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Evaluation of an injectable rhGDF-5/PLGA construct for minimally invasive periodontal regenerative procedures: a histological study in the dog

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Abstract

Aim: To evaluate the injectability, biocompatibility, safety, and periodontal wound healing/regeneration following application of a novel bioresorbable recombinant human growth/differentiation factor-5 (rhGDF-5)/poly(lactic-*co*-glycolic acid) (PLGA) construct.

Material and Methods: Periodontal pockets (3 × 6 mm, width × depth) were surgically created over the buccal roots of the second and fourth mandibular premolars in eight adult Hound Labrador mongrel dogs. Surgeries including injection of the rhGDF-5/PLGA construct into the pockets were sequenced that four animals provided 2-/4-week and four animals 6-/8-week observations of sites receiving rhGDF-5/PLGA or serving as sham-surgery control.

Results: The rhGDF-5/PLGA construct was easy to prepare and apply. Approximately 0.2 ml (93 μg rhGDF-5)/tooth was used. Clinical and radiographic healing was exemplary without adverse events. Healing was characterized by a non-specific connective tissue attachment, acellular/cellular cementum, periodontal ligament (PDL), bone regeneration, and a junctional epithelium. PLGA fragments were observed in 4/7, 2/8, and 1/8 sites at 2, 4, and 6 weeks, respectively. Associated inflammatory reactions exhibited no limiting effect on periodontal wound healing/regeneration. Root resorption/ankylosis was not observed. Bone formation showed apparent increased maturity (lamellar bone) at 6 weeks in sites receiving rhGDF-5/PLGA compared with the control. Both protocols exhibited significant increases in PDL, cementum, and bone regeneration over time, without significant differences between treatments. In time, PDL and cementum regeneration was twofold greater for the control at 4 weeks ($p = 0.04$) while increased bone formation was observed at sites receiving rhGDF-5/PLGA ($p < 0.01$).

Conclusions: In conclusion, the rhGDF-5/PLGA construct appears to be a safe technology for injectable, ease-of-use application of rhGDF-5-stimulated periodontal wound healing/regeneration. Additional work to optimize the polymer carrier and rhGDF-5 release kinetics/dose might be required before evaluating the efficacy of this technology in clinical settings using minimally invasive approaches.

Key words: bone; cementum; GDF-5; periodontal ligament; periodontal regeneration; PLGA; tissue engineering

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Conflict of interest and source of funding statement

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Ever since the discovery, purification, cloning, and characterization of bone morphogenetic proteins (BMPs) (Urist 1965, Wang et al. 1988, 1990, 1993, Wozney et al. 1988, Celeste et al. 1990, Özkaynak et al. 1990, Sampath et al. 1992), constructs including purified or recombinant BMPs have been evaluated in support of bone formation in the axial and appendicular skeleton (for a review, see Bishop & Einhorn 2007, Hsu & Wang 2008, Huang et al. 2008). Several studies in pre-clinical and clinical settings have focused on periodontal wound healing/regeneration following surgical implantation of primarily recombinant human BMP-2 (rhBMP-2) and rhBMP-7, also known as recombinant human osteogenic protein-1, in combination with a variety of candidate biomaterials used as carriers (for a review, see Lee et al. 2010). However, surgical implantation of rhBMP-2 and rhBMP-7 appears to be associated with root resorption/ankylosis when evaluated in discriminating large animal models, suggesting a potential limitation of their clinical utility for periodontal indications.

An intriguing alternative to rhBMP-2 or rhBMP-7 for periodontal indications is recombinant human growth/differentiation factor-5 (rhGDF-5), also known as cartilage-derived morphogenetic protein-1 (Hötten et al. 1994, 1996). This concept is based on several lines of evidence. Expression of GDF-5, -6, and -7 genes in bovine and rat tooth germs at the root-forming stage suggests that GDFs may play important regulatory roles in the development of the periodontal attachment (Morotome et al. 1998, Sena et al. 2003). In vitro, GDF-5 stimulates human periodontal ligament (PDL) cells by affecting extracellular matrix metabolism in a dose-dependent manner (Nakamura et al. 2003). In vivo, GDF-5 induces cartilage and bone formation when implanted in muscular tissues in rodents (Hötten et al. 1996). In addition, GDF-5 has been associated with tendon and ligament formation in rat models (Wolfman et al. 1997, Forslund et al. 2003, Bolt et al. 2007, Dines et al. 2007).

