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A phase IIa randomized controlled clinical and histological pilot study evaluating rhGDF-5/β-TCP for periodontal regeneration

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Abstract

Aim: The primary objective of this study was to clinically and histologically evaluate periodontal wound healing/regeneration following surgical implantation of recombinant human growth/differentiation factor-5 (rhGDF-5) adsorbed onto a particulate β-tricalcium phosphate (β-TCP) carrier rhGDF-5/β-TCP into periodontal defects in man.

Material & Methods: Twenty chronic periodontitis patients, each with at least one tooth scheduled for extraction exhibiting a probing depth $\geq$6 mm and an associated intra-bony defect $\geq$4 mm participated in the study upon written informed consent. Subjects (one defect/patient) were randomized to receive open flap debridement (OFD) + rhGDF-5/β-TCP (n = 10) or OFD alone (control; n = 10). Block biopsies of the defect sites were collected at 6 months post-surgery and prepared for the histological evaluation. Two masked examiners evaluated the deepest aspect of each defect site relative to bone (height/area), periodontal ligament (PDL) and cementum regeneration, and residual β-TCP.

Results: Sites receiving rhGDF-5/β-TCP showed numerically greater PD reduction (3.7 ± 1.2 *versus* 3.1 ± 1.8 mm; $p = 0.26$), less gingival recession (0.5 ± 0.8 *versus* 1.4 ± 1.0 mm; $p < 0.05$) and greater clinical attachment level (CAL) gain (3.2 ± 1.7 *versus* 1.7 ± 2.2 mm; $p = 0.14$) at the deepest aspect of the defect compared with OFD alone. One biopsy in the rhGDF-5/β-TCP and four biopsies in the OFD group were deemed as not evaluable. Histologically, bone regeneration height was almost threefold greater for the rhGDF-5/β-TCP treatment compared with OFD alone (2.19 ± 1.59 *versus* 0.81 ± 1.02 mm; $p = 0.08$). Similarly an almost twofold increase was observed for PDL (2.16 ± 1.43 *versus* 1.23 ± 1.07 mm; $p = 0.26$), cementum (2.16 ± 1.43 *versus* 1.23 ± 1.07 mm; $p = 0.26$) and bone regeneration area (0.74 ± 0.69 *versus* 0.32 ± 0.47 mm^2; $p = 0.14$). Root resorption/ankylosis was not observed. Residual β-TCP occupied 8.4 ± 11.5% of the area of interest in biopsies of patients receiving rhGDF-5/β-TCP. Five biopsies (one rhGDF-5/β-TCP, four OFD) were deemed unsuitable to allow a meaningful histological or histometrical evaluation.

Conclusions: Descriptive statistics showed greater PD reduction and CAL gain, and greater alveolar bone regeneration and periodontal regeneration at sites that received rhGDF-5/β-TCP compared to control. However, these differences were not statistically significant. Future studies with larger sample sizes will have to be conducted to verify these findings.

Key words: bone; bone substitutes; histology; human; intra-bony; periodontal regeneration; recombinant human growth/dierentiation factor-5; β-tricalcium phosphate; surgical debridement; tissue engineering

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Conflict of Interest and source of funding

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Conventional periodontal therapy usually results in healthy periodontal tissues, including reduced probing depths and maintained attachment levels for decades, provided adequate oral hygiene standards are adopted and maintained (Lindhe & Nyman 1984, Axelsson et al. 2004). However, conventional periodontal therapy rarely translates into periodontal regeneration characterized by cementum formation, inserting functionally oriented collagen fibres and alveolar bone formation resulting in establishment of a periodontal ligament (PDL) of physiological width and composition (Wikesjö & Selvig 1999).

A number of treatment concepts have been introduced with the hypothesis that they somehow induce/support periodontal regeneration. These comprise various surgical approaches, including root surface conditioning schemes and/or implantation of a range of biomaterials, barrier devices, and matrix, growth and differentiation factors used as stand-alone protocols or in combinations (Bosshardt 2008, Trombelli & Farina 2008, Stavropoulos & Wikesjö 2010). In perspective, advances in cell and molecular biology have unveiled a number of biological mediators critical to development that potentially also support wound healing/regeneration and/or induce de novo tissue formation in conducive environments. Growth and differentiation factors associated with periodontal tissues and considered as candidate agents in support of periodontal wound healing/regeneration include platelet-derived growth factor, insulin-like growth factors-I and -II, acidic and basic fibroblast growth factors, transforming growth factor-β, and bone morphogenetic proteins (BMPs) (Lee et al. 2010a, Stavropoulos & Wikesjö 2010).

Recombinant human BMP-2 (rhBMP-2) and rhBMP-7 have been evaluated extensively for periodontal wound healing/regeneration in various settings using a variety of candidate carriers (Stavropoulos & Wikesjö 2010). Recent attention has been drawn to growth/differentiation factor-5 (GDF-5), another member of the BMP family (Hötten et al. 1994). The GDF-5 has been recognized as an important factor in limb development (Hötten et al. 1996, Buxton et al. 2001). The GDF-5 has also been expressed in bovine and rat tooth germs in cells associated with PDL formation and cells located along the alveolar bone and cementum surfaces during the course of root formation, suggesting that GDF-5 may play regulatory roles in the development of the periodontal attachment (Morotome et al. 1998, Sena et al. 2003). In vitro, GDF-5 enhances human PDL cell proliferation, affecting extracellular matrix metabolism in a dose-dependent order (Nakamura et al. 2003). In vivo application of GDF-5 is shown to induce and/or enhance tendon/ligament, cartilage, and/or bone tissue formation in rodent, porcine, canine and human clinical studies (Moore et al. 2010).

The rhGDF-5 adsorbed onto a particulate porous β-tricalcium phosphate (β-TCP) biomaterial (Pöhling et al. 2006), but also using other substrates, has been evaluated in several craniofacial settings using rodent screening and discriminating large animal models (Gruber et al. 2008, 2009, Schwarz et al. 2008, Kim et al. 2009, Weng et al. 2009, Kwon et al. 2010a,b,c, Lee et al. 2010b, Polimeni et al. 2010, Min et al. 2011), and in clinical trials (Koch et al. 2010, Stavropoulos et al. 2011). Implantation of rhGDF-5/β-TCP into intra-bony critical-size defects in dogs resulted in significant periodontal regeneration compared with β-TCP carrier controls without root resorption/ankylosis (Lee et al. 2010b). Collectively, these pre-clinical observations may indicate that rhGDF-5/β-TCP exhibits significant potential to support/induce periodontal wound healing/regeneration also in clinical settings. Thus, the primary objective of this study was to histologically evaluate periodontal wound healing/regeneration following surgical implantation of rhGDF-5/β-TCP into deep intra-bony periodontal defects in man.

Material and Methods

Patients

Twenty chronic periodontitis patients (mean age 48.8 ± 10.6 years; 16 males; four females) presenting to the Department of Periodontology, Semmelweis University, Budapest, Hungary, each exhibiting at least one tooth scheduled for extraction with a probing depth ≥ 6 mm and an associated intra-bony defect ≥ 4 mm following basic periodontal therapy, volunteered to participate in this phase IIa, stratified, randomized, open, controlled, parallel-group clinical trial (first patient enrolled in July 2007, last patient completed in August 2008). All patients received oral and written information about the research protocol, including treatment plan, clinical/surgical procedures and further treatment. The patients signed an informed consent form that provided the possibility of withdrawing from the study at any time and without any consequence for their further treatment. The study was planned and carried out in compliance with the Declaration of Helsinki and Good Clinical Practice, and was monitored by a Contract Research Organization (FGK Clinical Research GmbH, Münich, Germany). The study protocol was approved by the National Committee of Science and Research Ethics Of Medical Research Council and the Institutional Ethics Committee of Semmelweis University.

