J Clin Periodontol 2011; 38: 261–268 doi: 10.1111/j.1600-051X.2010.01691.x

Journal of Clinical Periodontology

# Wound healing/regeneration using recombinant human growth/differentiation factor-5 in an injectable poly-lactide-co-glycolide-acid composite carrier and a one-wall intra-bony defect model in dogs

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*Min C-K, Wikesjö UME, Park J-C, Chae G-J, Pippig SD, Bastone P, Kim C-S, Kim C-K. Wound healing/regeneration using recombinant human growth/differentiation factor-5 in an injectable poly-lactide-co-glycolide-acid composite carrier and a one-wall intra-bony defect model in dogs. J Clin Peridontol 2011; 38: 261–268. doi: 10.1111/j.1600-051X.2010.01691.x.*

***Abstract***

**Objective:** The purpose of this study was to evaluate the effect of recombinant human growth/differentiation factor-5 (rhGDF-5) on periodontal wound healing/regeneration using an injectable poly-lactide-co-glycolide-acid (PLGA) composite carrier and an established defect model.

**Methods:** Bilateral 4 × 5 mm (width × depth) one-wall, critical-size, intra-bony periodontal defects were surgically created at the second and the fourth mandibular pre-molar teeth in 15 Beagle dogs. The animals were randomized to receive (using a split-mouth design; defect sites in the same jaw quadrant getting the same treatment) rhGDF-5 high dose (188 μg/defect) *versus* sham-surgery control (five animals), rhGDF-5 mid dose (37 μg/defect) *versus* carrier control (five animals), and rhGDF-5 low dose (1.8 μg/defect) *versus* treatment reported elsewhere (five animals). The animals were euthanized for histometric analysis following an 8-week healing interval.

**Results:** Clinical healing was uneventful. The rhGDF-5/PLGA construct was easy to assemble and apply. The rhGDF-5 high dose supported significantly increased bone formation compared with the low-dose, sham-surgery, and carrier controls ($p < 0.05$) and induced significantly increased cementum formation compared with the controls ($p < 0.05$). Root resorption/ankylosis or other aberrant healing events were not observed.

**Conclusion:** rhGDF-5 appears to effectively support periodontal wound healing/regeneration in a dose-dependent order; the PLGA composite appears to be an effective ease-of-use candidate for carrier technology.

Key words: dog; periodontal regeneration; PLGA; rhGDF-5; tissue engineering

Accepted for publication 30 November 2010

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

Prof. Ulf M. E. Wikesjö serves as a consultant to Scil Technology. Dr. Susanne D. Pippig and Dr. Patrizia Bastone are the employees of Scil Technology GmbH. All other authors have no conflict of interests. The research reported herein was funded by a grant from Scil Technology GmbH (Martinsried, Germany) to Prof. Chong-Kwan Kim and by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (R13-2003-013-04002-0).

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Biomedical research has focused on a number of novel approaches to periodontal regeneration. An intriguing alternative is the application of polypeptide growth factors including bone morphogenetic proteins (BMPs) [reviewed by Lee et al. (2010a)]. BMPs have received intensive study ever since their discovery (Urist 1965, Wozney et al. 1988) and in particular BMP-2 and BMP-7 have been locally applied to initiate/stimulate the cascade of wound healing events that lead to regeneration of the periodontal attachment (Ripamonti et al. 1994, 1996, 2001, 2002, Sigurdsson et al. 1995, 1996, Giannobile et al. 1998, Wikesjö et al. 1999, 2003a, b, c, 2004, Choi et al. 2002, Selvig et al. 2002, Saito et al. 2003). However, observations of aberrant healing events including root resorption/ankylosis have questioned the utility of BMP-2 and BMP-7 for periodontal regeneration (Sigurdsson et al. 1995, 1996, Giannobile et al. 1998, Wikesjö et al. 1999, 2003a, b, c, 2004, Choi et al. 2002, Selvig et al. 2002).

