J Clin Periodontol 2010; 37: 759–768 doi: 10.1111/j.1600-051X.2010.01579.x

Journal of Clinical Periodontology

# Alveolar ridge augmentation using implants coated with recombinant human growth/differentiation factor-5: histologic observations

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*Polimeni G, Wikesjö UME, Susin C, Qahash M, Shanaman RH, Prasad HS, Rohrer MD, Hall J. Alveolar ridge augmentation using implants coated with recombinant human growth/differentiation factor-5: histologic observations. J Clin Periodontol 2010; 37: 759–768. doi: 10.1111/j.1600-051X.2010.01579.x.*

***Abstract***

**Objectives:** In vitro and in vivo preclinical studies suggest that growth/differentiation factor-5 (GDF-5) may induce local bone formation. The objective of this study was to evaluate the potential of recombinant human GDF-5 (rhGDF-5) coated onto an oral implant with a purpose-designed titanium porous oxide surface to stimulate local bone formation including osseointegration and vertical augmentation of the alveolar ridge.

**Materials and Methods:** Bilateral, critical-size, 5 mm, supraalveolar peri-implant defects were created in 12 young adult Hound Labrador mongrel dogs. Six animals received implants coated with 30 or 60 $\mu$g rhGDF-5, and six animals received implants coated with 120 $\mu$g rhGDF-5 or left uncoated (control). Treatments were alternated between 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 weeks 3, 4, 7, and 8 post-surgery when they were euthanized for histologic evaluation.

**Results:** The clinical examination showed no noteworthy differences between implants coated with rhGDF-5. The cover screw and implant body were visible/palpable through the alveolar mucosa for both rhGDF-5-coated and control implants. There was a small increase in induced bone height for implants coated with rhGDF-5 compared with the control, induced bone height averaging ($\pm$ SD) $1.6 \pm 0.6$ mm for implants coated with 120 $\mu$g rhGDF-5 *versus* $1.2 \pm 0.5$, $1.2 \pm 0.6$, and $0.6 \pm 0.2$ mm for implants coated with 60 $\mu$g rhGDF-5, 30 $\mu$g rhGDF-5, or left uncoated, respectively ($p < 0.05$). Bone formation was predominant at the lingual aspect of the implants. Narrow yellow and orange fluorescent markers throughout the newly formed bone indicate relatively slow new bone formation within 3–4 weeks. Implants coated with rhGDF-5 displayed limited peri-implant bone remodelling in the resident bone; the 120 $\mu$g dose exhibiting more advanced remodelling than the 60 and 30 $\mu$g doses. All treatment groups exhibited clinically relevant osseointegration.

**Conclusions:** rhGDF-5-coated oral implants display a dose-dependent osteoinductive and/or osteoconductive effect, bone formation apparently benefiting from local factors. Application of rhGDF-5 appears to be safe as it is associated with limited, if any, adverse effects.

Key words: alveolar augmentation; dental/oral implants; dogs; GDF-5; growth/differentiation factor-5; osseointegration; tissue engineering; titanium porous oxide; titanium

Accepted for publication 13 March 2010

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**Conflict of interest and source of funding statement**

The authors declare that they have no conflicts of interest in this study.
This study was supported by a grant from Nobel Biocare AB, Göteborg, Sweden. Jan Hall is an employee of Nobel Biocare AB. Ulf ME Wikesjö was an employee of Wyeth Research, and serves as a consultant to Wyeth Research and Nobel Biocare.

Growth/differentiation factor 5 (GDF-5) is a protein belonging to the bone morphogenetic protein (BMP) family and the transforming growth factor-$\beta$ (TGF-$\beta$) superfamily. Members of the BMP family and the TGF-$\beta$ superfamily are regulators of cell growth and differentiation in development and post-foetal life. Evidence suggests essential roles for GDF-5 in skeletal and joint development (Storm & Kingsley 1996, Buxton et al. 2001, Settle et al. 2003) as well as development of teeth (Morotome et al. 1998) and the periodontal ligament (Sena et al. 2003). The complete amino acid sequence of human GDF-5 has been determined through initial degenerate PCR and subsequent cloning and nucleotide sequencing of genomic DNA and cDNA (Hötten et al. 1994). In vitro studies demonstrate the ability of GDF-5 to stimulate the differentiation of stromal cells into osteogenic lineages and the promotion of their angiogenic activity by increasing the vascular endothelial growth factor gene (Zeng et al. 2006, 2007). Others observed that application of GDF-5 induced the proliferation and differentiation of mouse calvaria cells to produce ectopic bone formation (Yoshimoto et al. 2006). Local application of GDF-5 has resulted in increased bone formation in a number of small and large animal preclinical models under different experimental conditions and using a variety of carrier technologies (for a review, see Moore et al. 2010).

