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# Periodontal wound healing/regeneration following implantation of recombinant human growth/differentiation factor-5 (rhGDF-5) in an absorbable collagen sponge carrier into one-wall intrabony defects in dogs: a dose-range study

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***Abstract***

**Aim:** Recombinant human growth/differentiation factor-5 (rhGDF-5) is being evaluated as a candidate therapy in support of periodontal regeneration. The objective of this study was to evaluate cementum and alveolar bone formation, and aberrant healing events following surgical implantation of rhGDF-5 in an absorbable collagen sponge (ACS) carrier using an established periodontal defect model.

**Materials and Methods:** Bilateral 4 × 5 mm (width × depth), one-wall, critical-size, intrabony periodontal defects were surgically created at the mandibular second and fourth pre-molar teeth in 15 Beagle dogs. Five animals received 1 μg/defect and five animals 20 μg/defect rhGDF-5 in unilateral defect sites. Contralateral sites received treatments reported elsewhere. Five animals received rhGDF-5/ACS with 0 (buffer control) and 100 μg/defect rhGDF-5 in contralateral defect sites. The animals were euthanized at 8 weeks post-surgery for histologic and histometric evaluation.

**Results:** Surgical implantation of rhGDF-5 stimulated significant periodontal regeneration. Cementum formation was significantly enhanced in sites implanted with rhGDF-5 (1 and 100 μg) compared with control ($p < 0.05$). Similarly, bone formation height was significantly greater in sites receiving rhGDF-5 (1 and 100 μg) compared with control ($p < 0.05$). There were no significant or remarkable differences in bone and cementum formation within the selected dose interval (1, 20 and 100 μg rhGDF-5). None of the control or the rhGDF-5 sites exhibited root resorption, ankylosis, or other aberrant tissue reactions.

**Conclusion:** Surgical implantation of rhGDF-5/ACS may be used safely to support periodontal wound healing/regeneration in intrabony periodontal defects without complications.

Key words: absorbable collagen sponge; dog; periodontal regeneration; rhGDF-5; tissue engineering

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**Conflict of interests and source of funding statement**

Dr. Susanne Pippig is an employee of Scil Technology GmbH. Dr. Michael Siedler was an employee of Scil Technology at the time of the study was conducted. Dr. Chong-Kwan Kim receives grants for conduct of studies from Scil Technology. Dr. Ulf M. E. Wikesjö is a consultant to Scil Technology. The research reported herein, funded by Scil Technology, was conducted at Scil Technology, Martinsried, Germany, and at Yonsei University, Seoul, Korea.

Critical biologic directives for periodontal regeneration include unobstructed space provision allowing formation of a periodontal regenerate; wound stability allowing uneventful maturation of the periodontal regenerate; and conditions for primary intention healing disallowing infection of the periodontal regenerate (Polimeni et al. 2006). The current clinical protocol, including the use of occlusive barrier devices, appears to be unnecessary and may complicate periodontal wound healing (Danesh-Meyer & Wikesjö 2001, Wikesjö et al. 2003c). Moreover, placement of slowly or non-resorbing biomaterials into a defect site may obstruct periodontal regeneration (Trombelli et al. 1999, Wikesjö et al. 2003a). Nevertheless, clinical shortcomings do not always allow optimized conditions for periodontal wound healing/regeneration used in experimental models. Thus, adjunctive measures acknowledging critical biologic directives and the native potential for periodontal wound healing/regeneration may provide a clinical benefit.

Urist (1965) discovered a group of proteins in the bone matrix capable of inducing cartilage, bone, and marrow in ectopic rodent models and named them bone morphogenetic proteins (BMPs). Recombinant human BMPs were eventually made available following extensive purification and molecular cloning (Wozney et al. 1988, Celeste et al. 1990, Özkaynak et al. 1990) identifying the specific bone-inducing proteins (Wang et al. 1990, Sampath et al. 1992, Hötten et al. 1996). Recombinant human BMP-2 (rhBMP-2), recombinant human osteogenic protein-1 (rhOP-1/rhBMP-7), recombinant human growth/differentiation factor-5 (rhGDF-5), and rhBMP-12 (rhGDF-7) have been evaluated for a number of indications in the axial and appendicular skeleton including spine fusion, hip arthroplasty, fracture repair (Friedlaender 2001, Valentin-Opran et al. 2002), and craniofacial indications including congenital defects (cleft palate), orthognatic and resection defects, plastic procedures, alveolar augmentation, implant fixation, and for periodontal defects (Wozney & Wikesjö 2008). rhBMP-2 and rhOP-1 have received FDA approval for clinical use including orthopaedic and maxillofacial indications.