Teeth included in the study (one tooth/patient) exhibited an advanced, primarily 1- or 2-wall intra-bony periodontal defect (probing depth ≥ 6 mm – radiographic defect depth ≥ 4 mm) and were scheduled for extraction (i.e. deemed irrational/hopeless to treat) or hemisection due to advanced periodontal destruction and/or restorative considerations. The decision for extraction or hemisection was part of a treatment plan established by clinicians not involved in, or otherwise related to the study. Additional inclusion criteria were: (a) intra-bony defects had to be associated with maxillary/mandibular single-rooted teeth (mandibular incisors excluded)

or mandibular molars with a mesial or distal defect adjacent to an edentulous space; and (b) high oral hygiene standards, that is, absence of plaque at the defect site and full-mouth plaque and bleeding on probing scores $\leq 20\%$. Main exclusion criteria were: (a) teeth with narrow (<2 mm) inter-proximal spaces precluding surgical manipulations; (b) teeth with root concavities/furrows or furcation involvement degree II or greater; (c) teeth with periodontal abscess; d) teeth with endodontic pathology; (e) women of child-bearing potential, pregnant or lactating; (f) smoking; (g) previous (within last 2 months before screening) or current treatment with systemic corticosteroids of more than 5 mg/day prednisone equivalent; (h) previous or current therapy with drugs having any influence on bone metabolism, such as calcitonin, parathormone, bisphosphonates or fluoride within the last 12 months before screening; (i) common contra-indications for periodontal surgery and (j) clinically relevant cardiovascular, hepatic and renal diseases.

rhGDF-5/β-TCP

The investigated product consisted of two components: rhGDF-5 coated onto β-TCP. The coating density for the rhGDF-5/β-TCP construct was 500 μg rhGDF-5/g β-TCP (Pöhling et al. 2006).

Surgical procedures

Basic periodontal therapy, including oral hygiene instructions, scaling and root planing was completed within 8 weeks before final screening for inclusion in the study; if necessary, splinting of mobile teeth was completed prior to periodontal surgery. An experienced periodontal surgeon (PW) performed all surgeries using microsurgical instrumentation and magnification. Intra-crevicular incisions were made and the flaps were horizontally extended without vertical releasing incisions to accommodate the defect location and configuration and to ensure tension-free wound closure for primary intention healing. The surgical protocol, open flap debridement (OFD), was identical for all sites except for implantation of rhGDF-5/β-TCP into test sites (Fig. 1). The deepest site (mesiobuccal, buccal, distobuccal, mesiolingual, lingual or distolingual) of the intra-bony defect was determined using a periodontal probe (PCP 15; Hu-Friedy, Chicago, IL, USA) after debridement of the granulation tissue and involved root surfaces (Fig. 1). Thus determined deepest site was chosen as the site of analysis for all clinical and histological evaluations. For reasons of orientation/standardization during the histological procedures and evaluation, a vertical notch indicating the deepest site was prepared into the crown of the tooth coronal to the anticipated post-surgery position of the gingival margin using a flame-shaped diamond bur. A horizontal notch outlining the most apical extent of root planing (i.e. the base of the defect) was made onto the root surface using a Ø 1-mm diamond bur. A randomization code was used to assign defects to receive rhGDF-5/β-TCP or OFD. The randomization code was opened after defect preparation was completed to avoid clinical bias. Randomization was stratified according to the intra-bony depth of the periodontal defect. One random list was generated for intra-bony defect depth 4–6 mm, the other for intra-bony defect depth >6 mm. Defects receiving rhGDF-5/β-TCP were filled to the level of the alveolar crest using minimal compression. The mucoperiosteal flaps were positioned and stabilized for primary intention healing using monofilament sutures (B. Braun Melsungen AG, Melsungen, Germany), and the level of the gingival margin at the deepest site was recorded using the periodontal probe. Please refer to supporting information (Fig. S1) for additional radiographic and clinical, including surgical, images from representative cases. Post-surgery care included pain control (Nurofen, 200 mg, 3–4 times per day; Reckitt Benckiser, Slough, UK), systemic (Augmentin 625 mg, TID/7 days; GlaxoSmithKline, London, UK London, UK) and local (twice daily 0.2% chlorhexidine rinses, BID/4 weeks) antimicrobial control, and a series of recall appointments (biweekly the first 6 weeks and then monthly until the end of the

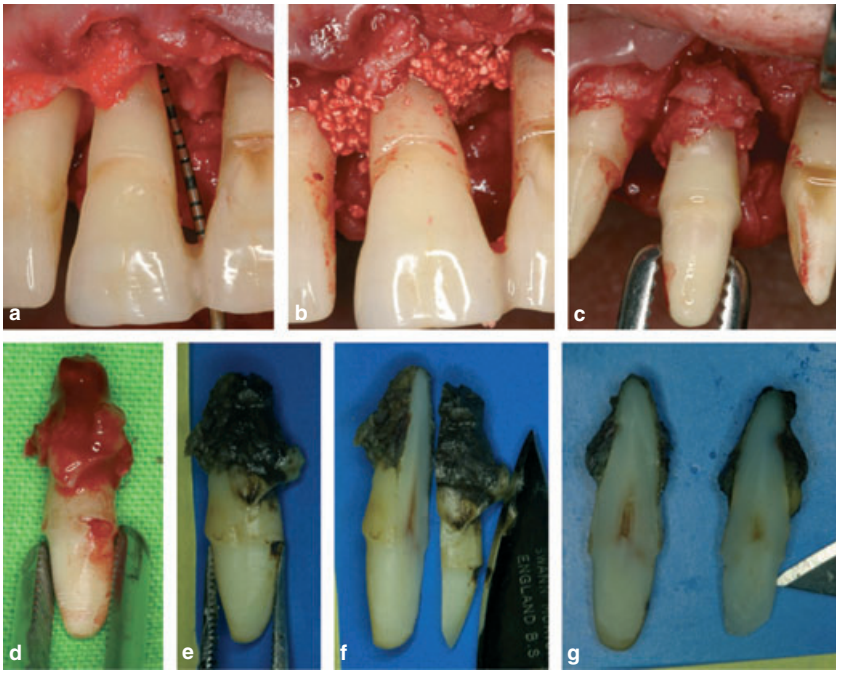

Fig. 1. (a) Intra-bony defect site (≥ 4 mm) implanted with recombinant human growth/differentiation factor-5/β-tricalcium phosphate (rhGDF-5/β-TCP). (b) The defect is implanted with loosely packed rhGDF-5/β-TCP to the level of the adjoining alveolar bone. (c, d) A limited block biopsy including, the defect site and adjoining tissues is removed following 6 months healing interval. (e–g) Upon completed demineralization, the block biopsy is split into two along long axis of the tooth exactly at the vertical notch, indicating the deepest aspect of the intra-bony component, providing two specimen blocks.

study) including, professional supragingival plaque control. Sutures were removed at day 14. Additional appointments including professional supragingival tooth cleaning were scheduled.

Surgical biopsies

The investigator recording the clinical parameters at baseline, re-recorded the same parameters at 6 months post-surgery without having access to patient journal material except study tooth number; and then, upon local anaesthesia, mucoperiosteal flaps were raised and the experimental roots/teeth and immediately adjoining soft and hard tissues, that is, the periodontal tissues including, the margins of the original intra-bony defect, were carefully removed without damage to neighbouring teeth and vital anatomical structures (Fig. 1). Resulting defects were managed to restore hard and soft tissue contours and to install dental implants as indicated to support fixed prosthetic rehabilitation according to individualized treatment plans.

