.
- *Osseointegration (resident bone)*: the percent of BIC within resident bone measured from the reference notch to the apex of the implant.

Statistical analysis

Examiner reliability for the histometric evaluation was assessed using the Concordance correlation coefficient. This coefficient ranges between 0 and 1; the higher the coefficient, the greater the reliability. Concordance correlation coefficient for linear measurements of bone height was 0.96 showing high reliability.

All implants were included in the analysis. The animal was used as the unit of analysis. Generalized estimating equations were used to perform the analysis. Measurements at site level were used and estimates were adjusted for the clustering of sites into animals using a robust variance estimator. Wald tests were used for multiple comparisons and the level of significance was set at 5%. All analysis was performed using a computer-based statistical software (Stata 7.0 for Windows, Stata Corporation, College Station, TX, USA).

Results

Clinical observations

Early healing events were generally uneventful; suture lines remained intact without evidence of tissue necrosis or infection; however, all jaw quadrants exhibited significant swelling (Fig. 1). In general, jaw quadrants receiving implants coated with rhBMP-7 exhibited swelling that gradually regressed and became hard to palpation disguising the contours of the implants. Healing through week 8 progressed uneventfully. There were no noteworthy differences relative to the number of visible, palpable, and exposed implants coated with rhBMP-7 at 1.5 or 3.0 mg/ml. Cover screws were visible through the mucosa; however, new tissue formation, hard to palpation, completely covered the implant body. None of the implants was lost. One implant coated with rhBMP-7 at 3.0 mg/ml exhibited signs of seroma formation. One implant coated with rhBMP-7 at 1.5 mg/ml and three implants coated with rhBMP-7 at 3.0 mg/ml exhibited exposures limited to the cover screw.

Histological observations

Implants coated with rhBMP-7 at 1.5 and 3.0 mg/ml exhibited robust, clinically relevant bone formation reaching or exceeding the implant platform without apparent differences between rhBMP-7 concentrations; bone formation generally being more consistent at the lingual aspect of the implants (Figs 2 and 3). The newly formed bone exhibited characteristics of the resident bone and showed significant osseointegration within newly formed and resident bone. Exposed implants in one animal (rhBMP-7 at 1.5 mg/ml) exhibited limited bone formation. Three implants in a second animal (rhBMP-7 at 1.5 or 3.0 mg/ml) exhibited an evidence of previous seroma formation. There were no other remarkable differences in appearance between the newly formed and the adjoining resident bone. Implants coated with rhBMP-7 at 1.5 mg/ml exhibited limited or no appreciable peri-implant bone remodelling, whereas implants coated with rhBMP-7 at 3.0 mg/ml showed bone remodelling extending up to or beyond 300 μm from the implant surface in several animals. None of the implants coated with rhBMP-7 at 1.5 mg/ml exhibited remodelling of the buccal plate. Several implants coated with rhBMP-7 at 3.0 mg/ml showed evidence of considerable remodelling of the buccal plate. Wide yellow and orange fluorescence labels throughout the new bone indicated rapid early bone formation.

Histometric observations

Implants coated with rhBMP-7 at 1.5 and 3.0 mg/ml exhibited robust bone formation (height and area) averaging 4.1 ± 1.0 *versus* 3.6 ± 1.7 mm, and 3.6 ± 1.9 *versus* $3.1 \pm 1.8\,mm^2$, respectively (Fig. 4). The density of the induced bone averaged $56 \pm 12\%$ and $50 \pm 8\%$ for implants coated with rhBMP-7 at 1.5 and 3.0 mg/ml, respectively (Fig. 5). The corresponding BIC values were $51 \pm 15\%$ and $47 \pm 12\%$. There were limited differences in resident bone density immediately outside the implant threads,