The potential of rhBMP-2 and rhOP-1 to support periodontal regeneration has been evaluated extensively using discriminating large animal models (Ishikawa et al. 1994, Sigurdsson et al. 1995, 1996, Ripamonti et al. 1996, 2001, Kinoshita et al. 1997, Giannobile et al. 1998, Wikesjö et al. 1999, 2003b–e, Blumenthal et al. 2002, Choi et al. 2002, Selvig et al. 2002, Sorensen et al. 2004a). These studies show that greater bone and cementum formation may be observed following application of rhBMP-2 or rhOP-1 compared with controls. They clearly indicate the critical importance of the carrier/delivery system (Sigurdsson et al. 1995, 1996, Sorensen et al. 2004a). Dose ranging of rhBMP-2 has yielded only minor differences between treatments (Wikesjö et al. 1999). Notably, rhBMP-2 applications frequently result in root resorption/ankylosis (Sigurdsson et al. 1995, 1996, Wikesjö et al. 1999, 2003b–e, 2004, Selvig et al. 2002, Sorensen et al. 2004a), whereas root resorption/ankylosis appears to be an infrequent observation in more limited periodontal defects (Ishikawa et al. 1994, Kinoshita et al. 1997, Blumenthal et al. 2002, Choi et al. 2002) and in non-human primate clinical modelling (Ripamonti et al. 1996, 2001, Blumenthal et al. 2002). Typically, the loose fibrovascular tissue observed following application of rhBMP-2, which has often been characterized as a periodontal ligament (PDL), in a longer perspective matures into fatty marrow rather than a viable periodontal ligament. (Wikesjö et al. 2003e). Thus, it appears that rhBMP-2 and to some extent rhOP-1 may not optimally support regeneration of the periodontal attachment.

GDF-5, also known as cartilage-derived morphogenetic protein-1 (CDMP-1), exhibits a close structural relationship with the other BMP members of the transforming growth factor-$\beta$ superfamily (Chang et al. 1994). GDF-5 plays a crucial role in skeletal morphogenesis. Briefly, mice with brachypodism (reduced limb size) exhibit a missense mutation in the GDF-5 gene (Storm et al. 1994, Storm & Kingsley 1996, Wolfman et al. 1997, Mikic et al. 2001, 2002). GDF-5 promotes mesenchymal cell recruitment and skeletal processes such as endochondreal ossification, synovial joint formation, tendon/ligament development, and odontogenesis (Buxton et al 2001). GDF-5 enhances sGAG synthesis in human periodontal ligament cells (Nakamura et al. 2003). GDF-5 enhances chondrogenic or osteogenic differentiation in mesenchymal cell cultures via the BMP receptor-Smads signal pathway (Nishitoh et al. 1996, Akiyama et al. 2000, Baur et al. 2000, Aoki et al. 2001). rhGDF-5 in suitable carriers induces tendon/ligament-like, cartilage-like, and/or bone-like tissue formation in rodents, ruminants, and primates (Wolfman et al. 1997, Takaoka et al. 1988, Pöhling et al. 2006, Yoshimoto et al. 2006). As it appears, GDF-5, in addition to being associated with formation of bone, cartilage, and tendons in development, also supports formation of these tissues in post-foetal life. The objective of this dose-range study was to evaluate periodontal regeneration including a functionally oriented PDL, cementum and bone formation, and any aberrant healing sequela following surgical implantation of rhGDF-5 in an established one-wall intrabony periodontal defect model in dogs.

## Materials and Methods

### Animals

Fifteen male Beagle dogs, approximately 18 months old, weight 10–15 kg, bred exclusively for biomedical research purposes, were used. The animals exhibited an intact dentition with a healthy periodontium. Animal selection and management, surgical protocol, and preparation followed routines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea. The animals had ad libitum access to water and a pelleted laboratory diet, with the exception of 1 week immediately post-surgery, when they were fed a canned soft dog food diet (Prescription Diet Canine i/d, Hill's Pet Nutrition Inc., Topeka, KS, USA).

### rhGDF-5/ACS

rhGDF-5 (Scil Technology GmbH, Martinsried, Germany) was reconstituted and

diluted in glutamic acid buffer into concentrations of 0.0139, 0.28, and 1.39 $\mu$g/$\mu$l. A sterile 0.75 × 1.5 in. absorbable collagen sponge (ACS; CollaCote, Zimmer Dental, Carlsbad, CA, USA) was removed from the outer tray and placed on a sterile field. The ACS, cut to pieces to fill the 4 × 5 mm intrabony periodontal defect, was soak-loaded with 72 $\mu$l of rhGDF-5 at 0 (buffer control), 0.0139, 0.28, and 1.39 $\mu$g/$\mu$l, and was thus implanted within 30 min. All materials were supplied by Scil Technology and stored at −80°C until use.

#### Surgical protocol

Food was withheld the night preceding surgery. The surgical procedure was performed under general anaesthesia induced by an intravenous (i.v.) injection of atropine (0.04 mg/kg; Kwangmyung Pharmaceutical Ind. Co. Ltd., Seoul, Korea) and an intramuscular injection of a combination of xylazine (Rompun, Bayer Korea Co., Seoul, Korea) and ketamin (Ketara, Yuhan Co., Seoul, Korea), followed by inhalation anaesthesia (Gerolan, Choongwae Pharmaceutical Co., Seoul, Korea). Routine dental infiltration anaesthesia was used at the surgical sites.