Histotechnical procedures

The block biopsies were fixed in 10% buffered formalin, decalcified in EDTA, dehydrated in graded ethanol series and embedded in paraffin. Immediately prior to embedding, the roots/teeth were split in half along their long axis at the vertical notch indicating the deepest aspect of the intra-bony defect (Fig. 1). Thus, histological sections representing the deepest aspect of the defect were obtained. Twenty sections from each of the two specimen blocks were obtained using a microtome set at 8 μm, the sections subsequently stained using van Giesons' picro fuchsin or the oxone-aldehyde-fuchsin-Halmi stain (Fullmer et al. 1974).

Histological and histometrical analysis

Two experienced examiners (A. Stavropoulos, U. Wikesjö), masked in regard with biopsy/treatment group relation, conducted the histological and histometrical analysis using a computer-assisted toolbox (VIS; Visiopharm A/S, Hørsholm, Denmark) while viewing the section (s) with the highest technical quality on an LCD flat screen with live stream of images captured by a digital camera (Olympus DP 71; Olympus Denmark AS, Ballerup, Denmark) adapted to a light microscope (Olympus DH 50; Olympus Denmark AS). The following parameters were recorded:

- Epithelial attachment (mm): distance between the gingival margin and the apical extent of the junctional epithelium.
- Cementum regeneration (mm): distance between the apical extension of root planing and coronal extension of a continuous layer of new cementum or cementum-like deposit on the planed root.
- Periodontal ligament regeneration (mm): distance between the apical extension of root planing and coronal extension of a functionally oriented PDL on the planed root.
- Bone regeneration height (mm): distance between the apical extension of root planing and coronal extension of regenerated alveolar bone along the planed root.
- Bone regeneration area (mm^2): area represented by new alveolar bone (including marrow spaces) along the planed root within a standardized area of interest (AoI). The AoI was defined as an open rectangle (2.5 × 1 mm, height × width) juxtaposed to the root with its base located immediately coronally to the apical extension of the root planing and its side paralleling the root surface.
- Bone regeneration density (%): fraction mineralized bone within AoI.
- Residual β-TCP density (%): fraction residual biomaterial within bone or AoI.
- Root resorption (mm): sum of linear distances of distinct resorption lacunae on the planed root.
- Ankylosis (mm): sum of linear distances of ankylotic union between the regenerated alveolar bone and the planed root.

In addition, the maturity of the regenerated bone was estimated using a six-point scale: 0 = no bone; 1 = few woven bone trabeculae with minimal contact to β-TCP; 2 = woven bone trabeculae with osteoblasts and broad osteoid, β-TCP present; 3 = lamellar bone trabeculae, narrow osteoid, β-TCP present; 4 = areas of Haversian bone, β-TCP grossly degraded and 5 = bone completely remodelled, β-TCP not present. Evidence of inflammation, foreign body reaction associated with the β-TCP particles or multinuclear osteoclast-like cells within the regenerated bone were estimated using a six-point scale: 0 = not present; 1 = minimal; 2 = slight; 3 = moderate; 4 = marked and 5 = severe.

Statistical analysis

The data were analysed using descriptive methods. Comparisons between treatments were conducted using parametric (two-sided *t*-test) or non-parametric (two-sided Wilcoxon test) statistics for the clinical and histometrical data, respectively. The 95% confidence intervals were calculated as well. For all tests, a significance level of 5% was applied. Due to the explorative character of the study, no adjustment of type I error was performed. Safety parameters were analysed descriptively. SAS Version 9.1.3© 2002–2003 (SAS Institute Inc., Cary, NC, USA) was used for the statistical analysis.

Results

Baseline and post-surgery clinical recordings are shown in Table 1. There were no relevant clinical differences between the treatment groups at baseline. Full-mouth plaque scores averaged 7.3 ± 6.7% for the rhGDF-5/β-TCP and 8.9 ± 4.8% for the OFD control. Full-mouth bleeding scores averaged 11.2 ± 4.1% and 12.7 ± 5.4% for the rhGDF-5/β-TCP and the OFD control, respectively. In each group, five patients presented with an intra-osseous component of 4–6 mm and five patients with an intra-osseous component >6 mm.

Healing progressed without complications, including suture-line or flap dehiscences. Minor adverse events, such as mild post-surgery swelling and pain – apparently unrelated to the rhGDF-5/β-TCP – were observed. Both rhGDF-5/β-TCP and

Table 1. Clinical (baseline and 6 months) and histometrical recordings for patients/sites receiving rhGDF-5/β-TCP and OFD

Group	Patient no.	Tooth	PD baseline	CAL baseline	IC	PD 6 months	CAL gain 6 months	JE	NC	PDL	Bone			β-TCP (%)
						mm					height (mm)	area (mm^2)	density (%)	
rhGDF-5/β-TCP	2	35	8	12	7	3	5	2.84	2.98	2.98	3.79	0.72	95.00	2.00
	3	36*	9	13	5	5	4	2.60	1.45	1.45	2.77	2.20	80.00	0.00
	6	11	9	12	4	6	4	3.48	2.91	2.91	2.53	1.38	85.00	8.00
	10	34	9	11	5	5	3	2.78	0.95	0.95	2.17	0.79	95.00	2.00
	13	12	8	11	4	5	6	2.05	4.99	4.99	5.02	0.43	99.00	37.00
	14	23	11	12	11	7	3		1.81	1.81	1.88	0.26	100.00	7.00
	16	21	10	12	7	7	2	3.94	1.80	1.80	1.03	0.72	78.00	9.00
	17	23	11	15	11	5	0	3.35	0.00	0.00	0.0	0.00	0.00	0.00
	18	21	7	10	4	5	2	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
	19	45	9	14	9	6	3	1.42	2.52	2.52	0.48	0.12	75.00	11.00
	Mean ± SD		9.1 ± 1.3	12.2 ± 1.5	6.7 ± 2.8	5.4 ± 1.2	3.2 ± 1.7	2.8 ± 0.8	2.2 ± 1.4	2.2 ± 1.4	2.2 ± 1.6	0.7 ± 0.7	78.6 ± 30.9	8.4 ± 11.5
OFD	1	21	9	10	4	7	1	4.29	0.32	0.32	0.69	0.32	93.0	-
	4	12	9	11	7	5	4	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	-
	7	11	12	13	7	6	4	4.04	1.48	1.48	2.20	0.37	100.0	-
	8	23	8	9	5	5	1	1.57	0.96	0.96	0.00	0.00	0.0	-
	9	22	8	10	5	5	0	1.96	0.00	0.00	0.00	0.00	0.0	-
	11	35	7	8	5	6	−1	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	-
	12	44	13	17	9	7	5	3.00	2.99	2.99	1.96	1.20	78.0	-
	15	34	8	15	9	5	3		1.60	1.60	0.00	0.00	0.0	-
	20	35	12	15	9	10	1	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	-
	21	11	6	6	4	5	−1	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	-
	Mean ± SD		9.2 ± 2.3	11.4 ± 3.5	6.4 ± 2.1	6.1 ± 1.6	1.7 ± 2.2	3.0 ± 1.2	1.2 ± 1.1	1.2 ± 1.1	0.8 ± 1.0	0.3 ± 0.5	45.2 ± 50.0	-
	p-value**		0.79	0.43	0.97	0.44	0.14	0.74	0.26	0.26	0.08	0.14	0.26	-

CAL, clinical attachment level; IC, intra-bony defect depth; JE, junctional epithelium; NC, cementum regeneration; OFD, open flap debridement; PD, probing depth; PDL, periodontal ligament regeneration; rhGDF-5/β-TCP, recombinant human growth/differentiation factor-5/β-tricalcium phosphate.
*Distal side.
**rhGDF-5/β-TCP *versus* OFD.

