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Periodontal wound healing/regeneration following the application of rhGDF-5 in a β-TCP/PLGA carrier in critical-size supra-alveolar periodontal defects in dogs

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Abstract

Aim: The objective of this study was to evaluate the effect of a novel recombinant human GDF-5 (rhGDF-5) construct intended for onlay and inlay indications on periodontal wound healing/regeneration.

Methods: Contralateral, surgically created, critical-size, 6-mm, supra-alveolar periodontal defects in five adult Hound Labrador mongrel dogs received rhGDF-5 coated onto β-tricalcium phosphate (β-TCP) particles and immersed in a bioresorbable poly(lactic-*co*-glycolic acid) (PLGA) composite or the β-TCP/PLGA carrier alone (control). The rhGDF-5 and control constructs were moulded around the teeth and allowed to set. The gingival flaps were then advanced; flap margins were adapted 3–4 mm coronal to the teeth and sutured. The animals were euthanized at 8 weeks post-surgery when block biopsies were collected for histometric analysis.

Results: Healing was generally uneventful. A few sites exhibited minor exposures. Three control sites and one rhGDF-5 site (in separate animals) experienced more extensive wound dehiscencies. The rhGDF-5 and control constructs were easy to apply and exhibited adequate structural integrity to support the mucoperiosteal flaps in this challenging onlay model. Limited residual β-TCP particles were observed at 8 weeks for both rhGDF-5/β-TCP/PLGA and β-TCP/PLGA control sites. The rhGDF-5/β-TCP/PLGA sites showed significantly greater cementum (2.34 ± 0.44 *versus* 1.13 ± 0.25 mm, $p = 0.02$) and bone (2.92 ± 0.66 *versus* 1.21 ± 0.30 mm, $p = 0.02$) formation compared with the carrier control. Limited ankylosis was observed in four of five rhGDF-5/β-TCP/PLGA sites but not in control sites.

Conclusions: Within the limitations of this study, the results suggest that rhGDF-5 is a promising candidate technology in support of periodontal wound healing/regeneration. Carrier and rhGDF-5 dose optimization are necessary before further advancement of the technology towards clinical evaluation.

Key words: β-tricalcium phosphate; bone; cementum; growth/differentiation factor-5; periodontal ligament; poly(lactic-*co*-glycolic acid); tissue engineering, periodontal regeneration

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Conflict of interest and source of funding statement

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Tissue elements sequestered within the periodontal ligament (PDL) appear critical to periodontal wound healing/regeneration (Melcher 1976). However, a triad of clinical conditions, i.e. wound stability, space provision, and wound closure for primary intention healing, must also be met to release this innate regenerative potential (Polimeni et al. 2009). Following the discovery, purification, cloning, and characterization of bone morphogenetic proteins (BMPs) (Urist 1965, Wozney et al. 1988, Wang et al. 1988, 1990, 1993, Celeste et al. 1990, Özkaynak et al. 1990, Sampath et al. 1992, Hötten et al. 1994, 1996), BMPs have been evaluated as therapeutic agents in several settings. Using preclinical models, recombinant human BMP-2 (rhBMP-2), rhBMP-7, and other members of the BMP family of proteins have been shown to stimulate clinically significant regeneration of alveolar bone and cementum or a cementum-like tissue when implanted into periodontal defects (Jin 1989, Bowers et al. 1991, Ishikawa et al. 1994, Ripamonti et al. 1994, 1996, 2001, 2002, Sigurdsson et al. 1995, 1996, Kinoshita et al. 1997, Giannobile et al. 1998, Kuboki et al. 1998, Wikesjö et al. 1999, 2003a–c, 2004, Blumenthal et al. 2002, Choi et al. 2002, Selvig et al. 2002, Saito et al. 2003, Sorensen et al. 2004, Takahashi et al. 2005, Bergenholtz et al. 2006). However, application of rhBMP-2 and rhBMP-7 has also been associated with root resorption/ankylosis when evaluated in large animal models (Sigurdsson et al. 1995, 1996, Giannobile et al. 1998, Wikesjö et al. 1999, 2003a–c, Selvig et al. 2002, Saito et al. 2003, Sorensen et al. 2004, Takahashi et al. 2005).