In a previous report, we outlined the development of a candidate carrier for rhGDF-5 including poly(lactic-*co*-glycolic acid) (PLGA), a well-described bioresorbable synthetic polymer, dissolved within a biocompatible organic solvent and functionally supplemented with different additives (Herberg et al. 2008). The resulting rhGDF-5/PLGA construct is designed as a two-component system and requires simple admixing of the lyophilized rhGDF-5 and the paste-like PLGA carrier before administration. When brought in contact with aqueous media and/or body fluids like blood, the rhGDF-5/PLGA construct immediately solidifies and forms a highly porous scaffold in situ. These specially tailored characteristics (e.g., blood interaction, matrix porosity, drug delivery, and biodegradation) of the rhGDF-5/PLGA construct are expected to broaden the clinical utility of rhGDF-5-induced tissue engineering (Herberg et al. 2008). The injectability of this novel rhGDF-5/PLGA construct is considered to support minimally invasive regenerative procedures (Cortellini & Tonetti 2001, 2007) and ease-of-use application in contained and non-contained periodontal defects. The primary objective of this study was to evaluate the clinical injectability and biocompatibility of the rhGDF-5/PLGA construct. The effect on periodontal wound healing/regeneration with a focus on safety was also evaluated using a surgically created periodontal pocket model in dogs.

Material and Methods

Animals

Eight male Hound Labrador mongrel dogs, age 18–24 months, weight 25–30 kg, obtained from a USDA-licensed vendor were used. The animals were acclimated and accustomed to a canned soft dog-food diet. One oral prophylaxis (tooth cleaning) was performed under sedation (telazol 5 mg/kg–xylazine 1 mg/kg; i.m.) using aseptic techniques within 2 weeks before experimental surgeries.

rhGDF-5/PLGA construct

The test rhGDF-5/PLGA construct was prepared as instructed by the manufacturer (Scil Technology GmbH, Martinsried, Germany). Before application, one pre-filled syringe of the PLGA carrier was homogeneously mixed with one vial of the rhGDF-5 lyophilisate. After filling the rhGDF-5/PLGA construct into a 1.0 ml disposable syringe, the syringe was equipped with a 0.9 × 23 mm/20 G standard dental needle to allow for application to the defect site.

Experimental surgery

Food was withheld the night preceding surgery. The animals were pre-anaesthetized with atropine (0.02–0.04 mg/kg; i.m.), buprenorphine HCl (0.01–0.03 mg/kg; i.m.), and acepromazine (0.2–0.3 mg/kg; i.m.). After tranquilization, a 20–23 G catheter was placed into the foreleg for induction with propofol (5–7 mg/kg; i.v.). Animals were then moved to the operating room and maintained on inhalation 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; i.v.) to maintain hydration during surgery. The depth of anaesthesia was monitored by a lack of response to toe pinch, lack of a corneal reflex, and by monitoring the depth of respiration.

One experienced surgeon (U. M. E. W.) performed all surgical procedures. Periodontal buccal dehiscence defects were prepared in the mandibular pre-molar region to create standardized surrogate periodontal pockets much like they may appear following non-surgical instrumentation removing the cementum and the pocket epithelium (Fig. 1). Briefly, following routine dental infiltration anaesthesia (lidocaine HCl 2%, epinephrine 1:100,000), local buccal mucoperiosteal flaps were reflected over the roots of the second and the fourth pre-molars and alveolar bone was removed over the mesial and distal roots. The distance from the cemento-enamel junction (CEJ) to the apical extent of the defect approximated 6 mm. The width of the defect encompassed the width of the respective roots approximating 3–4 mm. The exposed root surfaces were instrumented with the intent to remove the cementum. A distinct instrumentation notch was created in the most apical and lateral extents of the defects. The flaps were then replaced and sutured (GORE-TEX™ Suture CV5, W.L. Gore & Associates Inc., Flagstaff, AZ, USA). Upon wound closure, the rhGDF-5/