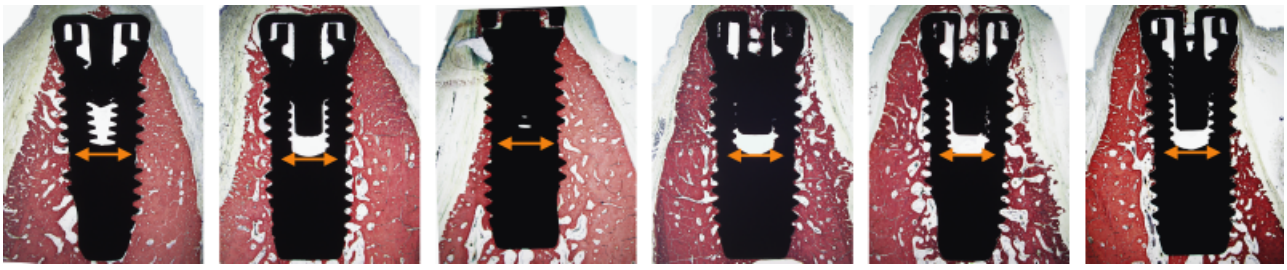

Fig. 2. Photomicrographs (incandescent light microscopy) showing contra-lateral jaw quadrants including $\varnothing 4.0 \times 10$ mm implants coated with recombinant human bone morphogenetic protein-7 (rhBMP-7) at 1.5 mg/ml (left) and 3.0 mg/ml (right), also shown in Fig. 3. Buccal implant surfaces face left and right for implants soak loaded with rhBMP-7 at 1.5 and 3.0 mg/ml, respectively. The orange arrows delineate the 5 mm notch placed level with the resident alveolar bone. Stain: Stevenel's blue and van Gieson's picro fuchsin.

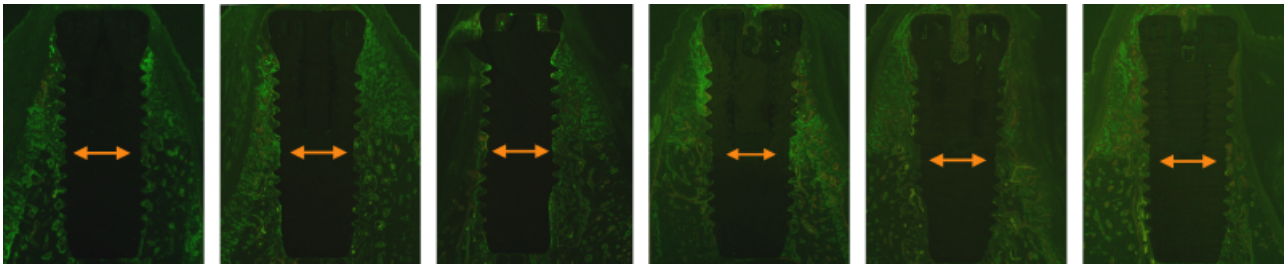

Fig. 3. Photomicrographs (fluorescence microscopy) showing contra-lateral jaw quadrants including ∅4.0 × 10 mm implants coated with recombinant human bone morphogenetic protein-7 (rhBMP-7) at 1.5 mg/ml (left) and 3.0 mg/ml (right), also shown in Fig. 2. Buccal implant surfaces face left and right for implants soak loaded with rhBMP-7 at 1.5 and 3.0 mg/ml, respectively. The orange arrows delineate the 5 mm notch placed level with the resident alveolar bone.

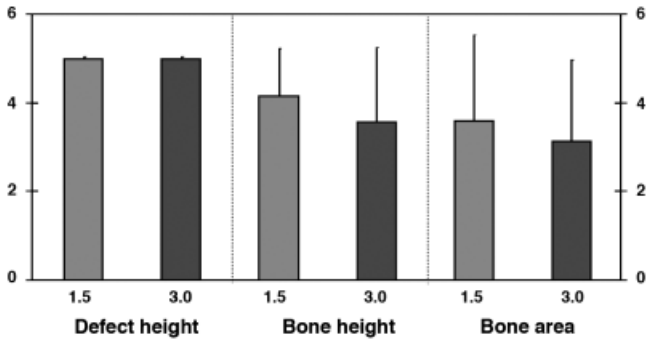

Fig. 4. Mean (± SD in mm/mm^2) induced bone formation for animals receiving implants coated with recombinant human bone morphogenetic protein-7 (rhBMP-7) at 1.5 or 3.0 mg/ml. There were no statistically significant differences between the groups.

bone density averaging 63 ± 10% *versus* 57 ± 10% for implants coated with rhBMP-7 at 1.5 and 3.0 mg/ml, respectively (Fig. 6). The corresponding values for bone density within the thread area was 44 ± 17% *versus* 40 ± 9%, and 68 ± 14% *versus* 67 ± 7% for BIC. There were no statistically significant differences between the groups for any parameter evaluated.

Discussion

The objective of this study was to evaluate the potential of a purpose-designed titanium porous-oxide implant surface coated with rhBMP-7 to stimulate alveolar ridge augmentation and osseointegration using the critical-size supraalveolar peri-implant defect model in dogs. Implants coated with rhBMP-7 at 1.5 and 3.0 mg/ml produced clinically relevant bone formation and osseointegration. No statistically significant differences in bone formation were observed among sites receiving rhBMP-7 at 1.5 or 3.0 mg/ml. Notably, the 3.0 mg/ml concentration was associated with some side effects. These findings suggest that rhBM-7-coated implants may induce clinically relevant local bone formation within the selected dosage interval.