Growth/differentiation factor-5 (GDF-5), a member of the BMP family of proteins, also known as cartilage-derived morphogenetic protein-1 (CDMP-1), shares 49–55% of protein sequence identity with BMP-2 and BMP-7 (Hötten et al. 1994, 1996). GDF-5 is required for skeletal patterning and vertebrate limb development (Storm & Kingsley 1996, Faiyaz-Ul-Haque et al. 2002a, b, Settle et al. 2003). GDF-5, as well as GDF-6 and -7, gene expression has been demonstrated in bovine and rat tooth germs at the root forming stage and has been associated with PDL formation (Morotome et al. 1998, Sena et al. 2003). Furthermore, GDF-5 may provide an environment conducive to periodontal wound healing/regeneration affecting PDL cell proliferation in a dose-dependent manner (Nakamura et al. 2003). Moreover, GDF-5 has been shown to support bone and tendon/ligament formation in the axial and appendicular skeleton including craniofacial indications (for a review, see Moore et al. 2010). Pre-clinical studies have evaluated rhGDF-5 in periodontal settings using a β-tricalcium phosphate (β-TCP) carrier, a Type I absorbable collagen sponge carrier, and a poly(lactic-*co*-glycolic acid) (PLGA) carrier (Kim et al. 2009, Kwon et al. 2010, Lee et al. 2010). However, these technologies used in initial proof-of-concept studies have limited structural integrity and may not be sufficiently versatile to be successfully applied also for challenging onlay indications. Thus, the objective of this study was to evaluate periodontal wound healing/regeneration following surgical implantation of rhGDF-5 coated onto β-TCP immersed in a PLGA composite, a structurally improved carrier potentially suitable for both inlay and onlay indications, using a critical-size supra-alveolar periodontal defect model in dogs.

Material and Methods

Animals

Five male Hound Labrador mongrel dogs, age 18–24 months, approximate weight 25–30 kg, obtained from USDA-licensed vendor were used following a protocol approved by the Medical College of Georgia Animal Care and Use Committee. The animals were accustomed to a canned soft dog-food diet during acclimatization to prevent unnecessary stress due to dietary alterations post-surgery. One oral prophylaxis was performed under sedation (telazol 5 mg/kg – xylazine 1 mg/kg IM) using aseptic techniques within 2 weeks before experimental surgeries.

rhGDF-5/β-TCP/PLGA Composite

The candidate rhGDF-5/β-TCP/PLGA composite (Scil Technology GmbH, Martinsried, Germany) was prepared according to the manufacturer's directions. The PLGA composite represents a novel mouldable biomaterial (Pompe 2008). Briefly, the composite consists of poly(D,L-lactic-co-glycolic acid) (Boehringer Ingelheim, Ingelheim, Germany) dissolved in polyethylene glycol 400 (Merck, Darmstadt, Germany) by heat treatment, calcium sulphate (Carl Roth, Karlsruhe, Germany), and β-TCP powder (Ceraver Osteal, Roissy, France) dispersed in the polymeric solution. Before application, rhGDF-5 coated onto β-TCP granules (Calciresorb, Ceraver Osteal) at a concentration of 500 μg/g β-TCP was homogeneously blended into the bioresorbable PLGA composite. The â-TCP biomaterial includes micro- and macro-porous irregular 500–100-μm-diameter granules of a phase purity $>95\%$ with an average pore diameter of 2 μm and a pore area of 0.7 m^2/g. The macro-pore diameter ranges between 100 and 400 μm and the pore area is estimated to be 1.2 m^2/g (Pöhling et al. 2006). For the control, an equivalent amount of β-TCP granules (Calciresorb, Ceraver Osteal) and PLGA composite were combined. All materials were stored at -80°C until use.