The concept of coating titanium implants with bioactive proteins arises from the hypothesis that the titanium implant itself may serve as a carrier technology for the proteins (Hall et al. 2007). The proteins will, upon installation, diffuse from the implant surface into the immediate peri-implant tissues in support of local bone formation and osseointegration. Previous studies have evaluated the potential of a purpose-designed titanium porous oxide oral implant surface (Hall & Lausmaa 2000) to serve as a carrier for rhBMP-2 and rhBMP-7 (Hall et al. 2007, Leknes et al. 2008a, b, Wikesjö et al. 2008a, b, c). Another recent study evaluated dicalcium phosphate-, GDF-5-, and GDF-5/dicalcium phosphate-coated titanium implants in a rabbit model (Simank et al. 2006). Mechanical stability was tested by applying a submaximal load, resulting in a micro-displacement between implant and bone, leaving the interface intact for additional analysis using micro-CT and histology. The dicalcium phosphate and GDF-5 coatings exhibited a positive effect on implant stability in this study. The objective of the present study was to evaluate the potential of GDF-5 coated onto the purpose-designed titanium porous oxide oral implant surface to stimulate local bone formation including osseointegration and vertical augmentation of the alveolar ridge.

## Materials and Methods

### Animals

Twelve male Hound Labrador mongrel dogs, age 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.

### Endosseous oral implants

Sterile endosseous oral implants with a titanium porous oxide surface, designed to serve as a carrier for bioactive proteins (TiUnite™, Ø4.0 × 10 mm; Nobel Biocare AB, Göteborg, Sweden) coated with 30, 60, or 120 $\mu$g rhGDF-5 (Scil Technology GmbH, Martinsried, Germany) using a proprietary process and placed into individual glass vials, were shipped overnight on dry ice to the surgical laboratory. Titanium porous oxide surface implants without the rhGDF-5 coating served as controls. The titanium implants, custom-made for the supraalveolar peri-implant defect model (Wikesjö et al. 2006), were manufactured with a reference notch 5 mm from the implant platform (Fig. 1). 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, histologic, and histometric analysis. Implants were stored at $-80^{\circ}$C until use. The implants were acclimatized to room temperature before implantation.

*Fig. 1.* Custom-made titanium porous oxide surface-modified implant (TiUnite™, Ø4.0 × 10 mm; Nobel Biocare AB).

### Surgery protocol

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

One experienced surgeon (U. M. E. W.) performed all the surgical procedures. Bilateral, critical-size, supraalveolar peri-implant defects were created in the mandibular pre-molar region (Fig. 2; Wikesjö et al. 2006). Briefly, buccal and lingual mucoperiosteal flaps were reflected and alveolar bone was removed around the circumference of the pre-molar teeth to a level approximately 6 mm apical to the cemento-enamel junction using water-cooled rotating burs. The pre-molar teeth were extracted and the first molar was amputated at the level of the reduced alveolar crest. Three implants were placed into osteotomies prepared into the extraction sites of the third and fourth pre-molar teeth in each jaw quadrant. A few implants were placed into osteotomies prepared into the reduced alveolar process when placement into extraction sites was not possible. Five mm of the implant was placed within the

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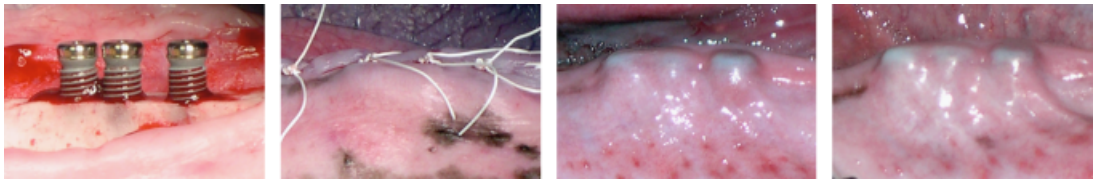

*Fig. 2*. Critical-size, supraalveolar peri-implant defect (left) following wound closure advancing the mucogingival flaps to cover the implants (left centre), and following 4 (right centre) and 8 (right) weeks of healing. This defect received implants coated with 30 μg rhGDF-5. GDF-5 growth/differentiation factor-5; rhGDF, recombinant human GDF-5.

*Table 1*. Distribution of treatments among animals receiving implants coated with rhGDF-5

| | Group 1 | Group 2 |
|---|---|---|
| No. of animals | 6 | 6 |
| Test item | rhGDF-5 30 *versus* 60 μg | rhGDF-5 120 μg *versus* control |
| Implants per jaw quadrant | 3 | 3 |
| Healing interval | 8 weeks | 8 weeks |

GDF-5, growth/differentiation factor-5; rhGDF, recombinant human GDF-5.

surgically reduced alveolar ridge to the level of the reference notch, creating 5 mm, critical-size, supraalveolar, peri-implant defects.

Six animals received implants coated with rhGDF-5 at a dosage of 30 and 60 μg/implant in contralateral jaw quadrants and six animals received implants coated with rhGDF-5 at a dosage of 120 μg/implant or uncoated implants (control) in contralateral jaw quadrants (Table 1). Treatments were alternated between the left and the right jaw quadrants. 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). Photographic registrations were obtained following implant placement and wound closure.