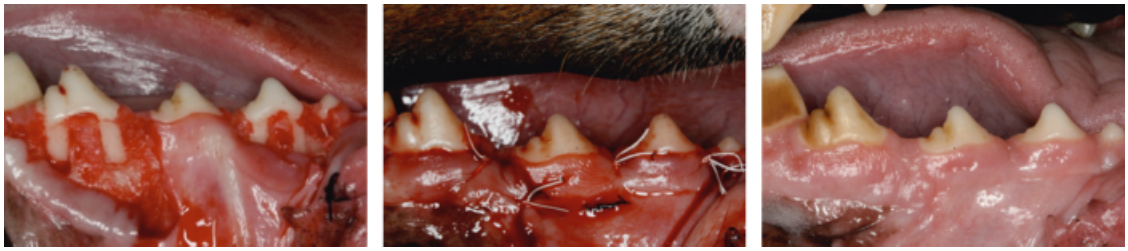

Fig. 1. Representative, surgically created, buccal dehiscence defects over the roots of the second and fourth mandibular pre-molar teeth using local mucoperiosteal flaps for each defect site (left), wound closure/pocket creation (centre), and healing at euthanasia (right).

Table 1 Distribution of treatments among defect sites and animals, and healing intervals

Animal #	First surgery (weeks)	rhGDF-5/PLGA	Sham-surgery	Second surgery (weeks)	rhGDF-5/PLGA	Sham-surgery
1	8	LP2	LP4	6	RP2	RP4
2	8	RP4	RP2	6	LP4	LP2
3	8	LP2	LP4	6	RP2	RP4
4	8	RP4	RP2	6	LP4	LP2
5	4	LP2	LP4	2	RP2	RP4
6	4	RP4	RP2	2	LP4	LP2
7	4	LP2	LP4	2	RP2	RP4
8	4	RP4	RP2	2	LP4	LP2

RP, right pre-molar; LP, left pre-molar; rhGDF-5, recombinant human growth/differentiation factor-5; PLGA, poly(lactic-*co*-glycolic acid).

PLGA construct was injected into the surgically created periodontal pocket over each root in one pre-molar per jaw quadrant; the second pre-molar served as the sham-surgery control. The volume of rhGDF-5/PLGA to be injected determined using open defects approximated 0.2 ml/tooth (0.1 ml/root). Using a time-lapse protocol, surgeries/treatments were sequenced as shown in Table 1. A PLGA carrier control was not used because preliminary studies had demonstrated its limited effect in periodontal defects (Herberg et al. 2008). Photographs of the experimental sites were taken following defect induction, wound closure and application of the rhGDF-5/PLGA construct, and at euthanasia.

Post-surgery procedures

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

Euthanasia

The animals were anaesthetized and euthanized at week 4 or 8 post-surgery by an injection of concentrated sodium pentobarbital (Euthasol® 150 mg/kg i.v.; Delmarva Laboratories Inc., Midlothian, VA, USA). Block sections including teeth, alveolar bone, and surrounding mucosa were collected following euthanasia and radiographed. The tissue blocks 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

The block sections 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 one masked experienced examiner (U. M. E. W.) 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, residual biomaterial, and associated tissue reactions.

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 a PC-based image analysis system (Image-Pro Plus™, Media Cybernetic, Silver Spring, MD, USA). The most central section, based on the buccal–lingual extension of the root canal, from each tooth/root was used for the histometric analysis. The following measurements were recorded for the experimental buccal surfaces of each tooth:

- Defect height: distance between the apical extension of the root planing and the CEJ.
- Junctional epithelium: distance from the CEJ to the apical extension of an epithelial attachment along the root surface.
- Cementum regeneration (height): distance between the apical extension of the root planing and the coronal extension of a continuous layer of new cementum or cementum-like deposit on the planed root.
- PDL regeneration (height): distance between the apical extension of the root planing and the coronal exten-

sion of a functionally oriented PDL on the planed root.
- Bone regeneration (height): distance between the apical extension of root planing and the coronal extension of regenerated alveolar bone along the planed root.
- Bone regeneration (area): area represented by new alveolar bone along the planed root.
- Bone regeneration (density): ratio of regenerated bone/marrow spaces.