Leknes et al. (2008b) in a radiographic assessment of the present material observed that most implant sites showed void spaces within the newly formed bone at 4 weeks that resolved within 8 weeks. These findings are in accordance with bone remodelling observed using fluorescent light microscopy, especially at the higher rhBMP-7 dose. At 8 weeks, the newly formed bone exhibited characteristics similar to the resident bone indicating the maturation of the tissues. Robust alveolar bone formation was observed in the histological and radiographic analysis. Implants coated with rhBMP-7 at 1.5 and 3.0 mg/ml averaged, respectively, 4.1 ± 1.0 and 3.6 ± 1.7 mm new bone height in the histologic analysis of buccal/lingual bone formation, and 4.4 ± 0.8 and 4.2 ± 1.1 mm in the radiographic analysis of inter-proximal bone formation at week 8. Thus, rhBMP-7-coated implants (Leknes et al. 2008b) similar to rhBMP-2-coated implants (Leknes et al. 2008b) show robust bone formation occurring as early as 4 weeks, resolution within few weeks of the biological complications related to the rapid bone formation, and remodelling of the newly formed bone yielding similar characteristics of the resident bone.

In parallel studies, we evaluated the potential of rhBMP-2 coated onto titanium porous oxide surface implants to induce alveolar bone formation implants using the critical-size supraalveolar peri-implant defect model (Leknes et al. 2008a, Wikesjö et al. 2008b). New bone formation (height) amounted to 4.2 ± 0.7 and 4.2 ± 1.2 mm for sites receiving implants coated with rhBMP-2 at 1.5 and 3.0 mg/ml, respectively (Wikesjö et al. 2008b) *versus* 4.1 ± 1.0 and 3.6 ± 1.7 mm for implants coated with rhBMP-7 at 1.5 and 3.0 mg/ml, respectively, in the present study. rhBMP-2 yielded somewhat increased bone area compared with rhBMP-7 amounting to 5.6 ± 2.2 *versus* 3.6 ± 1.9 mm^2 at 1.5 mg/ml and 7.4 ± 3.5 *versus* 3.1 ± 1.8 mm^2 at 3.0 mg/ml. Notably, for both rhBMP-2- and rhBMP-7-coated implants, local factors, in particular space provision, appear to influence bone formation. Whereas the more prominent lingual shelf of the defect sites consistently supports bone formation, the considerably narrower buccal aspect appears more inconsistent. Moreover, similar to that observed for rhBMP-2-coated implants, implants coated with rhBMP-7 exhibited dose-dependent seroma formation and resident bone peri-implant remodelling, the overall appreciation of these effects appearing more tempered at sites evaluating rhBMP-7-coated implants. Importantly, these studies suggest that both rhBMP-2 and rhBMP-7 may induce clinically relevant new bone formation and osseointegration of the carrier implants.

The potential of rhBMP-7 to support craniofacial bone formation and/or periodontal wound healing/regeneration has been evaluated in a vast variety of large animal pre-clinical models including calvarial defects (Ripamonti et al.

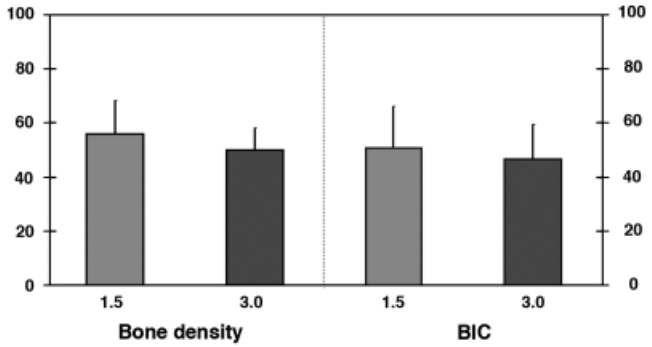

Fig. 5. Mean (± SD in %) new bone density and bone–implant contact (BIC) for animals receiving implants coated with recombinant human bone morphogenetic protein-7 (rhBMP-7) at 1.5 or 3.0 mg/ml. There were no statistically significant differences between the groups.

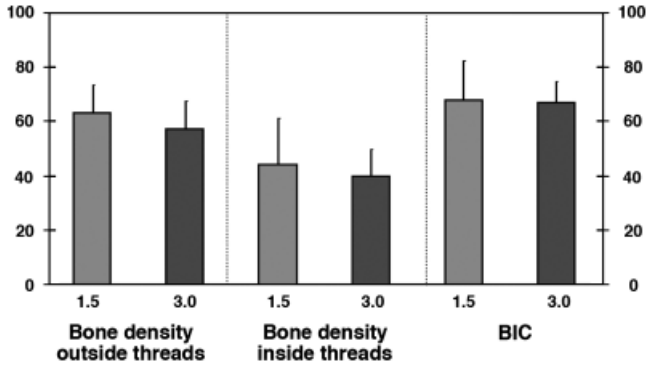

Fig. 6. Mean (± SD in %) resident bone density and bone–implant contact (BIC) for animals receiving implants coated with recombinant human bone morphogenetic protein-7 (rhBMP-7) at 1.5 or 3.0 mg/ml. There were no statistically significant differences between the groups.