Growth/differentiation factor-5 (GDF-5), also known as cartilage-derived morphogenetic protein-1, a member of the BMP family of proteins (Chang et al. 1994, Hötten et al. 1994, 1996), has been shown to induce/enhance local bone formation in cranial and craniofacial settings, for spine fusion and long bone fracture indications, and to support cartilage and tendon/ligament repair using a variety of small and large animal models and in initial clinical studies [reviewed by Moore et al. (2010)]. For example, Kuniyasu et al. (2003) reported the successful bone augmentation induced by 1, 10, and 100 $\mu$g recombinant human GDF-5 (rhGDF-5) in a collagen composite using a rat calvaria onlay model. rhGDF-5 coated onto a particulate $\beta$-tricalcium phosphate ($\beta$-TCP) micro/macro-porous carrier initiated/simulated significantly greater bone formation compared with conventional bone biomaterials including $\beta$-TCP as a stand-alone therapy and bovine bone mineral-derived products in critical-size rat calvarial through-and-through osteotomy defects (Pöhling et al. 2006). A prospective, randomized pilot clinical study on the safety and efficacy of rhGDF-5 coated onto $\beta$-TCP for sinus lift augmentation concluded the rhGDF-5/$\beta$-TCP to be as effective and safe as the control treatment, i.e., an autologous bone/$\beta$-TCP composite (Koch et al. 2010). Moreover, GDF-5 genes are expressed in bovine and rat tooth germs at the root-forming stage and may thus represent potent regulatory molecules in the development of the periodontal attachment and potentially, in extension, support periodontal wound healing/regeneration (Morotome et al. 1998, Nakamura et al. 2003, Sena et al. 2003).

Although GDF-5 *per se* induces *de novo* bone formation, a well-aligned delivery system may typically enhance GDF-5 bioactivity. Numerous biomaterials have been investigated as such; desired characteristics including biocompatibility, biodegradability, and surface characteristics for cell attachment and differentiation. Additional criteria include retention of incorporated molecules, and longer retention might result in increased osteoinductive activity (Uludag et al. 2000). Biodegradable biocompatible polymers appear to be preferred carriers. Previous studies have shown that biodegradable synthetic polymers combined with BMPs induce bone formation in animal models; polylactic-co-glycolic-acid (PLGA) microsphere or capsule assemblies have been considered for BMP-2; however, insufficient mechanical properties limits their use to inlay indications (Kenley et al. 1994, Sigurdsson et al. 1995, 1996, Isobe et al. 1996). More recently, injectable, ease-of-use micro/macro-porous PLGA composites featuring structural integrity upon setting have been considered and combined with rhGDF-5 (Herberg et al. 2008, Kwon et al. 2010a, b). The purpose of this study was to evaluate the effect of rhGDF-5 on periodontal wound healing/regeneration using an injectable PLGA composite carrier and an established defect model.

## Materials and Methods

### Animals

Fifteen male Beagle dogs (approximate age 15 months, and weight 9–13 kg) bred exclusively for biomedical research were used. The animals exhibited an intact dentition with a healthy periodontium. Animal selection and management, surgical protocol, and preparation followed routines approved for his study by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea. The animals were fed a canned soft dog food diet (Prescription Diet Canine i/d, Hill's Pet Nutrition Inc., Topeka, KS, USA).

### Biomaterials

The novel rhGDF-5/PLGA construct (Scil Technology GmbH, Martinsried, Germany) consisted of rhGDF-5 in an injectable bioresorbable PLGA composite carrier (Herberg et al. 2008, Pompe 2008). To avoid incompatibilities between the active drug and the carrier, the two components were supplied separately. The final pharmaceutical formulation consisted of the paste-like PLGA composite in a pre-filled glass syringe and the lyophilized rhGDF-5 in a special glass vial, to be mixed immediately before use. rhGDF-5/PLGA constructs included rhGDF-5 at a high (188 $\mu$g/defect), medium (37 $\mu$g/defect), and low (1.8 $\mu$g/defect) dose; each defect received approximately 80 µl of the rhGDF-5/PLGA construct or the PLGA composite control.

### Surgical protocol

With minor modifications, the surgical protocol and post-surgery procedures followed established routines (Kim et al. 2004, 2005). Food was withheld the night preceding surgery. The surgical procedures were performed under general anaesthesia induced by a subcutaneous injection of atropin (0.05 mg/kg; Kwangmyung Pharmaceutical Ind. Co. Ltd, Seoul, Korea) and an intravenous injection of a combination of xylazine (Rompun®, Bayer Korea Co., Seoul, Korea) and Zoletil® (Virbac Laboratories, Carros, France) followed by inhalation anaesthesia. Routine dental infiltration anaesthesia was used at the surgical sites.

The mandibular third pre-molars were surgically extracted before the experimental surgery and the extraction sites were allowed to heal for 2 months. The remaining dentition received oral prophylaxis in conjunction with the extractions.

