Journal of Clinical Periodontology
Official Journal of The European Federation of Periodontology
Founded by the British, Dutch, French, German, Scandinavian, and Swiss Societies of Periodontology

J Clin Periodontol 2008; 35: 976–984 doi: 10.1111/j.1600-051X.2008.01323.x

Development of an injectable composite as a carrier for growth factor-enhanced periodontal regeneration

Samuel Herberg[1], Michael Siedler[2], Susanne Pippig[1], Andreas Schuetz[1], Carola Dony[1], Chong-Kwan Kim[3] and Ulf M. E. Wikesjö[4]

[1]Scil Technology GmbH, Martinsried, Germany; [2]Private Practice, München, Germany; [3]Department of Periodontology, College of Dentistry, Yonsei University, Seoul, Korea; [4]Department of Periodontics, Medical College of Georgia School of Dentistry, Augusta, GA, USA

Herberg S, Siedler M, Pippig S, Schuetz A, Dony C, Kim C-K, Wikesjö UME. Development of an injectable composite as a carrier for growth factor-enhanced periodontal regeneration. J Clin Periodontol 2008; 35: 976–984. doi: 10.1111/j.1600-051X.2008.01323.x.

Abstract

Aim: Biomaterials are often applied in periodontal therapy; however, not always well adapted for tissue regeneration. The objective of this study was to evaluate the physico-chemical properties and biocompatibility of an injectable, in situ setting composite for growth factor-enhanced periodontal regeneration.

Material and Methods: The composite constitutes bioresorbable poly(lactic-*co*-glycolic acid) (PLGA) and additives forming in situ a matrix designed as a carrier for recombinant human growth/differentiation factor-5 (rhGDF-5). In vitro characterization included the porosity, biointeraction, biodegradation, injectability, and biological activity of released rhGDF-5. Biocompatibility was compared with granular β-tricalcium phosphate and an absorbable collagen sponge using a canine periodontal defect model.

Results: The PLGA composite showed a highly porous (500–1000 μm) space-providing structure. It effectively induced coagulation exhibiting an intimate interaction with the fibrin clot. The biphasic biodegradation was complete within 4 weeks. The composite was conveniently injectable (90.4 $\pm$ 3.6 N) for ease of use. It exhibited a sustained rhGDF-5 release over 4 weeks (40.8%) after initial burst (3.4%) detected by ALP activity. Sites receiving the composite showed limited, if any, residuals and had no appreciable negative effect on periodontal wound healing. There were no noteworthy inflammatory lesions in sites receiving the PLGA composite.

Conclusion: Characteristics of the PLGA composite makes it an attractive matrix to support native wound healing and rhGDF-5-enhanced periodontal regeneration.

Key words: bioresorbable; blood clot; in situ setting; minimally invasive; periodontal regeneration; PLGA; rhGDF-5; space providing

Accepted for publication 17 August 2008

Conflict of interest and source of funding statement

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

Effective periodontal therapy comprises regeneration of the various components of the periodontal attachment, including alveolar bone, cementum, periodontal ligament, and gingiva lost due to periodontal disease. Results from pre-clinical studies have shown the critical importance of the unimpeded adsorption/adhesion and maturation of a blood clot formed during surgery between the gingival flap and the tooth for periodontal wound healing/regeneration (for a review, see Wikesjö & Selvig 1999). However, this native blood clot commonly exhibits insufficient mechanical stability to ensure uneventful wound maturation of the interface towards structural integrity (Linghorne & O'Connell 1950, Hiatt et al. 1968, Polson & Proye 1983, Wikesjö et al. 1991). Furthermore, it has been demonstrated that space provision is a critical factor for periodontal regeneration, including alveolar bone; a statistically significant direct relationship has been shown between space provision and bone formation in periodontal sites (Haney et al. 1993, Sigurdsson et al. 1994, Trombelli et al. 1999, Polimeni et al. 2005). Regardless of the finding that

periodontal regeneration can predictably be obtained in the absence of tissue occlusion (Wikesjö et al. 2003b), technology limitations have been associated with space-providing biomaterials and devices used adjunctive to periodontal regenerative procedures, in particular, slow resorption rates interfering with tissue formation (Wikesjö et al. 2001). Thus, it remains a challenge to develop adequate biomaterials that meet necessary prerequisites, including biocompatibility, space provision, clot stabilization, and biodegradation to provide a balanced support for regeneration of the periodontal attachment.

Current treatment protocols aimed at periodontal regeneration all include surgical procedures. The ultimate goal, however, would be to develop treatment protocols that support periodontal regeneration using minimally invasive techniques and technology (Harrel 1999, Cortellini & Tonetti 2001, 2007). To date conventional non-surgical periodontal therapy has been focused on mechanical supra- and subgingival tooth debridement to reduce the bacterial load in periodontal sites. In turn, these microbiologic alterations have resulted in resolution of associated inflammatory processes and generally secure the stability of the periodontal attachment (Sbordone et al. 1990, Petersilka et al. 2002, Adriaens & Adriaens 2004). Products developed in support of this non-surgical treatment concept, for example Atridox™, Elyzol® Dental Gel, and Arestin®, focus on local delivery of antibiotics (e.g., doxycycline, metronidazol, minocycline) directly to a periodontal site. These products may aid to reduce subgingival bacterial load, thereby significantly control associated inflammatory processes; however, their application may only result in unintentional regeneration of involved tissues (Hanes & Purvis 2003). The only apparent product used in conjunction with non-surgical periodontal therapy to support regeneration of the periodontal attachment is Emdogain®, a combination product of porcine-derived enamel matrix proteins in a propylene glycol alginate gel (Gestrelius et al. 1997, Hammarström 1997). Emdogain® has been suggested to support periodontal wound healing/regeneration in surgical settings (Cochran et al. 2003, Sanz et al. 2004). However, the effect appears limited following non-surgical minimally invasive protocols (Wennström & Lindhe 2002, Gutierrez et al. 2003, Sculean et al. 2003).

An alternative periodontal regenerative technique may be the minimally invasive application of suitable growth factor(s). The basis for this origin in the discovery that demineralized bone induces bone formation when implanted in ectopic rodent models (Urist 1965). In the late 1980s, the responsible factors, bone morphogenetic proteins (BMPs), were identified and became available for pre-clinical and clinical evaluation (Celeste et al. 1990, Özkayanak et al. 1990, Wozney et al. 1988). Pre-clinical studies have evaluated the surgical application of recombinant human BMP-2 (rhBMP-2), recombinant human osteogenic protein-1 (rhOP-1/rhBMP-7), and recombinant human growth/differentiation factor-7 (rhGDF-7/rhBMP-12) using a variety of carrier technologies in periodontal settings. Implantation of rhBMP-2 and rhBMP-7 induced significant bone formation, including ankylosis and root resorption, but not a periodontal ligament (Sigurdsson et al. 1995, 1996, Giannobile et al. 1998, Wikesjö et al. 1999, 2003a, c, d, 2004, Sorensen et al. 2004), whereas implantation of rhGDF-7 supported regeneration of the periodontal attachment (Wikesjö et al. 2004). Another promising candidate for a combination product is rhGDF-5, also known as cartilage-derived morphogenetic protein-1 (CDMP-1) (Hötten et al. 1996). As rhGDF-5, like rhGDF-7, induces bone less aggressively compared with rhBMP-2 and rhBMP-7, it may allow regeneration of all periodontal tissues without ankylosis and root resorption when delivered in a suitable carrier.