The mandibular first and third premolars were extracted before the experimental surgery, and the extraction sites were allowed to heal for 8 weeks. The remaining dentition received oral prophylaxis in conjunction with the extractions.

Experimental surgeries were performed under general anaesthesia. Buccal and lingual mucoperiosteal flaps were elevated to create critical-size, ''box-type'', 4 × 5 mm (width × height), one-wall intrabony defects at the distal aspect of the second and the mesial aspect of the fourth mandibular pre-molar teeth in the right and left jaw quadrants (Fig. 1) (Kim et al. 2004). Following root planing to remove the root cementum, a reference notch was made with a round bur into the root surface at the base of the defects. Five animals received rhGDF-5/ACS with 0 (buffer control) and 100 $\mu$g rhGDF-5 in contralateral defect sites. Five animals received 1 $\mu$g and five animals received 20 $\mu$g rhGDF-5 in unilateral defect sites. The defects were filled to the level of the alveolar crest. Intrabony defects within each jaw quadrant received the same treatment. Contralateral sites received treatments reported elsewhere. The mucogingival flaps were then advanced, adapted, and sutured using a resorbable suture material (Vicryl 5.0 Polyglactin 910, Ethicon, Johnson & Johnson, Somerville, NJ, USA).

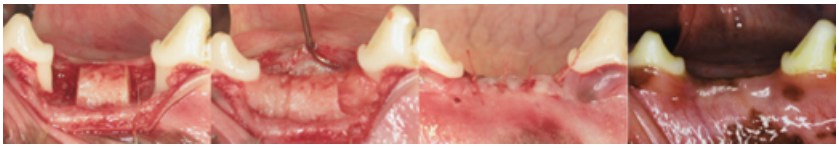

*Fig. 1.* Surgically created, critical-size, one-wall, intrabony periodontal defects at the distal aspect of the second and mesial aspect of the fourth mandibular pre-molar teeth (left). Application of human growth/differentiation factor-5-5/absorbable collagen sponge (left centre). Mucoperiosteal flaps adapted and sutured for primary intention healing (right centre). Healing at week 8 post-surgery (right).

#### Post-operative management

The animals received an intramuscular administration of a broad-spectrum antibiotic (Cefazoline Sodium 20 mg/kg, Yuhan Co.) and daily topical application of a 0.2% chlorhexidine solution (Hexamedin®, Bukwang Pharmaceutical Co., Seoul, Korea) for infection control. Observations of experimental sites with regard to gingival health, maintenance of suture line closure, oedema, and evidence of tissue necrosis or infection were made daily until suture removal, and at least twice weekly thereafter. The animals were euthanized at 8 weeks post-surgery using an overdose of pentobarbital sodium (90–120 mg/kg; i.v.). Block sections including defect sites and surrounding alveolar bone and mucosal tissues were then collected. Photographic and radiographic recordings were completed pre/intra-surgery, immediately post-surgery, and at 8 weeks post-surgery.

#### Histological processing

The block specimens were rinsed in sterile saline and immersed in 10% neutral-buffered formalin at a volume 10 times that of the block section for 10 days. After rinsing in sterile water, the sections were decalcified in 5% formic acid for 14 days, trimmed, dehydrated in a graded ethanol series, and embedded in paraffin. Step-serial sections, 5 $\mu$m thick, were cut in a mesial-distal vertical plane, at approximately 80-$\mu$m intervals. The sections were stained using haematoxylin/eosin and Masson's trichrome stains. The three most central sections of each defect site selected based on the width of the root canal were used for the histological and histometric analysis.

#### Histological analysis

One experienced masked examiner performed the histopathologic evaluation of the tissue specimens using incandescent and polarized light microscopy (Olympus Multi-view microscope BH2, Tokyo, Japan). Three central sections stained with haematoxylin/eosin and one section stained with Masson's trichrome were evaluated including observations of bone regeneration (lamellar and woven bone), residual biomaterial and associated tissue reaction(s), cementum regeneration (cellular/acellular cementum; cementoid/cementum-like layer; and intrinsic/extrinsic/mixed fibre cementum), PDL orientation/density (0, no PDL fibres; 1, low-density PDL fibres; 2, moderate-density PDL fibres; and 3, high-density PDL fibres or same as the native adjoining PDL), ankylosis, and undermining root resorption.