Experimental Surgery

Food was withheld the night preceding surgery. The animals were pre-anaesthetized with atropine (0.02–0.04 mg/kg; IM), buprenorphine HCl (0.01–0.03 mg/kg; IM), and acepromazine (0.2–0.3 mg/kg; IM). After tranquilization, a 20–23-gauge catheter was placed in the foreleg for induction with propofol (5–7 mg/kg; IV). Animals were then moved to the surgical theatre and maintained on gas anaesthesia (1.5–2% isoflurane/O_2 to effect). The animals received a slow constant rate infusion of lactated Ringer's solution (10–20 ml/kg/h; IV) to maintain hydration during surgery. Depth of anaesthesia was monitored by lack of response to toe pinch, lack of corneal reflex, and by monitoring the depth of respiration.

Three experienced surgeons (U. M. E. W., F. C. B., and R. W. H.) performed the surgical procedures. Supra-alveolar, critical-size, periodontal defects were created around the third and fourth mandibular premolar teeth in right and left jaw quadrants in each animal (Wikesjö & Nilvéus 1991, Wikesjö et al. 1994). Briefly, buccal and lingual mucoperiosteal flaps were reflected following buccal and lingual sulcular incisions from the canine tooth to the second molar. The first and second premolar teeth were extracted and the first molar was surgically reduced to the level of the alveolar crest. Alveolar bone was removed around the circumference of the remaining premolar teeth using chisels and water-cooled rotating burs. The root surfaces were instrumented with cur-

ettes, chisels, and water-cooled rotating diamonds to remove the cementum. The crowns of the teeth were reduced to approximately 2 mm coronal to the cemento-enamel junction (CEJ) and the cut surfaces were smoothed. Exposed pulpal tissues were sealed (Cavit®, ESPE, Seefeld/Oberbayern, Germany). The clinical defect height from the CEJ to the surgically reduced alveolar crest was set at 6 mm as measured using a periodontal probe.

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

Contralateral supra-alveolar periodontal defects received the same amount of the test rhGDF-5/β-TCP/PLGA (500 μg rhGDF-5/site) and the β-TCP/PLGA control composite. The constructs were placed to cover the roots to replace the removed alveolar bone and were allowed to harden in situ by exchange of polyethylene glycol with body fluid while absorbing bleeding. Treatments were alternated between left and right jaw quadrants in subsequent animals.

Following placement of the rhGDF-5/β-TCP/PLGA or the β-TCP/PLGA control composite, the periostea were fenestrated at the base of the flaps to allow wound closure with tension-free flap apposition for primary intention healing. The flaps were advanced; the flap margins were adapted 3–4 mm coronal to the teeth and sutured (GORE-TEX™ Suture CV5, W.L. Gore & Associates Inc., Flagstaff, AZ, USA). Intrasurgery photographs were taken before and immediately after placement of the rhGDF-5/β-TCP/PLGA or the β-TCP/PLGA control composite, and after wound closure.

Post-surgery Procedures

A long-acting opioid (buprenorphine HCl, 0.01–0.03 mg/kg; IM, BID/3 days) was administered for pain control. A broad-spectrum antibiotic (enrofloxacin; 2.5 mg/kg; IM, SID/7 days) was administered for infection control. Plaque control was maintained by flushing of the oral cavity with chlorhexidine gluconate (Xttrium Laboratories Inc., Chicago, IL, USA; 20–30 ml of a 2% solution; BID) until completion of the study. The animals continued the canned soft dog-food diet throughout the study. Sutures were removed under sedation (telazol 5 mg/kg – xylazine 1 mg/kg; IM) at approximately 10 days. The experimental areas were monitored daily until suture removal for wound swelling/dehiscencies/infection and were reviewed thereafter at least weekly. Radiographic recordings were made at suture removal (at approximately 10 days), and at 4 and 8 weeks.

Euthanasia

The animals were anaesthetized (telazol 5 mg/kg – xylazine 1 mg/kg; IM) and euthanized at week 8 by an injection of concentrated sodium pentobarbital (Euthasol® 150 mg/kg; IV, Delmarva Laboratories Inc, Midlothian, VA, USA). Block sections including teeth, alveolar bone, and surrounding mucosa were collected after euthanasia and radiographed. The specimens were rinsed in sterile saline and transferred to 10% neutral-buffered formalin at a volume 10 times that of the individual block section.