Experimental surgeries were performed under general anaesthesia by one experienced surgeon (C. K. K.). Buccal and lingual mucoperiosteal flaps were elevated to create critical-size, ''box-type'', 4 × 5 mm (width × height) one-wall intra-bony defects at the distal aspect of the second and the mesial aspect of the fourth mandibular premolar teeth in right and left jaw quadrants (Fig. 1). Instrumentation with curettes was used to remove the periodontal ligament and root cementum and to establish a distinct landmark at the

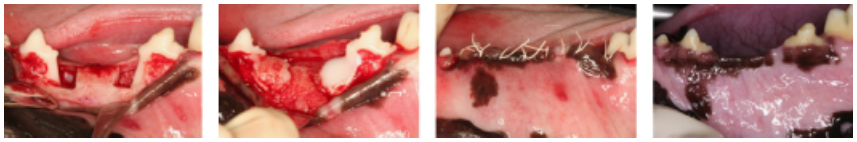

*Fig. 1.* Surgically created, critical-size, one-wall, intra-bony periodontal defect at the distal aspect of the second and mesial aspect of the fourth mandibular premolar teeth (left). Application of rhGDF-5/PLGA (left centre). Mucoperiosteal flaps adapted and sutured for primary intention healing (right centre). Healing at 8 weeks (right). rhGDF-5, recombinant human growth/differentiation factor-5; PLGA, poly-lactide-co-glycolide-acid.

base of the defect. The animals were randomized to receive (using a split-mouth design; defect sites in the same jaw quadrant getting the same treatment) rhGDF-5/PLGA high dose *versus* sham-surgery control (five animals), rhGDF-5/PLGA mid dose *versus* carrier (PLGA composite) control (five animals), and rhGDF-5/PLGA low dose *versus* treatment reported elsewhere (five animals). The approximately 80 µl defects were filled to the level of the alveolar crest. Next, the mucogingival flaps were advanced, adapted, and sutured using expanded polytetrafluoroethylene sutures (Gore Tex® Suture CV-5, W.L. Gore & Associates Inc., Flagstaff, AZ, USA).

#### Post-surgery procedures

The animals received a broad-spectrum antibiotic (20 mg/kg i.m./3 days; Cefazoline Sodium, Yuhan Co., Seoul, Korea) 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 (day 10), and at least twice weekly thereafter. The animals were euthanized at 8 weeks 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 collected. Photographic and radiographic (not shown) recordings were completed intra-surgery, immediately post-surgery, and at 8 weeks.

#### 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 tissue block 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 µm thick, were cut in a mesial–distal vertical plane at approximately 80 µm intervals. The sections were stained using haematoxylin/eosin. The four most central sections of each defect site selected based on the width of the root canal were used for histological and histometric analysis.

#### Histological analysis

One experienced masked examiner (G. J. C.) performed the histopathologic evaluation using incandescent and polarized light microscopy (Olympus multi-view microscope BH2, Tokyo, Japan) including observations of bone regeneration, residual biomaterial and associated tissue reaction(s), cementum regeneration, periodontal ligament regeneration, ankylosis, and undermining root resorption.

#### Histometric analysis

One calibrated masked examiner (G. J. C.) 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 four central sections (Fig. 2):

- defect height: distance from the apical extension of the root planing to the cemento-enamel junction (CEJ);
- epithelial attachment: distance from the CEJ to the termination of an epithelial attachment on the root surface. This parameter included any gingival recession;
- cementum regeneration: distance from the apical extension of the root planing to the coronal extension of newly formed cementum or a cementum-like substance on the root surface;
- connective tissue attachment: distance from the apical extension of the junctional epithelium to the coronal extension of cementum formation;
- bone regeneration (height): distance from the apical extension of the root planing to the coronal extension of newly formed bone along the root surface;
- bone regeneration (area): newly formed bone within a 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 the root planing;
- root resorption: combined linear heights of distinct resorption lacunae on the planed root; and
- ankylosis: combined linear heights of ankylotic union between the regenerated alveolar bone and the planed root.

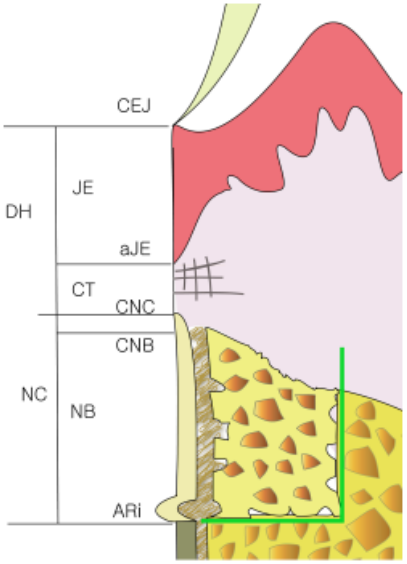


*Fig. 2.* Schematic diagram of landmarks and parameters for histometric analysis. CEJ, cemento-enamel junction; ARi, apical extension of root instrumentation; CNB, coronal extension of new bone formation; CNC, coronal extension of new cementum formation; aJE, apical termination of the junctional epithelium; DH, defect height; NC, new cementum; JE, junctional epithelium; CT, connective tissue attachment; and NB, new bone.