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

#### Post-surgery care

A long-acting opioid, buprenorphine HCl (0.01–0.03 mg/kg i.m.) was administered immediately post-surgery and re-dosed twice daily for 3 days. A broad-spectrum antibiotic (enrofloxacin; 2.5 mg/kg i.m.) was administered immediately post-surgery and re-dosed twice daily for 7 days. Sutures were removed under sedation (propofol; 5–7 mg/kg i.v.) at approximately 10 days. Radiographs were obtained under sedation (propofol, 5–7 mg/kg i.v. 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 (20–30 ml of a 2% solution; Xttrium Laboratories Inc., Chicago, IL, USA) 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

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

#### Euthanasia

The animals were anaesthetized (see above) and euthanized at week 8 post-surgery by an i.v. 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 histopathologic and histometric analysis using incandescent, polarized, and fluorescent light microscopy.

#### Clinical analysis

One examiner (G. P.) re-capped 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.

#### Histopathologic analysis

Two masked experienced examiners (U. M. E. W., M. Q.) performed the histopathologic evaluation including observations of bone formation and resorption,

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cortex formation, seroma formation, fibrovascular tissue and marrow, and inflammatory reactions using computer-enhanced images, and 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): 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): area of newly formed bone along the implant above the reference notch, excluding bone formation exceeding the implant platform.
- Bone density (new bone): ratio bone/marrow spaces in newly formed bone.
- Osseointegration (new bone): per cent 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): ratio bone/marrow spaces in a $300 \times 1800\,\mu m$ area (width × height) immediately outside the implant threads in resident bone.
- Bone density inside the implant threads (resident bone): ratio bone/marrow spaces within the implant threads in resident bone.
- Osseointegration (resident bone): per cent BIC within resident bone measured from the reference notch to the apex of the implant.

#### Statistical analysis

Examiner reliability was assessed using the concordance correlation coefficient (Lin 1989, 2000), which ranges between 0 and 1. The 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. All measurements at site level were averaged for each jaw quadrant. A general linear model including a population-averaged panel-data methodology to account for the split-mouth design was used. A robust variance estimation was used in these models. Analysis of differences between doses was performed using Wald tests adjusted for multiple comparisons. 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

Healing was uneventful. No implant was lost. None of the implant exhibited signs of seroma formation. There were no dramatic or meaningful differences between implants coated with 30, 60, or 120 $\mu$g rhGDF-5 relative to the number of visible, palpable, and exposed implants (Fig. 2). In the control group, all implants were visible and palpable through the mucosa at 8 weeks; 8 implants were exposed (data not shown). The radiographic observations of this study will be detailed elsewhere.

#### Histologic observations

Jaw quadrants receiving implants coated with 30 or 60 $\mu$g rhGDF-5 exhibited limited new bone formation predominantly located to their lingual aspect (Fig. 3). There were no remarkable differences in the appearance between the newly formed and the immediately adjoining resident bone. None of the implants showed evidence of seroma formation. The fluorescence microscopy revealed limited peri-implant resident bone remodelling (data not shown). Most implants exhibited some initial resorption of the thin buccal plate, in nearly all sites, but partly replaced by newly formed bone.

Jaw quadrants receiving implants coated with 120 $\mu$g rhGDF-5 showed relatively robust bone formation primarily located to their lingual aspect (Fig. 4). There were no remarkable differences in the appearance between the newly formed and the immediately adjacent resident bone. No implant showed evidence of seroma formation. The fluorescence microscopy showed peri-implant resident bone remodelling encompassing replacement of bone inside the thread area (data not shown). A few implants exhibited resorption of the thin buccal plate replaced, at least in part, by newly formed bone.

Jaw quadrants receiving uncoated control implants showed limited, if any, new bone formation (Fig. 4). None of the implants showed evidence of seroma formation or peri-implant remodelling in the resident bone. Most implants exhibited resorption of the buccal plate without recovery of the resorbed bone.

#### Histometric observations

The results of the histometric analysis are summarized in Figs 5–7, and Tables 2 and 3. There was a small increase in induced bone height for implants coated with rhGDF-5 compared with control ($p < 0.05$). The induced bone height averaged ($\pm$ SD) $1.6 \pm 0.6$ mm for implants coated with 120 $\mu$g rhGDF-5 *versus* $1.2 \pm 0.5$, $1.2 \pm 0.6$, and $0.6 \pm 0.2$ mm for implants coated with 60 or 30 $\mu$g rhGDF-5, or uncoated controls, respectively. Implants coated with 120 $\mu$g rhGDF-5 exhibited significantly greater induced bone area than the control ($p < 0.01$). No other significant differences were observed among the experimental groups regarding bone area.

Induced bone density ranged from 62% to 69% for implants coated with rhGDF-5, being somewhat lower than that of the resident bone (78%; $p < 0.05$). Differences in bone density were reflected in the extent of osseointegration, BIC ranging from 35% to 51% for implants coated with rhGDF-5 *versus* 79% for the control ($p < 0.01$). Implants coated with 120 $\mu$g rhGDF-5 exhibited lower BIC than implants coated with 60 and 30 $\mu$g rhGDF-5 ($p < 0.05$).

