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Bone regeneration in dehiscence-type defects at chemically modified (SLActive®) and conventional SLA titanium implants: a pilot study in dogs

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

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

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Abstract

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

Material and Methods: Standardized buccal dehiscence defects (height: 3 mm, width: 3 mm) were surgically created following implant site preparation in both the upper and lower jaws of four beagle dogs. modSLA and SLA implants were inserted bilaterally according to a split-mouth design. The animals were sacrificed after 2 and 12 weeks ($n = 2$ animals each). Dissected blocks were processed for histomorphometrical analysis: defect length, new bone height (NBH), percent linear fill (PLF), percent of bone-to-implant contact (BIC-D) and area of new bone fill (BF).

Results: 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. In particular, histomorphometrical analysis revealed the following mean values at 12 weeks: NBH (3.2 ± 0.3 mm), PLF (98%), BIC-D (82%) and BF (2.3 ± 0.4 mm^2).

Conclusion: Within the limits of the present study, it was concluded that modSLA titanium surfaces may promote bone regeneration in acute-type buccal dehiscence defects at submerged implants.

Key words: animal study; dehiscence-type defect; histomorphometrical analysis; sand-blasted and acid-etched surface; titanium surface

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Conflict of interest and source of funding statement

The authors Frank Schwarz, Monika Herten, Martin Sager and Jürgen Becker declare that they have no conflict of interests. The co-authors Marco Wieland and Michel Dard provided an experimental implant design.

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The replacement of missing teeth by means of endosseous titanium implants has been proven to be a successful treatment modality for both completely and partially edentulous patients (Jemt et al. 1996, Lindquist et al. 1996, Buser et al. 1997, Lambrecht et al. 2003). This concept is mainly based on the biologic phenomenon of osseointegration, which has been characterized as a direct structural and functional connection between ordered, living bone and the surface of a load-bearing implant (Brånemark 1985). A prerequisite for a successful osseointegration, however, is the establishment of a direct bone-to-implant contact (BIC) without the inter-position of non-bone or connective tissue (Brånemark et al. 1969, Albrektsson 1983, Davies 1998). In the past few years, several modifications of specific surface properties such as topography, structure, chemistry, surface charge and wettability have been investigated to predictably improve the osseointegration of titanium implants (Albrektsson 1983). Most recently, a chemically modified sand-blasted, large grit and acid-etched (modSLA) titanium surface has been introduced in order to enhance bone apposition (Buser et al.

2004, Zhao et al. 2005). The specific production process used for modSLA surfaces (i.e. rinsing the titanium surface after the etching process under N_2 protection and continous storage in an isotonic NaCl solution) has been reported to produce a chemically active surface with a small amount of hydrocarbons and carbonates. The resulting hydroxylated/hydrated modSLA surface was shown to have an initial advancing water contact angle of 0°, indicating its immediate wettability and ultra-hydrophilic character. Furthermore, an increased surface free energy (SFE) has been reported by Rupp et al. (2006), resulting in an increased water/biomaterial contact area. Moreover, the results obtained from a recent in vitro study have indicated that osteoblasts grown on modSLA surfaces exhibited a more differentiated phenotype characterized by increased alkaline phosphatase activity (ALP) and osteocalcin and generated an osteogenic microenvironment through higher production of prostanglandine E2 (PGE2) and transforming growth factor (TGF)-β1 (Zhao et al. 2005). Histological observations in miniature pigs have shown that modSLA implants exhibited a significantly greater mean percentage of BIC as compared with conventional SLA surfaces at 2 and 4 weeks of healing (Buser et al. 2004). However, a successful osseointegration of endosseous implants can be compromised by bone defects due to small anatomic jawbone configurations. Particularly, alveolar bone dehiscences and fenestrations in association with oral implant installation may result from either insufficient vestibulo-oral alveolar width or inadvertent misdirection of implant placement. Although the technique of guided bone regeneration (GBR) was also successfully used for bone augmentation at dehisced dental implants, the cumulative implant survival rate has been shown to be clearly lower than those regularly reported for implants placed into sufficiently dimensioned alveolar ridges (Jovanovic et al. 1992, Dahlin et al. 1995). Hence, it has to be considered that augmenting bone at exposed implant threads may involve additional risk factors for the longevity of the implants placed (van Steenberge et al. 2003). So far, these risk factors have not yet been fully classified. Particularly, the extent of a dehisced implant surface to serve as a sufficient base to establish new BIC is still unknown. Based on the above-summarized data, however, it might be hypothesized that the hydrophilic properties noted for modSLA implant surfaces can also improve bone formation in dehiscence-type defects.

Therefore, the aim of the present study was to evaluate histomorphometrically in a beagle dog model bone regeneration in acute-type buccal dehiscence-type defects at submerged modSLA and SLA titanium implants.