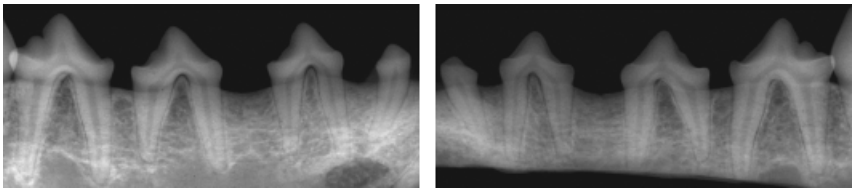

Fig. 2. Representative radiographs of second and fourth mandibular pre-molar sites implanted with the recombinant human growth/differentiation factor-5/poly(lactic-*co*-glycolic acid) construct or serving as sham-surgery control. There are no appreciable differences between pre-molar sites in this case representing 4- (right) and 2-week (left) observations. The radiographs of the experimental sites show conditions comparable to that of the untreated third mandibular pre-molars.

Statistical analysis

Examiner reliability for the histometric evaluation was assessed using the Concordance Correlation Coefficient. This coefficient ranges between 0 and 1; the higher the coefficient the greater the reliability. Thus, duplicate measurements, at least 2 weeks apart, were completed using a randomly selected sample (30%) of the specimens. The concordance correlation coefficient for the histometric measurements ranged from 0.96 to 0.99, demonstrating high reliability for all the parameters assessed.

The animal was used as the unit of analysis. Generalized estimating equations were used to perform the analysis. Measurements at the site level were used and estimates were adjusted for the clustering of sites into animals using a robust variance estimator. Wald tests were used for multiple comparisons and the level of significance was set at 5%. The entire 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/PLGA construct was easy to prepare and apply to the defect sites. Evaluation of open defects allowed estimation of the amount of the rhGDF-5/PLGA construct to be used at the experimental sites. Approximately 0.2 ml of the rhGDF-5/PLGA construct/tooth (0.1 ml/root) was used in this study. Clinical healing was generally exemplary in all defect sites without signs of swelling or adverse events (Fig. 1). The chlorhexidine plaque control programme maintained overall healthy gingival conditions. The gingival margins were located covering the CEJ without appreciable recession.

Radiographs obtained at euthanasia did not provide evidence for any appreciable adverse or other reactions in sites receiving the rhGDF-5/PLGA construct (Fig. 2). There were no appreciable differences in the radiographic appearance between sites receiving the rhGDF-5/PLGA construct and the sham-surgery control at 2, 4, 6, and 8 weeks post-surgery. Moreover, the radiographic appearance of the experimental sites did not differ from that observed at the untreated third pre-molar.

Histologic observations

Representative photomicrographs for the rhGDF-5/PLGA construct and sham-surgery control-treated sites at 2, 4, 6, and 8 weeks post-surgery are shown in Fig. 3. Healing along the defect root surface was characterized by a non-specific connective tissue attachment, acellular and cellular cementum, PDL, bone regeneration, and junctional epithelium. Fragments of the rhGDF-5/PLGA construct were observed in four of seven sites at the 2-week observation interval, two of eight sites at 4 weeks, and in one site within newly formed bone at 6 weeks. No residual PLGA was observed at the 8-week time point in any defect. Root resorption and ankylosis were not observed. Bone formation in sites receiving the rhGDF-5/PLGA construct presented an apparent increased maturity (lamellar bone) at the 6-week observation interval. There were no remarkable differences in cementum formation between treatments, both exhibiting similar patterns of cellular and acellular cementum formation, intrinsic, extrinsic, and mixed fibre cementum. None of the sites exclusively exhibited extrinsic fibre cementum characteristic of the native cementum. The density of the fibrous attachment was low, with collagenous fibres rarely organized perpendicular or oblique to the root surface. A junctional epithelium was present in some specimens without noteworthy differences between sites receiving the rhGDF-5/PLGA construct or control. Residual biomaterial was associated with inflammatory reactions including multinucleated cells surrounding the fragmented material. The inflammatory reactions had no obvious limiting effect on periodontal wound healing/regeneration.

