J Clin Periodontol 2008; 35: 64–75 doi: 10.1111/j.1600-051X.2007.01159.x

Journal of Clinical Periodontology

Bone regeneration in dehiscence-type defects at non-submerged and submerged chemically modified (SLActive®) and conventional SLA titanium implants: an immunohistochemical study in dogs

Frank Schwarz[1], Martin Sager[2], Daniel Ferrari[1], Monika Herten[1], Marco Wieland[3] and Jürgen Becker[1]

[1]Department of Oral Surgery; [2]Animal Research Institute, Heinrich Heine University, Düsseldorf, Germany; [3]Institut Straumann AG, Basel, Switzerland

Schwarz F, Sager M, Ferrari D, Herten M, Wieland M, Becker J. Bone regeneration in dehiscence-type defects at non-submerged and submerged chemically modified (SLActive®) and conventional SLA titanium implants: an immunohistochemical study in dogs. J Clin Periodontol 2008; 35: 64–75. doi: 10.1111/j.1600-051X.2007.01159.x.

Abstract

Objectives: The aim of the present study was to evaluate bone regeneration in dehiscence-type defects at non-submerged and submerged titanium implants with chemically modified (mod) and conventional sandblasted/acid-etched (SLA) surfaces.

Material and Methods: Standardized buccal dehiscence defects were surgically created following implant site preparation in both the upper and lower jaws of 12 beagle dogs. Both types of implants were randomly assigned to either a non-submerged or a submerged healing procedure. After 1, 2, 4, and 8 weeks, dissected blocks were processed for histomorphometrical [e.g. new bone height (NBH), per cent linear fill (PLF), percentage of bone to implant contact (BIC-D), area of new bone fill (BF)] and immunohistochemical analysis.

Results: At 8 weeks, non-submerged and submerged SLA implants revealed significantly lower mean NBH (1.1 ± 0.8–1.9 ± 1.2 mm), PLF (27.7 ± 20.3–46.0 ± 28.5%), BIC-D (26.8 ± 10.4–46.2 ± 16.2%), and BF (1.3 ± 0.9–3.4 ± 2.8 mm^2) values than respective modSLA implants [NBH (2.6 ± 0.8–4.3 ± 0.1 mm), PLF (64.2 ± 19.4–107.2 ± 4.7%), BIC-D (67.5 ± 18.8–82.1 ± 14.8%), BF (2.9 ± 1.0–6.7 ± 1.1 mm^2)]. Within modSLA groups, significantly highest BF values were observed at submerged implants.

Conclusion: It was concluded that (i) modSLA titanium surfaces promoted bone regeneration in acute-type buccal dehiscence defects and (ii) a submerged healing procedure improved the outcome of healing additionally.

Key words: animal study; bone regeneration; dehiscence-type defect; hydrophilic titanium surface; immunohistochemistry

Accepted for publication 5 October 2007

Conflict of interest and source of funding statement

Frank Schwarz, Martin Sager, Daniel Ferrari, Monika Herten and Jürgen Becker declare that they have no conflict of interests. Dr. Wieland is an employee of Institute Straumann AG, the manufacturer of the tested implant systems.

This study was funded by a grant (454-2006) of the ITI Foundation, an independent non-profit organization financially supported by Institute Straumann AG, Basel, Switzerland. The study materials were kindly provided by Institut Straumann AG.

Nowadays, there is considerable evidence supporting the view that the technique of guided bone regeneration (GBR) may also predictably support bone augmentation at dehisced dental implants (Becker et al. 1990, Mayfield et al. 1998, Oh et al. 2003). The basic concept originally described for GBR involves the placement of a barrier membrane to protect the blood clot and create a secluded space around the bone defect, thus enabling access for bone-forming cells without competition from other tissues (Dahlin et al. 1988). Because osteogenic cells have also been observed to arise from pericytes adjacent to small blood vessels in connective tissue, it was hypothesized that early vascularization of the wound area may also play a crucial role in GBR (Long et al. 1995, Rickard et al. 1996, Reilly et al. 1998). Indeed, the results from a recent animal study have indicated that barrier membranes supporting an early transmembraneous angiogenesis exhibited an enhanced bone regeneration in a peripheral compartment of dehiscence-type defects (Schwarz et al. 2007e). Recently, the specific surface properties noted for a chemically modified sandblasted, large grit, and acid-etched (modSLA) titanium surface have also been shown to enhance angiogenesis during very early stages of osseointegration (Schwarz et al. 2007a, d). In particular, it was observed that within 24 h the stabilized blood clot adjacent to hydroxylated/hydrated modSLA surfaces was organized by proliferating blood vessels, and homogeneously substituted by a well-organized collagen-rich connective tissue at day 4. In contrast, conventional SLA surfaces revealed a partial collapse of the blood clot at day 1, resulting in a delayed vascularization and mainly formation of an undifferentiated granulation tissue (Schwarz et al. 2007a, d). Accordingly, modSLA surfaces exhibited a significantly higher mean percentage of bone to implant contact (BIC) as compared with conventional SLA surfaces at 7, 14, and 28 days of both non-submerged and submerged healing (Buser et al. 2004, Bornstein et al. 2007, Schwarz et al. 2007a, d). These observations might be explained by the higher surface free energy and increased wettability (initial advancing water contact angle of 0°) of hydrophilic modSLA surfaces (Rupp et al. 2006). The specific production process used for modSLA surfaces (i.e. rinsing the titanium surface after the etching process under N_2 protection and continuous storage in an isotonic NaCl solution) has been reported to retain the high surface energy of the uncontaminated TiO_2 surface by preventing the adsorption of potential contaminants from the atmosphere (e.g. hydrocarbons and carbonates) (Zhao et al. 2005). Recent studies have shown that osteoblasts grown on modSLA surfaces exhibited a more differentiated phenotype characterized by increased alkaline phosphatase activity and osteocalcin (OC) and generated an osteogenic microenvironment through higher production of PGE2 and TGF-β1 (Zhao et al. 2005, 2007). Based on these findings, it was hypothesized that the hydrophilic surface properties noted for modSLA implants might also support bone formation at deficient jaw bone sites. Indeed, most recent histological data have demonstrated that modSLA titanium surfaces promoted bone regeneration in acute-type buccal dehiscence defects at submerged implants without the additional use of GBR. In contrast, wound healing at SLA implants was predominantly characterized by the formation of a dense connective tissue, without any signs of bone regeneration (Schwarz et al. 2007c). So far, however, it remains unknown to what extent a submerged healing procedure might have prevented a collapse of the mucoperiosteal flap into the defect area and subsequently influenced bone regeneration.

Therefore, the aim of the present study was to immunohistochemically evaluate bone regeneration in dehiscence-type defects at either non-submerged or submerged modSLA and SLA titanium implants in a dog model.

Material and Methods

Animals

Twelve beagle dogs (age 12–15 months, mean weight 10.6 ± 1.2 kg) were used in the study. All animals exhibited a fully erupted permanent dentition. During the experiment, the dogs were fed once per day with soft-food diet and water. Animal selection, management, and surgery protocol were approved by the Animal Care and Use Committee of the Heinrich Heine University and the Bezirksregierung Düsseldorf. The experimental segment of the study started after an adaption period of 4 weeks.

Study design

The study was performed in two surgical phases. In the first phase, extraction of the mandibular and maxillary second, third, fourth pre-molar as well as the first and second molar (P2–M2) was performed bilaterally in all dogs. After 3 months of healing, standardized buccal dehiscence defects were surgically created following implant site preparation in both the upper ($n = 8$ defects per animal) and the lower jaws ($n = 8$ defects per animal). Subsequently, a total of two modSLA and two SLA implants were randomly placed in each quadrant of both the upper and lower jaws. According to a split-mouth design, the quadrants were randomly allocated to either a submerged or a non-submerged healing procedure (total $n = 16$ implants per animal).

Randomization was based on a computer-generated list (RandList®, DatInf GmbH, Tübingen, Germany). The animals were sacrificed after 1, 2, 4, and 8 weeks of healing, including three animals each.

Surgical procedure

Following intra-muscular sedation with 0.17 mg/kg acepromazine (Vetranquil 1%, Ceva Tiergesundheit, Düsseldorf, Germany), anaesthesia was initiated using 21.5 mg/kg thiopental-sodium (Trapanal 2.5%, Altana GmbH, Konstanz, Germany). During all the surgical procedures, inhalation anaesthesia was performed using oxygen and nitrous oxide and isoflurane. To maintain hydration, all animals received a constant-rate infusion of lactated Ringer's solution while anaesthetized. Intra-operative analgesia was performed

by an intravenous injection of 0.4 mg/kg piritramid (Dipidolor®, Janssen-Cilag GmbH, Neuss, Germany) and 4.5 mg/kg carprofene (Rimadyl®, Pfitzer Pharma GmbH, Karlsruhe, Germany). For post-operative treatment, piritramid and carprofene were applied subcutaneously for 3 days at the same dose as described above.

In the first surgery, P2–M2 were carefully removed bilaterally in both jaws after reflection of mucoperiosteal flaps and tooth separation. After wound closure by means of mattress sutures, the sites were allowed to heal for 3 months. Prophylactic administration of clindamycine (11.0 mg/kg body weight, Cleorobe®, Pharmacia Tiergesundheit, Erlangen, Germany) was performed intra- and post-operatively for 10 days.