#### Statistical analysis

Summary statistics (mean ± SD) based on animal means for the experimental treatments were calculated using the

four central sections from each defect. The statistical comparison between the treatments rhGDF-5/PLGA high, mid, and low dose, carrier control, and sham-surgery control was performed for the paired comparisons in the split-mouth design for the fixed factor treatment and the hierarchical random factors, animal, side (right, left), position (1, 2 respective 3, 4), and replication (1, 2, 3, 4) as an incomplete block design using a mixed linear model. Two-sided *p*-values are reported for the treatment differences for each individual endpoint without multiplicity adjustment. All calculations were performed in R using the library (nlme) and library (multcomp). The level of significance was set at 5%.

## Results

### Clinical observations

The rhGDF-5/PLGA composite was easy to prepare and apply to the surgical sites. The composite immediately solidified as it was brought in contact with blood. The PLGA formulation also facilitated space formation and maintenance. All sites healed uneventfully with minimal signs of inflammation and some gingival recession.

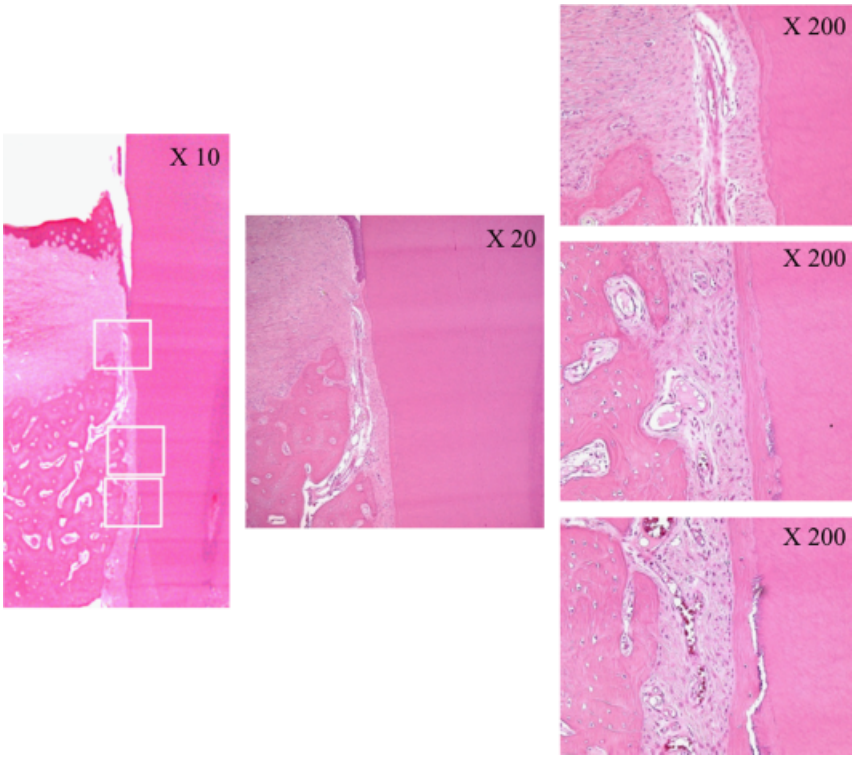


*Fig. 3*. Photomicrograph from the site receiving the rhGDF-5 high dose in the PLGA composite carrier displaying the coronal extension of the newly formed bone and cementum and the apical termination of the epithelial attachment. Large amount of bone was observed and newly formed cementum tissue was covering the root surface. Inset boxes in lower magnification (× 10) represent the area in higher magnification (× 20/ × 200). rhGDF-5, recombinant human growth/differentiation factor-5; PLGA, poly-lactide-co-glycolide-acid.

### Histopathologic analysis

Photomicrographs of typical experimental sites are shown in Figs. 3–7. The observations appeared similar within and among treatments. The PLGA composite did not appear to have obstructed or otherwise compromised periodontal wound healing/regeneration. There were no remarkable inflammatory lesions in sites implanted with the PLGA biomaterial. Moreover, the PLGA composite appeared almost completely resorbed irrespective of rhGDF-5 dose.

All experimental sites showed new bone and cementum formation along the planed root surface, the amount varying within each group. Both cellular cementum and thin acellular cementum were observed. Differential deposition of cellular and acellular cementum was not found for any group. The newly formed, high-density, predominantly woven bone with primary osteons (and minimal presence of lamellar bone) appeared hypercellular, noticeably different from the native alveolar bone. The newly formed periodontal ligament included extrinsic/intrinsic (mixed) fibre cementum. Root resorption/ankylosis was not observed in any of the intra-bony periodontal defects.