Bone density immediately outside the implant threads was significantly greater at uncoated implants (81%) than at implants coated with 30 $\mu$g rhGDF-5

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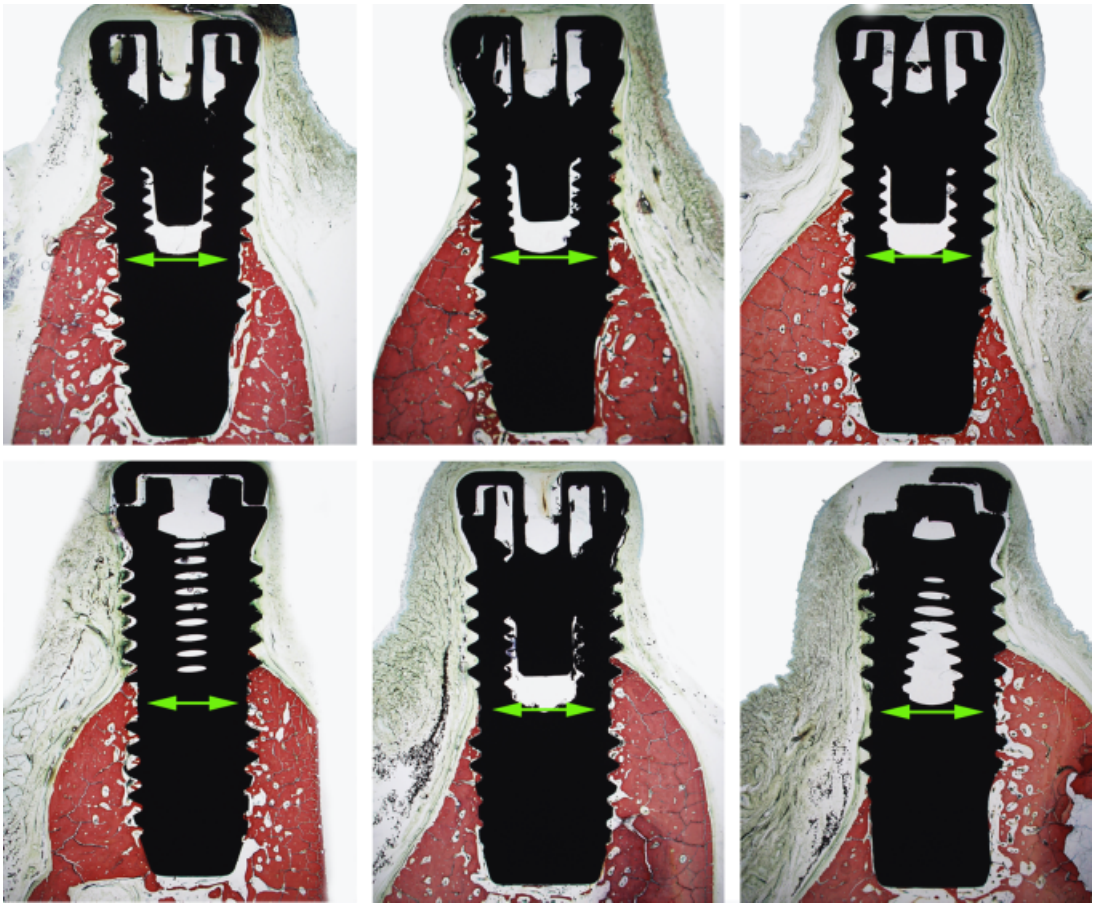

*Fig. 3*. Photomicrographs from one animal showing Ø4.0 × 10 mm titanium porous oxide implants coated with 60 μg rhGDF-5 (top row; buccal surfaces facing right) and 30 μg rhGDF-5 (bottom row; buccal surfaces facing left) placed in contralateral jaw quadrants. Left implants represent the most anterior implants and right implants represent the most posterior implants. The implants display limited bone formation generally confined to their lingual aspect, whereas the buccal aspect shows a net loss of bone following crestal remodelling. Green arrows delineate the 5 mm notch placed level with the resident alveolar bone. GDF-5, growth/differentiation factor-5; rhGDF, recombinant human GDF-5.

(77%) and 60 μg rhGDF-5 (73%) ($p < 0.05$). Implants coated with 120 μg rhGDF-5 exhibited significantly greater bone density (80%) outside the implant threads than implants coated with 60 μg rhGDF-5 ($p < 0.01$). There were marked differences in bone density inside the thread area, averaging 43%, 42%, and 44% for implants coated with 30, 60, or 120 μg rhGDF-5, respectively, *versus* 53% for the control ($p < 0.01$). Differences in bone density inside the thread area apparently resulted in significant differences in BIC ranging from 38% to 44% for implants coated with rhGDF-5 *versus* 75% for the control ($p < 0.01$).