Histological Processing

Tissue blocks including premolar teeth, bone, and soft tissue were fixed in 10% buffered formalin for 3–5 days, decalcified in 5% formic acid for 8–10 weeks, trimmed, dehydrated, and embedded in paraffin. Serial sections (7 μm) were produced in a buccal–lingual plane throughout the mesial–distal extension of the teeth. Every 14th section was stained for observations at 100 μm intervals. Haematoxylin/eosin stains were used.

Histologic and Histometric Analysis

The histopathologic evaluation by two masked, experienced examiners (U. M. E. W., D. H. K.) included observations of bone formation/resorption, woven and lamellar bone, cortex formation, seroma formation, fibrovascular tissue and marrow, vascularity, cementum formation, fibrous attachment, epithelial attachment, root resorption, ankylosis, and residual biomaterial and associated tissue reactions. PDL fibre orientation was observed and PDL fibre density was scored (score 0: no PDL fibres, score 1: low-density PDL fibres, score 2: moderate-density PDL fibres, score 3: high-density PDL fibres, same as the native adjoining PDL).

One masked, calibrated examiner (D. H. K.) performed the histometric analysis using incandescent and polarized light microscopy (BX 51, Olympus America Inc., Melville, NY, USA), a microscope digital camera system (Retiga 4000R QImaging, Burnaby, BC, Canada), and an image analysis software (Image-Pro Plus™, Media Cybernetic, Silver Spring, MD, USA) including a custom macro for analysis of the critical-size supra-alveolar periodontal defect model. The most central section based on the buccal–lingual extension of the root canal from each tooth/root was used for the histometric analysis (Koo et al. 2004). The following measurements were recorded for the experimental buccal surfaces of each tooth:

- Defect height: distance between the apical extension of root planing and the CEJ.
- Junctional epithelium: distance from the CEJ to the apical extension of an epithelial attachment along the root surface.
- Cementum formation (height): distance between the apical extension of root planing and the coronal extension of a continuous layer of new cementum or cementum-like deposit on the planed root.
- PDL formation (height): distance between the apical extension of root planing and the coronal extension of a functionally oriented PDL on the planed root.
- Bone formation (height): distance between the apical extension of root planing and the coronal extension of regenerated alveolar bone along the planed root.
- Bone formation (area): area represented by new alveolar bone along the planed root.
- Bone formation (density): ratio of regenerated bone/marrow spaces.
- Root resorption: combined linear heights of distinct resorption lacunae on the planed root.
- Ankylosis: combined linear heights of ankylotic union between the regenerated alveolar bone and the planed root.

Statistical Analysis

Examiner reliability for the histometric evaluation was assessed using the

concordance correlation coefficient. Within the framework of this study, the concordance correlation coefficient ranges between 0 and 1; the higher the coefficient, the greater the reliability. The concordance correlation coefficient for the histometric measurements ranged from 0.96 to 0.99, demonstrating high reliability for all parameters assessed but showing moderate reliability for ankylosis (0.69).

The animal was used as the unit of analysis. Linear models were used to perform the analysis. Measurements at site level were used and estimates were adjusted for the clustering of sites into animals using a robust variance estimator. Wald's tests were used for multiple comparisons and the level of significance was set at 5%. All analysis was performed using a computer-based statistical software (Stata 9.2 for Windows, Stata Corporation, College Station, TX, USA).

Results

Clinical and Radiographic Observations

The rhGDF-5/β-TCP/PLGA composite was easy to prepare and to adapt to the surgical sites. At suture removal, approximately 10 days post-surgery, three control sites were partially exposed. At 4 weeks, four control and one rhGDF-5/β-TCP/PLGA site were partially exposed. At 8 weeks, four control and one rhGDF-5/β-TCP/PLGA site were exposed, and one rhGDF-5/β-TCP/PLGA site showed the top surface of the teeth, while three sites remained submerged (Fig. 1).

Both rhGDF-5/β-TCP/PLGA and control sites exhibited radiopacity, consistent with residual β-TCP particles encompassing almost the entire defect at suture removal. At 4 weeks, the radiopacity appeared reduced, consistent with resorption of the β-TCP biomaterial. At 8 weeks, there were significant differences in radiopacity/bone formation between control and experimental sites. Whereas bone formation was limited to the apical third of the roots in the control sites, bone formation commonly encompassed almost the complete furcation area and adjacent interproximal surfaces in the rhGDF-5/β-TCP/PLGA sites (Fig. 2). Radiographic suggestions of root resorption were observed in one animal at 8 weeks, including one experimental and one control premolar tooth. All other teeth appeared unaffected by root resorption.