This study describes the development of an injectable, in situ setting composite biomaterial consisting of a well-known bioresorbable polymer, poly(lactic-*co*-glycolic acid) (PLGA), and various additives designed to serve as a carrier for rhGDF-5 for minimally invasive regenerative procedures and ease-of-use surgical application in non-contained periodontal defects. The physico-chemical properties of the PLGA composite were characterized relative to space-providing porosity and bioadhesion for blood clot stabilization, biodegradation for rapid clearance, and injectability. The feasibility of the PLGA composite as a carrier for sustained delivery of rhGDF-5 was determined in vitro. Biocompatibility of the PLGA composite was compared with other common biomaterials used to deliver growth factors in the surgical treatment of periodontal or osseous defects in a well-characterized intra-bony periodontal defect model in the Beagle dog using histologic and histometric analysis.

Material and Methods

Injectable PLGA composite

The injectable composite consists of poly (D,L-lactic-*co*-glycolic acid) (20.0 wt%; PLGA Resomer® RG502H; Boehringer Ingelheim, Ingelheim, Germany) and polyethylene glycol 1500 (2.0 wt%; Merck, Darmstadt, Germany) dissolved in polyethylene glycol 300 (47.0 wt%; Merck) by heat treatment. Calcium sulphate (15.0 wt%; Carl Roth, Karlsruhe, Germany), D(−)-mannitol (13.0 wt%; Carl Roth), and cellulose ether (3.0 wt%; Hercules, Alizay, France) were dispersed in the polymeric solution.

The rhGDF-5 lyophilisate was manufactured by dissolving D(−)-mannitol (71.4 wt%), D(+)-trehalose (17.8 wt%; Merck), and L-methionine (5.0 wt%; Sigma, St. Louis, MO, USA) in 50 mM acetic acid (Merck). The rhGDF-5 stock solution (5.8 wt%; Biopharm, Heidelberg, Germany) diluted to suitable concentrations with 50 mM acetic acid was added to the dissolved bulking materials. The final drug solution was lyophilized in glass vials vented with pure nitrogen before sealing.

The injectable PLGA composite was mixed with the lyophilized rhGDF-5 before administration.

Porosity

After application, the porosity of the injectable PLGA composite was evaluated using scanning electronic microscopy (SEM) following in vitro incubation of the composite in a large volume of phosphate-buffered saline (PBS) at 37°C for 2 h and drying for 24 h at 4°C. For the SEM investigation, samples were sputter-coated with gold (Edwards S150B, Edwards, Crawley, West Sussex, UK). The gas pressure was 6 mbar, the current 10 mA, the voltage 1.5 kV, and the coating-time 90 s. SEM analysis of samples was performed using a LEO 1455 (Carl Zeiss Inc., Thornwood, NJ, USA) at 10 kV.

Biointeraction

Biointeraction with blood was evaluated in vitro by incubation of the PLGA composite in citrate phosphate dextran-

buffered human whole blood. The composite was allowed to set for 12 h at 37°C. After preparation, the clotted samples were embedded (Peel-A-Way® Disposable Embedding Molds T-12; Polysciences Inc., Warrington, PA, USA) in Tissue Freezing Medium (Jung, Leica Instruments, Nussloch, Germany) and allowed to freeze on dry ice plates. Sectioning (10–12 μm) was carried out at -20°C using a Leica cryotom CM 3050 (Leica, Bensheim, Germany). After drying and fixation in ethanol, the sections were counterstained with Mayer's hematoxylin (Bio Optica, Milano, Italy) and mounted using Shandon ImmuMount (Thermo Electron Corp., Pittsburgh, PA, USA). The interaction of the injectable composite with blood was determined using light microscopy (Olympus BX41; Olympus, Hamburg, Germany).

Biodegradation

Biodegradation studies were performed in vitro by incubation of the PLGA composite in PBS for 4 weeks at 37°C. At each analytical time-point, specimens were dried for mass balance and afterwards solubilized in tetrahydrofuran anhydrous (Merck) for gel permeation chromatography to determine the weight average polymer molecular weight (M_w) of PLGA. M_w polystyrenes in the range of 5.6–34.0 kDa (PSS, Mainz, Germany) were used as standards. The GPC conditions were as follows: Tosoh TSKgel G3000H_{HR} (5 μm, 7.8 mm × 30 cm) column (Tosoh Biosciences, Stuttgart, Germany) maintained at 40°C, Dionex P580 series isocratic pump, autosampler, column oven (Dionex Corp., Sunnyvale, CA, USA), miniDAWN™ multi-angle laser light scattering detector (Wyatt Technology Corp., Santa Barbara, CA, USA), RI 2000-F refractive index detector (Schambeck SFD, Bad Honnef, Germany), and 80 μl injection volume. Tetrahydrofuran at a flow rate of 1 ml/min. was used as the mobile phase. The average M_w was calculated by Astra® GPC software (Wyatt Technology Corp.). The relative M_w was expressed as percentage of the M_w of the sample to the initial M_w of PLGA within the injectable composite.

Injectability

Injectability experiments were carried out using a material testing machine (TH 2730; Thuemler, Nuernberg, Germany) and an aluminium syringe rack (Lang, Bad Aibling, Germany). Measurements were performed using 1 ml glass syringes (Hypak™ SCF; BD Medical, Heidelberg, Germany) filled with the injectable PLGA composite in combination with 0.9 × 23 mm/20G standard dental needles (Transcoject, Neumuenster, Germany) and displayed as load–displacement diagrams. Empty 1 ml glass syringes supplemented with standard dental needles served for blank subtraction. The following settings were used: (1) test modus: compression; (2) feed rate: 10 mm/min.; (3) distance: 30 mm; (4) F_0: 0.1 N; (5) F_{limit}: 150 N; (6) fill volume: 0.7 ml; (7) analysis: mean (± SD) of triplicate recordings of blank and injectable composite within linear measurement range; and (8) injectability data displayed as ejection force (in N) as deviation of test and blank force at a concrete time point. The injectable PLGA composite was compared with Emdogain® (Straumann, Basel, Switzerland) and Atridox™ (Block Drug Corporation Inc., Jersey City, NJ, USA).

rhGDF-5 release and biological activity

rhGDF-5 release studies were performed by incubation of rhGDF-5-loaded PLGA composites in physiological cell-culture medium plus 10% foetal calf serum (FCS) (α-MEM; Gibco®; Invitrogen, Karlsruhe, Germany) over a period of 4 weeks at 37°C in vitro. The biological activity of released rhGDF-5 was quantified using an alkaline phosphatase (ALP) assay. MCHT-1/26 cells in α-MEM/10% FCS were incubated at 3×10^4 cells/ml in a microtitre plate (Nunc, Roskilde, Denmark) for 24 h at 37°C, 10% CO_2, 95% RH. After removal of the supernatant, fresh medium with rhGDF-5 standard or sample dilution was added to the wells (quadruples). Following a further incubation for 3 days at 37°C, 5% CO_2, 95% RH, cells were washed with 0.9% NaCl and lysed (1 mM $MgCl_2$, 1% NP-40; Sigma). ALP activity was determined in the cleared lysate using 0.3 mM *p*-nitrophenylphosphate in diethanolamine buffer (Pierce, Rockford, IL, USA) as substrate followed by incubation for 45 min. at 37°C, 5% CO_2, 95% RH. Absorbance at 405 nm was recorded after stopping with 750 mM NaOH (Merck).