## Discussion

Preclinical studies have shown that GDF-5 is safe and can induce relevant bone formation using a variety of carrier technologies in a broad range of experimental settings including rat calvaria osteotomy defects (Kuniyasu et al. 2003, Pöhling et al. 2006), rabbit or sheep spine fusion models (Jahng et al. 2004, Magit et al. 2006), minipig osteochondral femoral defects (Jung et al. 2006), and canine alveolar ridge or minipig sinuslift models for endosseous oral implant site development (Gruber et al. 2008, Schwarz et al. 2008). The present study demonstrated dose-dependent bone formation and osseointegration using a titanium porous oxide oral implant surface as a carrier for rhGDF-5 in a well-characterized critical-size canine large animal model (Wikesjö et al. 2006) in concert with that observed in previous studies. For example, observations from the critical-size calvaria through-and-through osteotomy defect model in adult Sprague–Dawley rats demonstrate significantly greater bone formation at sites implanted with rhGDF-5 in a β-tricalcium phosphate (β-TCP) carrier compared with controls (Pöhling et al. 2006). In fact, bone formation, assessed histometrically, was approximately fivefold greater for the rhGDF-5/β-TCP construct compared with two different manufacture β-TCP biomaterials, a commercially available bovine bone mineral biomaterial used as a stand-alone technology or combined with a collagen matrix, or a commercially available bovine bone mineral biomaterial combined with a synthetic peptide. Only sites treated with rhGDF-5/β-TCP showed complete osseous bridging of the defect. Similar advantageous effects of rhGDF-5 were reported in a study using a Göttingen minipig maxillary sinus bone augmentation model (Gruber et al. 2008). Six animals received equal volumes 1.2 mg rhGDF-5 in a β-TCP carrier *versus* β-TCP

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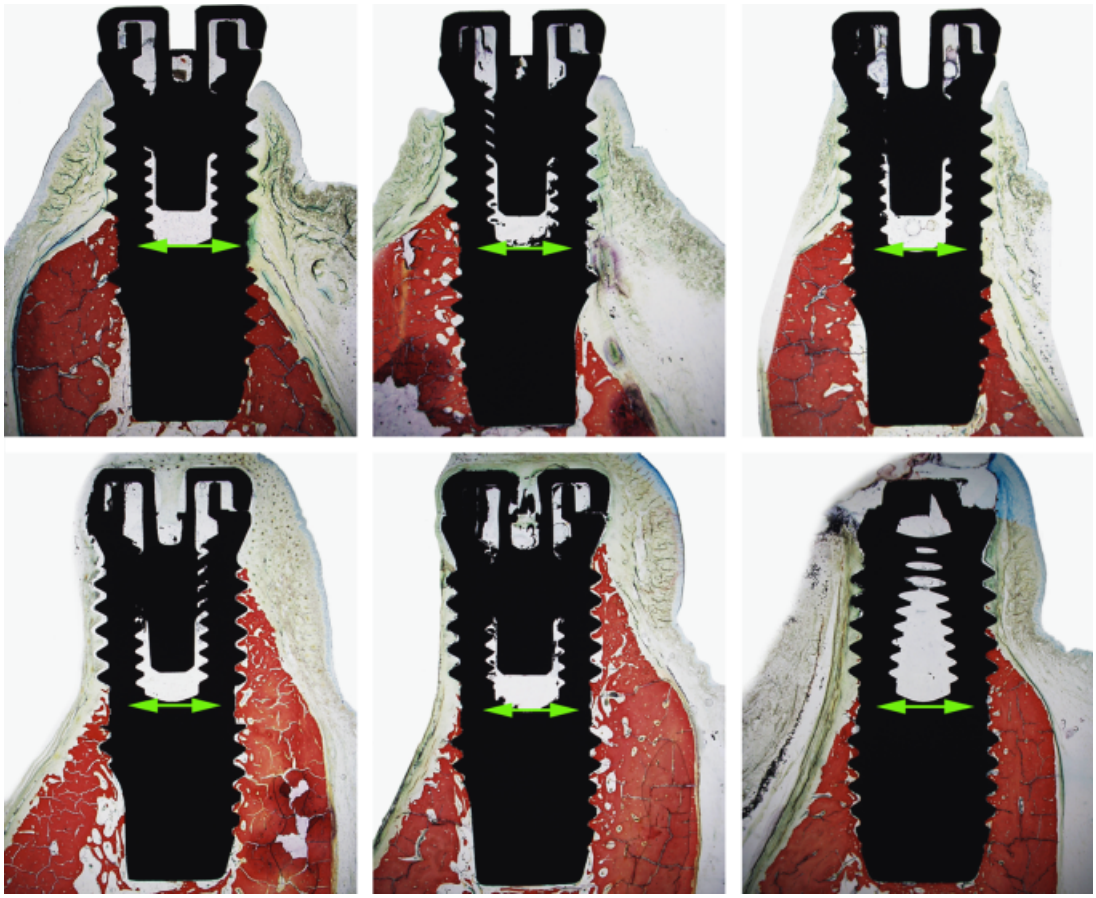

*Fig. 4*. Photomicrographs from one animal showing Ø4.0 × 10-mm-uncoated control titanium porous oxide implants (top row; buccal surfaces facing right) and implants coated with 120 μg rhGDF-5 (bottom row; buccal surfaces facing left) placed in contralateral jaw quadrants. Left implants represent the most anterior implants and right implants represent the most posterior implants. Controls show limited bone formation confined to the lingual aspect of the implants, whereas the buccal aspect shows a net loss of bone following crestal remodelling. Implants coated with 120 μg rhGDF-5 show significant bone formation on their lingual aspects. The buccal alveolar crest is maintained without additional bone formation following crestal remodelling. Green arrows delineate the 5 mm notch placed level with the resident alveolar bone. GDF-5, growth/differentiation factor-5; rhGDF, recombinant human GDF-5.