In the second surgery, midcrestal incisions were made and full-thickness mucoperiosteal flaps were reflected to expose the respective sites for implant insertion in both the upper and lower jaws. Surgical implant sites were prepared bilaterally, at a distance 10 mm apart, using a low-trauma surgical technique under copious irrigation with sterile 0.9% physiological saline (surgery protocol by Institut Straumann AG, Basel, Switzerland).

Following implant site preparation, standardized dehiscence-type defects, approximately 4 mm in height from the crestal bone, 3 mm in depth from the surface of the buccal bone, and 3 mm in width mesiodistally, were created with a straight fissure carbide bur. The osteotomy procedures were performed under copious irrigation with sterile 0.9% physiological saline. The defect sizes were standardized using a periodontal probe (PCP12, Hu-Friedy Co., Chicago, IL, USA). Thereafter, both modSLA and SLA implants (Standard Plus, regular neck, ∅3.3 mm, length 8 mm, Institut Straumann AG, Basel, Switzerland, commercial name of modSLA is SLActive®) were inserted with good primary stability (i.e. lack of clinical implant mobility) in such a way that the borderline between the transmucosal (1.8 mm) and the bony part of the implant (BTB) coincided with the bone crest (Fig. 1a and b). In case of SLA, the implants were thoroughly rinsed with sterile saline before insertion. Following irrigation, the mucoperiosteal flaps were either repositioned, or advanced to allow for either a non-submerged or a submerged healing procedure, respectively. In both groups, wound closure was achieved with resorbable 5.0 polyglygolic acid mattress sutures (Resorba®, Nürnberg, Germany) (Fig. 1c and d). All surgical procedures were performed by the same experienced operator.

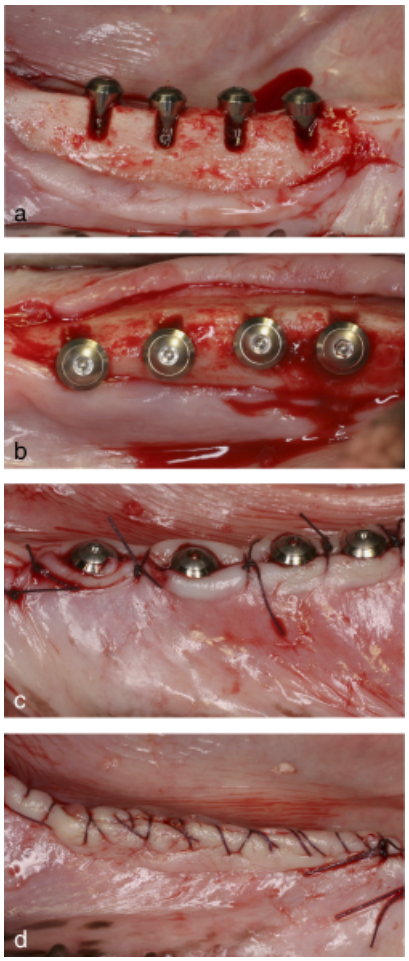

Fig. 1. (a) Following implant site preparation, four standardized dehiscence-type defects, approximately 4 mm in height from the crestal bone, and 3 mm in width mesiodistally, were created in each quadrant of both upper and lower jaws. Both modSLA and SLA implants were inserted in a way so that the borderline between the transmucosal (1.8 mm) and bony part of the implant (BTB) coincided with the bone crest. (b) All defects revealed a depth of 3 mm, as measured from the surface of the buccal bone. The mucoperiosteal flaps were either repositioned, or advanced to allow for a non-submerged (c) or submerged (d) healing procedure, respectively. SLA, sandblasted/acid-etched; modSLA, modified SLA.

Animal sacrifice and retrieval of specimens

The animals were killed (overdose of sodium pentobarbital 3%) after a healing period of 1, 2, 4, and 8 weeks including three animals each, respectively, and the oral tissues were fixed by perfusion with 10% buffered formalin administered through the carotid arteries. The jaws were dissected and blocks containing the experimental specimens were obtained. All specimens

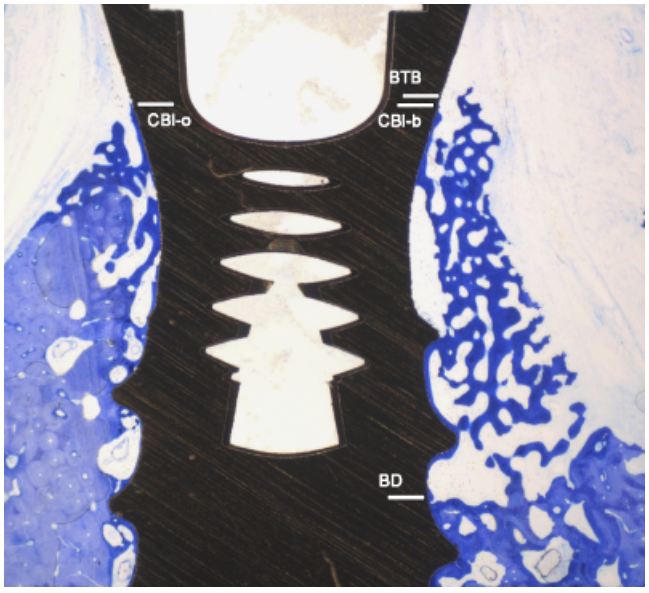

Fig. 2. Landmarks for histomorphometrical analysis: borderline between the transmucosal and bony part of the implant (BTB), the bottom of the bone defect (BD), the most coronal level of bone in contact with the implant at both buccal and oral sites (CBI-b/o). (modSLA, 2 weeks, submerged, lower jaw, central aspect, TB stain, original magnification × 25).

Table 1. Histomorphometrical results (± SD) of non-submerged implants in the upper jaw at 1, 2, 4, and 8 weeks ($n = 12$ dogs)

	modSLA		SLA		p value*	
	central	lateral	central	lateral	central	lateral
DL (mm)						
1 weeks	4.0 ± 0.2	4.1 ± 0.3	4.2 ± 0.1	4.1 ± 0.1	NS	NS
2 week	4.1 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	4.1 ± 0.2	NS	NS
4 weeks	4.0 ± 0.1	4.1 ± 0.2	4.2 ± 0.1	4.2 ± 0.1	NS	NS
8 weeks	4.0 ± 0.1	4.1 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	NS	NS
NBH (mm)						
1 week	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS	NS
2 weeks	1.1 ± 0.3†	2.7 ± 0.7	0.7 ± 0.7	1.0 ± 0.8	NS	$p<0.05$
4 weeks	2.6 ± 0.2†	3.2 ± 0.3	1.5 ± 0.3	2.0 ± 1.4	NS	$p<0.05$
8 weeks	4.3 ± 0.1	3.2 ± 0.9	1.8 ± 1.7	1.9 ± 1.2	$p<0.01$	$p<0.05$
PLF (%)						
1 week	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS	NS
2 weeks	27.8 ± 8.0‡	64.3 ± 15.7	18.3 ± 18.3	23.6 ± 18.8	NS	$p<0.05$
4 weeks	66.2 ± 7.0†	77.0 ± 3.3	35.9 ± 7.2	46.3 ± 32.9	$p<0.05$	$p<0.05$
8 weeks	107.2 ± 4.7	77.9 ± 21.2	43.8 ± 41.2	45.8 ± 30.5	$p<0.01$	$p<0.05$
BIC-D (%)						
1 week	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS	NS
2 weeks	27.6 ± 7.3†	68.0 ± 18.3	12.5 ± 12.5	24.2 ± 19.4	NS	$p<0.05$
4 weeks	60.8 ± 2.0†	77.4 ± 3.2	28.4 ± 2.9	40.6 ± 28.4	$p<0.05$	$p<0.05$
8 weeks	78.8 ± 11.0	70.4 ± 7.8	35.2 ± 34.0	45.2 ± 30.2	$p<0.05$	$p<0.05$
BF (mm^2)						
1 week	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS	NS
2 weeks	1.8 ± 0.2‡	5.5 ± 1.5	1.1 ± 1.1	1.5 ± 1.1†	NS	$p<0.01$
4 weeks	4.5 ± 1.1	10.9 ± 1.7‡	1.9 ± 0.1	5.8 ± 5.6	$p<0.05$	$p<0.01$
8 weeks	2.9 ± 1.0‡	3.3 ± 1.7†	2.2 ± 1.8	1.3 ± 0.9	NS	$p<0.05$
D-CBI (mm)						
1 week	4.2 ± 0.2	3.9 ± 0.5	4.1 ± 0.1	4.2 ± 0.4	NS	NS
2 weeks	2.9 ± 0.4‡	1.2 ± 0.4	3.4 ± 0.8	3.0 ± 1.8	NS	$p<0.05$
4 weeks	1.9 ± 0.7†	0.7 ± 0.1	2.7 ± 0.3	2.1 ± 1.2	$p<0.01$	$p<0.05$
8 weeks	0.2 ± 0.1	0.6 ± 0.3	2.5 ± 1.2	1.9 ± 1.3	$p<0.05$	NS

*Comparisons between groups (unpaired t-test).
Comparisons within (non-submerged to submerged) groups (paired t-test):
†$p<0.05$, ‡$p<0.01$.
SLA, sandblasted/acid-etched; modSLA, modified SLA; DL, defect length; NBH, new bone height; PLF, per cent linear fill; BIC-D, percentage of bone to implant contact; BF, area of new bone fill; D-CBI, difference in buccal and oral dimension of CBI.

were fixed in 10% neutral-buffered formalin solution for 4–7 days.