OFD protocols resulted in statistically significant clinical improvements reflected by PD reduction ($p < 0.001$) and CAL gain ($p < 0.001$ and $p = 0.03$). Sites receiving rhGDF-5/β-TCP showed numerically greater PD reduction (3.7 ± 1.2 *versus* 3.1 ± 1.8 mm; $p = 0.26$), less gingival recession (0.5 ± 0.8 *versus* 1.4 ± 1.0 mm; $p < 0.05$) and greater CAL gain (3.2 ± 1.7 *versus* 1.7 ± 2.2 mm; $p = 0.14$) at the deepest aspect of the defect compared with OFD alone.

Five biopsies (one rhGDF-5/β-TCP, four OFD) were deemed unsuitable for histological or histometrical evaluation; either the tissues approximating the site of interest were missing, or the apical extent of the defect was missing, or the apical extent of root instrumentation could not with certainty be determined. The results of the histometrical evaluation of 15 samples (nine rhGDF-5/β-TCP, six OFD) – average values and for each individual site – are shown in Table 1. Representative photomicrographs from sites receiving rhGDF-5/β-TCP or OFD alone are shown in Figs 2–5.

Table 2 shows the results of the histopathological evaluation of the biopsies regarding the maturity of newly formed bone and presence of any inflammatory reactions, foreign body reactions associated with the β-TCP implant, or multinuclear osteoclast-like cells. No apparent qualitative differences between the treatments were detected – except the presence of residual β-TCP particles in some specimens receiving rhGDF-5/β-TCP. In most cases, there were no or only minor remarkable observations.

New cellular cementum with inserting functionally oriented collagen fibres and various amounts of new bone were observed coronally to the apical extent of root instrumentation in all but one site receiving rhGDF-5/β-TCP. With the one exception exhibiting a non-specific connective tissue attachment, an epithelial attachment immediately adjoined the coronal extension of the new cementum; new cementum/PDL formation averaging 2.2 ± 1.4 mm. Regenerated bone was observed in eight of nine evaluable biopsies from defect sites receiving rhGDF-5/β-TCP, new bone formation height averaging 2.2 ± 1.6 mm.

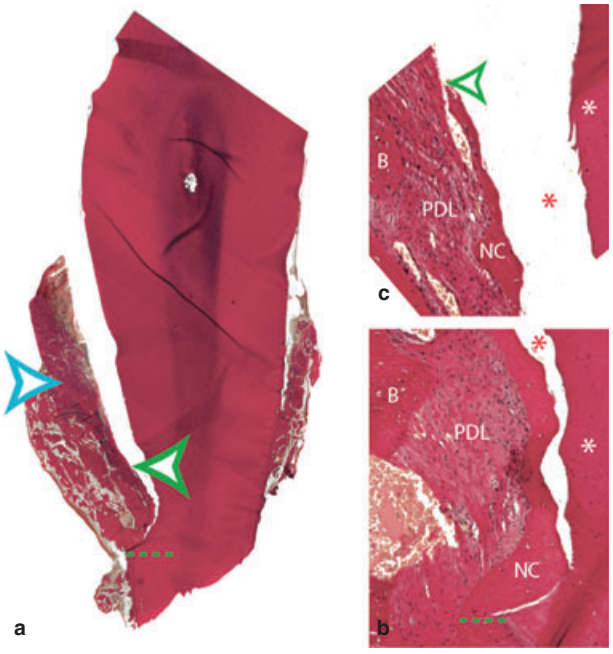

Fig. 2. Photomicrograph of defect site receiving recombinant human growth/differentiation factor-5/β-tricalcium phosphate (rhGDF-5/β-TCP) [overview (a) and 90× magnifications of the apical (b) and coronal (c) aspects of the defect site] showing new cellular cementum (NC) gradually thinning in a coronal direction. New bone (B) formation reaches a somewhat higher than visible cementum formation. Residual β-TCP particles cannot be observed. The green line indicates the apical termination of root instrumentation; arrowheads indicate the coronal extension of new cementum (green) and bone (blue) formation. The white asterisk (*) indicates the root; the red asterisk (*) indicates an artefactual split between the new cementum and the root (van Gieson's pichro fuchsin).

The newly formed alveolar bone appeared rather dense including trabecular woven bone with few areas of lamellar bone and few osteoclast-like cells. Root resorption/ankylosis was not observed. Residual β-TCP was limited (8.4 ± 11.5%), in many cases, completely embedded within the newly formed alveolar bone (Fig. 4) and apparently, did not compromise periodontal or bone regeneration. Nevertheless, β-TCP particles surrounded by non-specific connective tissue with apparently limited relation to new bone formation were occasionally observed (Fig. 4). Residual β-TCP was not associated with signs of inflammation or foreign body reactions. Cementum regeneration averaged 1.2 ± 1.1 mm in sites receiving OFD alone. Regenerated bone was seen in three of six evaluable specimens, mean bone regeneration height averaging 0.8 ± 1.0 mm. The new alveolar bone appeared rather dense and consisted mostly of woven bone with a few areas of lamellar bone (Fig. 5). Root resorption/ankylosis was not observed. Only minimal evidence of inflammation was observed. The analysis did not reveal any statistically significant differences between the two groups regarding any histometrical parameter.

Discussion

The results from this study suggest that surgical implantation of rhGDF-5/β-TCP into intra-bony periodontal defects might support/enhance periodontal wound healing/regeneration. Mean cementum and bone regeneration was approximately twofold and threefold greater for the sites receiving rhGDF-5/β-TCP compared with OFD alone. With one exception, periodontal regeneration was observed in all evaluable specimens from sites implanted with rhGDF-5/β-TCP, whereas only half of the evaluable sites receiving OFD showed some amounts of regeneration. The lack of statistically significant differences between the groups may likely reflect the reduced number of patients/evaluable biopsies. Furthermore, considering that only severely compromised teeth slated for extraction in the overall treatment plan were included in the study and that such teeth might be expected to have a compromised regenerative potential, the results are encouraging.

The assumption that rhGDF-5/β-TCP supports/enhances periodontal regeneration corroborates pre-clinical studies using discriminating large animal models (Kwon et al. 2010b,c, Lee et al. 2010b). The rhGDF-5/β-TCP was evaluated using a critical-size, intra-bony periodontal defect model in dogs. Histometrical evaluation following an 8-week healing interval showed that sites receiving rhGDF-5/β-TCP exhibited statistically significantly enhanced bone and cementum regeneration compared with β-TCP and sham-surgery controls (Lee et al. 2010b). In a second study employing the same model, rhGDF-5/β-TCP and a clinical benchmark recombinant, human platelet-derived growth factor in a β-TCP carrier (rhPDGF/β-TCP; GEM21S, Osteohealth®, Shirley, NY, USA) were compared (Kwon et al. 2010c). Sites receiving rhGDF-5/β-TCP exhibited statistically significantly enhanced bone and cementum regeneration compared with sites receiving the rhPDGF/β-TCP benchmark. Individually and collectively, these pre-clinical studies and the present first clinical/histological study in man, point to the potential of rhGDF-5/β-TCP to support/enhance periodontal regeneration.