#### Histometric analysis

One calibrated masked examiner performed the histometric analysis using a PC-based image analysis system (Image-Pro Plus™, Media Cybernetic, Silver Spring, MD, USA) and incandescent and polarized light microscopy (Olympus Multi-view microscope BH2). The following parameters were analysed for the three central sections (Fig. 2):

- Defect height: distance from the apical extension of the root surface notch to the cemento-enamel junction (CEJ);
- Epithelial attachment: distance from the CEJ to the apical extension of an epithelial attachment on the root surface. This parameter included any gingival recession;
- Connective tissue attachment: the extent of a non-specific connective tissue attachment was calculated as the defect height minus the sum of cementum regeneration and the epithelial attachment;

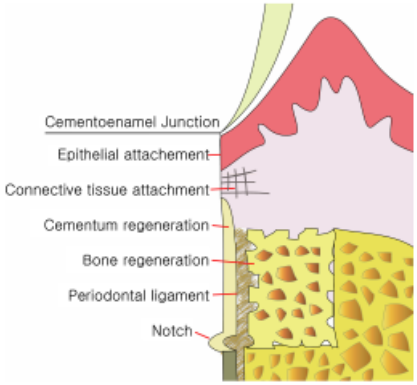


*Fig. 2*. Landmarks/parameters used in the histometric analysis. The green template served as a proxy for the defect site for estimation of bone regeneration area.

- Cementum regeneration: distance from the apical extension of the root surface notch to the coronal extension of newly formed cementum or a cementum-like substance on the root surface;
- Bone regeneration (height): distance from the apical extension of the root surface notch to the coronal extension of newly formed bone along the root surface; and
- Bone regeneration (area): new alveolar bone within the template that served as a standardized proxy for the defect site. The template was aligned parallel to the root surface interfacing the apical extension of defect at the root surface notch.

#### Statistical analysis

Summary statistics (mean ± SD) based on animal means for the experimental treatments were calculated using the three central sections from each defect, defects being averaged for each site. Animal means were used to test for differences between experimental conditions using one-way analysis of variance and a post hoc test. The level of significance was set at 5%.

## Results

#### Clinical observations

Clinical healing following surgical implantation of rhGDF-5/ACS was uneventful (Fig. 1). Healing appeared to be similar for the various rhGDF-5 and control sites.

#### Radiographic observations

Pre-surgery, immediately post-surgery, and 8-week radiographic observations showed variable bone formation among and within the experimental groups, groups receiving rhGDF-5 generally showing greater bone formation than that in the control.

#### Histologic observations

Photomicrographs of experimental sites are shown in Figs 3 through 5. The observations appeared to be similar within and among treatments. Inflammatory cell infiltrates and remnants of the ACS were scarce, if at all detected. However, there were apparent differences in the extent of cementum formation among sites receiving rhGDF-5 and the control. The newly formed cementum appeared to be a predominantly cellular intrinsic and/or mixed fibre cementum. Moreover, PDL fibre density appeared to be low in sites receiving the control and 100 $\mu$g rhGDF-5 treatments compared with that observed for the 1 and 20 $\mu$g rhGDF-5 treatments that approached moderate density (Fig. 4).

Alveolar bone formation varied in extension along the root surfaces. The newly formed bone was predominantly woven bone including many primary osteons, hypercellularity, and high density distinguishing it from the resident alveolar bone (Fig. 5). Root resorption and ankylosis were not observed in any of the defect sites.

#### Histometric analysis

The results from the histometric analysis are shown in Table 1 and Fig. 6. Induced defect height averaged ( ± SD) 4.46 ± 0.68, 4.87 ± 0.69, 5.01 ± 0.51, and 4.58 ± 0.69 mm for sites receiving 0 (buffer control), 1, 20, and 100 $\mu$g rhGDF-5, respectively, without significant differences between the sites. The corresponding observations for the epithelial attachment amounted to 1.86 ± 0.76, 1.29 ± 0.82, 1.55 ± 1.18, and 1.05 ± 0.72 mm and for the non-specific connective tissue attachment 0.10 ± 0.09, 0.09 ± 0.12, 0.43 ± 0.85, and 0.05 ± 0.05 mm, without significant differences between the sites.

Cementum regeneration averaged 2.49 ± 0.71, 3.50 ± 0.91, 3.03 ± 1.18, and 3.49 ± 0.80 mm for sites receiving 0, 1, 20, and 100 $\mu$g rhGDF-5, respectively, sites receiving 1 and 100 $\mu$g rhGDF-5 showing significantly greater cementum formation than control ($p < 0.05$). The corresponding observations for alveolar bone regeneration (height) averaged 1.44 ± 0.39, 2.09 ± 0.78, 2.22 ± 0.82, and 2.19 ± 0.42 mm, sites receiving 1 and 100 $\mu$g rhGDF-5 showing significantly greater bone formation than control ($p < 0.05$). Alveolar bone regeneration (area) averaged 4.17 ± 1.28, 4.92 ± 3.00, 5.61 ± 2.39, and 6.59 ± 2.05 mm$^2$ for sites receiving 0, 1, 20, and 100 $\mu$g rhGDF-5, respectively, with no significant differences. Ankylosis and root resorption were not observed for any treatment.