Histological preparation

The specimens were dehydrated using ascending grades of alcohol and xylene, infiltrated, and embedded in methylmethacrylate (Technovit 9100 NEU, Heraeus Kulzer, Wehrheim, Germany) for non-decalcified sectioning. During this procedure, any negative influence of polymerization heat was avoided due to a controlled polymerization in a cold atmosphere (−4°C). After 20 h, the specimens were completely polymerized. Each implant site was cut in the bucco-oral direction along with the long axis of the implant using a diamond wire saw (Exakt®, Apparatebau, Norderstedt, Germany). Serial sections were prepared from the respective defect areas, resulting in four sections approximately 300 μm in thickness each (Donath 1985). In particular, implant sections showing an inner thread were chosen for the evaluation of central defect areas, while respective sections showing no inner thread of the implant were chosen for the evaluation of lateral defect areas. Subsequently, all specimens were glued with acrylic cement (Technovit 7210 VLC, Heraeus Kulzer) to silanized glass slides (Super Frost, Menzel GmbH, Braunschweig, Germany) and ground to a final thickness of approximately 40 μm. One part of the sections was scheduled for histomorphometrical analysis and stained with toluidine blue (TB) to evaluate new bone formation. With this technique, old bone stains light blue, whereas newly formed bone stains dark blue because of its higher protein content (Schenk et al. 1984). The other part of the sections was prepared for immunohistochemical labelling. Additionally, sections obtained at 1 week were stained with Masson–Goldner trichrome (MG).

Immunohistochemical labelling

For immunohistochemistry, all tissue section were deplasted in xylol (2 × 30 min.) followed by a treatment in 2-methoxyethylacetate (2 × 20 min.) and acetone (2 × 5 min.). After rehydration in phosphate-buffered saline (PBS), antigen unmasking was performed by incubating the slides for 15 min. in trypsin (0.05% in PBS, PAA Laboratories GmbH, Pasching, Austria) at 37°C. After washing with PBS, the activity of endogenous peroxidase was quenched with 0.9% hydrogen peroxide in PBS for 10 min. at room temperature, the specimens were washed, and non-specific binding sites were blocked with a blocking solution for 30 min. (DakoCytomation, Hamburg, Germany). The primary mouse monoclonal antibody to transglutaminase II (angiogenesis) (1:40 dilution, Labvision, Fremont, CA, USA) as well as OC (1:40 dilution, Acris Antibodies GmbH, Hiddenhausen, Germany) and corresponding unspecific antibodies (mouse IgG_1) (DakoCytomation), respectively, as negative control were applied to tissue sections in a humidified chamber and incubated overnight at 8°C. The slides were washed in PBS, and incubated with secondary biotinylated anti-mouse antibody (1:50 dilution, DakoCytomation) for 90 min. at room temperature. After washing in PBS, the presence of antibody–antigen complexes was visualized using a streptavidin-peroxidase solution (1:250 dilution, DakoCytomation) and AEC (3-amino-9-ethylcarbazole) as the chromogen (DakoCytomation).

Histomorphometrical analysis

Histomorphometrical analyses as well as microscopic observations were performed by one experienced investigator masked to the specific experimental conditions. For image acquisition, a colour CCD camera (Color View III, Olympus, Hamburg, Germany) was mounted on a binocular light microscope (Olympus BX50, Olympus). Digital images (original magnification × 200) were evaluated using a software program (analySIS FIVE docu®, Soft Imaging System, Münster, Germany).

The following landmarks were identified in the stained sections: BTB by

drawing a perpendicular line of 1.8 mm from the implant shoulder in the apical direction parallel to the long axis of the implant, the bottom of the bone defect (BD), the most coronal level of bone in contact with the implant at both buccal and oral sites (CBI-b/o). Defect length (DL) was measured from BTB to BD (mm), new bone height (NBH) was measured from BD to CBI-b (mm), per cent linear fill (PLF) was defined as NBH divided by DL, the amount of new BIC in the defect area was measured as the percentage of the distance from BD to BTB (BIC-D), and the difference in buccal and oral dimension of CBI (D-CBI) was defined as CBI-o − CBI-b (mm). Additionally, the area (mm^2) of new bone fill (BF) was measured from BD to CBI-b (Fig. 2).

Statistical analysis

The statistical analysis was performed using a commercially available software program (SPSS 15.0, SPSS Inc., Chicago, IL, USA). The mean values and standard deviations among animals were calculated for each variable and group. The data rows were examined with the Kolmogorow–Smirnow test for normal distribution. For the statistical evaluation of the changes within groups (i.e. non-submerged to submerged), the paired *t*-test was used. For comparisons between groups (i.e. either central or lateral aspects), the unpaired *t*-test was used. The α error was set at 0.05.

Results

The post-operative healing was uneventful in all dogs. There were no signs of any wound dehiscence or exposure of the transmucosal part of the implant body in respective submerged modSLA and SLA groups.

Histological observations/ histomorphometrical analysis

The mean values and percentages of DL, NBH, PLF, BIC-D, BF, and D-CBI for each group at 1, 2, 4, and 8 weeks are presented in Tables 1–4. In general, there were no significant differences in the mean DL values between non-submerged and submerged implants in different groups over time ($p > 0.05$; unpaired *t*-test, respectively).

At 1 week, histomorphometrical analysis failed to reveal any increases in mean NBH, PLF, BIC-D, or BF values in all groups. MG stain demonstrated a homogeneous stabilization of the blood clot at the both central and lateral aspects of modSLA implants (Fig. 3a). The area of stained fibrin, however, was thinner at the coronal aspect of the defect area. This was particularly true for non-submerged modSLA implants (Fig. 3b). In contrast, a partial or even full collapse of the blood clot was commonly observed at both non-submerged and submerged SLA implants (Fig. 3c). Immunohistochemical observation exhibited a pronounced OC antigen reactivity mainly along BD. In the basal compartment of the defect area, all groups demonstrated a primary spongework of newly formed blood vessels obviously originating from open marrow spaces at BD. However, the mean cross- and longitudinal sectional area of blood vessels at the lateral aspect of the defect area was significantly highest at non-submerged and submerged modSLA implants (upper jaw: 0.64–0.97 mm^2; lower jaw: 0.61–0.80 mm^2), compared with the respective SLA groups (upper jaw: 0.19–0.24 mm^2; lower jaw: 0.22–0.26 mm^2) ($p < 0.01$; unpaired *t*-test, respectively). The connective tissue surrounding the vascular structures was also demarcated by an intense OC staining (Fig. 3d). In these areas, TB stain revealed some tiny spots of mineralization (Fig. 3e). In both non-submerged and submerged modSLA groups, the formation of vascular structures was also observed within the central compartment of the wound area (upper jaw: 0.29–0.34 mm^2; lower jaw: 0.19–0.37 mm^2), mainly along the implant surface (Fig. 3f). In contrast, SLA implants revealed a significantly lower mean cross- and longitudinal sectional area of blood vessels at the central

Table 2. Histomorphometrical results (± SD) of non-submerged implants in the lower jaw at 1, 2, 4, and 8 weeks ($n = 12$ dogs)

	modSLA		SLA		*p* value*	
	central	lateral	central	lateral	central	lateral
DL (mm)						
1 week	4.1 ± 0.2	4.2 ± 0.1	4.0 ± 0.2	4.2 ± 0.3	NS	NS
2 weeks	4.0 ± 0.2	4.2 ± 0.1	4.2 ± 0.1	4.1 ± 0.2	NS	NS
4 weeks	4.1 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	NS	NS
8 weeks	4.1 ± 0.1	4.1 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	NS	NS
NBH (mm)						
1 week	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS	NS
2 weeks	1.5 ± 0.5	2.0 ± 0.7†	0.3 ± 0.2	0.4 ± 0.3	NS	$p<0.05$
4 weeks	2.7 ± 0.9	3.2 ± 0.7	1.5 ± 0.7	1.6 ± 1.1	$p<0.05$	$p<0.05$
8 weeks	3.1 ± 1.1	2.6 ± 0.8	1.1 ± 0.8	1.7 ± 1.6	$p<0.05$	NS
PLF (%)						
1 week	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS	NS
2 weeks	36.6 ± 11.6	48.9 ± 17.1†	10.3 ± 7.6	10.8 ± 8.6	$p<0.05$	$p<0.01$
4 weeks	65.2 ± 20.6	77.7 ± 17.1	37.9 ± 18.2	37.8 ± 27.0	$p<0.05$	$p<0.05$
8 weeks	75.8 ± 28.3	64.2 ± 19.4	27.7 ± 20.3	42.1 ± 39.7	$p<0.01$	$p<0.05$
BIC-D (%)						
1 week	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS	NS
2 weeks	38.2 ± 8.8	41.7 ± 12.9†	7.4 ± 4.5	5.0 ± 3.5	$p<0.01$	$p<0.01$
4 weeks	70.0 ± 15.0	77.0 ± 14.8	27.7 ± 11.9	37.3 ± 25.6	$p<0.01$	$p<0.05$
8 weeks	71.2 ± 20.3	67.5 ± 18.8	29.1 ± 17.1	39.0 ± 38.4	$p<0.01$	$p<0.05$
BF (mm^2)						
1 week	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS	NS
2 weeks	1.8 ± 0.8‡	3.7 ± 0.8	0.7 ± 0.7	0.8 ± 0.5	NS	$p<0.01$
4 weeks	4.7 ± 1.1	8.4 ± 4.2	2.9 ± 1.4	4.8 ± 2.6	$p<0.05$	$p<0.05$
8 weeks	4.3 ± 1.5†	3.9 ± 1.6†	1.9 ± 1.1	3.4 ± 2.8	$p<0.01$	NS
D-CBI (mm)						
1 week	3.8 ± 0.4	4.0 ± 0.3	4.2 ± 0.2	4.0 ± 0.2	NS	NS
2 weeks	2.6 ± 0.4	1.9 ± 0.8†	3.8 ± 0.4	4.1 ± 0.2†	NS	$p<0.05$
4 weeks	1.2 ± 1.2	0.9 ± 0.7	2.5 ± 0.7	2.5 ± 1.0	$p<0.05$	$p<0.05$
8 weeks	0.6 ± 0.4	1.3 ± 0.7	2.3 ± 0.9	2.7 ± 1.2	$p<0.05$	$p<0.05$