In the present study, conventional periodontal flap surgery (OFD) was chosen as the control. Previous reports from human histological studies (Listgarten & Rosenberg 1979, Bowers et al. 1989a,b) suggest that wound healing following OFD is characterized by the formation of an epithelial attachment over the periodontally involved portion of the tooth, while some new bone formation may occur. However, recently accumulated observations from a series of experimental studies indicate that periodontal wound healing, provided: wound stability allowing uneventful adsorption/adhesion and unimpeded maturation of a fibrin clot onto the instrumented root sur-

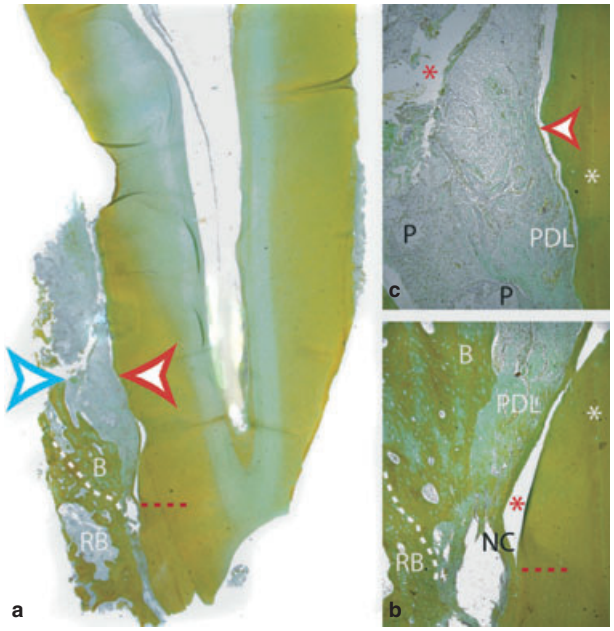

Fig. 3. Photomicrograph of defect site receiving recombinant human growth/differentiation factor-5/β-tricalcium phosphate (rhGDF-5/β-TCP) [overview (a) and 90× magnifications of the apical (b) and coronal (c) aspects of the defect site] showing a thin new cellular cementum (NC) throughout the biopsy and β-TCP particles (P) without apparent association with bone formation. The red line indicates the apical termination of root instrumentation; arrowheads indicate the coronal extension of new cementum (red) and bone (blue) formation. The white asterisk (*) indicates the root; the red asterisk (*) indicates an artefactual split between the new cementum and the root; RB indicates resident bone (Halmi).

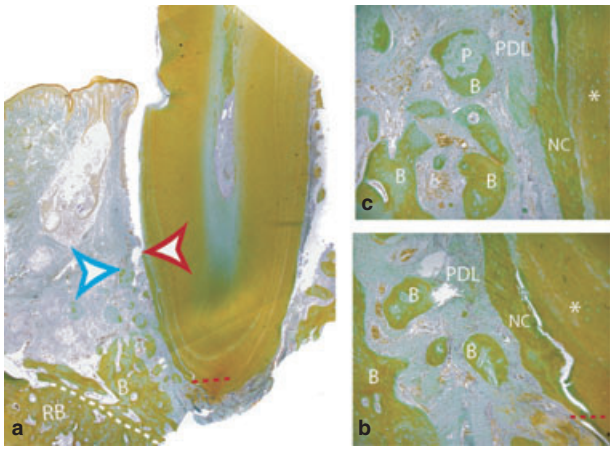

Fig. 4. Photomicrograph of defect site receiving recombinant human growth/differentiation factor-5/β-tricalcium phosphate (rhGDF-5/β-TCP) [overview (a) and 90× magnifications of apical (b) and central (c) aspects of the defect site], showing a new cellular cementum (NC) throughout the biopsy and bone formation (B) approaching the level of the newly formed cementum. A few β-TCP particles (P) completely embedded in new bone are shown while in the more lateral aspects of the defect, β-TCP without apparent association with bone formation are shown. The red line indicates the apical termination of root instrumentation; arrowheads indicate the coronal extension of new cementum (red) and bone (blue) formation. The white asterisk (*) indicates the root; RB indicates resident bone (Halmi).

face; unobstructed space provision allowing formation and maturation of a periodontal regenerate; and measures favoring primary intention healing, that is, prohibiting infection of the periodontal regenerate, may well support periodontal regeneration including, an intrinsic/mixed fibre cementum, a functionally oriented PDL and alveolar bone (Polimeni et al. 2006, 2009). Indeed, three of six evaluable biopsies in the present study showed some amounts of periodontal regeneration, supporting the notion that careful wound manipulation and post-surgery follow-up thus appear to be sufficient to support periodontal regeneration also following OFD. In perspective, it has been shown that using a minimally invasive surgical technique, for example, M-MIST (Cortellini & Tonetti 2011), that addresses the above biological directives, substantial clinical and radiographic resolution of deep intra-bony periodontal defects can be achieved. In this study, the addition of an enamel matrix derivative or the enamel matrix derivative in combination with a bovine bone biomaterial did not further enhance treatment outcomes (Cortellini & Tonetti 2011).

Lack of a β-TCP control might be considered a weak point in the present study, however, a β-TCP control was not deemed necessary due to the pilot nature of this human clinical/histological study. As discussed earlier, the results from pre-clinical studies using a critical-size, intra-bony periodontal defect model (Kim et al. 2004) evaluating rhGDF-5/β-TCP *versus* β-TCP and sham-surgery (Lee et al. 2010b), and rhGDF-5/β-TCP *versus* the rhPDGF/β-TCP benchmark (Kwon et al. 2010c) clearly demonstrate that rhGDF-5/β-TCP significantly enhances periodontal regeneration, whereas β-TCP limitedly contributes to periodontal wound healing/regeneration (Lee et al. 2010a). Human histological case reports have shown no or only limited periodontal regeneration following implantation of β-TCP into intra-bony defects (Stahl & Froum 1986, Froum & Stahl 1987, Stavropoulos et al. 2010)) In a five-case report, only limited new cellular cementum with inserting collagen fibres and bone formation was observed, while the majority of β-TCP particles remained embedded in connective tissue (Stavropoulos et al. 2010). Collectively these observations point to the limited regenerative potential of β-TCP in periodontal settings. Nevertheless, a β-TCP con-

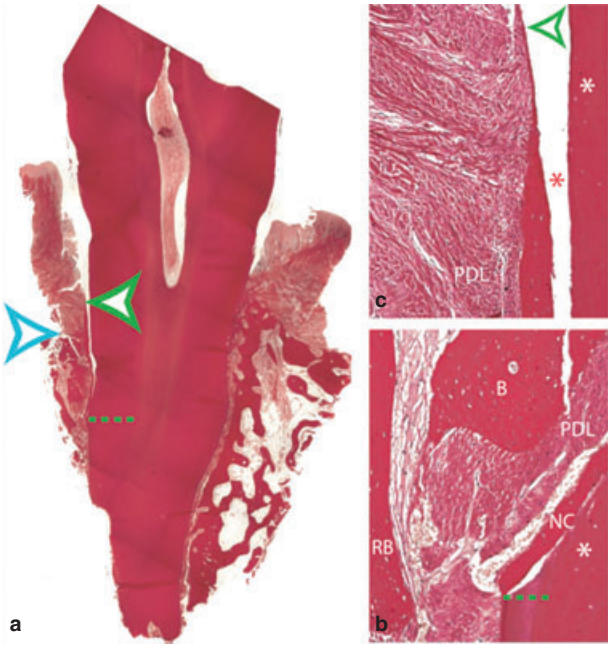

Fig. 5. Photomicrograph of defect site receiving open flap debridement [overview (a) and 90× magnifications of the apical (b) and coronal (c) aspects of the defect site], showing new cellular cementum (NC) reaching a significantly higher level than the adjoining regenerated alveolar bone (B). The green line indicates the apical termination of root instrumentation; arrowheads indicate the coronal extension of new cementum (green) and bone (blue) formation. The white asterisk (*) indicates the root; the red asterisk (*) indicates an artefactual split between the new cementum and the root; RB indicates resident bone (van Giesons' pichro fuchsin).

trol would appear desirable, if not required, when evaluating rhGDF-5/β-TCP in clinical pivotal studies.