## Discussion

The objective of this dose-range study was to evaluate periodontal regeneration including a functionally oriented PDL, cementum and bone formation, and any aberrant sequela following surgical implantation of rhGDF-5/ACS using an established canine one-wall intrabony periodontal defect model. The rhGDF-5/ACS treatment significantly augmented periodontal regeneration including cementum and bone formation; PDL fibre density appeared to be higher for the lower compared with the high rhGDF-5 dose and the control. Importantly, wound healing following the rhGDF-5 treatments was not accompanied by adverse events including root resorption/ankylosis. Thus, the observations herein suggest that surgical implantation of rhGDF-5/ACS may be used safely to support periodontal regeneration in intrabony periodontal defects. Nevertheless, longer-term studies are necessary to confirm an uneventful maturation of the newly formed periodontal attachment.

rhGDF-5 was delivered to the intrabony experimental defects using an ACS. ACSs have been used in a multitude of preclinical and clinical studies as carriers for BMPs, in particular, in combination with rhBMP-2 in various settings in the axial and appendicular skeleton (Valentin-Opran et al. 2002, Wozney & Wikesjö 2008). These studies suggest that the structural integrity of the rhBMP-2 soak-loaded ACS is not ideal for onlay indications, but appears to be satisfactory for inlay indications including the maxillary sinus (Hanisch et al. 1997, Boyne et al. 2005); the rhBMP-2/ACS construct readily becomes victim