Histometric analysis

The results of the histometric analysis are shown in Table 2. Because fragments of the rhGDF-5/PLGA construct observed at 2–6 weeks were too small and infrequent to measure and root resorption and ankylosis were not observed, the histometric evaluation was limited to defect height, cementum, PDL, bone regeneration (height, area, density), and junctional epithelium. Both protocols exhibited significant increases in cementum, PDL, and bone regeneration over time, without consistent significant differences between treatments. However, there were significant differences between treatments in cementum, PDL, and bone regeneration in time. Cementum and PDL regeneration were twofold greater for the sham-surgery control compared with the rhGDF-5/PLGA construct at the 4-week observation interval ($p = 0.04$). In contrast, there was a significant increase in bone regeneration (height: $p = 0.002$; area: $p = 0.009$; and density: $p = 0.008$) at sites implanted with rhGDF-5/PLGA compared with control at this observation interval.

Discussion

The main objectives of this study were to evaluate the clinical injectability and biocompatibility of a novel rhGDF-5/PLGA construct. The effect on periodontal wound healing/regeneration

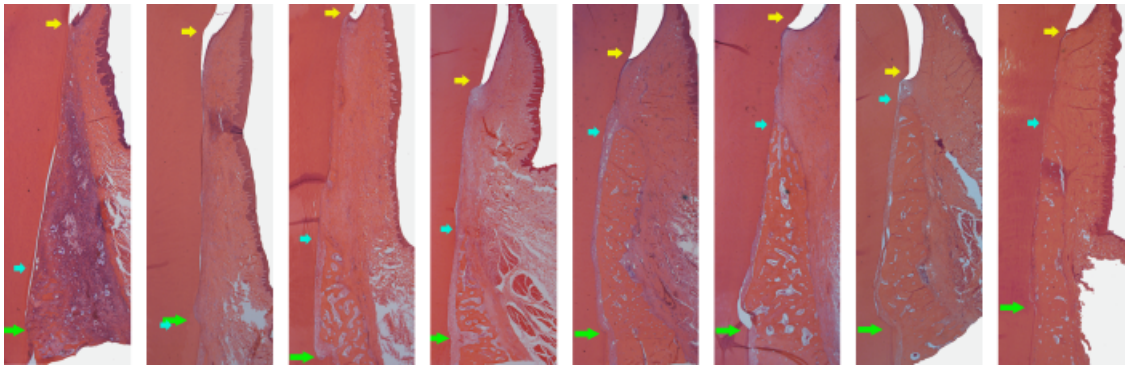

Fig. 3. Representative photomicrographs of experimental pre-molar sites implanted with recombinant human growth/differentiation factor-5 (rhGDF-5)/poly(lactic-*co*-glycolic acid) (PLGA) construct or serving as sham-surgery control at 2 (left), 4 (left centre), 6 (right centre), and 8 (right) weeks post-surgery. The left photomicrograph in each pair represents the rhGDF-5/PLGA construct. An inflammatory reaction likely assorted with biodegradation of the rhGDF-5/PLGA construct can be seen at 2 weeks. Generally, there are no other appreciable differences between the pre-molar site pairs. Green arrows represent the apical extension of the defects, blue arrows represent the coronal extension of newly formed alveolar bone, and the yellow arrows represent the location of the cemento-enamel junction.