The significance of the carrier material for the outcomes of periodontal wound healing/regeneration or bone formation *per se* has been demonstrated in studies evaluating rhBMP-2 (Sigurdsson et al. 1996, Sorensen et al. 2004). The composition (ceramic or polymer), structural integrity, bioresorption/biodegradation, and release kinetics, all represent factors that significantly may influence new tissue formation. Whereas ceramic non-resorbable bone derivatives or synthetic ceramics may displace and/or obstruct bone formation, polymeric biomaterials belonging to the poly(α-hydroxy ester) grouping may accumulate inflammatory reactions resorbing newly formed and resident bone (Polimeni et al. 2008). The irregular micro/macroporous β-TCP granules in the present study apparently releases rhGDF-5 to support bone formation and maturation within a relatively short interval without remarkably interfering with periodontal regeneration or supporting adverse events including, root resorption/ankylosis or significant foreign body inflammatory reactions. The amount of residual β-TCP carrier juxtaposed the root surface in the present group of biopsies was generally small (mean 8.4%), suggesting that this carrier would completely degrade and/or resorb within a relatively short interval (LeGeros 1993).

The rhGDF-5 dosing was not addressed in the present study. Dosing of any biological relates to properties of the agent, properties of the carrier (buffer/matrix) including release kinetics, and related bioresorption/biodegradation, as well as unique characteristics of the recipient site, that is, periodontal, alveolar/peri-implant, sinus, etc. Dosing also implies temporal considerations, what is the most advantageous time for release of the biological agent relative to the target tissues – bone and periodontal ligament – following wounding. A dose range study evaluating rhGDF-5 in an absorbable collagen sponge (ACS) carrier using the periodontal intra-bony defect model herein, did not reveal significant differences over a large rhGDF-5 dose range (including the dose used herein), however, all effective compared with control (Kim et al. 2009). The coating density of rhGDF-5/β-TCP in the present study was 500 μg rhGDF-5/g β-TCP. When rhGDF-5/β-TCP has been used at this concentration for sinus augmentation (Koch et al. 2010, Stavropoulos et al. 2011) similar amounts of bone formation were produced as those following an autogenous bone/β-TCP composite. Notably, no differences in bone formation following sinus augmentation with rhGDF-5/β-TCP at 400 or 800 μg/g β-TCP were observed using a mini-pig model, a species with a bone metabolic rate similar to humans (Gruber et al. 2009). Although no claims can be made regarding optimal dosing of rhGFD-5 for periodontal indications on the basis of the present or previous studies, they all with no exception point to an undisputable potential of rhGDF-5 to enhance/accelerate wound healing/regeneration in craniofacial settings and elsewhere in the appendicular and axial skeleton (Moore et al. 2010).

Consistent with preceding preclinical evaluations (Kwon et al. 2010b,c, Lee et al. 2010b), no significant adverse events were observed following application of rhGDF-5/β-TCP. This is of particular importance for the continued clinical evaluation, acceptance and use of rhGDF-5/β-TCP for periodontal indications. In perspective, root resorption/ankylosis appears a common observation evaluating other BMP technologies, which in turn suggest a limitation for their potential use for periodontal indications (Stavropoulos & Wikesjö 2010). In contrast to the preceding pre-clinical studies (Kwon et al. 2010b,c, Lee et al. 2010b) and the present study evaluating rhGDF-5/β-TCP, a PDL did not develop following application of rhBMP-2 in various carriers. Rather, new tissue formation included a cellular, cementum-like tissue, alveolar bone and interposing loose fibrovascular tissue/marrow with little, if any, evidence of functionally oriented collagen fibres, and limited root resorption/ankylosis eventually morphing into bone, fatty marrow and associated advancing

Table 2. Histopathological assessment by treatment, rhGDF-5/β-TCP *versus* OFD

	rhGDF-5/β-TCP	OFD
Evaluable biopsies	9/10	6/10
Maturity of newly formed bone		
0 = no new bone formation	1	3
1 = few woven bone trabeculae with minimal contact to residual β-TCP	0	0
2 = woven bone trabeculae with active osteoblasts and broad osteoid, residual β-TCP present	1	1
3 = lamellar bone trabeculae, narrow osteoid, residual β-TCP present	3	0
4 = few areas of Haversian bone, residual β-TCP grossly degraded	3	1
5 = completely remodelled, no residual β-TCP present	1	1
Histological signs of inflammation		
0 = not present	0	0
1 = minimal	6	5
2 = slight	1	0
3 = moderate	1	0
4 = marked	1	1
5 = severe	0	0
Foreign body reaction around β-TCP particles		
0 = not present	8	6
1 = minimal	1	0
2 = slight	0	0
3 = moderate	0	0
4 = marked	0	0
5 = severe	0	0
Multinuclear osteoclastic cells in the regenerated bone		
0 = not present	9	6
1 = minimal	0	0
2 = slight	0	0
3 = moderate	0	0
4 = marked	0	0
5 = severe	0	0

OFD, open flap debridement; rhGDF-5/β-TCP, recombinant human growth/differentiation factor-5/β-tricalcium phosphate.

root resorption/ankylosis replacing the tooth structures (Wikesjö et al. 2003). One could speculate that a similar situation might apply to rhGDF-5/β-TCP. This seems, however, unlikely as in contrast to the above-mentioned studies, the newly formed tissues in the present set of biopsies – similar to that observed in the pre-clinical evaluations – included a PDL exhibiting obliquely or perpendicularly oriented collagenous fibres inserting into a newly formed cellular cementum and alveolar bone, that is, exhibited features of a native PDL.

When evaluating the outcome of regenerative procedures using human biopsies, one has to bear in mind that the clinical and laboratory handling of the samples, including the biopsy harvesting procedure, may play important roles for the unbiased evaluation. First, defect sites at near terminal teeth vary considerably in extent, configuration, and soft tissue coverage in itself contributing a significant variability to any histometrical evaluation. Then, depending on defect dimensions, presence/absence of neighbouring teeth, as well as tooth localization within the mouth (anterior *versus* posterior), the ability to place an adequate root notch – indicating the apical extent of the defect – to be used for the histometrical evaluation is also variable. Next, in addition to the dexterity/proficiency of the surgeon, anatomical (presence of neighbouring teeth and narrow *versus* wide inter-proximal space), aesthetic (anterior *versus* posterior) and prosthetic concerns (need *versus* no need to preserve portion of the tooth) dictate the "topographical" region (i.e. the biopsy represents all or part of the original intra-bony defect) and the extent of tissues available for evaluation. In the present study, the surgeon had extensive experience with human periodontal biopsy procedures and the various steps during clinical/histological handling were arranged that the obtained sections represented the deepest aspect of the defect prior to treatment. In this context, one may argue that histometrical measurements performed on one section chosen out of twenty 8-μm sections obtained from each of the two specimen blocks, introduced some bias in the present evaluation. However, section selection was based solely on the technical quality of the section (limited or no artifacts) and not on preferential selection of the section with the best/greatest histological outcome. In addition, rather limited variation in the regenerative response should be expected within this narrow (320-μm) fraction of the defect space representing its deepest aspect. Finally, teeth included in human biopsy studies are usually deemed irrational to treat – among other reasons – due to advanced periodontal disease; thus, as already mentioned, the regenerative potential at such sites might be limited and may not at all represent the target population considered for the candidate technology under evaluation. The teeth included in the present study despite advanced disease, were all deemed as having reasonable potential for regeneration due to the deep intra-bony component and the 1-/2-wall configuration. Indeed, variable amounts of cementum and periodontal ligament regeneration were observed in five of six control biopsies, indicating the significant innate potential of the periodontium for regeneration.