1996b, 2001b, c, Springer et al. 2005a), maxillary sinus augmentation (Margolin et al. 1998, McAllister et al. 1998, Terheyden et al. 1999a, Roldán et al. 2004), mandibular segmental defects (Abu-Serriah et al. 2004, Wang et al. 2004), periodontal defects (Ripamonti et al. 1996a, 2001a, Giannobile et al. 1998, Springer et al. 2005b), extraction sites (Cook et al. 1995), and in the development of heterotopically engineered vascular bone grafts for mandibular reconstruction (Terheyden et al. 1999b, 2001a, b, 2004, Warnke et al. 2004, 2006). Direct comparisons among these studies are difficult to draw due to different study designs, model, length of follow-up, dose, and carrier(s) used. Nevertheless, with few exceptions, these pre-clinical studies point a clinically relevant effect of rhBMP-7 in a multiple of applications. The remarkable development and clinical implementation of heterotopically engineered vascular bone grafts by the Terheyden group (Terheyden et al. 1999b, 2001a,b, 2004, Warnke et al. 2004, 2006) underscores one of the first practical possibilities of what rhBMP-7 and other BMP technologies may offer.

The present study did not include an internal control in which the critical-size defect was created and uncoated implants were used. Critical-size defect models have been developed and used extensively to assess biological potential, efficacy and safety of candidate bone biomaterials, devices, and growth factor-based technologies intended for indications in the axial and appendicular skeleton before clinical evaluation and public release (Einhorn 1999, Buma et al. 2004, Liebschner 2004). Our laboratory has specifically developed and characterized the critical-size supraalveolar peri-implant defect model for the assessment of alveolar bone regenerative technologies (Wikesjö et al. 2006, Qahash et al. 2008, Lee et al. 2009). For this model, buccal control sites exhibit bone loss averaging (± SD) 0.4 ± 0.2 mm and lingual control sites bone gain averaging 0.4 ± 0.1 mm for a net gain of 0.0 ± 0.1 mm over an 8-week healing interval. The overall bone area gain averages 0.3 ± 0.1 mm^2 encompassing 0.0 ± 0.0 mm^2 for buccal and 0.3 ± 0.1 mm^2 for lingual sites (Wikesjö et al. 2006). It becomes evident that this critical-size defect model does not regenerate spontaneously and that any bone formation observed may only be credited to an implanted osteoconductive or osteoinductive technology, in this study of rhBMP-7. Thus, adding an internal control would indeed represent unnecessary duplication, breaching the shaping principles *Refinement–Reduction–Replacement* that provide a systematic framework to achieve the goal of humane experimental techniques (Russell & Burch 1959, Institute of Laboratory Animal Resources 1996).

rhBMP-7 or rhOP-1 was first used to treat a tibial non-union defect more than 15 years ago and it has been approved for clinical use for almost a decade (White et al. 2007). The number of studies evaluating its clinical use and safety for a variety of orthopaedic indications including tibial non-unions, tibial fractures, scaphoid non-unions, atrophic long bone non-unions, and spinal fusion increases steadily and shows promising results (Garrison et al. 2007, White et al. 2007). In perspective, our observations support this growing body of evidence of a significant clinical value of rhBMP-7 in support of bone regeneration. Nevertheless, dosing, carriers, application and long-term prognosis remain areas that need to be further addressed. In conclusion, rhBMP-7 coated onto titanium porous oxide surface implants exhibits a convincing potential to stimulate clinically relevant local bone formation including osseointegration and vertical augmentation of the alveolar ridge. Notably, higher concentrations were associated with some local side effects.

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Clinical Relevance

Scientific rationale for the study: The objective of this study was to evaluate the potential of rhBMP-7 also known as rhOP-1 coated onto oral implants featuring a purpose-designed titanium porous oxide surface to stimulate local bone formation including osseointegration and vertical alveolar ridge augmentation.

Principal findings: Using the supraalveolar peri-implant defect model, we show that rhBMP-7 coated oral implants display a robust osteoinductive and/or osteoconductive effect; bone formation apparently benefiting from local factors. Application of rhBMP-7 appears safe as it is associated with limited, if any, adverse effects.

Practical implications: Implants coated with rhBMP-7 exhibited clinically relevant bone formation without use of bone grafting, biomaterials or devices for GBR.