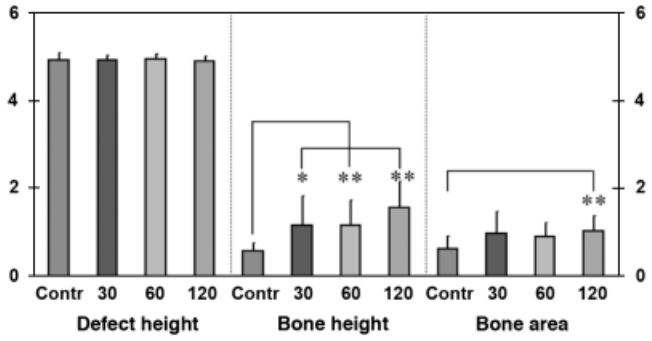


*Fig. 5*. Mean (± SD in mm/mm$^2$) induced bone formation (height and area) for animals receiving uncoated implants (control) or implants coated with 30, 60, or 120 μg rhGDF-5. GDF-5 growth/differentiation factor-5; rhGDF, recombinant human GDF-5.

alone, and six animals 2.4 mg rhGDF-5 in a β-TCP carrier *versus* β-TCP alone implanted into contralateral sinus sites in conjunction with placing a titanium screw-type implant into each site. Three animals from each group were euthanized at 4 and at 12 weeks. The histometric analysis showed significantly greater mean bone volume density for the 1.2 mg rhGDF-5 dose (22.8%) compared with control (8%) at 4 weeks ($p \leqslant 0.05$). BIC rates were also significantly enhanced for the rhGDF-5 treatments at 4 weeks (1.2 mg rhGDF-5: 41.9%; 2.4 mg rhGDF-5: 40.6%) compared with the control (7.8% and 16.4%, respectively; $p \leqslant 0.05$).

Different experimental models have been utilized to evaluate the biologic and clinical potential of various candidate therapies for alveolar augmentation and osseointegration. This and several other studies in our laboratories have used the critical-size, supraalveolar, peri-implant defect model (Caplanis et al. 1997, Sigurdsson et al. 1997, 2001, Tatakis et al. 2002, Wikesjö et al. 2002, 2003, 2004, 2006, 2008c, Polimeni et al. 2004, Qahash et al. 2007, 2008, Leknes et al. 2008a, b). Critical-size, supraal-

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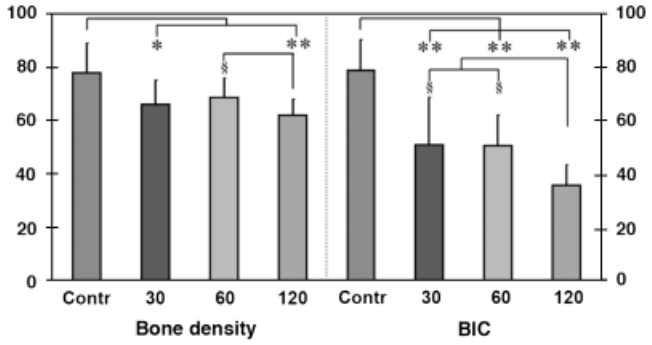


*Fig. 6.* Mean (± SD in %) induced bone density and bone–implant contact (BIC) for animals receiving uncoated implants (control) or implants coated with 30, 60, or 120 μg rhGDF-5. GDF-5, growth/differentiation factor-5; rhGDF, recombinant human GDF-5.

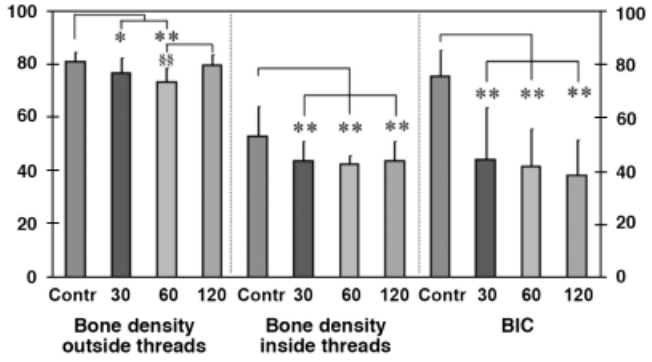


*Fig. 7.* Mean (± SD in %) resident bone density and bone–implant contact (BIC) for animals receiving uncoated implants (control) or implants coated with 30, 60, or 120 μg rhGDF-5. GDF-5, growth/differentiation factor-5; rhGDF, recombinant human GDF-5.