*Comparisons between groups (unpaired *t*-test).

Comparisons within (non-submerged to submerged) groups (paired *t*-test):
†$p<0.05$, ‡$p<0.01$.

SLA, sandblasted/acid-etched; modSLA, modified SLA; DL, defect length; NBH, new bone height; PLF, per cent linear fill; BIC-D, percentage of bone to implant contact; BF, area of new bone fill; D-CBI, difference in buccal and oral dimension of CBI.

Table 3. Histomorphometrical results (± SD) of submerged implants in the upper jaw at 1, 2, 4, and 8 weeks ($n = 12$ dogs)

	modSLA		SLA		p value*	
	central	lateral	central	lateral	central	lateral
DL (mm)						
1 week	4.0 ± 0.2	4.1 ± 0.2	4.1 ± 0.1	4.2 ± 0.2	NS	NS
2 weeks	4.1 ± 0.1	3.9 ± 0.1	4.2 ± 0.1	4.2 ± 0.2	NS	NS
4 weeks	4.2 ± 0.1	4.1 ± 0.2	4.1 ± 0.2	4.3 ± 0.2	NS	NS
8 weeks	4.1 ± 0.2	4.1 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	NS	NS
NBH (mm)						
1 week	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS	NS
2 weeks	3.3 ± 0.5†	3.0 ± 0.7	1.4 ± 1.2	1.5 ± 1.3	$p<0.05$	$p<0.05$
4 weeks	4.0 ± 0.1†	3.5 ± 0.4	2.0 ± 1.2	2.0 ± 1.8	$p<0.05$	NS
8 weeks	3.7 ± 0.1	3.6 ± 0.8	1.4 ± 1.0	1.9 ± 1.2	$p<0.05$	$p<0.05$
PLF (%)						
1 week	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS	NS
2 weeks	79.4 ± 13.0‡	76.1 ± 15.6	29.1 ± 24.9	36.5 ± 31.7	$p<0.01$	$p<0.01$
4 weeks	96.1 ± 3.2†	84.1 ± 10.9	49.1 ± 29.7	47.6 ± 42.2	$p<0.01$	$p<0.05$
8 weeks	91.2 ± 1.9	87.8 ± 18.4	34.5 ± 24.5	46.0 ± 28.5	$p<0.01$	$p<0.05$
BIC-D (%)						
1 week	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS	NS
2 weeks	67.6 ± 12.0†	61.3 ± 14.2	30.9 ± 30.2	36.8 ± 33.5	$p<0.05$	$p<0.05$
4 weeks	98.0 ± 11.1†	82.2 ± 10.1	40.5 ± 21.6	45.0 ± 38.2	$p<0.01$	$p<0.01$
8 weeks	81.4 ± 12.6	75.8 ± 22.3	34.4 ± 20.7	44.2 ± 24.6	$p<0.01$	$p<0.05$
BF (mm^2)						
1 week	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS	NS
2 weeks	10.5 ± 0.5‡	8.4 ± 3.8	1.9 ± 1.0	4.3 ± 1.1†	$p<0.01$	$p<0.05$
4 weeks	4.5 ± 1.0	6.4 ± 2.9‡	1.5 ± 0.8	3.4 ± 2.0	$p<0.05$	$p<0.01$
8 weeks	6.6 ± 0.9‡	6.7 ± 1.1†	2.6 ± 0.6	2.3 ± 0.7	$p<0.01$	$p<0.01$
D-CBI (mm)						
1 week	4.0 ± 0.3	4.0 ± 0.1	3.9 ± 0.2	4.3 ± 0.2	NS	NS
2 weeks	1.0 ± 0.5‡	1.2 ± 0.7	3.0 ± 1.3	2.6 ± 1.3	NS	$p<0.05$
4 weeks	0.2 ± 0.1†	0.4 ± 0.2	2.0 ± 1.2	2.5 ± 1.5	$p<0.05$	$p<0.05$
8 weeks	0.4 ± 0.1	0.3 ± 0.2	2.5 ± 0.7	2.1 ± 1.1	$p<0.05$	$p<0.05$

*Comparisons between groups (unpaired *t*-test).
Comparisons within (non-submerged to submerged) groups (paired *t*-test):
†$p<0.05$, ‡$p<0.01$.
SLA, sandblasted/acid-etched; modSLA, modified SLA; DL, defect length; NBH, new bone height; PLF, per cent linear fill; BIC-D, percentage of bone to implant contact; BF, area of new bone fill; D-CBI, difference in buccal and oral dimension of CBI.

aspect of the defect area (upper jaw: 0.07–0.12 mm^2; lower jaw: 0.06–0.09 mm^2) ($p<0.01$; unpaired *t*-test, respectively).

At 2 weeks, newly formed trabeculae of woven bone, arising from open marrow spaces at BD, invaded the defect area in coronal and lateral directions. While this trabecular bone established a close BIC in the modSLA groups (Fig. 4a and b), the respective SLA groups frequently revealed a layer of non-mineralized tissue separating the implant surface in the regenerated area (Fig. 4c and d). Immunohistochemical analysis revealed a pronounced OC antigen reactivity mainly within the connective tissue adjacent to modSLA implants (Fig. 5a and b). A partial collapse of the mucoperiosteal flap within the defect area was commonly observed for non-submerged SLA and modSLA as well as submerged SLA implants (Fig. 5c). The extent of bone formation varied considerably within and between groups. In particular, significantly the highest mean NBH, PLF, BIC-D, and BF values were observed at the central and lateral aspects of modSLA implants in both the upper and lower jaws. While at the lateral aspect, these values appeared to be comparable for non-submerged and submerged implants, the central aspect exhibited significantly higher mean NBH, PLF, BIC-D, BF, and subsequently lower D-CBI values for submerged implants. This was particularly true for modSLA implants placed in the upper jaws (Tables 1–4).

At 4 weeks, wound healing was mainly characterized by an ongoing bone formation in all groups. In general, the subsequently formed primary spongework of woven bone covered the defect area in coronal and lateral directions, primarily along and in close contact to both implant surfaces. Significantly highest mean NBH, PLF, BIC-D, and BF values were observed at the central and lateral aspects of modSLA implants in both the upper and lower jaws (Tables 1–4). However, in the upper jaw, submerged healing of modSLA implants resulted in significantly higher NBH, PLF, BIC-D, and subsequently lower D-CBI values than the respective non-submerged healing procedure. In particular, histological observation revealed a partial collapse of the mucoperiosteal flap at the central aspect of the defect area at non-submerged modSLA implants, thus compromising the space for bone regeneration in the lateral direction (Fig. 6a and b). A similar pattern of wound healing was also observed for non-submerged as well as submerged SLA implants, because in both groups, the mucoperiosteal flap had completely occupied the central compartment of the defect areas (Fig. 6c and d).

At 8 weeks, histological observation revealed a continuous filling of the intertrabecular spaces in all groups and subsequently a transformation into a firmly attached mature, parallel-fibred woven bone. Early signs of remodelling, replacing the primary bone by secondary osteons, were apparent. Again, the amount of bone regeneration varied considerably within and between groups. In particular, modSLA implants revealed significantly highest NBH, PLF, BIC-D, and BF values at both the central and lateral aspects of non-submerged and submerged implants (Tables 1–4). Commonly, the newly formed buccal aspects of the alveolar bone even reached the level of the respective oral aspects. However, submerged healing of modSLA implants resulted in significantly higher BF values at both central and lateral aspects than the respective non-submerged healing approach (Tables 1–4). This difference was more pronounced at the central aspect of the defect area, indicating that a submerged healing procedure supported space maintenance in this area (Fig. 7a and b). In contrast, non-submerged and submerged SLA implants commonly exhibited a collapse of the mucoperiosteal flap at the central aspect of the defect area, thus compromising the space for bone regeneration (Fig. 7c and d).