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

	Defect height	Cementum regeneration	PDL regeneration	Bone regeneration (height)	Bone regeneration (area)	Bone regeneration (density)	Junctional epithelium
2-week							
rhGDF-5/PLGA	5.90 ± 0.20	0.34 ± 0.13	0.26 ± 0.10	0.76 ± 0.22	0.29 ± 0.08	36.79 ± 7.50	0.10 ± 0.10
Sham-surgery	6.22 ± 0.22	0.33 ± 0.16	0.28 ± 0.15	0.67 ± 0.44	0.46 ± 0.32	26.96 ± 5.55	0.11 ± 0.08
p-value	**0.03**	0.95	0.92	0.73	0.26	0.16	0.96
4-week							
rhGDF-5/PLGA	6.05 ± 0.32	0.63 ± 0.07	0.56 ± 0.07	3.52 ± 0.33	2.62 ± 0.46	60.74 ± 1.14	1.04 ± 0.36
Sham-surgery	5.67 ± 0.34	1.31 ± 0.69	1.24 ± 0.68	2.03 ± 0.82	1.47 ± 0.76	45.47 ± 10.78	0.50 ± 0.24
p-value	0.07	**0.04**	**0.04**	**0.002**	**0.009**	**0.008**	0.21
6-week							
rhGDF-5/PLGA	5.50 ± 0.41	1.88 ± 0.58	1.87 ± 0.58	3.90 ± 0.42	2.86 ± 0.25	78.86 ± 1.54	0.89 ± 0.30
Sham-surgery	5.63 ± 0.26	1.89 ± 0.63	1.92 ± 0.62	3.63 ± 0.30	2.40 ± 0.53	82.21 ± 1.46	0.45 ± 0.19
p-value	0.60	0.98	0.92	0.38	0.29	0.12	0.22
8-week							
rhGDF-5/PLGA	5.86 ± 0.37	1.62 ± 0.59	1.56 ± 0.61	4.67 ± 0.52	3.02 ± 0.45	79.45 ± 1.55	0.21 ± 0.14
Sham-surgery	5.63 ± 0.12	1.89 ± 0.56	1.92 ± 0.55	4.06 ± 0.27	3.29 ± 1.24	82.62 ± 2.81	0.55 ± 0.30
p-value	0.21	0.36	0.28	**0.047**	0.56	0.14	0.32

p-values < 0.05 signify bold numerals. PDL, periodontal ligament; rhGDF-5, recombinant human growth/differentiation factor-5; PLGA, poly(lactic-*co*-glycolic acid).

with an emphasis on safety evaluating rhGDF-5/PLGA-associated aberrant healing events was also examined using surgically induced periodontal pocket modelling in dogs. The observations suggest that the rhGDF-5/PLGA construct is easy to prepare and apply subgingivally. The rhGDF-5/PLGA construct appeared to be largely resorbed within 2 weeks, and it was not associated with appreciable adverse events. Importantly, the rhGDF-5/PLGA construct did not support the formation of an epithelial attachment or otherwise compromise periodontal wound healing/regeneration.

Buccal dehiscence defects have been used in earlier studies to evaluate aspects of periodontal wound healing/regeneration (Gottlow et al. 1994, Lundgren et al. 1995, Graziani et al. 2005). The present study used a canine buccal dehiscence defect/periodontal pocket model. The distance from the CEJ to the apical extent of the defect approximated 6 mm; the width of the defect encompassed the width of the respective roots, approximately 3–4 mm. There were no significant differences between rhGDF-5/PLGA and the sham-surgery control in regeneration of the periodontal attachment over 8 weeks. However, there were particular differences in cementum, PDL, and bone regeneration at 4 weeks. Bone formation height averaged 3.5 and 2.0 mm (58% and 35% of the defect height), respectively, for sites receiving rhGDF-5/PLGA and sham-surgery control. This observation points to significant early/accelerated bone formation following implantation of rhGDF-5/PLGA. This observation is consistent with that observed in several other skeletal settings including spine and long bones following application of rhGDF-5 constructs (for a review, see Moore et al. 2010) and may have importance for periodontal wound healing/regeneration as well.

Previous studies suggest that biomaterials, also including biodegradable polymers and ceramics, may obstruct bone formation and periodontal regen-

eration (Wikesjö & Nilvéus 1990, Trombelli et al. 1999, Pöhling et al. 2006). A recent study by Koo et al. (2007), however, showed that recombinant human transforming growth factor-β_1 accelerated degradation of the calcium carbonate biomaterial while not affecting periodontal wound healing/regeneration. In the present study, fragments of the PLGA biomaterial were observed at 2 weeks but could no longer be observed at 8 weeks. Residual biomaterial was associated with inflammatory reactions including multinucleated cells, a reaction that is typical for biomaterials based on poly-α-hydroxy acid technologies. However, importantly, the rhGDF-5/PLGA combination did not appear to exhibit appreciable limiting effects on periodontal wound healing/regeneration.