Biocompatibility

Biocompatibility was analysed using the critical-size, one-wall, intra-bony periodontal defect model in the Beagle dog (Kim et al. 2004). The PLGA composite was compared with β-tricalcium phosphate (β-TCP) and an absorbable collagen sponge (ACS), biomaterials commonly used as implantable carriers for growth factors. Fifteen young adult male/female Beagle dogs, approximate weight 15 kg, exhibiting intact dentition and healthy periodontal tissues, were used. Animal selection, management, and surgical protocol followed a routines approved by the Institutional Animal Care and Use Committee, Yonsei University, Seoul, Korea.

In brief, bilateral, surgically created, critical-size, mandibular, one-wall, intra-bony periodontal defects, two defects/jaw quadrant, five animals/treatment, were implanted with: (1) ACS (CollaCote®, Zimmer Dental, Carlsbad, CA, USA) cut into 4 × 4 × 5 mm specimens and soak-loaded with L-glutamatic buffer (0.5% sucrose, 2.5% glycine, 30 mM L-glutamatic acid, 0.01% polysorbate 80, pH 4.5; provided by Scil Technology GmbH); (2) β-TCP (Calciresorb®, Ceraver-Osteal, France) soak-loaded with sterile saline; (3) the injectable PLGA composite without rhGDF-5 (Scil Technology GmbH); or (4) served as sham-surgery controls. The mucoperiosteal flaps were advanced, adapted, and sutured to allow primary intention healing. The animals were euthanized at week 8 post-surgery when biopsies of the defect sites were collected and processed for light microscopy histopathologic and histometric analysis using routine procedures (Kim et al. 2004).

Results

Porosity

Figure 1a shows an overview of the coherent structure of the injectable PLGA composite following a 2 h in vitro incubation in PBS at 37°C. The material exhibits a sponge-like structure as a result inter-connected macropores in the range of 500–1000 μm. The texture of the material was uniformly rough and showed no indication of any particles from the powdery raw material components such as calcium sulphate suggesting that the inorganic components were homogeneously integrated

into the PLGA-matrix. Figure 1b also shows micropores below 500 μm integrated into the walls of the macropores, further increasing the inter-connectivity of the material.

Biointeraction

To simulate the application of the injectable PLGA composite in a periodontal site, the composite was incubated in stabilized human whole blood in vitro. The PLGA composite effectively induced the coagulation process shortly upon injection in the non-coagulating medium. A representative photomicrograph of a cryo-section following processing and staining is shown in Fig. 2. The blood clot discernable by the specifically stained leucocytes nuclei exhibits an intimate binding to the colourless composite material at the 12 h observation. This experimental set-up was chosen to evaluate only the passive distribution of blood cells during the initial phase of the in situ setting process and provides no information of active cell migration in the following stages of tissue regeneration.

Biodegradation

Biodegradation of the injectable PLGA composite should be tailored to the regenerative rate of the tissues. The degradation profile of the PLGA composite determined in vitro can be divided into two phases. Phase 1 was characterized by rapid breakdown of molecular mass (54.5%) and polymer weight (23.2%) by week 1. During phase 2, the degradation rate decreased significantly leading to nearly complete weight loss by week 4 (2.1%), as shown in Fig. 3. This result was confirmed by the determination of the M_w, which decreased to 44.3% by week 3. After 4 weeks, no PLGA could be detected by using gel permeation chromatography due to limitations of the analytical method and the rapid degradation profile described.

Injectability

Figure 4 shows the ejection force of the injectable PLGA composite compared with market products in pre-filled syringes (Emdogain® and Atridox™) intended to be applied through suitable needles. The determined ejection force to apply Emdogain® using standard needles (0.9 × 23 mm/20 G) in the

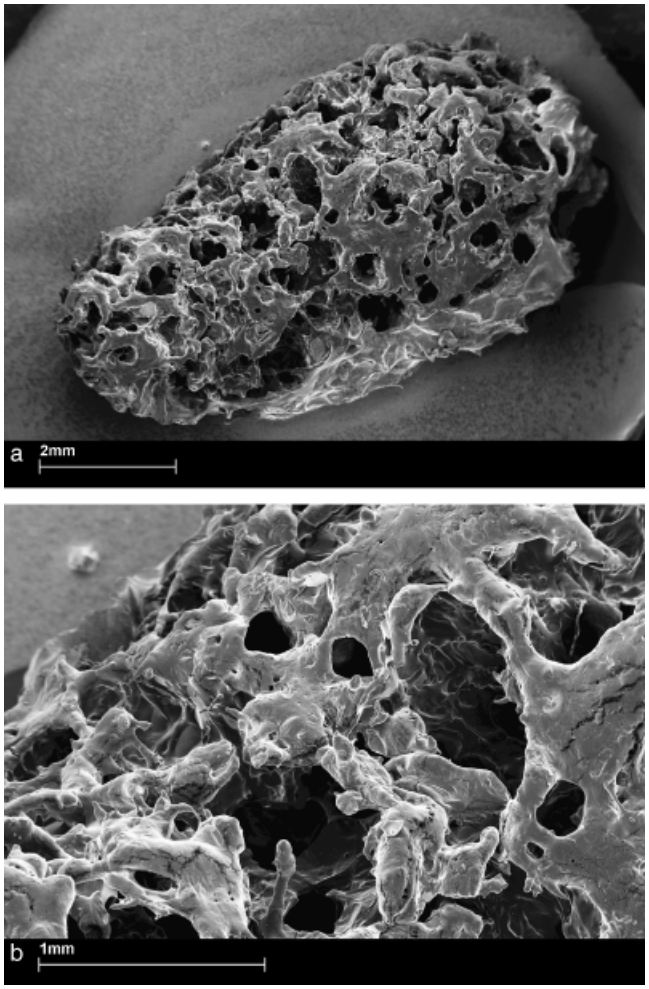

Fig. 1. Scanning electron microscopy photomicrographs of the macroporous PLGA composite following a 2-h incubation at 37°C in phosphate-buffered saline and drying. (a) Overview of the coherent sponge-like structure with inter-connected macropores. (b) Smaller micropores below 1000 μm integrated into the walls of the macropores are shown.