Histologic Observations

Photomicrographs of rhGDF-5/β-TCP/PLGA and control sites are shown in Figs 3 and 4. Sites receiving rhGDF-5/β-TCP/PLGA and control composites both exhibited new bone formation including lamellar and woven bone. Cementum formation was predominantly acellular or cellular intrinsic or mixed fibre cementum, with no remarkable differences between sites. Extrinsic fibre cementum was rare. The density of the PDL was generally similar for rhGDF-5/β-TCP/PLGA and control sites, ranging from absence of appreciable PDL fibres to a moderately dense PDL without reaching the density of the native PDL. The formation of a junctional epithelium, a consequence of wound dehiscencies, was observed in two rhGDF-5/β-TCP/PLGA and four control sites. Root resorption was observed in four rhGDF-5/β-TCP/PLGA and three control sites, and ankylosis in four rhGDF-5/β-TCP/PLGA sites and in none of the control sites. Limited β-TCP/PLGA residues were observed in two rhGDF-5/β-TCP/PLGA and four control sites. One site (control) showed significantly more residual β-TCP/PLGA than that observed in all other sites.

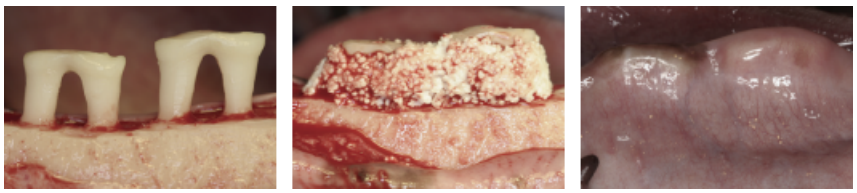

Fig. 1. Representative, surgically created, 6-mm, critical-size, supra-alveolar, periodontal defects over the roots of the third and fourth mandibular premolar teeth (left), application of the rhGDF-5/β-TCP/PLGA composite (centre), and healing at euthanasia at 8 weeks post-surgery (right).

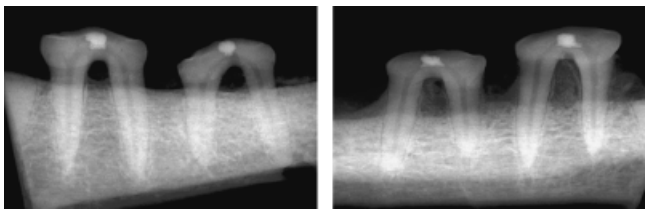

Fig. 2. Representative radiographs of contralateral, critical-size, supra-alveolar, periodontal defects implanted with rhGDF-5/PLGA/β-TCP (right) or the PLGA/β-TCP carrier control (left) at 8 weeks. The rhGDF-5/PLGA/β-TCP test sites exhibit bone formation reaching the cemento-enamel junction and displaying a periodontal ligament space *versus* more modest bone formation in the control.

Histometric Analysis

The results of the histometric analyses are shown in Table 1. As fragments of the β-TCP/PLGA biomaterial were too small and infrequent to be measured, the histometric evaluation was limited to defect height, junctional epithelium, cementum, PDL, bone formation (height, area, and density), root resorption, and ankylosis. The rhGDF-5/β-TCP/PLGA group showed 2.4 times greater bone formation (height) and 2.1 times greater cementum formation than the control group ($p = 0.02$). Increased PDL formation, bone area, and density were also observed in the rhGDF-5/β-TCP/PLGA group compared with the control group; however, these differences did not attain statistical significance. No statistically significant differences were observed for root resorption between groups. However, significantly increased ankylosis was observed in the rhGDF-5/β-TCP/PLGA group compared with the control group ($p = 0.006$).