Table 4. Histomorphometrical results (± SD) of submerged implants in the lower jaw at 1, 2, 4, and 8 weeks ($n = 12$ dogs)

	ModSLA		SLA		p value*	
	central	lateral	central	lateral	central	lateral
DL (mm)						
1 week	4.2 ± 0.2	4.1 ± 0.2	4.1 ± 0.3	4.2 ± 0.1	NS	NS
2 weeks	4.3 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	4.2 ± 0.2	NS	NS
4 weeks	4.1 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	NS	NS
8 weeks	4.2 ± 0.2	4.1 ± 0.1	4.0 ± 0.2	4.1 ± 0.2	NS	NS
NBH (mm)						
1 week	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS	NS
2 weeks	1.8 ± 0.6	3.4 ± 1.1†	0.8 ± 0.7	1.6 ± 1.2	NS	$p<0.05$
4 weeks	2.8 ± 1.2	3.8 ± 0.5	1.3 ± 0.4	1.9 ± 1.0	$p<0.05$	$p<0.05$
8 weeks	3.7 ± 0.1	3.6 ± 0.3	1.2 ± 0.6	1.7 ± 0.5	$p<0.05$	$p<0.05$
PLF (%)						
1 week	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS	NS
2 weeks	41.5 ± 14.4	82.6 ± 27.9†	18.3 ± 15.2	37.8 ± 29.2	$p<0.05$	$p<0.01$
4 weeks	69.5 ± 29.8	91.9 ± 12.7	32.0 ± 10.7	46.6 ± 24.3	$p<0.05$	$p<0.05$
8 weeks	92.4 ± 2.4	88.3 ± 6.7	28.9 ± 15.1	40.8 ± 12.0	$p<0.01$	$p<0.01$
BIC-D (%)						
1 week	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS	NS
2 weeks	39.7 ± 12.0	75.9 ± 14.7†	8.6 ± 7.3	35.6 ± 24.8	$p<0.01$	$p<0.05$
4 weeks	67.1 ± 26.4	89.4 ± 10.6	32.0 ± 10.7	46.6 ± 24.3	$p<0.05$	$p<0.01$
8 weeks	82.1 ± 14.8	71.0 ± 20.3	26.8 ± 10.4	46.2 ± 16.2	$p<0.01$	$p<0.05$
BF (mm^2)						
1 week	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS	NS
2 weeks	5.2 ± 2.9‡	7.6 ± 2.5	1.7 ± 0.9	3.5 ± 1.9	$p<0.05$	$p<0.05$
4 weeks	5.2 ± 0.8	6.9 ± 2.3	2.3 ± 2.0	3.3 ± 2.4	$p<0.01$	$p<0.05$
8 weeks	6.4 ± 0.7†	5.4 ± 1.2†	1.9 ± 0.3	2.6 ± 0.7	$p<0.01$	$p<0.01$
D-CBI (mm)						
1 week	4.2 ± 0.4	4.1 ± 0.2	4.2 ± 0.2	4.0 ± 0.4	NS	NS
2 weeks	2.5 ± 0.6	0.6 ± 0.4†	3.6 ± 0.9	2.6 ± 1.3†	NS	$p<0.05$
4 weeks	1.3 ± 1.1	0.3 ± 0.2	2.9 ± 0.6	2.2 ± 1.1	$p<0.05$	$p<0.05$
8 weeks	0.3 ± 0.2	0.5 ± 0.3	2.6 ± 0.8	1.9 ± 0.3	$p<0.05$	$p<0.05$

*Comparisons between groups (unpaired *t*-test).
Comparisons within (non-submerged to submerged) groups (paired *t*-test):
†$p<0.05$, ‡$p<0.01$.
SLA, sandblasted/acid-etched; modSLA, modified SLA; DL, defect length; NBH, new bone height; PLF, per cent linear fill; BIC-D, percentage of bone to implant contact; BF, area of new bone fill; D-CBI, difference in buccal and oral dimension of CBI.

Discussion

The present study was designed to histomorphometrically evaluate bone regeneration in acute-type buccal dehiscence defects at either non-submerged or submerged modSLA and SLA titanium implants in a dog model. Within its limits, it was observed that new bone formation was mainly influenced by surface hydrophilicity in addition to microtopography. In particular, at 2, 4, and 8 weeks of healing, modSLA implants exhibited significantly higher mean NBH, PLF, BIC-D, BF, and subsequently lower D-CBI values than conventional SLA implants. In this context, it must be emphasized that the surgical creation of standardized buccal dehiscence-type defects in dogs is a commonly used model to evaluate bone regeneration at titanium implants (Becker et al. 1990, Zablotsky et al. 1991, Casati et al. 2002, Oh et al. 2003, Schwarz et al. 2007b, c, e). When interpreting the present results, however, one must keep in mind that acute-type defects have a certain tendency towards spontaneous healing, thus supporting bone regeneration in all groups investigated. Indeed, immunohistochemical analysis revealed a similar pattern of wound healing at the lateral aspects of the defect area in both modSLA and SLA groups. In particular, it was observed that angiogenesis, OC synthesis, and subsequently the formation of localized spots of mineralization mainly arose from open marrow spaces at the defect borders, thus explaining the improved bone regeneration at the lateral aspect of the wound area in all groups. However, in contrast to conventional SLA implants, an intense OC antigen reactivity was also commonly observed within the newly formed connective tissue along modSLA surfaces. Because OC is one of the most abundant non-collageneous proteins of the bone matrix that is exclusively synthesized by osteoblasts, odontoblasts, and hypertrophic chondrocytes (Gallop et al. 1980, Hauschka et al. 1989, Hopyan et al. 1999, Raymond et al. 1999), it might be supposed that osteoblastic differentiation was initiated within 1 week and mainly localized to the defect borders and along modSLA surfaces. Furthermore, after 1 week of healing, immunohistochemical analysis revealed a pronounced proliferation of blood vessels adjacent to modSLA surfaces, even reaching the central compartment of the defect area. In contrast, at SLA implants, the primary spongework of newly formed vascular structures was mainly localized to BD and the lateral aspects of the defect area. In this context, it must be emphasized that osteogenic cells have also been observed to arise from pericytes adjacent to small blood vessels in connective tissue (Long et al. 1995, Rickard et al. 1996, Reilly et al. 1998), thus explaining, at least in part, the pronounced bone regeneration at modSLA implants. The improved pattern of angiogenesis might be explained by the hydrophilic surface properties noted for hydroxylated/hydrated modSLA surfaces, resulting in a higher wettability when compared with conventional SLA surfaces (Rupp et al. 2006). Indeed, MG stain revealed a close adhesion of the blood clot to the hydrophilic modSLA implant surfaces at 1 week, thus promoting the ingrowth of new blood vessels from the adjacent alveolar bone. In contrast, the blood clot appeared to be partially or even fully collapsed at SLA implants. This observation is in agreement with previous studies, indicating an improved stabilization of the blood clot at hydrophilic modSLA surfaces during the initial stages of osseointegration (Schwarz et al. 2007a, d). Basically, the blood clot acts as a physical matrix that induces and amplifies the migration, proliferation, and differentiation of endothelial cells, subsequently leading to improved angiogenesis (Liu et al. 1990). Accordingly, the compromised bone regeneration at both non-submerged and submerged SLA implants might be explained by a partial or even full a destabilization of the blood clot, thus allowing a collapse of the mucoperiosteal flap within the wound area. This might also be