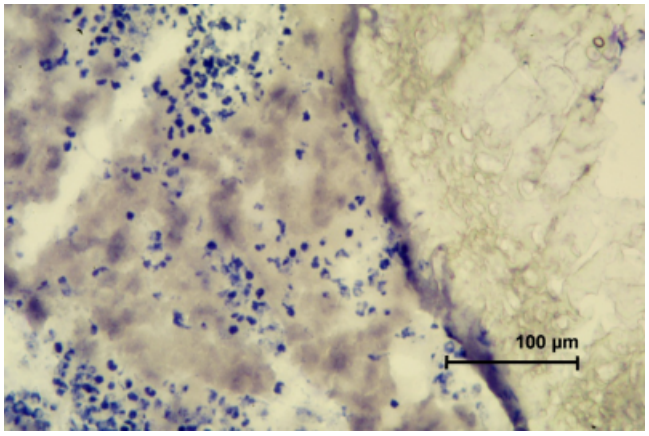

Fig. 2. Photomicrograph of the blood clot–PLGA composite interface following incubation in stabilized human whole blood in vitro after hematoxylin staining of a cryo-section. Specifically stained leucocytes nuclei within the formed blood clot are shown to the left; the colourless matrix of the PLGA composite is shown to the right. The crossing blue frontline shows the intimate binding of the blood clot to the PLGA composite. PLGA, poly(lactic-*co*-glycolic acid).

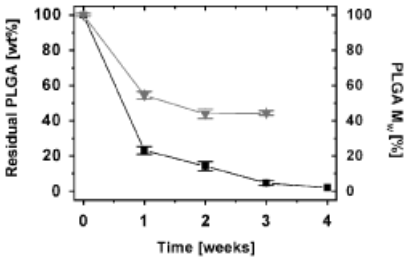

Fig. 3. In vitro degradation of the PLGA composite in phosphate-buffered saline at 37°C. Data are concentrated on the slowest degrading component. Left vertical axis represents the residual PLGA in weight percentage compared with t_0, determined by weight balance. Right vertical axis represents the PLGA molecular weight (M_w) in percentage compared with t_0, determined by GPC. Mean of duplicate measurements ± SD.

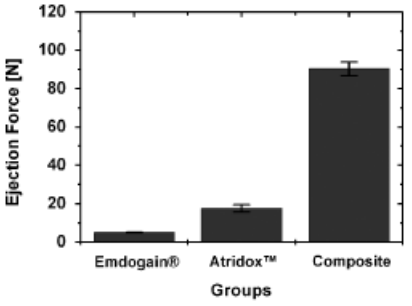

Fig. 4. Ejection forces (N) of the PLGA composite compared with Emdogain® and Atridox™ within pre-filled 1 ml glass syringes supplemented with standard needles (0.9 × 23 mm/20 G). Group means of triplicate measurements after blank subtraction ± SD.

experimental set-up was 5.2 ± 0.2 N, for Atridox™ 17.6 ± 2.0 N, and for the PLGA composite 90.4 ± 3.6 N.

rhGDF-5 release and activity

To evaluate the potential of the injectable PLGA composite as a carrier for local delivery of a growth factor, the cumulative release of rhGDF-5-loaded composites was analysed in vitro using an ALP activity assay. Relative biological activity of released rhGDF-5 was measured after the co-cultivation of MCHT-1/26 cells with the sample dilution. The obtained ALP data reveal a slow and sustained release profile of rhGDF-5 exhibiting a small initial burst of 3.4% after day 1 followed by a nearly linear release up to 40.8% after 4 weeks observation interval (Fig. 5).

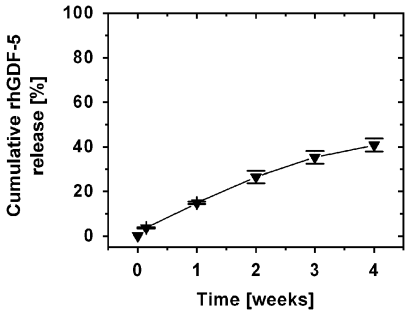

Fig. 5. In vitro release of recombinant human growth/differentiation factor-5 (rhGDF-5) from the PLGA composite in α-MEM/10% foetal calf serum (media exchange method) at 37°C. Data are expressing the relative biological activity of the cumulatively released growth factor after the co-cultivation with MCHT-1/26 cells, determined by alkaline phosphatase assay. Mean of duplicate measurements ± SD.

Biocompatibility

All but one defect site healed uneventfully with minimal signs of inflammation and some gingival recession. Photomicrographs of representative experimental sites are shown in Fig. 6. Healing appeared similar within and among treatments. Root surfaces were covered with cellular/acellular intrinsic fibre cementum (cementum without a functionally oriented periodontal ligament), or with cellular/acellular extrinsic/intrinsic (mixed) fibre cementum (cementum with a functionally oriented periodontal ligament). The fibre density varied along the root surface with no apparent preference with regards to location. The functionally oriented periodontal ligament appeared of low density in most sections on a scale from no fibres over low and mid-density to high density equating that of the native periodontal ligament. Functionally oriented periodontal fibres did not only traverse the periodontal ligament space but also were observed supracrestally.

Regenerated alveolar bone also varied in extension along the root surface. The newly formed bone was mostly woven bone with primary osteons. Regenerated cementum generally extended above the alveolar crest. Only a few teeth in the β-TCP group showed undermining root resorption. Sites having received β-TCP showed some residual biomaterial apparently undergoing resorption. Bone/osteoid formed onto aspects of the biomaterial. Bone formation from the alveolar crest apparently circumscribed the residual biomaterial. Sites receiving the PLGA composite showed limited, if any, residuals and no apparent negative effect on bone formation, indicating that this biomaterial did not appear to obstruct or otherwise compromise bone formation. There were no noteworthy inflammatory lesions in sites receiving the PLGA composite.

An epithelial attachment of variable length was observed in all sites irrespective of treatment with no clear distinction between treatments. The epithelium did not compromise the possibility to evaluate treatment effects on periodontal regeneration.

The results of the histometric analysis are shown in Table 1. There were no significant differences in defect height among the groups. The epithelial attachment was significantly greater in sites receiving β-TCP compared with sham-surgery ($p < 0.05$). Connective tissue attachment was significantly enhanced in sites receiving the PLGA composite and sham-surgery compared with ACS ($p < 0.05$). Connective tissue attachment was also significantly enhanced in the sham-surgery group compared with β-TCP ($p < 0.05$) (connective tissue attachment in this context represents an unspecific connective tissue attachment to a root surface previously deprived of its periodontal attachment observed in-between the coronal extension of new cementum formation on the root surface and the apical extension of the epithelial attachment). Cementum and bone regeneration (height) showed no significant differences among the groups, whereas bone regeneration (area) was significantly enhanced in sites receiving β-TCP compared with the sham-surgery and PLGA composite groups ($p < 0.05$); bone regeneration excluding residual β-TCP.