*Table 2.* Results of the histometric analysis of rhGDF-5-induced peri-implant bone for animals ($N = 6$) receiving implants coated with 30 *versus* 60 μg rhGDF-5 and animals ($N = 6$) receiving implants coated with 120 μg rhGDF-5 *versus* sham surgery in contralateral jaw quadrants (means ± SD)

| | Defect height (mm) | Bone Height (mm) | Bone area ($mm^2$) | Bone density (%) | BIC (%) |
|---|---|---|---|---|---|
| rhGDF-5 (30 μg) | 4.9 ± 0.1 | 1.2 ± 0.6* | 1.0 ± 0.5 | 66.0 ± 8.6* | 51.1 ± 17.2**§§ |
| rhGDF-5 (60 μg) | 5.0 ± 0.1 | 1.2 ± 0.5** | 0.9 ± 0.3 | 68.8 ± 6.7§ | 50.5 ± 10.8**§§ |
| rhGDF-5 (120 μg) | 4.9 ± 0.1 | 1.6 ± 0.6** | 1.0 ± 0.3** | 62.0 ± 5.3** | 35.5 ± 7.5** |
| Sham-surgery | 4.9 ± 0.1 | 0.6 ± 0.2 | 0.6 ± 0.3 | 77.9 ± 10.8 | 78.8 ± 10.8 |

Compared with sham surgery: $^{*}p < 0.05$; $^{**}p < 0.01$.
Compared with rhGDF-5(120 μg): $^{\S}p < 0.05$; $^{\S\S}p < 0.01$.
GDF-5, growth/differentiation factor-5; rhGDF, recombinant human GDF-5; BIC, bone–implant contact.

veolar, peri-implant defects can be reproducibly created. Post-surgery complications including suture line dehiscencies, exposure, and infection of implanted technology are rare with careful wound management. Local factors at least including conditions for primary intention healing and space-provision appear to influence bone formation. Whereas the more prominent lingual shelf of the defect sites consistently supports bone formation, the buccal considerably narrower aspect appears to be more inconsistent (Wikesjö et al. 2006). The radiographic and histometric analysis of this model consistently show limited, if any, regeneration of alveolar bone in sham-surgery control sites over an 8-week healing interval. In other words, the critical-size, supraalveolar peri-implant defect model, a genuine onlay defect model, displays a limited innate osteogenic potential under optimal conditions for healing. Thus, this discerning model represents a strict tool in the judicious evaluation of candidate bone biomaterials, devices for GBR, and implantable or injectable technologies using matrix, growth, or differentiation factors as stand-alone or combination technologies for alveolar augmentation and osseointegration of oral implants.

In the present study, the histological analysis showed new bone formation at implants coated with rhGDF-5 significantly exceeding that of the sham-surgery control in a dose-dependent order, the 120-μg dose exhibiting the most notable effect. There was a statistically significant increase in induced bone height for implants coated with rhGDF-5 compared with the control, induced bone height averaging 1.6 ± 0.6, 1.2 ± 0.5, and 1.2 ± 0.6 mm and for implants coated with 120, 60, and 30 μg rhGDF-5, respectively, *versus* 0.6 ± 0.2 mm for uncoated controls. Similar results have been observed following guided bone regeneration with or without adjunctive demineralized, freeze-dried, allogeneic bone matrix (Caplanis et al. 1997, Wikesjö et al. 2004). Whereas guided bone regeneration marginally supports osteogenic bone formation following 8- or 16-week healing intervals, rhBMP-2 at various dosages in an absorbable collagen sponge carrier supports significantly greater, but considerably variable, bone formation (Sigurdsson et al. 1997, Tatakis et al. 2002, Wikesjö et al. 2003). Sixteen-week healing intervals combined with higher concentrations apparently produce denser, more mature bone qualities (Sigurdsson et al. 1997). Shorter, 8-week healing intervals and lower rhBMP-2 concentrations reveal remarkably irregular, sparsely trabecular immature bone and osseointegration of negligible relevance (Tatakis et al. 2002, Wikesjö et al. 2003). Use of space-providing devices supports a more geometrically ordered bone formation but still of an immature sparsely trabecular nature (Wikesjö et al. 2003, 2004). Use of cadaver-derived or synthetic carrier technologies with innate structural integrity produces relevant rhBMP-2 induced bone formation exhi-

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*Table 3*. Results of the histometric analysis of resident peri-implant bone for animals ($N = 6$) receiving implants coated with 30 *versus* 60 $\mu$g rhGDF-5 and animals ($N = 6$) receiving implants coated with 120 $\mu$g rhGDF-5 *versus* sham surgery in contralateral jaw quadrants (means $\pm$ SD)

| | Bone density outside the threads (%) | Bone density inside the threads (%) | BIC (%) |
|---|---|---|---|
| rhGDF-5 (30 $\mu$g) | 76.6 $\pm$ 5.1* | 43.5 $\pm$ 7.0* | 44.12 $\pm$ 19.1** |
| rhGDF-5 (60 $\mu$g) | 73.3 $\pm$ 4.5**§§ | 42.2 $\pm$ 2.7* | 41.7 $\pm$ 13.5** |
| rhGDF-5 (120 $\mu$g) | 79.7 $\pm$ 3.3 | 43.8 $\pm$ 6.6** | 38.3 $\pm$ 12.6** |
| Sham-surgery | 81.1 $\pm$ 2.8 | 53.1 $\pm$ 10.5 | 75.3 $\pm$ 9.6 |

Compared with sham surgery: $^{*}p < 0.05$; $^{**}p < 0.01$.
Compared with rhGDF-5(120 $\mu$g): $^{\S\S}p < 0.01$.
GDF-5, growth/differentiation factor-5; rhGDF, recombinant human GDF-5; BIC, bone–implant contact.

biting the qualities and osseointegration of the adjoining resident bone (Sigurdsson et al. 2001, Wikesjö et al. 2002). All these studies demonstrate the distinct effects of the BMP constructs; however, variations in healing intervals, dosages, and carrier technologies make surefooted comparisons and cross-interpretation of the results exceedingly difficult even if the studies have been carried out using the same well-characterized defect model.