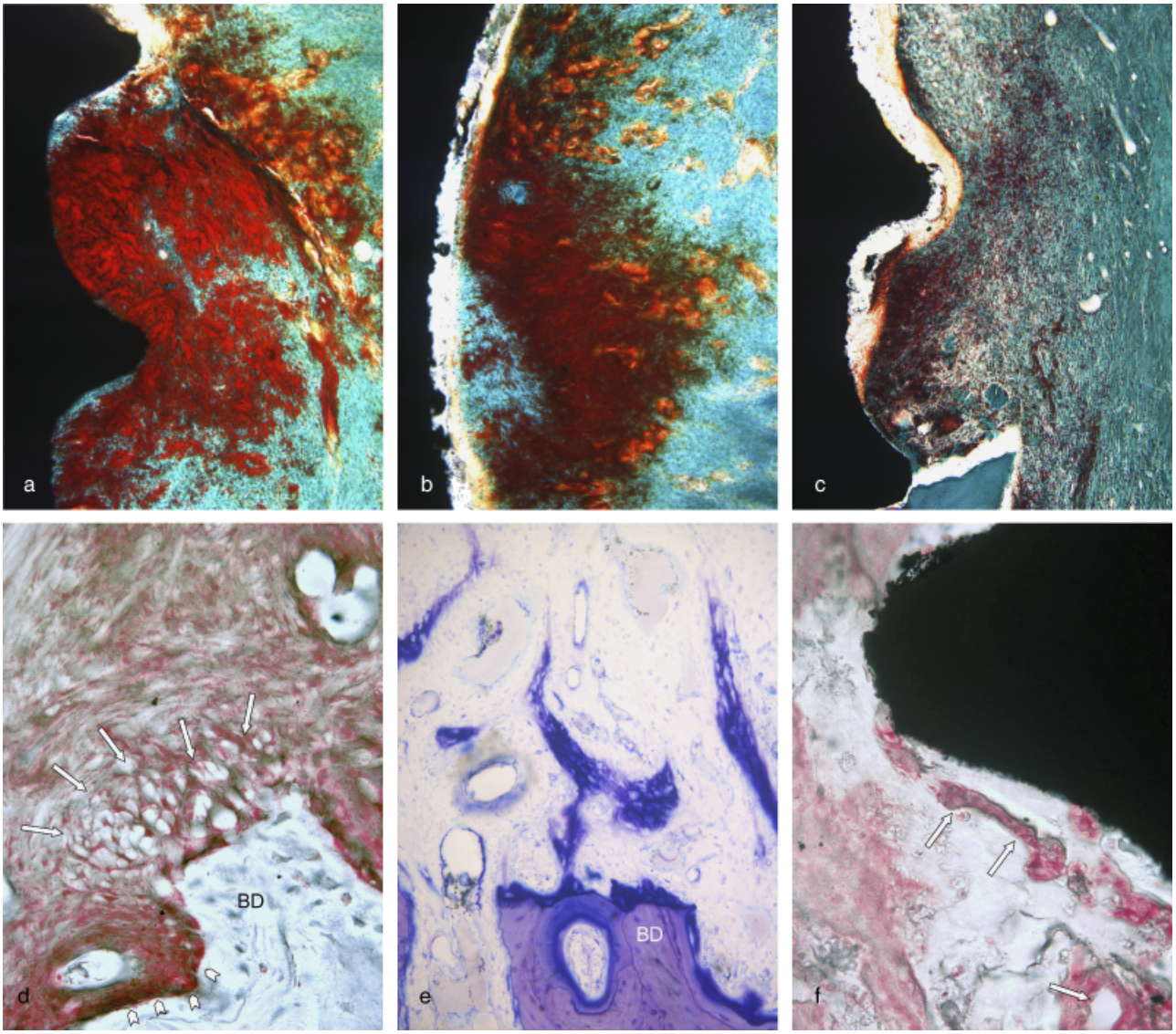

Fig. 3. (a) modSLA implants revealed a homogeneous stabilization of the blood clot at both central and lateral aspects of the defect area (1 week, submerged, lower jaw, central aspect, MG stain, original magnification × 100). (b) The area of stained fibrin was thinner at the coronal aspect of the defect (1 week, non-submerged, lower jaw, central aspect, MG stain, original magnification × 100). (c) Both non-submerged and submerged SLA implants commonly revealed a partial or even full collapse of the blood clot (1 week, submerged, lower jaw, central aspect, MG stain, original magnification × 100). (d) Pronounced OC antigen reactivity along to open marrow spaces at BD (arrowheads) and within the connective tissue surrounding a spongework of newly formed blood vessels (arrows) (SLA, 1 week, submerged, upper jaw, central aspect, original magnification × 400). (e) Some tiny spots of mineralization were mainly localized at the basal compartment of the defect area (modSLA, 1 week, non-submerged, upper jaw, central aspect, TB stain, original magnification × 400). (f) Non-submerged and submerged modSLA implants also revealed the formation of vascular structures (arrows) within the central compartment of the defect area (1 week, submerged, lower jaw, central aspect, TG antigen reactivity, original magnification × 400).

supported by the observation that submerged SLA implants revealed a slightly improved bone regeneration, suggesting that the implant shoulder supported the mucoperiosteal flap preserving its original position. Similar results were also observed in the modSLA group, because submerged implants exhibited significantly higher BF values than the non-submerged group. Indeed, histological analysis revealed a slight collapse of the mucoperiosteal flap and subsequently a thinner blood clot particularly at the coronal aspect of non-submerged modSLA implants. Another possible explanation for the improved bone regeneration at modSLA implants might be in part due to the stimulatory effects of high surface energy on osteogenic cells. As described above, recent results from an in vitro study have shown that osteoblasts grown on modSLA surfaces exhibited a more differentiated phenotype characterized by increased ALP activity and OC synthesis and generated an osteogenic microenvironment through higher production of PGE2 and TGF-β1 (Zhao et al. 2005, 2007). Indeed, recent animal studies provide clear evidence that modSLA surfaces enhanced bone apposition during early stages of wound healing at both non-submerged and submerged implant sites (Buser et al. 2004, Ferguson et al. 2006, Bornstein et al. 2007, Schwarz et al. 2007a, d). In general, the

amount of bone regeneration at SLA implants, as observed in the present study, is in agreement with most of the results obtained at untreated control defects, because exposed implant threads were mainly covered by a loosely adherent connective tissue and only minute amounts of NBH and BIC (Dahlin et al. 1989, Becker et al. 1990, Oh et al. 2003). The observation that submerged modSLA implants promoted bone regeneration in acute-type buccal dehiscence defects without the additional use of GBR or bone augmentation procedures is in agreement with recent results from a pilot study in dogs (Schwarz et al. 2007c). In particular, wound healing at SLA implants was predominantly characterized by the formation of a dense connective tissue at 2 and 12 weeks, without significant increases in mean NBH, PLF, BIC-D, or BF values. In contrast, modSLA implants exhibited a complete defect fill at 12 weeks following implant placement. At 2 and 12 weeks, SLA implants revealed significantly lower mean NBH (0 mm; 0.3–0.4 mm), PLF (0%; 7–10%), BIC-D (0%; 4–5%), BF (0 mm^2; 0.07–0.08 mm^2), and D-CBI (3.1–3.4 mm; 3.8–4.3 mm) values than modSLA implants [NBH (1.1–2.6 mm; 3.3–3.2 mm), PLF (34–80%; 97–98%), BIC-D (27–61%; 80–85%), BF (0.4–1.8 mm^2; 2.3–2.4 mm^2), and D-CBI (0.6–2.1 mm; 0.1–0.2 mm)]. However, the amount of BF reported in both groups appeared to be lower than the respective values obtained in the submerged healing groups of the present study. In this context, however, it must be emphasized that potential differences might be related to a smaller number ($n = 4$) and the higher age (20–24 months) of the animals as well as the reduced defect height of 3 mm (Schwarz et al. 2007c). A similar pattern and amount of bone regeneration was also observed when modSLA implants were combined with different types of barrier membranes or bone substitutes (Schwarz et al. 2007b, e). In particular, blood vessels and the subsequently

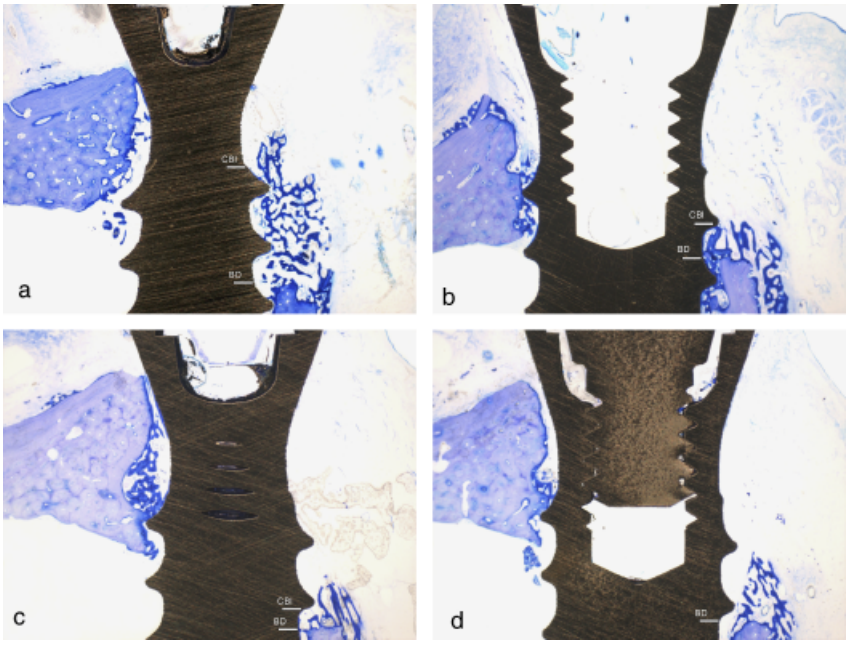

Fig. 4. Representative histological views of wound healing in different groups at 2 weeks. Bone regeneration in close contact to the implant surface was most pronounced at the lateral aspect of modSLA implants (upper jaws, TB stain, original magnification × 25). (a) modSLA, submerged, lateral aspect. (b) modSLA, submerged, central aspect. (c) SLA, submerged, lateral aspect. (d) SLA, submerged, central aspect.