Discussion

The objective of this study was to evaluate the effect of rhGDF-5 in a β-TCP/PLGA carrier on periodontal wound healing/regeneration using an

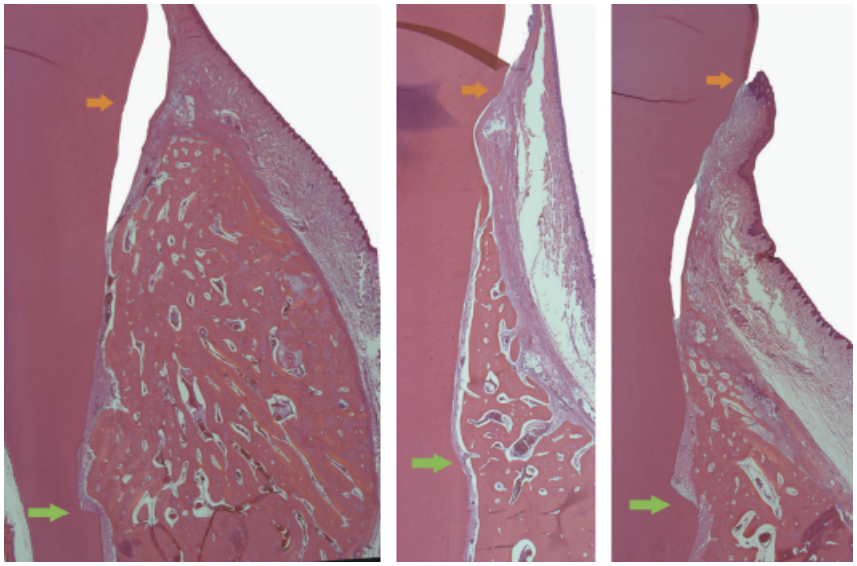

Fig. 3. Photomicrographs of 6-mm supra-alveolar periodontal defects implanted with rhGDF-5/PLGA/β-TCP (left/centre) or the PLGA/β-TCP carrier control (right) following an 8-week healing interval. The rhGDF-5/PLGA/β-TCP composite yielded greater bone formation compared with the control; bone formation reaching the cemento-enamel junction with (centre) or without (left) evidence of ankylosis. Green arrows indicate the apical extent of the defects and orange arrows indicate the cemento-enamel junction (haematoxylin/eosin).

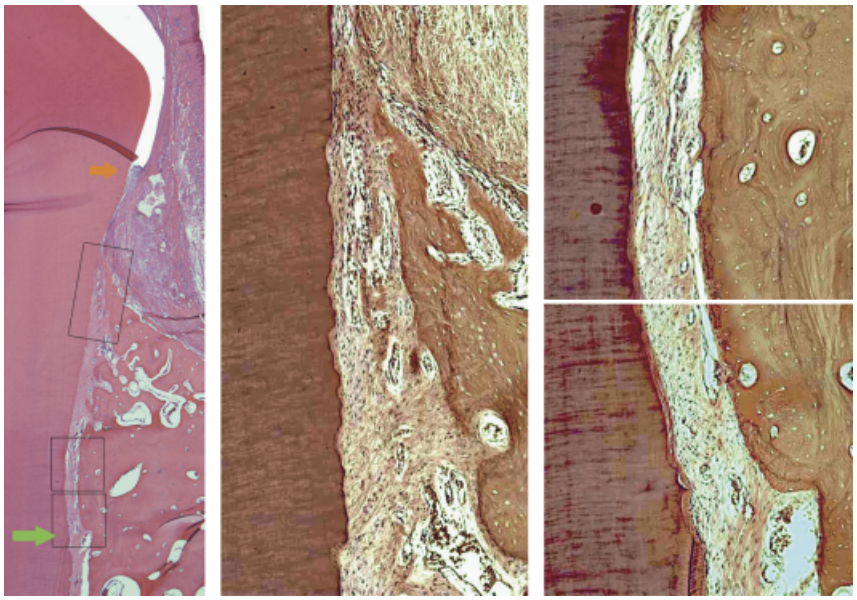

Fig. 4. Photomicrographs of 6-mm supra-alveolar periodontal defect implanted with rhGDF-5/PLGA/β-TCP (overview and high magnifications) following an 8-week healing interval. Regeneration of a periodontal ligament including in a coronal extension gradually thinning cementum formation may be observed. The green arrow indicates the apical extent of the defect and the orange arrow indicate the cemento-enamel junction (haematoxylin/eosin).