In conclusion, descriptive statistics showed greater CAL gain and PD reduction, and greater alveolar bone regeneration and periodontal regeneration at sites that received rhGDF-5/β-TCP compared to control. However, these differences were not statistically significant. Future studies with larger sample sizes will have to be conducted to verify these findings.

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References

Axelsson, P., Nyström, B. & Lindhe, J. (2004) The long-term effect of a plaque control program on tooth mortality, caries and periodontal disease in adults. Results after 30 years of maintenance. *Journal of Clinical Periodontology* **31**, 749–757.

Bosshardt, D. D. (2008) Biological mediators and periodontal regeneration: a review of enamel matrix proteins at the cellular and molecular levels.*Journal of Clinical Periodontology* **35** (8 Suppl), 87–105.

Bowers, G. M., Chadroff, B., Carnevale, R., Mellonig, J., Corio, R., Emerson, J., Stevens, M. & Romberg, E. (1989a) Histologic evaluation of new attachment apparatus formation in humans. Part I. *Journal of Periodontology* **60**, 664–674.

Bowers, G. M., Chadroff, B., Carnevale, R., Mellonig, J., Corio, R., Emerson, J., Stevens, M. & Romberg, E. (1989b) Histologic evaluation of new attachment apparatus formation in humans. Part III. *Journal of Periodontology* **60**, 683–693.

Buxton, P., Edwards, C., Archer, C. W. & Francis-West, P. (2001) Growth/differentiation factor-5 (GDF-5) and skeletal development. *The Journal of Bone and Joint Surgery* American Volume **83-A** Suppl. 1, S23–S30.

Cortellini, P. & Tonetti, M. (2011) Clinical and radiographic outcomes of the modified minimally invasive surgical technique with and without regenerative materials: a randomized-controlled trial in intra-bony defects. *Journal of Clinical Periodontology* **38**, 365–373.

Froum, S. & Stahl, S. S. (1987) Human intraosseous healing responses to the placement of tricalcium phosphate ceramic implants. II. 13 to 18 months. *Journal of Periodontology* **58**, 103–109.

Fullmer, H. M., Sheetz, J. H. & Narkates, A. J. (1974) Oxytalan connective tissue fibers: a review. *Journal of Oral Pathology* **3**, 291–316.

Gruber, R. M., Ludwig, A., Merten, H. A., Achilles, M., Pöhling, S. & Schliephake, H. (2008) Sinus floor augmentation with recombinant human growth and differentiation factor-5 (rhGDF-5): a histological and histomorphometric study in the Göttingen miniature pig. *Clinical Oral Implants Research* **19**, 522–529.

Gruber, R. M., Ludwig, A., Merten, H. A., Pippig, S., Kramer, F. J. & Schliephake, H. (2009) Sinus floor augmentation with recombinant human growth and differentiation factor-5 (rhGDF-5): a pilot study in the Göttingen miniature pig comparing autogenous bone and rhGDF-5. *Clinical Oral Implants Research* **20**, 175–182.

Hötten, G. C., Matsumoto, T., Kimura, M., Bechtold, R. F., Kron, R., Ohara, T., Tanaka, H., Satoh, Y., Okazaki, M., Shirai, T., Pan, H., Kawai, S., Pohl, J. S. & Kudo, A. (1996) Recombinant human growth/differentiation factor 5 stimulates mesenchyme aggregation and chondrogenesis responsible for the skeletal development of limbs. *Growth Factors* **13**, 65–74.

Hötten, G., Neidhardt, H., Jacobowsky, B. & Pohl, J. (1994) Cloning and expression of recombinant human growth/differentiation factor 5. *Biochemical and Biophysical Research Communications* **204**, 646–652.

Kim, C. S., Choi, S. H., Chai, J. K., Cho, K. S., Moon, I. S., Wikesjö, U. M. E. & Kim, C. K. (2004) Periodontal repair in surgically created intrabony defects in dogs: influence of the number of bone walls on healing response. *Journal of Periodontology* **75**, 229–235.

Kim, T-G., Wikesjö, U. M. E., Cho, K-S., Chai, J-K., Pippig, S.D., Siedler, M. & Kim, C-K. (2009) Periodontal wound healing/regeneration following application 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. *Journal of Clinical Periodontology* **36**, 589–597.

Koch, K. P., Becker, J., Terheyden, H., Capsius, B. & Wagner, W. (2010) A prospective, randomized clinical trial on the safety and efficacy of recombinant human growth and differentiation factor-5 coated onto β-tricalcium phosphate for sinus lift augmentation. *Clinical Oral Implants Research* **21**, 1301–1308.

Kwon, D. H., Bennett, W., Herberg, S., Bastone, P., Pippig, S., Rodriguez, N. A., Susin, C. & Wikesjö, U. M. E. (2010a) Evaluation of an injectable rhGDF-5/PLGA construct for minimally invasive periodontal regenerative procedures. A histological study in the dog. *Journal of Clinical Periodontology* **37**, 390–397.

Kwon, D. H., Bisch, F. C., Herold, R. W., Pompe, C., Bastone, P., Rodriguez, N. A., Susin, C. & Wikesjö, U. M. E. (2010b) Periodontal wound healing/regeneration following application of rhGDF-5 in a ß-TCP/PLGA carrier in critical-size supraalveloar periodontal defects in dogs. *Journal of Clinical Periodontology* **37**, 667–674.

Kwon, H-R., Wikesjö, U. M. E., Jung, U-W., Kim, Y-T., Bastone, P., Pippig, S. D. & Kim, C-K. (2010c) Growth/differentiation factor-5 significantly enhances periodontal wound healing/regeneration compared with platelet-derived growth factor-BB in dogs. *Journal of Clinical Periodontology* **37**, 739–746.

Lee, J., Stavropoulos, A., Susin, C. & Wikesjö, U. M. E. (2010a) Periodontal regeneration: focus on growth and differentiation factors. *Dental Clinics of North America*, **54**, 93–111.

Lee, J-S., Wikesjö, U. M. E., Jung, U-W., Choi, S-H., Pippig, S., Siedler, M. & Kim, C-K. (2010b) Periodontal wound healing/regeneration following implantation of recombinant human growth/differentiation factor-5 in a ß-tricalcium phosphate carrier into one-wall intrabony defects in dogs. *Journal of Clinical Periodontology* **37**, 382–389.

LeGeros, R. Z. (1993) Biodegradation and bioresorption of calcium phosphate ceramics. *Clinical Materials* **14**, 65–88.

Lindhe, J. & Nyman, S. (1984) Long-term maintenance of patients treated for advanced periodontal disease. *Journal of Clinical Periodontology* **11**, 504–514.