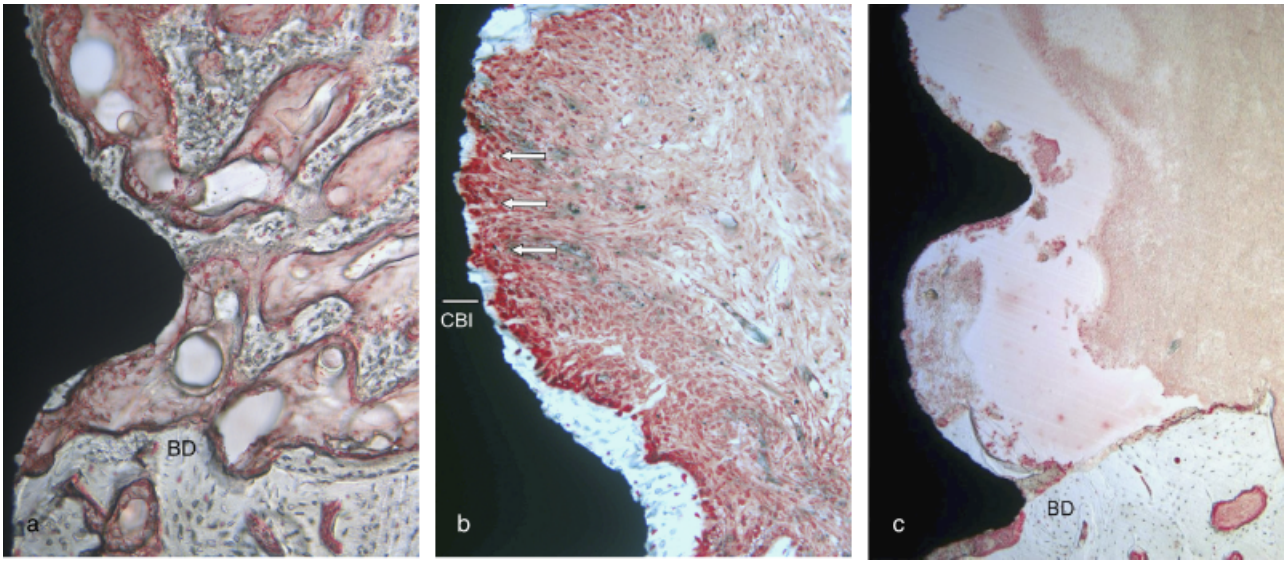

Fig. 5. (a) Bone regeneration in close contact to a modSLA surface. Newly formed blood vessels and trabecular bone appeared to be tightly interconnected (2 weeks, non-submerged, lower jaw, central aspect, TG antigen reactivity, original magnification × 200). (b) The OC antigen reactivity (arrows) was most pronounced within the connective tissue adjacent to modSLA implants (2 weeks, non-submerged, lower jaw, central aspect, original magnification × 200). (c) Partial collapse of the mucoperiosteal flap at the central aspect of the defect area. There were no signs of any bone regeneration (SLA, 2 weeks, non-submerged, lower jaw, central aspect, original magnification × 100).

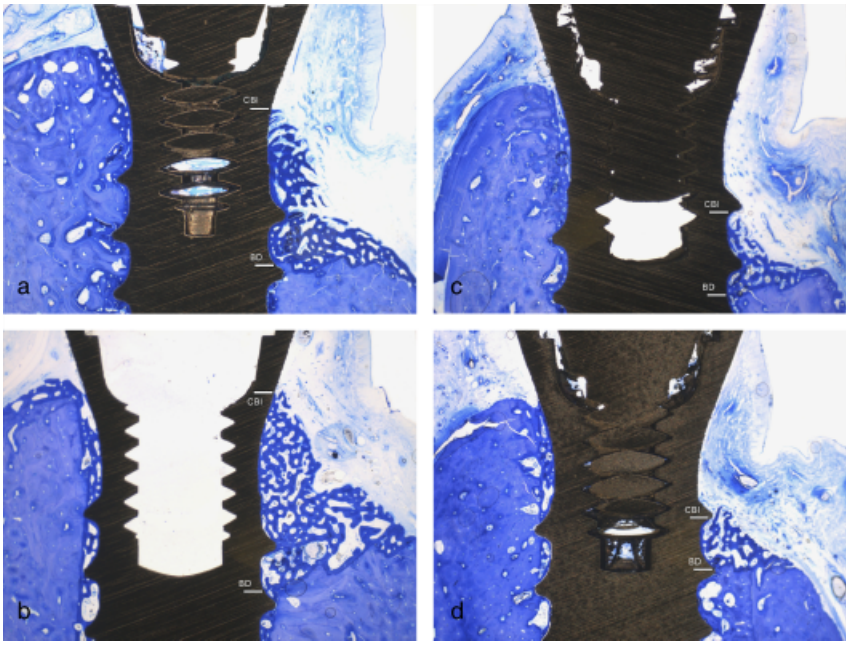

Fig. 6. Representative histological views of wound healing in different groups at 4 weeks. A primary spongework of woven bone covered the defect area in coronal and lateral directions. However, the extent of bone formation varied considerably within and between groups (lower jaws, TB stain, original magnification × 25). (a) modSLA, non-submerged, central aspect. (b) modSLA, submerged, central aspect. (c) SLA, non-submerged, central aspect. (d) SLA, submerged, central aspect.

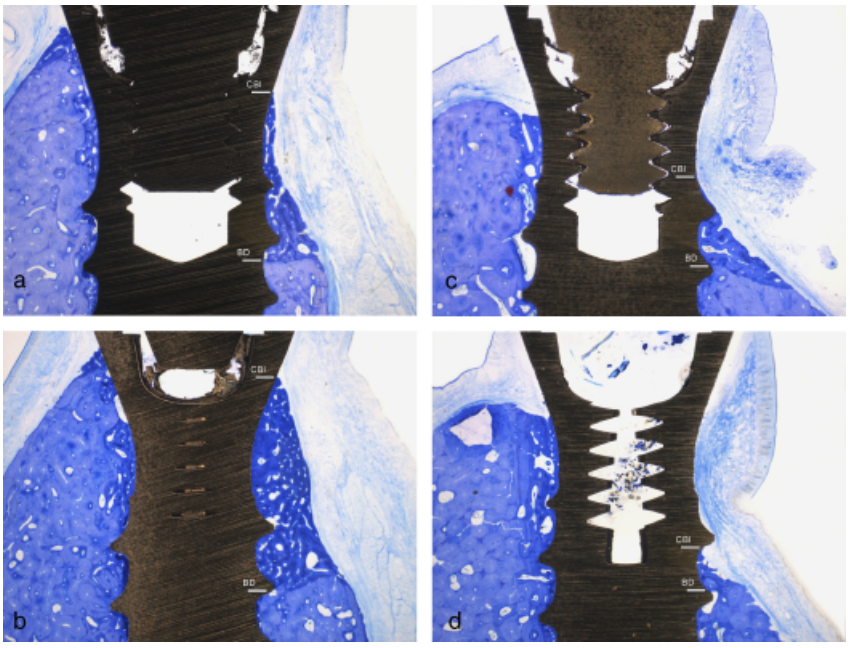

Fig. 7. Representative histological views of wound healing in different groups at 8 weeks. A partial collapse of the mucoperiosteal flap compromised bone regeneration at the central aspects of non-submerged modSLA as well as non-submerged and submerged SLA implants (lower jaws, TB stain, original magnification × 25). (a) modSLA, non-submerged, central aspect. (b) modSLA, submerged, lateral aspect. (c) SLA, non-submerged, central aspect. (d) SLA, submerged, central aspect.

formed primary spongework of woven bone invaded the defect area in the coronal direction, primarily along the surface of modSLA implants. After 12 weeks of healing, the amount of bone regeneration at acute-type buccal dehiscence defects varied between $1.2 \pm 0.3\,mm^2$ (control group: natural bone mineral – BDX) and $3.7 \pm 0.9\,mm^2$ (BDX+cross-linked collagen membrane) (Schwarz et al. 2007e). All these data, taken together with the results from the present study, seem to indicate that modSLA titanium implants might promote bone regeneration in dehiscence-type defects to a similar or even a higher level than GBR procedures using various types of bone grafts or barrier membranes. Indeed, previous studies revealed that the obturation of the space underneath a barrier device by slow or non-resorbable bone graft substitutes may delay bone formation (Trombelli et al. 1999, Stavropoulos et al. 2001). Moreover, from a clinical point of view, the present results corroborate recent data indicating that space provision seems to be a critical factor influencing the outcome of bone regeneration (Polimeni et al. 2004, Schwarz et al. 2007e). When interpreting the present study, however, it must be queried whether data obtained from a preclinical study performed in dogs can be applied to the clinical situation, because the turnover rate of bone remodelling in dogs has been reported to be approximately four times faster than the human turnover rate (Draper 1994).

Within the limits of the present study, it was concluded that (i) modSLA titanium surfaces promoted bone regeneration in acute-type buccal dehiscence defects and (ii) a submerged healing procedure improved the outcome of healing additionally.

Acknowledgements

We appreciate the skills and commitment of Ms. Brigitte Hartig, Mr. Phillip Kühn, and Mr. Vladimir Golubovic in the preparation of the histological specimens.

References

Becker, W., Becker, B. E., Handlesman, M., Celletti, R., Ochsenbein, C., Hardwick, R. & Langer, B. (1990) Bone formation at dehisced dental implant sites treated with implant augmentation material: a pilot study

in dogs. *International Journal of Periodontics and Restorative Dentistry* **10**, 92–101.

Bornstein, M. M., Valderrama, P., Jones, A., Wilson, T. G., Seibl, R. & Cochran, D. L. (2007) Bone apposition around two different sand-blasted and acid-etched titanium implant surfaces. A histomorphometric study in canine mandibles. *Clinical Oral Implants Research* (in press).