Discussion

The aim of the study was to analyse a novel, fully synthetic, composite material intended as a biodegradable carrier with distinct properties, which, combined with rhGDF-5, may support regeneration of the periodontal attachment. The composite was specially tailored to the dynamics of the early wound healing events in periodontal defect sites. The material was developed with the prospect of ease-of-use surgical

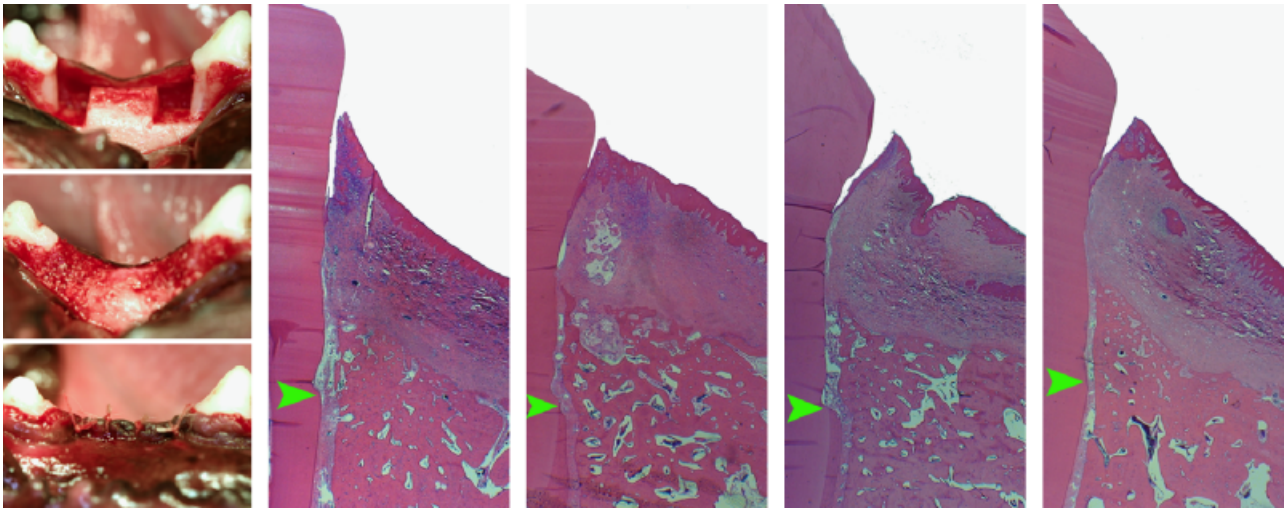

Fig. 6. Representative photomicrographs of defect sites implanted with the PLGA composite (left), β-TCP (left centre), ACS (right centre), or serving as sham-surgery control (right). Green arrowheads delineate the apical extent of the defect. The clinical series show the critical-size, one-wall, intra-bony defect model implanted with the PLGA composite.

Table 1. Results of the histometric analysis [group means ± SD (mm/mm^2)]

	PLGA composite	β-TCP	ACS	Sham-surgery
Defect height	4.75 ± 0.35	4.86 ± 0.38	4.46 ± 0.68	4.69 ± 0.36
Epithelial attachment	1.28 ± 0.76	2.64 ± 0.83*	1.86 ± 0.76	0.74 ± 0.50
Connective tissue attachment	1.28 ± 1.61†	0.57 ± 0.90	0.10 ± 0.09	1.47 ± 1.42‡
Cementum regeneration	1.99 ± 1.41	1.65 ± 0.82	2.49 ± 0.71	2.48 ± 1.28
Bone regeneration (height)	1.68 ± 0.33	1.70 ± 0.66	1.44 ± 0.39	1.68 ± 0.49
Bone regeneration (area)	3.39 ± 1.40	6.31 ± 2.41§	4.17 ± 1.28	3.00 ± 1.97

*β-TCP > sham-surgery; $p < 0.05$.
†PLGA > ACS; $p < 0.05$.
‡Sham-surgery > ACS and β-TCP; $p < 0.05$.
§β-TCP > sham-surgery and PLGA composite; $p < 0.05$.
PLGA, poly(lactic-*co*-glycolic acid); β-TCP, β-tricalcium phosphate; ACS, absorbable collagen sponge.

implantation in non-contained periodontal defects, and minimally invasive application directly to the defect site following non-surgical debridement.

A pre-requisite for a biomaterial intended to support periodontal regeneration is a suitable inter-connected porosity similar to native trabecular bone (e.g., pore size of approximately 100–1000 μm) to facilitate cell migration and proliferation from the surrounding tissue resources (e.g., residual periodontal ligament) into the space-providing matrix (Ruhé et al. 2005). The porosity of the PLGA composite after application was evaluated after in vitro incubation in phosphate buffer at 37°C and, upon drying, analysed by SEM. Inter-connected porosity is achieved by the special composition, which enables in situ pore forming as well as spreading of the material into the defect site. This pore forming process is completed after approximately 2 h in temperate, large-volume aqueous medium and leads to an average pore size of 500–1000 μm. However, this type of qualitative evaluation in vitro does not provide detailed information on the aimed clinical situation.

The blood clot serves as a provisionary matrix for periodontal regeneration, but lacks structural integrity (Baker et al. 2005). To overcome such limitations, interaction between the blood clot and a biomaterial is demanded. This was simulated by in vitro incubation of the PLGA composite in human whole blood. The studies revealed that the composite biomaterial is able to enhance the blood coagulation despite the repressed coagulation capability of the incubation medium leading to a close interaction of the composite with the blood clot. This noteworthy attribute of the biodegradable composite should effectively increase the on-site stability of the blood clot–PLGA composite construct within the defect site. However, this assay reflects the very first stage of the wound healing process and provides no information of cell migration and proliferation during later stages of clot maturation. Hence, the bioconductivity and biocompatibility of the composite was evaluated in vivo.

Bioresorbable polymers are well described as materials for the manufacturing of implants mainly for temporary fracture fixation and as matrices for drug delivery (Langer 1990, Hollinger & Leong 1996, Rokkanen et al. 2000). These materials degrade by hydrolytic chain secession to their monomers, which can be metabolized. In the periodontal field, they became of special interest when Nyman et al. (1982) introduced the concept of guided tissue regeneration and bioresorbable polymers were identified as candidate materials to overcome the necessity to replace the initially used non-resorbable barrier membranes (Falk et al. 1993). Membranes made of poly-lactic acid (PLA), poly-glycolic acid (PGA), and various co-polymers (PLGA) were developed and tested. Several authors described incompatibilities of these materials, in particular PLA-based technologies, most likely ascribed to the forming of toxic degradation products as well as prolonged and incomplete resorption (Wikesjö & Nilvéus 1990, Sigurdsson et al. 1996, Tatakis & Trombelli 1999, Schmitz et al. 2000, Polimeni et al. 2008). In contrast to

reports on PLA-based technologies, the one-wall intra-bony periodontal defect study showed excellent biocompatibility for the injectable PLGA composite comparable with that of the ACS and β-TCP technologies. Wound healing, in part, included a functionally oriented periodontal ligament. The composite exhibited timely biodegradation compared with β-TCP and no appreciable adverse reactions or residuals affecting bone formation making it a candidate matrix for the local delivery of a growth factor such as rhGDF-5.