Listgarten, M. A. & Rosenberg, M. M. (1979) Histological study of repair following new attachment procedures in human periodontal lesions. *Journal of Periodontology* **50**, 333–344.

Min, C-K., Wikesjö, U. M. E., Park, J-C., Chae, G-J., Pippig, S. D., Bastone, P., Kim, C-S. & Kim, C-K. (2011) Wound healing/regeneration using rhGDF-5 in an injectable composite carrier and a one-wall intrabony defect model in dogs. *Journal of Clinical Periodontology* **38**, 261–268.

Moore, Y., Dickinson, D. P. & Wikesjö, U. M. E. (2010) Growth/differentiation factor-5 (GDF-5): a candidate therapeutic agent for periodontal regeneration? Review of preclinical data. *Journal of Clinical Periodontology* **37**, 288–298.

Morotome, Y., Goseki-Sone, M., Ishikawa, I. & Oida, S. (1998) Gene expression of growth and differentiation factors-5, -6, and -7 in developing bovine tooth at the root forming stage. *Biochemical and Biophysical Research Communications* **244**, 85–90.

Nakamura, T., Yamamoto, M., Tamura, M. & Izumi, Y. (2003) Effects of growth/differentiation factor-5 on human periodontal ligament cells. *Journal of Periodontal Research* **38**, 597–605.

Pöhling, S., Pippig, S. D., Hellerbrand, K., Siedler, M., Schuetz, A. & Dony, C. (2006) Superior effect of MD05, beta-tricalcium phosphate coated with recombinant human growth/differentiation factor-5, compared to conventional bone substitutes in the rat calvarial defect model. *Journal of Periodontology* **77**, 1582–1590.

Polimeni, G., Koo, K-T., Pringle, G. A., Agelan, A., Safadi, F. F. & Wikesjö, U. M. E. (2008) Histopathological observations of a polylactic acid-based device intended for guided bone/tissue regeneration. *Clinical Implant Dentistry and Related Research* **10**, 99–105.

Polimeni, G., Susin, C. & Wikesjö, U. M. E. (2009) Regenerative potential and healing dynamics of the periodontium: a critical-size supra-alveolar periodontal defect study. *Journal of Clinical Periodontology* **36**, 258–264.

Polimeni, G., Wikesjö, U. M. E., Susin, C., Qahash, M., Shanaman, R. H., Prasad, H. S., Rohrer, M. D. & Hall, J. (2010) Alveolar ridge augmentation using implants coated with recombinant human growth/differentiation factor-5 (rhGDF-5). Histologic observations. *Journal of Clinical Periodontology* **37**, 759–768.

Polimeni, G., Xiropaidis, A. V. & Wikesjö, U. M. E. (2006) Biology and principles of periodontal wound healing/regeneration. *Periodontology 2000* **41**, 30–47.

Schwarz, F., Rothamel, D., Herten, M., Ferrari, D., Sager, M. & Becker, J. (2008) Lateral ridge augmentation using particulated or block bone substitutes biocoated with rhGDF-5 and rhBMP-2: an immunohistochemical study in dogs. *Clinical Oral Implants Research* **19**, 642–652.

Sena, K., Morotome, Y., Baba, O., Terashima, T., Takano, Y. & Ishikawa, I. (2003) Gene expression of growth differentiation factors in the developing periodontium of rat molars. *Journal of Dental Research* **82**, 166–171.

Sigurdsson, T. J., Nygaard, L., Tatakis, D. N., Fu, E., Turek, T. J., Jin, L., Wozney, J. M. & Wikesjö, U. M. E. (1996) Periodontal repair in dogs: evaluation of rhBMP-2 carriers. *The International Journal of Periodontics & Restorative Dentistry* **16**, 524–537.

Sorensen, R. G., Wikesjö, U. M. E., Kinoshita, A. & Wozney, J. M. (2004) Periodontal repair in dogs: evaluation of a bioresorbable calcium phosphate cement (Ceredex) as a carrier for rhBMP-2. *Journal of Clinical Periodontology* **31**, 796–804.

Stahl, S. S. & Froum, S. (1986) Histological evaluation of human intraosseous healing responses to the placement of tricalcium phosphate ceramic implants. I. Three to eight months. *Journal of Periodontology* **57**, 211–217.

Stavropoulos, A. & Wikesjö, U. M. E. (2010) Periodontal tissue engineering: focus on growth factors. In: Sculean, A. (ed). Periodontal Regenerative Therapy, pp. 193–214. Chicago: Quintessence Publishing Company.

Stavropoulos, A., Windisch, P., Szendröi-Kiss, D., Rosta, P., Gera, I. & Sculean, A. (2010) Clinical and histological evaluation of granular beta tricalcium phosphate for the treatment of

human intrabony periodontal defects. A report on five cases. *Journal of Periodontology* **81**, 325–334.

Stavropoulos, A., Becker, J., Capsius, B., Açil, Y., Wagner, W.& Terheyden, H. (2011) Histological evaluation of maxillary sinus floor augmentation with recombinant human growth and differentiation factor-5-coated β-tricalcium phosphate: results of a multicenter randomized clinical trial. *Journal of Cinical Periodontology* **38**, 966–974.

Trombelli, L. & Farina, R. (2008) Clinical outcomes with bioactive agents alone or in combination with grafting or guided tissue regeneration. *Journal of Clinical Periodontology* **35**(8 Suppl.), 117–135.

Weng, D., Pöhling, S., Pippig, S., Bell, M., Richter, E. J., Zuhr, O. & Hürzeler, M. B. (2009) The effects of recombinant human growth/differentiation factor-5 (rhGDF-5) on bone regeneration around titanium dental implants in barrier membrane-protected defects: a pilot study in the mandible of beagle dogs. *The International Journal of Oral & Maxillofacial Implants* **24**, 31–37.

Wikesjö, U. M. E., Lim, W. H., Thomson, R. C., Cook, A. D., Wozney, J. M. & Hardwick, W. R. (2003) Periodontal repair in dogs: evaluation of a bioabsorbable space-providing macroporous membrane with recombinant human bone morphogenetic protein-2. *Journal of Periodontology* **74**, 635–647.

Wikesjö, U. M. E. & Selvig, K. A.. (1999) Periodontal wound healing and regeneration. *Periodontology 2000* **19**, 21–39.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Radiographic and clinical, including surgical, images from representative cases.

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Clinical Relevance

Scientific rationale for the study: Compelling pre-clinical evidence indicates that recombinant human growth/differentiation factor 5 (rhGDF-5) supports periodontal wound healing/regeneration. The objective of this pilot study was to provide a histological, histometrical and clinical record of periodontal wound healing/regeneration in man following surgical implantation of rhGDF-5 adsorbed onto a particulate β-tricalcium phosphate (β-TCP) carrier into intra-bony periodontal defects compared with that following open flap debridement alone (OFD; control) using a 6-month healing interval.

Principal findings: An almost twofold greater CAL gain was observed at sites receiving rhGDF-5/β-TCP compared with OFD (3.2 *versus* 1.7 mm). Alveolar bone regeneration was almost threefold greater (2.2 *versus* 0.8 mm) and periodontal regeneration, almost twofold greater (2.2 *versus* 1.2 mm) at sites receiving rhGDF-5/β-TCP compared with OFD. Root resorption/ankylosis was not observed. Limited residual β-TCP was observed at 6 months post-surgery.

Practical implications: The rhGDF-5/β-TCP appears a promising candidate technology in support of periodontal wound healing/regeneration. The clinical relevance of this treatment should be evaluated in larger clinical studies.