However, a more immediate comparison of local bone formation and osseointegration between implants coated with rhGDF-5 in the present study and implants coated with rhBMP-2 at 0.75, 1.5, and 3.0 mg/ml (estimated to 30, 60, and 120 $\mu$g rhBMP-2/implant) in a parallel study (Wikesjö et al. 2008c) can be made due to the fact that both studies strictly adhered to an identical 8-week protocol using the critical-size supraalveolar peri-implant defect model, with the exception of dose and implant coating. Induced bone height averaged 1.2 $\pm$ 0.6, 1.2 $\pm$ 0.5, and 1.6 $\pm$ 0.6 mm for implants coated with 30, 60, and 120 $\mu$g rhGDF-5 *versus* 0.6 $\pm$ 0.2 mm for implants serving as sham-surgery controls without rhGDF-5. Corresponding bone formation at implants coated with rhBMP-2 averaged 4.4 $\pm$ 0.4, 4.2 $\pm$ 0.7, and 4.2 $\pm$ 1.2 *versus* 0.8 $\pm$ 0.3 mm for the sham-surgery control. rhGDF-5-induced bone density averaged 66%, 69%, and 62% for the 30, 60, and 120 $\mu$g dose, respectively, being significantly lower than that in resident bone (78%). Similarly, rhBMP-2 induced bone density averaged 63%, 61%, and 42% for the 0.75, 1.5, and 3.0 mg/ml solution, respectively. Bone density within the limited amount of new bone in the control group (73%) was significantly greater than that of the rhBMP-2-induced bone. Differences in bone density apparently resulted in significant differences in BIC ranging from 35% to 51% for implants coated with rhGDF-5 *versus* 79% for the control. In a similar order, implants coated with rhBMP-2 exhibited significantly smaller BIC values ranging from 30% to 39% *versus* 79% for the sham-surgery control. Apparently, differences in the magnitude of the newly formed bone induced by the rhBMP-2-coated implants did not result in a difference in the quality of the bone as shown by the magnitude of bone density and BIC. Nevertheless, it remains undetermined whether apparent differences between the rhBMP-2 and rhGDF-5 protocol in these studies relate to variance application of the proteins onto the titanium porous oxide implant surfaces or probably more likely due to genuine differences in bone inductive capacity.

Differences in bone formation among buccal and lingual sites or sites with a broad or a narrow alveolar base in the present study have also been observed in studies evaluating guided bone regeneration as well as rhBMP-2-coated implants (Polimeni et al. 2004, Wikesjö et al. 2008c) and in control sites (Wikesjö et al. 2006, Qahash et al. 2008). In this model, the tenting effect of the mucoperiosteal flaps supported by the implants and the alveolar base provides a primary unobstructed space for bone formation. Because the endosseous implants in this model are placed into extraction sockets behind a narrow buccal cortical plate, their position reduces the buccal alveolar base and in particular local endosteal tissue resources. Rather it appears that the buccal cortical plate remodels as a result of insults from the implant placement, resulting in net loss of bone and exposure of the implant bone-anchoring surface (Qahash et al. 2008). The broad lingual surgically reduced alveolar base, on the other hand, provides rich endosteal tissue resources supporting osteogenic bone formation and vascular elements supporting osteoinduction.

## Conclusion

In all, the results of this study suggest that rhGDF-5-coated oral implants display a dose-dependent osteoinductive and/or osteoconductive effect. Application of rhGDF-5 appears to be safe as it is associated with limited, if any, adverse effects.

## Acknowledgements

The authors thank Milton April, DVM, and Alexis Agelan, DVM, for veterinary care; Lewis Thomas Bright and Garen Lindsay for animal technical care and husbandry; and Ms Joanne Drew for administrative support. Scil Technology GmbH, kindly donated rhGDF-5 and prepared the rhGDF-5-coated implants. This study was supported by a grant from Nobel Biocare AB.

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**Clinical Relevance**

*Scientific rationale*: The objective of this study was to evaluate the potential of rhGDF-5 coated onto an oral implant with a purpose-designed titanium porous oxide surface to stimulate local bone formation including osseointegration and vertical augmentation of the alveolar ridge.
*Principal findings*: Using the supraalveolar peri-implant defect model, rhGDF-5-coated implants display a dose-dependent osteoinductive and/or an osteoconductive effect, bone formation apparently benefiting from local factors.
*Practical Implications*: Application of rhGDF-5 appears to be safe as it is associated with limited, if any, adverse effects.

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