### Histometric analysis

The results from the histometric analysis are shown in Table 1. Sites implanted with the rhGDF-5/PLGA high dose displayed significantly greater alveolar bone regeneration (height) than sites implanted with the low dose or serving as carrier and sham-surgery controls ($p<0.05$). Bone regeneration (area) was significantly greater in sites implanted with the rhGDF-5/PLGA high dose than sites implanted with the low dose. Cementum regeneration was significantly greater for the rhGDF-5/PLGA high dose compared with controls ($p<0.05$) and for the rhGDF-5/PLGA mid dose compared with the carrier control ($p<0.05$). The corresponding metrics showed an increased non-specific connective tissue attachment for the sham-surgery control compared with rhGDF-5 sites reaching statistical significance for the rhGDF-5 low and mid dose ($p<0.05$). Formation of the junctional epithelium ranged from $2.0 \pm 0.7$ to $1.2 \pm 0.4$ mm without significant differences between groups.

## Discussion

The aim of this study was to evaluate the effect of rhGDF-5 on periodontal wound healing/regeneration using an injectable PLGA composite carrier and an established one-wall intra-bony periodontal defect model. The rhGDF-5/PLGA construct significantly increased bone and cementum formation in a dose-dependent order in the absence of root resorption/ankylosis. To fully appreciate the effectiveness of rhGDF-5 on periodontal wound healing/regeneration, biocompatible, biodegradable carriers, preferably synthetic should be used; carrier prerequisites including porosity for optimized cell ingrowth, space provision, and provision(s) for clot/wound stabilization have been emphasized (Polimeni et al. 2006, Herberg et al. 2008). Recent studies have shown that rhGDF-5 soak loaded onto an absorbable collagen

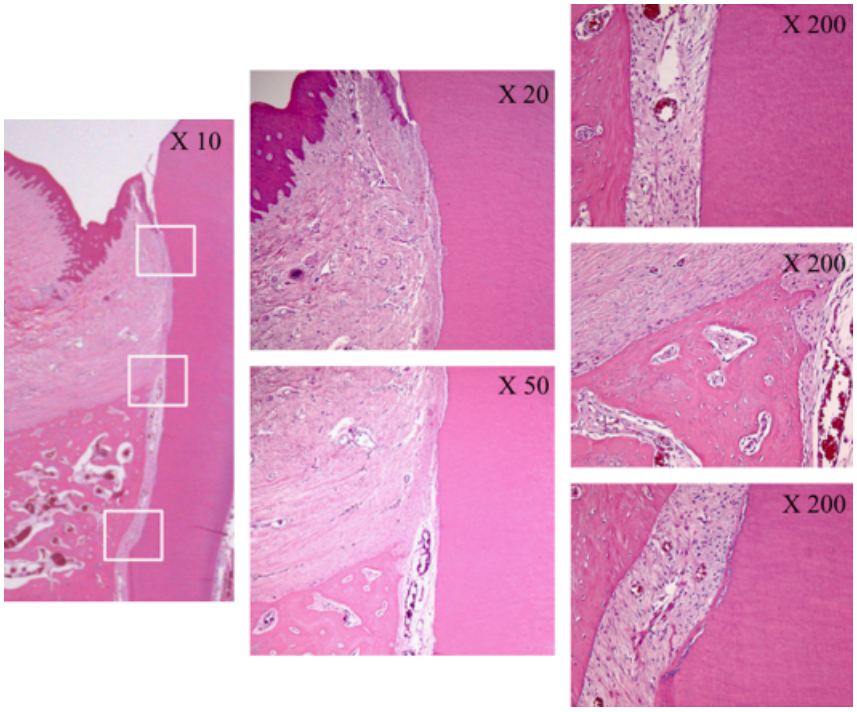


*Fig. 4*. Photomicrograph from the site receiving the rhGDF-5 mid dose in the PLGA composite carrier displaying the coronal extension of the newly formed bone and cementum and the apical termination of the epithelial attachment. Large amount of newly formed bone and cementum tissue was covering the root surface. Inset boxes in lower magnification (× 10) represent the area in higher magnification (× 20/ × 50/ × 200). rhGDF-5, recombinant human growth/differentiation factor-5; PLGA, poly-lactide-co-glycolide-acid.