To avoid obstruction of new tissue formation by a biomaterial as described for non- or slowly degradable calcium phosphate cements (Sorensen et al. 2004), the biodegradation of the composite was tailored to the regenerative rate of the tissues. For periodontal sites, a time frame of 2–4 weeks should not be exceeded (Wikesjö et al. 1991, 2003b). The present data suggest that the biomaterial meets the desired time frame as it was also confirmed in the in vivo evaluation.

One basic objective during the development of the PLGA composite was to ensure injectability through a standard needle for clinical ease-of-use and minimally invasive applications through prefilled syringes, as known from materials such as Emdogain® and Atridox™. The upper limit for the injection force by hand to apply those materials is about 200 N (Bohner & Baroud 2005). Thus, with a determined ejection force of 90.4 N, the injectability of the novel composite was demonstrated under the described conditions making it a highly convenient biomaterial for surgical and non-surgical indications.

Regardless of the well-known complexity of the comparability of in vitro and in vivo drug release data, a variety of analytical methods exist. Up to now ALP activity, which is a marker of osteoblast differentiation and BMP-induced osteogenic differentiation of mesenchymal cells, is the most widely and routinely used cell-based assay to measure BMP activity (Katagiri et al. 1994). As extensively described in the literature, BMPs apply their biological activity through the combination of type I and type II serine/threonine kinase receptors. Three distinct BMP type I receptors [activin receptor-like kinase (ALK)-2, ALK3, and ALK6) and type II receptors (BMPRII) in addition to two activin type II receptors (ActR-IIA and ActR-IIB) have been identified (for review, see Shi & Massague 2003). Hence, the ALP activity is a direct indication for the biological activity of the BMP in the sample that is determined by comparison of the induced ALP activity with the reference standard. The present data suggest that the novel PLGA composite exhibits the potential as a suitable carrier for the local delivery of a growth factor in a controlled manner. The detected relative biological activity of the cumulatively released recombinant protein after the co-cultivation with MCHT-1/26 cells clearly demonstrates that rhGDF-5 increases the ALP production in these cells in vitro.

About 20 currently known BMPs are directly involved in a wide range of cell regulatory processes, e.g., chemotaxis, differentiation, and proliferation (Pfeilschifter et al. 1990, Sampath et al. 1992, Bai et al. 2004) in many tissues, but still their main characteristic is induction of bone, cartilage, tendon, and ligament. This ability makes them lead candidates for tissue regeneration/engineering. Most studies performed have evaluated rhBMP-2 and rhBMP-7 in combination with additional biomaterials and/or carrier technologies in the axial and appendicular skeleton, including spine fusion, hip arthroplasty, and fracture repair and have been approved by the Food and Drug Administration and introduced to clinical practice (Bostrom & Camacho 1998, Kuslich et al. 1998, Spiro et al. 2000, 2001, Cook et al. 2001, Vaccaro et al. 2002, Valentin-Opran et al. 2002). Attempts to evaluate the potential of rhBMP-2 and rhBMP-7 in more complex tissues such as periodontal defects have revealed that both proteins effectively induce bone formation (Kang et al. 2004). Thus, it can be assumed that their massive support of the bone-forming process finally leads to ankylosis (Sigurdsson et al. 1995, 1996, Giannobile et al. 1998, Wikesjö et al. 1999, 2003a, c, d, 2004, Sorensen et al. 2004), whereby both proteins may not be suitable for this indication.

Recent studies have revealed the expression of a distinctive BMP subfamily, namely GDFs or CDMPs, in tooth formation during embryogenesis (Morotome et al. 1998, Sena et al. 2003) and regulation of human periodontal ligament cells in vitro (Nakamura et al. 2003). In contrast to rhBMP-2 or rhBMP-7, GDFs induce tendon/ligament-like tissue when implanted ectopically (Wolfmann et al. 1997). Their less-pronounced bone induction compared with rhBMP-2 or rhBMP-7 also make them ideal candidates to enhance periodontal wound healing while reducing the risk for aberrant healing events, such as root resorption and ankylosis (Wikesjö et al. 2004). However, rhGDF-5 coated onto β-TCP has also been shown to enhance local bone formation in a pre-clinical rodent model (Pöhling et al. 2006). Based on these findings, the novel injectable PLGA composite is expected to widen the utility of rhGDF-5 induced tissue engineering through ease-of-use application in non-contained defects and for minimally invasive applications.

References

Adriaens, P. A. & Adriaens, L. M. (2004) Effects of nonsurgical periodontal therapy on hard soft tissues. *Periodontology 2000* **36**, 121–145.

Bai, X., Xiao, Z., Pan, Y., Hu, J., Pohl, J., Wen, J. & Li, L. (2004) Cartilage-derived morphogenic protein-1 promotes the differentiation of mesenchymal stem cells into chondrocytes. *Biochemical and Biophysical Research Communications* **325**, 453–460.

Baker, D. L., Stanley Pavlow, S. A. & Wikesjö, U. M. E. (2005) Fibrin clot adhesion to dentin conditioned with protein constructs: an *in vitro* proof-of-principle study. *Journal of Clinical Periodontology* **32**, 561–566.

Bohner, M. & Baroud, G. (2005) Injectability of calcium phosphate pastes. *Biomaterials* **26**, 1553–1563.

Bostrom, M. P. & Camacho, N. P. (1998) Potential role of bone morphogenetic proteins in fracture healing. *Clinical Orthopaedics and Related Research* **355**, S274–S282.

Celeste, A. J., Iannazzi, J. A., Taylor, R. C., Hewick, R. M., Rosen, V., Wang, E. A. & Wozney, J. M. (1990) Identification of transforming growth factor β family members present in bone-inductive protein purified from bovine bone. *Proceedings of the National Academy of Sciences of the United States of America* **87**, 9843–9847.

Cochran, D. L., King, G. N., Schoolfield, J., Velasquez-Plata, D., Mellonig, J. T. & Jones, A. (2003) The effect of enamel matrix proteins on periodontal regeneration as determined by histological analyses. *Journal of Periodontology* **74**, 1043–1055.

Cook, S. D., Barrack, R. L., Shimmin, A., Morgan, D. & Carvajal, J. P. (2001) The use of osteogenic protein-1 in reconstructive surgery of the hip. *Journal of Arthroplasty* **16**, 88–94.

Cortellini, P. & Tonetti, M. S. (2001) Microsurgical approach to periodontal regeneration. Initial evaluation in a case cohort. *Journal of Periodontology* **72**, 559–569.

Cortellini, P. & Tonetti, M. S. (2007) A minimally invasive surgical technique with an

enamel matrix derivative in the regenerative treatment of intra-bony defects: a novel approach to limit morbidity. *Journal of Clinical Periodontology* **34**, 87–93.

Falk, H., Fornell, J. & Teiwik, A. (1993) Periodontal regeneration using a bioresorbable GTR device. *The Journal of the Swedish Dental Association* **85**, 673–681.

Gestrelius, S., Andersson, C., Johansson, A.-C., Persson, E., Brodin, A., Rydhag, L. & Hammarström, L. (1997) Formulation of enamel matrix derivative for surface coating. *Journal of Clinical Peridontology* **24**, 678–684.