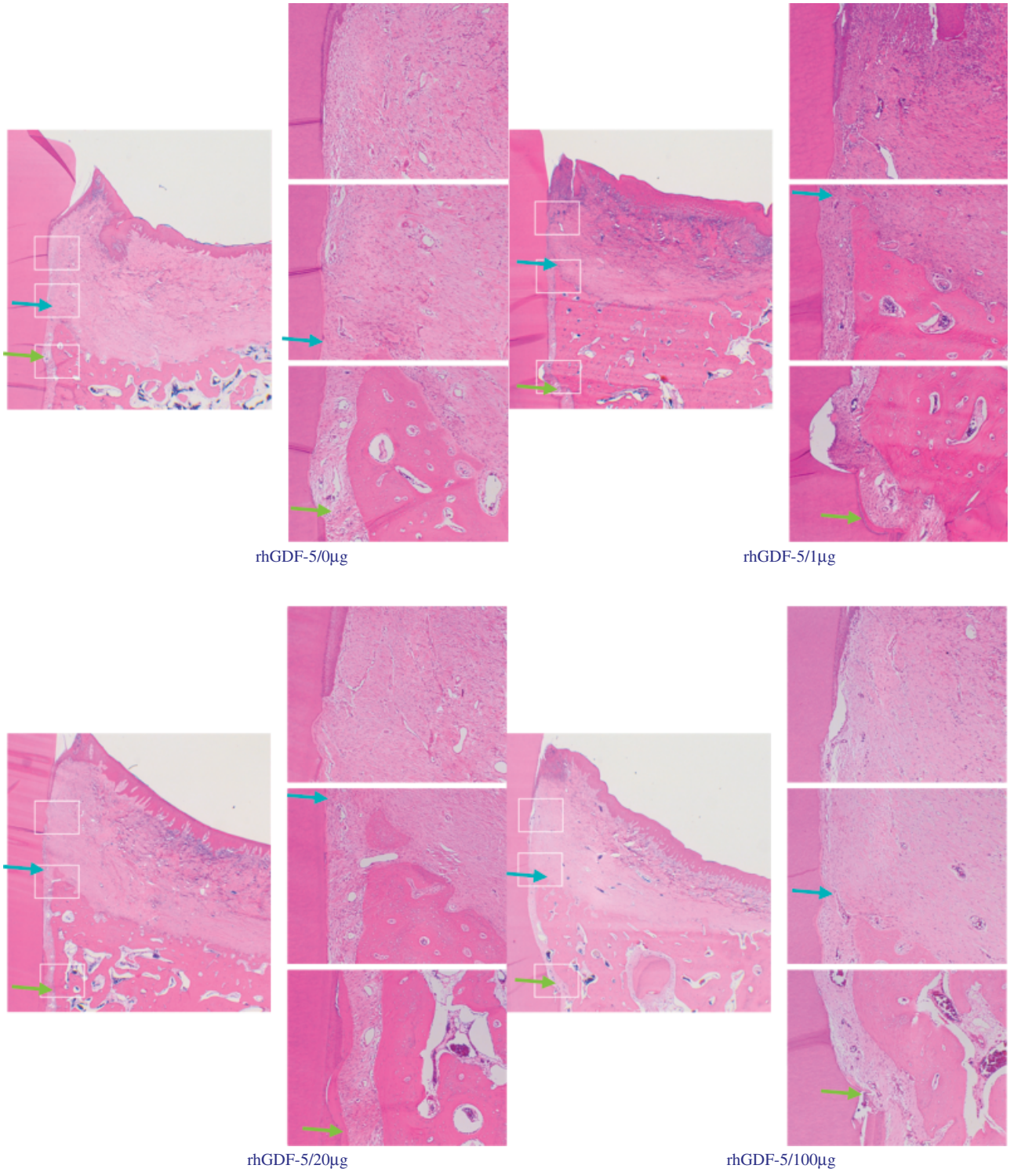


*Fig. 3*. Photomicrographs from sites implanted with human growth/differentiation factor-5 (rhGDF-5) in an absorbable collagen sponge carrier at 0 $\mu$g (top left), 1 $\mu$g (top right), 20 $\mu$g (bottom left), and 100 $\mu$g (bottom right) displaying the sites from the apical extension of the root planing (green arrow) along the root surface to the coronal extension of the newly formed alveolar bone (blue arrow) and cementum (mid-root inset) and the apical extension of an epithelial attachment (haematoxylin/eosin, original magnification $\times$ 10 and $\times$ 40).

to compression in onlay settings, producing less than desired bone volumes (Tatakis et al. 2002, Fiorellini et al. 2005). Combining the ACS carrier with structural elements such as porous space-providing devices or particulate bone biomaterials may, to some extent, alleviate this inherent deficiency (Wikesjö et al. 2003b, d e, Barboza et al. 2000, 2004). However, biomaterials may also compromise bone formation/maintenance, in particular, biomaterials

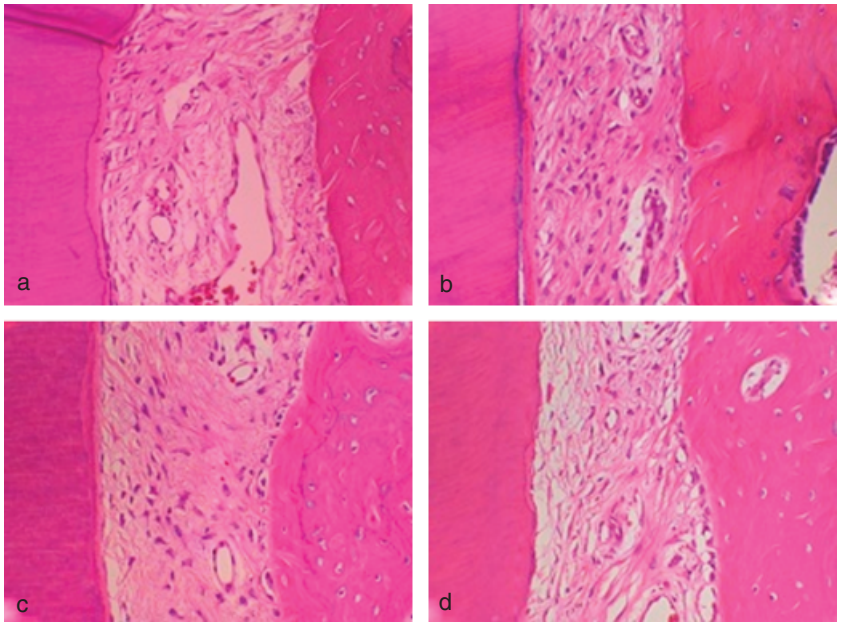


*Fig. 4*. 3Photomicrographs from sites implanted with human growth/differentiation factor-5 (rhGDF-5) in an absorbable collagen sponge (ACS) carrier at 0 $\mu$g (a), and sites receiving rhGDF-5/ACS at 1 $\mu$g (b), 20 $\mu$g (c), and 100 $\mu$g (d) displaying obliquely or perpendicularly oriented collagen fibres inserting into newly formed predominantly cellular cementum and bone (haematoxylin/eosin, original magnification $\times$ 200).