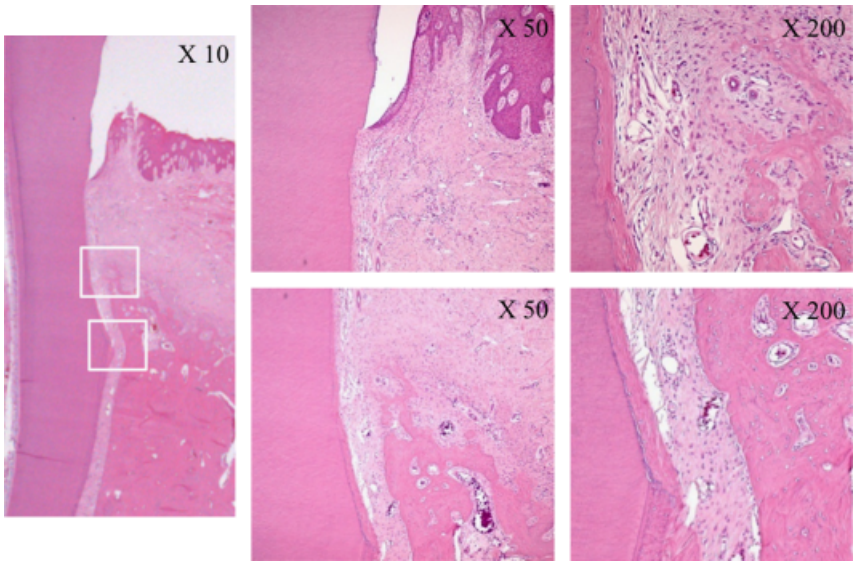


*Fig. 5*. Photomicrograph from site receiving the rhGDF-5 low dose in the PLGA composite carrier displaying the coronal extension of the newly formed bone and cementum and the apical termination of the epithelial attachment. Large amount of newly formed bone and cementum was observed. Inset boxes in lower magnification (× 10) represent the area in higher magnification (× 50/ × 200). rhGDF-5, recombinant human growth/differentiation factor-5; PLGA, poly-lactide-co-glycolide-acid.

sponge carrier (rhGDF-5/ACS) or adsorbed onto micro/macro-porous $\beta$-TCP particles (rhGDF-5/$\beta$-TCP) significantly enhances periodontal wound healing/regeneration compared with sham-surgery, carrier, and benchmark (rhPDGF/$\beta$-TCP) controls (Kim et al. 2009, Kwon et al. 2010c, Lee et al. 2010b). However, both the ACS and $\beta$-TCP carrier technologies are limited to inlay indications. The ease-of-use rhGDF-5/PLGA composite construct herein is intended for inlay and onlay indications as well as minimally invasive procedures (Herberg et al. 2008, Pompe 2008). A previous study, which focused on handling logistics, showed that the injectable rhGDF-5/PLGA construct offered a simple predicable application to subgingival sites using a non-critical-size defect model (Kwon et al. 2010a). However, the study did not offer conclusive evidence relative to the effectiveness of the rhGDF-5/PLGA construct to stimulate periodontal wound healing/regeneration shown in the present study, emphasizing the need for careful and appropriate selection of models to demonstrate disparate aspects of a novel technology before clinical release.

All sites receiving the rhGDF-5/PLGA construct or the PLGA composite carrier control showed trace amounts of the biomaterial. Moreover, root resorption/ankylosis was not observed in any of the intra-bony defects. These observations corroborate that observed in a previous study that the rhGDF-5/PLGA construct biodegrades timely without appreciably compromising periodontal wound healing/regeneration (Kwon et al. 2010a). The high-dose rhGDF-5/PLGA construct showed a statistically and clinically significant twofold improvement in bone and cementum formation including an intrinsic/extrinsic fibre cellular cementum over that in the controls emphasizing the favourable healing response. Although several studies have reported on PLGA constructs in craniofacial and periodontal settings, immediate comparisons between reports are not meaningful due to direct differences between constructs, defect models, animal platforms, and observation intervals. Of interest, however, is an in-depth in vivo analysis of rhGDF-5 release kinetics and wound healing dynamics to, if possible, elucidate factors critical to the enhanced outcomes.

This study showed increased bone formation in sites receiving the rhGDF-5 high dose compared with sites receiving the low dose and compared with carrier and sham-surgery controls. Bone density in sites implanted with rhGDF-5/PLGA appeared higher with