Giannobile, W. V., Ryan, S., Shih, M. S., Su, D. L., Kaplan, P. L. & Chan, T. C. (1998) Recombinant human osteogenic protein-1 (OP-1) stimulates periodontal wound healing in class III furcation defects. *Journal of Periodontology* **69**, 129–137.

Gutierrez, M. A., Mellonig, J. T. & Cochran, D. L. (2003) Evaluation of enamel matrix derivative as an adjunct to non-surgical periodontal therapy. *Journal of Clinical Periodontology* **30**, 739–745.

Hammarström, L. (1997) Enamel matrix, cementum development and regeneration. *Journal of Clinical Periodontology* **24**, 658–668.

Hanes, P. J. & Purvis, J. P. (2003) Local anti-infective therapy: pharmacological agents. A systematic review. *Annals of Periodontology* **8**, 79–98.

Haney, J. M., Nilvéus, R. E., McMillan, P. J. & Wikesjö, U. M. E. (1993) Periodontal repair in dogs: expanded polytetrafluoroethylene barrier membranes support wound stabilization and enhance bone regeneration. *Journal of Periodontology* **64**, 883–890.

Harrel, S. K. (1999) A minimally invasive surgical approach for periodontal regeneration: surgical technique and observations. *Journal of Periodontology* **70**, 1547–1557.

Hiatt, W. H., Stallard, R. E., Butler, E. D. & Badgett, B. (1968) Repair following mucoperiosteal flap surgery with full gingival retention. *Journal of Periodontology* **39**, 11–16.

Hollinger, J. O. & Leong, K. (1996) Poly(α-hydroxy acids) carriers for bone morphogenetic proteins. *Biomaterials* **17**, 187–194.

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.

Kang, Q., Sun, M. H., Cheng, H., Peng, Y., Montag, A. G., Deyrup, A. T., Jiang, W., Luu, H. H., Luo, J., Szatkowski, J. P., Vanichakarn, P., Park, J. Y., Li, Y., Haydon, R. C. & He, T.-C. (2004) Characterization of the distinct orthotopic bone-forming activity of 14 BMPs using recombinant adenovirus-mediated gene delivery. *Gene Therapy* **11**, 1312–1320.

Katagiri, T., Yamaguchi, A., Komaki, M., Abe, E., Takahashi, N., Ikeda, T., Rosen, V., Wozney, J. M., Fujisawa-Sehara, A. & Suda, T. (1994) Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage. *Journal of Cell Biology* **127** (Part 1), 1755–1766.

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 intrabony defects in dogs: influence of the number of bone walls on healing response. *Journal of Periodontology* **75**, 229–235.

Kuslich, S. D., Ulstrom, C. L., Griffith, S. L., Ahern, J. W. & Dowdle, J. D. (1998) The Bagby and Kuslich method of lumbar interbody fusion. History, techniques, and 2-year follow-up results of a United States prospective, multicenter trial. *Spine* **23**, 1267–1279.

Langer, R. (1990) New methods of drug delivery. *Science* **249**, 1527–1533.

Linghorne, W. J. & O'Connell, D. C. (1950) Studies in the regeneration and reattachment of supporting structures of teeth. I. Soft tissue reattachment. *Journal of Dental Research* **29**, 419–428.

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.

Nyman, S., Lindhe, J., Karring, T. & Rylander, H. (1982) New attachment following surgical treatment of human periodontal diseases. *Journal of Clinical Periodontology* **9**, 290–296.

Özkayanak, E., Rueger, D. C., Drier, E. A., Corbett, C., Ridge, R. J., Sampath, T. K. & Oppermann, H. (1990) OP-1 cDNA encodes an osteogenic protein in the TGF-beta family. *The EMBO Journal* **9**, 2085–2093.

Petersilka, G. J., Ehmke, B. & Flemmig, T. (2002) Antimicrobial effects of mechanical debridement. *Periodontology 2000* **28**, 56–71.

Pfeilschifter, J., Wolf, O., Naumann, A., Minne, H. N., Mundy, G. R. & Ziegler, R. (1990) Chemotactic response of osteoblast-like cells to transforming growth factor-β. *Journal of Bone and Mineral Research* **5**, 825–830.

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

Polimeni, G., Albandar, J. M. & Wikesjö, U. M. E. (2005) Prognostic factors for alveolar regeneration: effect of space provision. *Journal of Clinical Periodontology* **32**, 951–954.

Polimeni, P., 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.

Polson, A. M. & Proye, M. F. (1983) Fibrin linkage: a precursor for new attachment. *Journal of Periodontology* **54**, 141–147.

Rokkanen, P. U., Böstman, O., Hirvensalo, E., Mäkelä, E. A., Partio, E. K., Pätiälä, H., Vainionpää, S., Vihtonen, K. & Törmälä, P. (2000) Bioabsorbable fixation in orthopaedic surgery and traumatology. *Biomaterials* **21**, 2607–2613.

Ruhé, P. Q., Hedberg, E. L., Padron, N. T., Spauwen, P. H. M., Jansen, J. A. & Mikos, A. G. (2005) Biocompatibility and degradation of poly(D,L-lactic-*co*-glycolic acid)/calcium phosphate cement composites. *Journal of Biomedical Materials Research* **74A**, 533–544.

Sampath, T. K., Maliakal, J. C., Hauschka, P. V., Jones, W. K., Sasak, H., Tucker, R. F., White, K. H., Coughlin, J. E., Tucker, M. M., Pang, R. H., Corbett, C., Özkaynak, E., Oppermann, H. & Rueger, D. (1992) Recombinant human osteogenic protein-1 (hOP-1) induces new bone formation in vivo with a specific activity comparable with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation in vitro. *The Journal of Biological Chemistry* **267**, 20352–20362.

Sanz, M., Tonetti, M. S., Zabalegui, I., Sicilia, A., Blanco, J., Rebelo, H., Rasperini, G., Merli, M., Cortellini, P. & Suvan, J. E. (2004) Treatment of intrabony defects with enamel matrix proteins or barrier membranes: results from a multicenter practice-based clinical trial. *Journal of Periodontology* **75**, 726–733.

Sbordone, L., Ramaglia, L. & Guletta, E. (1990) Recolonization of the subgingival microflora after scaling and root planing in human periodontitis. *Journal of Periodontology* **61**, 579–584.

Schmitz, J. P., Lemke, R. R., Zardeneta, G., Hollinger, J. O. & Milam, SB. (2000) Isolation of particulate degradation debris 1 year after implantation of a Guidor membrane for guided bone regeneration: case report. *Journal of Oral and Maxillofacial Surgery* **58**, 888–893.

Sculean, A., Windisch, P., Keglevich, T. & Gera, I. (2003) Histologic evaluation of human intrabony defects following non-surgical periodontal therapy with and without application of an enamel matrix protein derivative. *Journal of Periodontology* **74**, 153–160.

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.

Shi, Y. & Massague, J. (2003) Mechanisms of TGF-bets signaling from cell membrane to the nucleus. *Cell* **113**, 685–700.