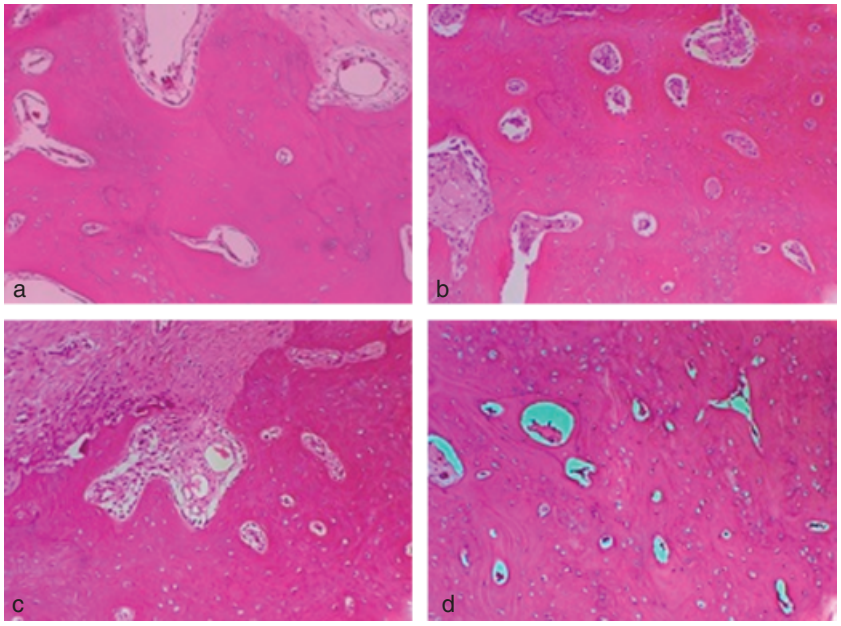


*Fig. 5*. Photomicrographs from sites receiving human growth/differentiation factor-5 (rhGDF-5) in an absorbable collagen sponge (ACS) carrier at 0 $\mu$g (a) showing mostly woven bone, and from sites receiving rhGDF-5/ACS at 1 $\mu$g (b), 20 $\mu$g (c), and 100 $\mu$g (d) showing mostly woven, high-density, hypercellular bone with many primary osteons (haematoxylin/eosin, original magnification $\times$ 100).

based on polylactic acid technologies (Sigurdsson et al. 1996, Polimeni et al. 2007). To circumvent added study variables, the present study used an established canine one-wall intrabony (inlay) periodontal defect model for this first large animal preclinical evaluation of rhGDF-5 for periodontal indications, the rigid space-providing skeletal defect, to some extent, protecting the rhGDF-5/ACS implant from tissue compression.

The innate regenerative potential of the periodontal attachment is substantial under optimal conditions for wound healing including space provision, wound stability, and primary intention healing as has been shown in a critical-size supraalveolar periodontal canine defect model, regeneration at times encompassing the entire 5-mm circumferential defect (Sigurdsson et al. 1994, Wikesjö et al. 2003c). The regenerative potential of the periodontium in patients should conceivably not be less under optimal conditions for periodontal wound healing/regeneration; however, such conditions are difficult to produce in the clinic. Thus, an interest in biologic implants such as in the present study that somehow may accelerate/facilitate/support periodontal wound healing/regeneration has emerged (Hammarström 1997, Yukna et al. 2002, Lynch et al. 2006). In this study, surgical implantation of rhGDF-5/ACS produced a statistically significant increase in cementum and bone regeneration over the native regenerative potential without significant or meaningful differences between the rhGDF-5 dosages evaluated.

The newly formed cementum was a predominantly cellular intrinsic and/or mixed fibre cementum without remarkable differences between control, and sites implanted with rhGDF-5 other than the rhGDF-5 treatment produced significantly increased cementum formation along the root surface. Similar structural observations of the regenerated periodontal attachment have been made in other settings evaluating periodontal wound healing/regeneration in the absence of a growth or a differentiation factor (Sigurdsson et al. 1994, Wikesjö et al. 2003c) and in a pilot study evaluating rhGDF-7/ACS (Wikesjö et al. 2004). The fibrous attachment also did not vary considerably in orientation among sites receiving the control and the experimental treatment; however, it appeared that the lower rhGDF-5 doses yielded a denser attachment than the high dose and the control. Similar observations have also been made in the study evaluating rhGDF-7/ACS (Wikesjö et al. 2004).

Alveolar bone formation (height) was significantly enhanced in defect sites implanted with rhGDF-5/ACS. It must be emphasized that bone and cementum formation was not associated with aberrant healing including root resorption/

*Table 1.* Histometric analysis (group means ± SD in mm/mm$^2$)

| Parameters | Doses | | | |
|---|---|---|---|---|
| | rhGDF-5 0 μg | rhGDF-5 1 μg | rhGDF-5 20 μg | rhGDF-5 100 μg |
| Defect height | 4.46 ± 0.68 | 4.87 ± 0.69 | 5.01 ± 0.51 | 4.58 ± 0.69 |
| Epithelial attachment | 1.86 ± 0.76 | 1.29 ± 0.82 | 1.55 ± 1.18 | 1.05 ± 0.72 |
| Connective tissue attachment | 0.10 ± 0.09 | 0.09 ± 0.12 | 0.43 ± 0.85 | 0.05 ± 0.05 |
| Cementum regeneration | 2.49 ± 0.71 (55.8%) | 3.50 ± 0.91* (71.9%) | 3.03 ± 1.18 (60.5%) | 3.49 ± 0.80* (76.2%) |
| Bone regeneration (height) | 1.44 ± 0.39 (32.3%) | 2.09 ± 0.78* (42.9%) | 2.22 ± 0.82 (44.3%) | 2.19 ± 0.42* (47.8%) |
| Bone regeneration (area) | 4.17 ± 1.28 | 4.92 ± 3.00 | 5.61 ± 2.39 | 6.59 ± 2.05 |

*$p < 0.05$ compared with rhGDF-5, 0 μg (ANOVA).
rhGDF-5, human growth/differentiation factor-5; ANOVA, analysis of variance.