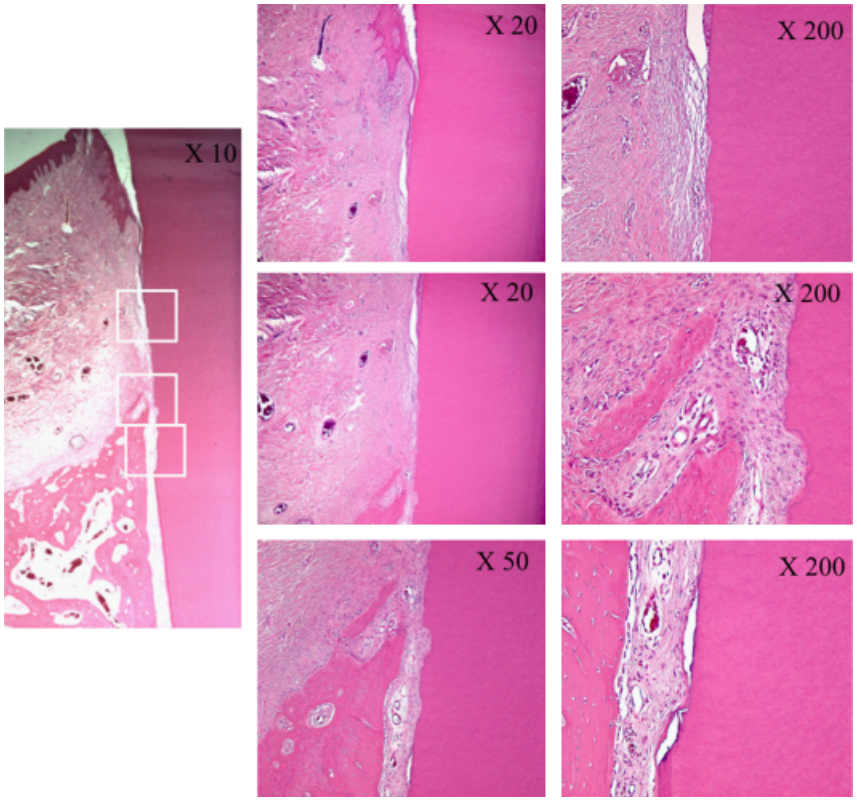


*Fig. 6*. Photomicrograph from site receiving the PLGA carrier control displaying the coronal extension of the newly formed bone and cementum and the apical termination of the epithelial attachment. Small amount of bone regeneration was observed with minimal extension of cementum regeneration. Inset boxes in lower magnification (× 10) represent the area in higher magnification (× 20/ × 50/ × 200). PLGA, poly-lactide-co-glycolide-acid.

*Table 1*. Results of the histometric analysis of periodontal wound healing/regeneration in one-wall intra-bony defects in dogs receiving rhGDF-5 at a high, mid, and low dose in a PLGA composite carrier *versus* carrier and sham-surgery controls (group means ± SD)

| | Defect height (mm) | Bone height (mm) | Bone area ($mm^2$) | Cementum (mm) | Connective tissue attachment (mm) | Epithelial attachment (mm) |
|---|---|---|---|---|---|---|
| High dose | 5.0 ± 0.8 | 2.2 ± 0.9* | 4.5 ± 2.1** | 2.6 ± 1.1¶ | 0.5 ± 0.4 | 1.2 ± 0.4 |
| Mid dose | 4.7 ± 0.5 | 1.7 ± 0.7 | 3.4 ± 1.5 | 2.7 ± 1.0¶¶ | 0.5 ± 0.4 | 1.4 ± 0.5 |
| Low dose | 4.6 ± 0.3 | 1.2 ± 0.6 | 1.8 ± 1.0 | 2.2 ± 0.9 | 0.3 ± 0.1 | 2.0 ± 0.7 |
| Carrier control | 4.3 ± 0.3# | 1.1 ± 0.6 | 2.3 ± 2.3 | 1.8 ± 0.4 | 1.1 ± 1.0 | 1.4 ± 0.2 |
| Sham surgery | 4.4 ± 1.4 | 1.1 ± 0.4 | 2.5 ± 1.2 | 1.6 ± 0.6 | 1.0 ± 0.9† | 1.8 ± 0.7 |

#$p<0.05$: carrier control *versus* high dose, mid dose, and sham-surgery control.
*$p<0.05$: high dose *versus* low dose, carrier and sham-surgery control.
**$p<0.05$: high dose *versus* low dose.
¶$p<0.05$: high dose *versus* carrier and sham-surgery control.
¶¶$p<0.05$: mid dose *versus* carrier control.
†$p<0.05$: sham-surgery control *versus* mid and low dose.
rhGDF-5, recombinant human growth/differentiation factor-5; PLGA, poly-lactide-co-glycolide-acid.

smaller marrow spaces than in resident bone, including a large number of osteocytes. These observations corroborate in vitro studies, suggesting that rhGDF-5 promotes an osteogenic differentiation in stromal cells (Shen et al. 2006, Zeng et al. 2007). In addition to immediate effects on differentiation and proliferation at cellular level, rhGDF-5 induces angiogenesis in vitro and in vivo (Yamashita et al. 1997, Zeng et al. 2007). Angiogenesis and vascular invasion appear important steps in the sequential cascade of bone formation (Reddi 1992). In the present study, bone formation might be caused by effects of both differentiation at cellular level and angiogenesis at tissue level. However, the woven nature of the new bone and its hypercellularity appeared different from the resident alveolar bone. Longer term observations will determine when this woven bone remodels into normal trabecular bone.