established critical-size, supra-alveolar periodontal defect model and a canine platform. Five animals received rhGDF-5/β-TCP/PLGA *versus* β-TCP/PLGA (control) in contralateral jaw quadrants. Sites receiving the rhGDF-5/β-TCP/PLGA composite exhibited significantly greater periodontal regeneration including cementum and bone formation compared with the control following an 8-week healing interval; however, newly formed periodontal tissues appeared compromised by ankylosis in the rhGDF-5/β-TCP/PLGA group.

Bone formation was significantly enhanced at sites receiving rhGDF-5/ß-TCP/PLGA compared with the β-TCP/PLGA control averaging 51% and 21% of the defect height encompassing 2.8 and 0.8 mm^2, respectively. Previous studies have used the supra-alveolar periodontal defect model to assess the effect of rhBMP-2 (Sigurdsson et al. 1995, 1996, Wikesjö et al. 1999, 2003a–c, 2004, Selvig et al. 2002, Sorensen et al. 2004). Wikesjö et al. (1999, 2004) demonstrated significantly enhanced bone formation following application of rhBMP-2 in various concentrations using a bovine Type I absorbable collagen sponge carrier. Sigurdsson et al. (1996) evaluated several other rhBMP-2 candidate carriers including canine demineralized bone matrix, bovine bone mineral matrix, absorbable collagen sponge, PLGA microparticles, and polylactic acid granules for their ability to support rhBMP-2-induced bone formation. Bone formation ranged from 71% to 100% of the defect height among these carriers. Ripamonti et al. (1996, 2001, 2002) evaluated rhBMP-7, rhBMP-2, and a combination of rhBMP-7 and rhBMP-2 in a collagenous bone matrix carrier in surgically induced Class II furcation defects in the baboon. rhBMP-2 and rhBMP-7 induced similar bone formation while the rhBMP-7/BMP-2 combination did not enhance bone formation further. The results of these studies and that of the present study collectively point to the significant potential of rhBMP-2, rhBMP-7, and rhGDF-5 to induce alveolar bone formation. Differences between proteins appear dose dependent, but may also be influenced by the carrier technology including polymer composition and release kinetics, as discussed below.

The rhGDF-5/β-TCP/PLGA treatment yielded increased cementum formation compared with the β-TCP/PLGA control averaging 40% and 20% of the defect height, respectively. Cementum formation encompassed acellular and/or cellular, intrinsic or mixed fibre cementum but rarely extrinsic fibre cementum without remarkable differences between treatments. Similarly, the PDL ranged from no appreciable PDL fibres to moderate density without reaching the density of the native adjoining PDL. The regenerative potential of the cementum in this study approaches that observed in a previous

Table 1. Results of the histometric analysis by observation interval (mean ± SE in mm, mm^2, and %)

	Defect height	Junctional epithelium	Cementum	PDL	Bone (height)	Bone (area)	Bone (density)	Root resorption	Ankylosis
Control	5.79 ± 0.07	1.57 ± 0.60	1.13 ± 0.25	0.80 ± 0.13	1.21 ± 0.30	0.84 ± 0.36	61.11 ± 5.33	0.14 ± 0.13	0.00
rhGDF-5	5.78 ± 0.07	0.69 ± 0.61	2.34 ± 0.44	1.46 ± 0.50	2.92 ± 0.66	2.77 ± 1.38	68.92 ± 5.91	0.24 ± 0.07	0.46 ± 0.13
p-value	0.87	0.39	**0.02**	0.19	**0.02**	0.22	0.20	0.56	**0.006**

Bold values signify $p < 0.05$.

study evaluating supra-alveolar periodontal defects implanted with rhBMP-2/ACS or rhGDF-7/ACS, the cementum regeneration averaging 43% and 41% of the defect height, respectively, within an 8-week perspective (Wikesjö et al. 2004). However, only sites receiving rhGDF-7 exhibited a functionally oriented PDL of high density whereas sites receiving rhBMP-2 included a fibrovascular tissue without noteworthy structural elements consistent with a PDL. It must be noted that other previous studies have reported increased cementum regeneration following the application of both rhBMP-2 and rhBMP-7, a functionally oriented PDL being observed both in confined intrabony and in furcation defects (Ishikawa et al. 1994, Ripamonti et al. 1996, 2001, 2002, Choi et al. 2002). Differences between studies may at least in part relate to the choice of experimental model and strategy of analysis, where proximity native tissue resources and plane of analysis may have influenced the outcomes of study.