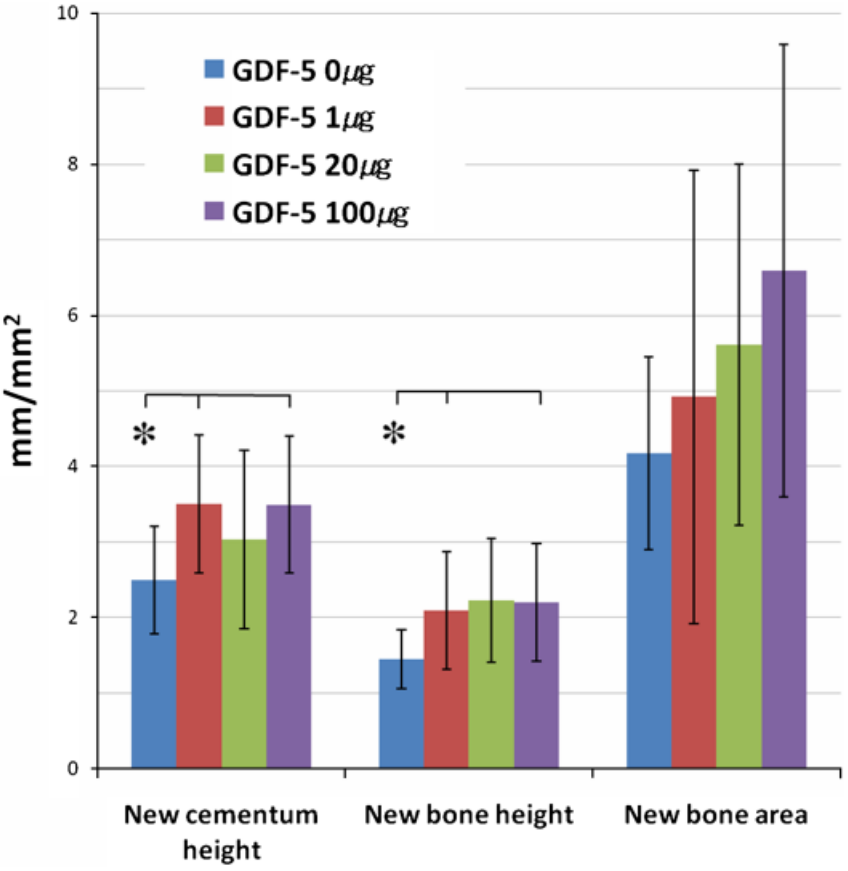


*Fig. 6.* Main results from the histometric analysis (*$p < 0.05$ compared with rhGDF-5 0 μg).

ankylosis for any site receiving rhGDF-5. This is particularly important since earlier studies evaluating in particular rhBMP-2 but also rhOP-1 in discriminating canine models all suggest that root resorption/ankylosis may be/become a terminating event for the use of these proteins for periodontal indications (Sigurdsson et al. 1995, 1996, Giannobile et al. 1998, Wikesjö et al. 1999, 2003b–e, 2004, Selvig et al. 2002, Sorensen et al. 2004a). Early 8-week observations displayed a regenerated periodontal attachment including newly formed cellular cementum, a fibrovascular tissue without obliquely or perpendicularly oriented collagenous fibres in a ''PDL'' space, and newly formed alveolar bone, often with limited evidence of root/resorption/ankylosis; however, longer-term 24-week observations showed maturation of the fibrovascular tissue into fatty marrow and the ''cementum'' and bone tissues associated with advanced root resorption/ankylosis gradually replacing the tooth structures (Wikesjö et al. 2003b). In contrast, in the present study, the same tissue set including cellular mixed fibre cementum, obliquely or perpendicularly oriented collagenous fibres, and newly formed alveolar bone was observed in sites receiving rhGDF-5 and in the control and should be expected to mature into a normal periodontal attachment without root resorption/ankylosis.

## Conclusions

Surgical implantation of rhGDF-5/ACS appears to be safe and may support periodontal wound healing/regeneration in intrabony periodontal defects without complications. Longer-term studies are necessary to secure the uneventful maturation of the newly formed periodontal attachment.

## Acknowledgement

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**Clinical Relevance**

*Scientific rationale for the study:* GDF-5 enhances chondrogenic or osteogenic differentiation in mesenchymal cell cultures. ACS is known to be a suitable carrier of growth factors. The objective of this study was to evaluate the dose-dependent periodontal regeneration potential of rhGDF-5 when combined with ACS.

*Principal findings:* Surgical implantation of rhGDF-5 showed significantly greater bone and cementum formation than control specimens. There were no remarkable differences in bone and cementum formation within the selected dose interval. rhGDF-5 sites exhibited no root resorption, ankylosis, or other aberrant tissue reactions in any defect site.

*Practical implications:* This study indicates that rhGDF-5/ACS may be used safely to support periodontal wound regeneration in periodontal defects without complications.