Cementum regeneration observed in this study included both cellular and acellular cementum. Newly formed cellular extrinsic/extrinsic (mixed) fibre cementum exhibited the same structure as that observed in the native cementum, whereas acellular cementum appeared similar only in density to native cementum. Previous studies have described a uniformly thin and acellular mineralized tissue regenerated onto the denuded dentin surface following periodontal therapy (Lindskog & Blomlöf 1992, Miyaji et al. 2006). Formation of this mineralized tissue appeared dependent on an initial resorption of dentin root surface, and that BMPs promoted this resorption and formation by facilitating osteoclastic effects. Although this study was not designed to detail initial healing events on the root surface, sites receiving the rhGDF-5/PLGA high dose showed considerable cementum regeneration (cellular and acellular) consistent with the native cementum in this animal model.

A newly formed periodontal ligament was observed along the root surface between the regenerated bone and cementum, and could be considered to be in an immature state. The periodontal ligament did not appear as dense as the native periodontal ligament over the uninvolved portions of the same root. These observations are consistent with what was observed following the application of rhGDF-5/$\beta$-TCP or rhGDF-5/ACS using the same defect model (Kim et al. 2009, Kwon et al. 2010c, Lee et al. 2010b) or following periodontal regeneration in the absence of an implanted biologic construct or biomaterial other than a space-providing device using the critical-size supraalveolar periodontal defect model (Sigurdsson et al. 1994, Wikesjö et al. 2003c) all following 8-week healing intervals. The results in this and parallel studies all collectively suggest that local application of rhGDF-5 increases regeneration of the periodontal attachment apparatus including alveolar bone, cementum, and the periodontal ligament.

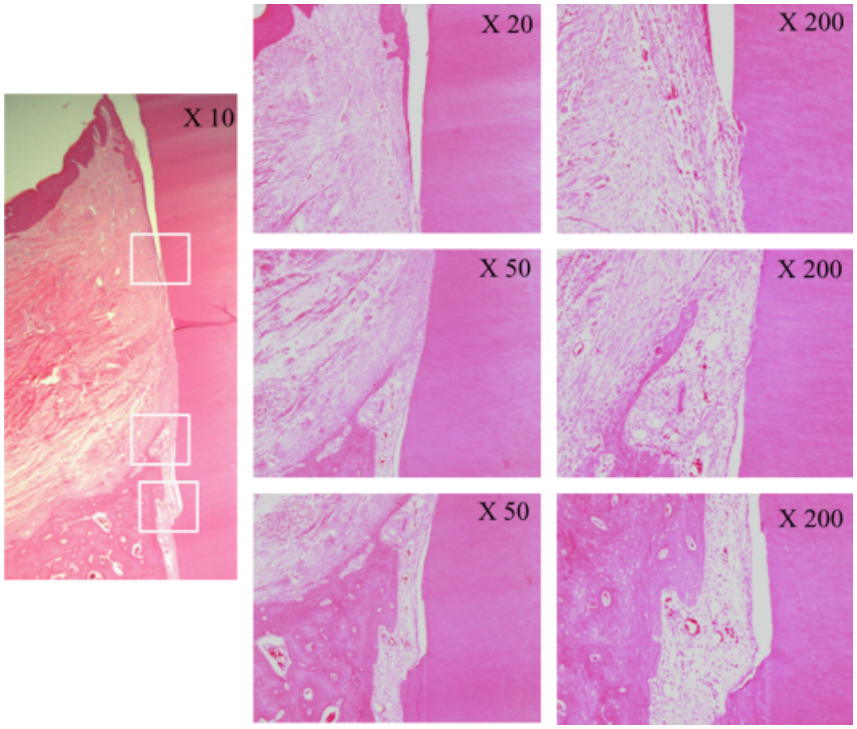


*Fig. 7.* Photomicrograph from site receiving the sham-surgery control displaying the minimal extension of newly formed bone and cementum and the apical termination of the epithelial attachment. Inset boxes in lower magnification (× 10) represent the area in higher magnification (× 20/ × 50/ × 200).

## Conclusion

rhGDF-5 appears to effectively support periodontal wound healing/regeneration in a dose-dependent order; the PLGA composite appears to be an effective ease-of-use candidate for carrier technology.

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**Clinical Relevance**

*Scientific rationale for the study*: rhGDF-5 is being evaluated as a candidate agent for periodontal wound healing/regeneration. The aim of this study was to evaluate the effect of rhGDF-5 on periodontal wound healing/regeneration using an injectable PLGA composite carrier and an established canine one-wall intra-bony defect model.

*Principal findings*: The rhGDF-5/PLGA construct was easy to prepare and apply. The PLGA composite biodegraded within 8 weeks without appreciable adverse events or compromised periodontal wound healing. The rhGDF-5 high dose (188 $\mu$g/defect) appeared to be the most effective dose supporting regeneration of cementum and alveolar bone in this model.

*Practical implications*: The rhGDF-5/PLGA construct appears to be a promising candidate for carrier technology in support of periodontal wound healing/regeneration.