Ankylosis in the cervical third of the defect was observed in four of five rhGDF-5/β-TCP/PLGA composite-treated animals or six of 10 teeth in the present study. This observation appears to be in contrast to that of recent studies evaluating rhGDF-5 using β-TCP, ACS, or PLGA carrier technologies. These studies, also using 8-week healing intervals, showed limited, or no root resorption or ankylosis (Kwon et al. 2009, Kim et al. 2009, Kwon et al. 2010, Lee et al. 2010). Differences in carrier technology possibly resulting in unfavourable rhGDF-5 release kinetics, or the biomaterials combination in itself may have elicited the healing response in the present study as aberrant healing events were also observed in carrier control sites.

Biomaterials used for periodontal reconstructive surgery should not obstruct bone formation or periodontal regeneration. In this study, limited β-TCP/PLGA residues were observed in two of five experimental sites, whereas residual biomaterial appeared in four of five control sites. Previous studies have reported large amounts of residual biomaterial including a bovine bone mineral (Sigurdsson et al. 1996) and a calcium phosphate cement (Sorensen et al. 2004), apparently obstructing periodontal regeneration including bone and cementum formation when used as carrier technologies for rhBMP-2. Koo et al. (2007) reported that sites implanted with rhTGF-β_1 in a Ca_2CO_3 carrier exhibited significantly smaller amounts of residual carrier biomaterial compared with the carrier control. This observation implies that the growth factor may have increased the biodegradation rate of the carrier. Possibly similar effects have occurred in the present study, indicating that rhGDF-5 induces remodelling of the carrier, thereby allowing tissue regeneration.

Controlled preclinical models with reproducible characteristics and biological reaction are critical for evaluation of safety and efficacy of periodontal reconstructive protocols before clinical evaluation and commercial introduction. This study utilized the critical-size supra-alveolar periodontal defect model including 6-mm supra-crestal circumferential periodontal defects and an adult Hound Labrador mongrel dog platform (Wikesjö & Nilvéus 1991, Wikesjö et al. 1994). This model is considered a critical-size defect because the surgically created defect will not regenerate within the lifetime of the animal without adjunctive measures. Sham-surgery and vehicle controls using 4- and 8-week healing intervals have demonstrated the limited regenerative potential in this model (Wikesjö & Nilvéus 1991, Sigurdsson et al. 1994, 1995, 1996, Wikesjö et al. 1999). In perspective, adding a sham-surgery control to the present study would most certainly represent unnecessary duplication violating the principles *Refinement–Reduction–Replacement*, key strategies of humane experimental techniques (Russell & Burch 1959, [11]Institute of Laboratory Animal Resources 1996). Therefore, this study did not include a sham-surgery control.

Conclusion

Within the limitations of this study, the results suggest that rhGDF-5 is a promising candidate technology in support of periodontal wound healing/regeneration. Carrier and rhGDF-5 dose optimization are necessary before further advancement of the technology towards clinical evaluation.

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Clinical Relevance

***Scientific rationale for the study*:** Recombinant human growth/differentiation factor-5 (rhGDF-5) is being evaluated as a candidate therapy for periodontal wound healing/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 a β-TCP PLGA composite carrier using an established periodontal defect model.

***Principal findings*:** Surgical implantation of the rhGDF-5/β-TCP/PLGA formulation showed significantly greater bone and cementum formation than the carrier control. Periodontal wound healing also included ankylosis.

***Practical implications*:** rhGDF-5 appears to be a promising technology to support periodontal wound healing/regeneration. Additional evaluation under optimal conditions for wound healing including variations in rhGDF-5 dosage and carrier optimization, is necessary before clinical evaluation.