Buser, D., Broggini, N., Wieland, M., Schenk, R. K., Denzer, A. J., Cochran, D. L., Hoffmann, B., Lussi, A. & Steinemann, S. G. (2004) Enhanced bone apposition to a chemically modified SLA titanium surface. *Journal of Dental Research* **83**, 529–533.

Casati, M. Z., Sallum, E. A., Nociti, F. H. Jr., Caffesse, R. G. & Sallum, A. W. (2002) Enamel matrix derivative and bone healing after guided bone regeneration in dehiscence-type defects around implants. A histomorphometric study in dogs. *Journal of Periodontology* **73**, 789–796.

Dahlin, C., Linde, A., Gottlow, J. & Nyman, S. (1988) Healing of bone defects by guided tissue regeneration. *Plastic and Reconstructive Surgery* **81**, 672–676.

Dahlin, C., Sennerby, L., Lekholm, U., Linde, A. & Nyman, S. (1989) Generation of new bone around titanium implants using a membrane technique: an experimental study in rabbits. *International Journal of Oral and Maxillofacial Implants* **4**, 19–25.

Donath, K. (1985) The diagnostic value of the new method for the study of undecalcified bones and teeth with attached soft tissue (Säge-Schliff (sawing and grinding) technique). *Pathology Research and Practice* **179**, 631–633.

Draper, H. H. (1994) Bone loss in animals. In: Draper, H. H. (ed). *Advances in Nutritional Research*, Vol. **9**, pp. 53–71. New York: Plenum Press.

Ferguson, S. J., Broggini, N., Wieland, M., de Wild, M., Rupp, F., Geis-Gerstorfer, J., Cochran, D. L. & Buser, D. (2006) Biomechanical evaluation of the interfacial strength of a chemically modified sandblasted and acid-etched titanium surface. *Journal of Biomedical Materials Research A* **78**, 291–297.

Gallop, P. M., Lian, J. B. & Hauschka, P. V. (1980) Carboxylated calcium-binding proteins and vitamin K. *New England Journal of Medicine* **302**, 1460–1466.

Hauschka, P. V., Lian, J. B., Cole, D. E. & Gundberg, C. M. (1989) Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. *Physiological Reviews* **69**, 990–1047.

Hopyan, S., Gokgoz, N., Bell, R. S., Andrulis, I. L., Alman, B. A. & Wunder, J. S. (1999) Expression of osteocalcin and its transcriptional regulators core-binding factor alpha 1 and MSX2 in osteoid-forming tumours. *Journal of Orthopaedic Research* **17**, 633–638.

Liu, H. M., Wang, D. L. & Liu, C. Y. (1990) Interactions between fibrin, collagen and endothelial cells in angiogenesis. *Advances in Experimental Medicine and Biology* **281**, 319–331.

Long, M. W., Robinson, J. A., Ashcraft, E. A. & Mann, K. G. (1995) Regulation of human bone marrow-derived osteoprogenitor cells by osteogenic growth factors. *Journal of Clinical Investigation* **95**, 881–887.

Mayfield, L., Skoglund, A., Nobreus, N. & Attstrom, R. (1998) Clinical and radiographic evaluation, following delivery of fixed reconstructions, at GBR treated titanium fixtures. *Clinical Oral Implants Research* **9**, 292–302.

Oh, T. J., Meraw, S. J., Lee, E. J., Giannobile, W. V. & Wang, H. L. (2003) Comparative analysis of collagen membranes for the treatment of implant dehiscence defects. *Clinical Oral Implants Research* **14**, 80–90.

Polimeni, G., Koo, K. T., Qahash, M., Xiropaidis, A. V., Albandar, J. M. & Wikesjö, U. M. (2004) Prognostic factors for alveolar regeneration: bone formation at teeth and titanium implants. *Journal of Clinical Periodontology* **31**, 927–932.

Raymond, M. H., Schutte, B. C., Torner, J. C., Burns, T. L. & Willing, M. C. (1999) Osteocalcin: genetic and physical mapping of the human gene BGLAP and its potential role in postmenopausal osteoporosis. *Genomics* **60**, 210–217.

Reilly, T. M., Seldes, R., Luchetti, W. & Brighton, C. T. (1998) Similarities in the phenotypic expression of pericytes and bone cells. *Clinical Orthopaedics and Related Research* **346**, 95–103.

Rickard, D. J., Kassem, M., Hefferan, T. E., Sarkar, G., Spelsberg, T. C. & Riggs, B. L. (1996) Isolation and characterization of osteoblast precursor cells from human bone marrow. *Journal of Bone and Mineral Research* **11**, 312–324.

Rupp, F., Scheideler, L., Olshanska, N., de Wild, M., Wieland, M. & Geis-Gerstorfer, J. (2006) Enhancing surface free energy and hydrophilicity through chemical modification of microstructured titanium implant surfaces. *Journal of Biomedical Materials Research A* **76**, 323–334.

Schenk, R. K., Olah, A. J. & Herrmann, W. (1984) Preparation of calcified tissues for light microscopy. In: Dickson, G. R. (ed). *Methods of Calcified Tissue Preparation*, pp. 1–56. Amsterdam: Elsevier.

Schwarz, F., Ferrari, D., Herten, M., Mihatovic, I., Wieland, M., Sager, M. & Becker, J. (2007a) Effects of surface hydrophilicity and microtopography on early stages of soft and hard tissue integration at nonsubmerged titanium implants. An immunohistochemical study in dogs. *Journal of Periodontology* **78**, 2171–2184.

Schwarz, F., Herten, M., Ferrari, D., Wieland, M., Schmitz, L. & Becker, J. (2007b) Guided bone regeneration at dehiscence-type defects using a biphasic hydroxyapatite/beta tricalcium phosphate (Bone Ceramic®) or a collagen coated natural bone mineral (BioOss® Collagen). An immunohistochemical study in dogs. *International Journal of Oral and Maxillofacial Implants*, doi: 10.1016/j.ijom.2007.07.014.

Schwarz, F., Herten, M., Sager, M., Wieland, M., Dard, M. & Becker, J. (2007c) Bone regeneration in dehiscence-type defects at chemically modified (SLActive) and conventional SLA titanium implants: a pilot study in dogs. *Journal of Clinical Periodontology* **34**, 78–86.

Schwarz, F., Herten, M., Sager, M., Wieland, M., Dard, M. & Becker, J. (2007d) Histological and immunohistochemical analysis of initial and early osseous integration at chemically modified and conventional SLA® titanium implants: preliminary results of a pilot study in dogs. *Clinical Oral Implants Research* **18**, 481–488.

Schwarz, F., Rothamel, D., Herten, M., Sager, M., Ferrari, D. & Becker, J. (2007e) Immunohistochemical characterization of guided bone regeneration at dehiscence-type defect using different barrier membranes. An experimental study in dogs. *Clinical Oral Implants Research* (in press).

Stavropoulos, A., Kostopoulos, L., Mardas, N., Nyengaard, J. R. & Karring, T. (2001) Deproteinized bovine bone used as an adjunct to guided bone augmentation: an experimental study in the rat. *Clinical Implant Dentistry and Related Research* **3**, 156–165.

Trombelli, L., Lee, M. B., Promsudthi, A., Guglielmoni, P. G. & Wikesjö, U. M. (1999) Periodontal repair in dogs: histologic observations of guided tissue regeneration with a prostaglandin E1 analog/methacrylate composite. *Journal of Clinical Periodontology* **26**, 381–387.

Zablotsky, M., Meffert, R., Caudill, R. & Evans, G. (1991) Histological and clinical comparisons of guided tissue regeneration on dehisced hydroxylapatite-coated and titanium endosseous implant surfaces: a pilot study. *International Journal of Oral and Maxillofacial Implants* **6**, 294–303.

Zhao, G., Raines, A. L., Wieland, M., Schwartz, Z. & Boyan, B. D. (2007) Requirement for both micron- and submicron scale structure for synergistic responses of osteoblasts to substrate surface energy and topography. *Biomaterials* **28**, 2821–2829.

Zhao, G., Schwartz, Z., Wieland, M., Rupp, F., Geis-Gerstorfer, J., Cochran, D. L. & Boyan, B. D. (2005) High surface energy enhances cell response to titanium substrate microstructure. *Journal of Biomedical Materials Research A* **74**, 49–58.

Address:
Priv. Doz. Dr. Frank Schwarz
Department of Oral Surgery
Westdeutsche Kieferklinik
Heinrich Heine University
D-40225 Düsseldorf
Germany
E-mail: Frank.Schwarz@med.uni-dueseldorf.de

Clinical Relevance

Scientific rationale for the study: Recent histological data have demonstrated that submerged modSLA titanium implants promote bone regeneration in acute-type buccal dehiscence defects without the additional use of GBR or bone augmentation procedures. However, the impact of the healing procedure (i.e. non-submerged or submerged) on bone regeneration at modSLA implants is unknown.

Principal findings: At 2, 4, and 8 weeks of healing, both non-submerged and submerged modSLA implants revealed significantly higher mean values of NBH, PLF, BIC-D, and BF than corresponding conventional SLA surfaces. In both groups, the submerged healing procedure promoted bone regeneration additionally, even reaching statistical significance in the modSLA group.

Practical implications: The most predictable outcome of bone regeneration in acute-type buccal dehiscence defects might be expected for submerged modSLA titanium implants.