Sigurdsson, T. J., Hardwick, R., Bogle, G. C. & Wikesjö, U. M. E. (1994) Periodontal repair in dogs: space provision by reinforced ePTFE

membranes enhances bone and cementum regeneration in large supraalveolar defects. *Journal of Periodontology* **65**, 350–356.

Sigurdsson, T. J., Lee, M. B., Kubota, K., Turek, T. J., Wozney, J. M. & Wikesjö, U. M. E. (1995) Periodontal repair in dogs: recombinant human bone morphogenetic protein-2 significantly enhances periodontal regeneration. *Journal of Periodontology* **66**, 131–138.

Sigurdsson, T. J., Nygaard, L., Tatakis, D. N., Fu, E., Turek, T. J., Wozney, J. M. & Wikesjö, U. M. E. (1996) Periodontal repair in dogs: evaluation of rhBMP-2 carriers. *The International Journal of Periodontics and Restorative Dentistry* **16**, 525–537.

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.

Spiro, R. C., Liu, L.-S., Heidaran, M. A., Thompson, A. Y., Ng, C. K., Pohl, J. & Poser, J. W. (2000) Inductive activity of recombinant human growth and differentiation factor-5. *Biochemical Society Transactions* **28**, 362–368.

Spiro, R. C., Thompson, A. Y. & Poser, J. W. (2001) Spinal fusion with recombinant human growth and differentiation factor-5 combined with a mineralized collagen matrix. *The Anatomical Record* **263**, 388–395.

Tatakis, D. N. & Trombelli, L. (1999) Adverse effects associated with a bioabsorbable guided tissue regeneration device in the treatment of human gingival recession defects. A clinicopathologic case report. *Journal of Periodontology* **70**, 542–547.

Trombelli, L., Lee, M. B., Promsudthi, A., Guglielmoni, P. G. & Wikesjö, U. M. E. (1999) Periodontal repair in dogs: histologic observations of guided tissue regeneration with a prostaglandin E_1 analog/methacrylate composite. *Journal of Clinical Periodontology* **26**, 381–387.

Urist, M. R. (1965) Bone: formation by autoinduction. *Science* **150**, 893–899.

Vaccaro, A. R., Anderson, D. G. & Toth, C. A. (2002) Recombinant human osteogenic protein-1 (bone morphogenetic protein-7) as an osteoinductive agent in spinal fusion. *Spine* **27**, 59–65.

Valentin-Opran, A., Wozney, J., Csimma, C., Lilly, L. & Riedel, G. E. (2002) Clinical evaluation of recombinant human bone morphogenetic protein-2. *Clinical Orthopaedics and Related Research* **395**, 110–120.

Wennström, J. L. & Lindhe, J. (2002) Some effects of enamel matrix proteins on wound healing in the dento-gingival region. *Journal of Clinical Periodontology* **29**, 9–14.

Wikesjö, U. M. E., Crigger, M., Nilvéus, R. & Selvig, K. A. (1991) Early healing events at the dentin–connective tissue interface. Light and transmission electron microscopy observations. *Journal of Periodontology* **62**, 5–14.

Wikesjö, U. M. E., Guglielmoni, P., Promsudthi, A., Cho, K.-S., Trombelli, L., Selvig, K. A., Jin, L. & Wozney, J. M. (1999) Periodontal repair in dogs: effect of rhBMP-2 concentration on regeneration of alveolar bone and periodontal attachment. *Journal of Clinical Periodontology* **26**, 392–400.

Wikesjö, U. M. E. & Nilvéus, R. (1990) Periodontal repair in dogs: effect of wound stabilization on healing. *Journal of Periodontology* **61**, 719–724.

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

Wikesjö, U. M. E., Sorensen, R. G., Kinoshita, A., Li, X. J. & Wozney, J. M. (2004) Periodontal repair in dogs: effect of rhBMP-12 on regeneration of alveolar bone and periodontal attachment. A pilot study. *Journal of Clinical Periodontology* **31**, 662–670.

Wikesjö, U. M. E., Sorensen, R. G. & Wozney, J. M. (2001) Augmentation of alveolar bone and dental implant osseointegration: clinical implications of studies with rhBMP-2. A comprehensive review. *The Journal of Bone and Joint Surgery. American Volume* **83-A** (Suppl. 1, Part 2), 136–145.

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

Wikesjö, U. M. E., Lim, W. H., Thomson, R. C. & Hardwick, W. R. (2003b) Periodontal repair in dogs: gingival tissue exclusion, a critical requirement for guided tissue regeneration? *Journal of Clinical Periodontology* **30**, 655–664.

Wikesjö, U. M. E., Xiropaidis, A. V., Thomson, R. C., Cook, A. D., Selvig, K. A. & Hardwick, W. R. (2003c) Periodontal repair in dogs: rhBMP-2 significantly enhances bone formation under provisions for guided tissue regeneration. *Journal of Clinical Periodontology* **30**, 705–714.

Wikesjö, U. M. E., Xiropaidis, A. V., Thomson, R. C., Cook, A. D., Selvig, K. A. & Hardwick, W. R. (2003d) Periodontal repair in dogs: space-providing ePTFE devices increase rhBMP-2/ACS-induced bone formation. *Journal of Clinical Periodontology* **30**, 715–725.

Wolfmann, N. M., Hattersley, G., Cox, K., Celeste, A. J., Nelson, R., Yamaji, N., Dube, J. L., DiBlasio-Smith, E., Nove, J., Song, J. J., Wozney, J. M. & Rosen, V. (1997) Ectopic induction of tendon and ligament in rats by growth and differentiation factors 5, 6 and 7, members of the TGF-β gene family. *The Journal of Clinical Investigation* **100**, 321–330.

Wozney, J. M., Rosen, V., Celeste, A. J., Mitsock, L. M., Whitters, M. J., Kriz, R. W., Hewick, R. M. & Wang, E. A. (1988) Novel regulators of bone formation: molecular clones and activities. *Science* **242**, 1528–1534.

Address:
Dr. Carola Dony
Scil Technology GmbH
Fraunhoferstraße 15
82152 Martinsried
Germany
E-mail: carola.dony@sciltechnology.com

Clinical Relevance

Scientific rationale for the study: As the periodontium encompasses several tissues, it remains a challenge to develop biomaterials that fulfil the necessary requirements to achieve a balanced support of periodontal regeneration. The objective of this study was to evaluate the physico-chemical properties and biocompatibility of a novel injectable, in situ setting composite for growth factor-enhanced periodontal regeneration.
Principal findings: The physico-chemical properties of the composite were adapted to meet the aimed requirements, including controlled release of rhGDF-5 over a period of 4 weeks in vitro. The material is completely synthetic, biodegradable, and can be produced as a convenient easy-to-use formulation. In a pre-clinical dog study, the composite showed excellent biocompatibility.
Practical implications: The characteristics of the novel composite are promising making it a model candidate for the minimally invasive delivery of rhGDF-5 to allow for a regeneration of all relevant tissues in periodontal and alveolar (peri-implant) defects.