PLGA is a synthetic biodegradable polymer that has been used for surgical sutures, drug delivery systems, orthopaedic fixation devices, and tissue engineering scaffolds (Athanasiou et al. 1996, Guzman et al. 1996, Kang et al. 2005). All biodegradable polymers contain hydrolysable bonds. Therefore, their most important degradation mechanism is chemical degradation via hydrolysis or enzyme-catalysed hydrolysis. The latter effect is often referred to as biodegradation because the degradation process is mediated at least partially by a biological system (Vert et al. 1992). The process of degradation describes the chain scission process during which polymer chains are cleaved to form oligomers and monomers. Erosion means the loss of material leading to monomers and oligomers, which can leave the polymer. Polymer degradation is the key process of erosion (Tamada & Langer 1993). The processes involved in the erosion of a degradable polymer are fairly complicated and influenced by various factors such as the polymer properties (e.g., morphology, device dimensions, porosity, hydrophobicity, molecular weight, and molecular weight distribution) and the degrading environment (e.g., fluid supply, ionic strength, pH value) (Göpferich 1996, Anderson & Shive 1997). Degradation might also be influenced by an incorporated growth factor (Koo et al. 2007), which in turn may influence the local environment. PLGA dissolved in an organic material was chosen as the carrier for rhGDF-5 to approximate an appropriate degradation rate and protein release kinetics to support periodontal regeneration. Additional work to optimize the polymer carrier and rhGDF-5 release kinetics/dose might, however, be required before clinical application.

The rhGDF-5/PLGA construct and sham-surgery control sites were not associated with aberrant healing events including root resorption and ankylosis; in other words, rhGDF-5 did not induce root resorption/ankylosis in this defect model. A previous study evaluated periodontal wound healing/regeneration following surgical implantation of rhGDF-7 and rhBMP-2 soak-loaded onto an absorbable collagen sponge using the critical-size supra-alveolar periodontal defect model (Wikesjö et al. 2004). The rhGDF-7 treatment supported significant periodontal regeneration compared with that following application of rhBMP-2. In contrast to that observed following surgical application of rhGDF-5 herein and rhGDF-7 (Wikesjö et al. 2004), root resorption/ankylosis appears to be a common sequel of periodontal wound healing/regeneration in studies evaluating application of rhBMP-2 and rhBMP-7 using rodent, canine, and non-human primate defect models and a variety of candidate carriers (Sigurdsson et al. 1995, 1996, Ripamonti et al. 1996, Giannobile et al. 1998, Wikesjö et al. 1999, 2003a, b, c, King & Hughes 1999, Sorensen et al. 2004).

In conclusion, the rhGDF-5/PLGA construct appears to be a safe technology for injectable, ease-of-use application of rhGDF-5-stimulated periodontal wound healing/regeneration. Additional work to optimize the polymer carrier and rhGDF-5 release kinetics/dose might be required before evaluating the efficacy of this technology in clinical settings using minimally invasive approaches.

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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 the injectability, biocompatibility, safety, and periodontal wound healing/regeneration following application of rhGDF-5 in a bioresorbable poly(lactic-*co*-glycolic acid) (PLGA) carrier intended for minimally invasive therapy.

Principal findings: The rhGDF-5/PLGA construct was easy to prepare and apply, and rapidly biodegrades without appreciable adverse events or compromises periodontal wound healing/regeneration.

Practical implications: The rhGDF-5/PLGA construct appears to be a safe technology for injectable, ease-of-use application of rhGDF-5-stimulated periodontal wound healing/regeneration. Additional work to optimize the polymer carrier and rhGDF-5 release kinetics/dose might be required before evaluating the efficacy of this technology in clinical settings using minimally invasive approaches.