Material and Methods

Animals

Four beagle dogs (age 20–24 months, mean weight 16.4 ± 0.4 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 and first molar (P2-M1) was performed bilaterally in all dogs. After 4 months of healing, standardized buccal dehiscence defects were surgically created following implant site preparation in both the upper ($n = 2$ defects per animal) and lower jaws ($n = 4$ defects per animal). Thereafter, three modSLA and three SLA implants were randomly inserted according to a split-mouth design (total $n = 24$ implants). Randomization was based on a computer-generated list (RandList®, DatInf GmbH, Tübingen, Germany). Accordingly, each animal received four implants bilaterally in the the lower (two modSLA, two SLA, respectively) jaw and two implants bilaterally in the upper jaw (one modSLA, one SLA, respectively). Radiographs were obtained before and immediately after tooth extraction as well as immediately after implant insertion. The animals were sacrificed after 2 and 12 weeks of submerged healing, including two animals each.

Surgical procedure

After sedation with acepromazine (0.17 mg/kg body weight), the dogs were anaesthetized with 21.5 mg/kg thiopental sodium. For all surgical procedures, inhalation anaesthesia was performed by use of oxygen and nitrous oxide and isoflurane. To maintain hydration, all animals received a constant rate infusion of lactated Ringer's solution while anaesthetized.

In the first surgery, P2-M1 were carefully removed bilaterally in both jaws after reflection of full-thickness mucoperiosteal flaps and tooth separation. After wound closure by means of matress sutures, the sites were allowed to heal for 4 months. Prophylactic administration of clindamycine (11.0 mg/kg body weight, Cleorobe®, Pharmacia Tiergesundheit, Erlangen, Germany) was performed intra- and postoperatively for 10 days.

In the second surgery, midcrestal incisions were made and full-thickness mucoperiosteal flaps 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 of 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 3 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 by the use of a periodontal probe (PCP12, Hu-Friedy Co., Chicago, IL, USA). Thereafter, both modSLA and SLA implants (regular neck, sand-blasted, large grit and acid-etched endosseous and transmucosal parts, ∅ 3.3 mm, length 8 mm, Institut Straumann AG, commercial name of modSLA is SLActive®) were inserted by a low-trauma surgical technique with good primary stability. ModSLA implants were produced as described by Rupp et al. (2006). The implants were inserted in a way that the implant shoulder was located 2 mm coronally to the bone crest at both buccal and oral aspects (BTB) (Fig. 1a). In case of SLA,

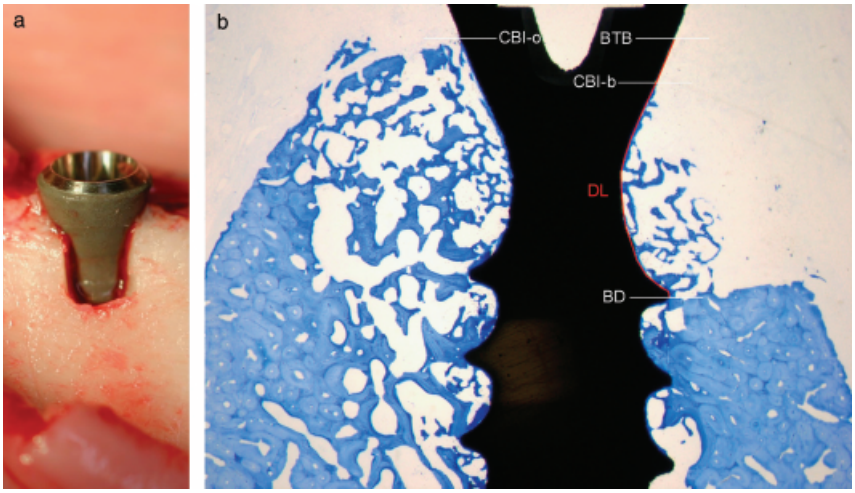

Fig. 1. (a) Following implant site preparation, standardized dehiscence-type defects, approximately 3 mm in height from the crestal bone, 3 mm in depth from the surface of the buccal bone and 3 mm in width mesiodistally, were created with a straight fissure carbide bur. (b) Landmarks for histomorphometrical analysis: 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). Defect length (DL) was measured from BTB to BD (mm), new bone height (NBH) was measured from BD to CBI-v (mm), percent linear fill (PLF) was defined as NBH divided by DL, the amount of new BIC was measured as percentage of the distance from BD to BTB bone-to-implant contact (BIC-D) and for the non-defect area of the implant surface as percentage of the distance from BD at the buccal aspect to BTB at the oral aspect (BIC-ND), the difference in buccal and oral dimension of CBI (D-CBI) was defined as CBI-o–CBI-v (mm); the area (mm^2) of new bone fill (BF) was measured from BD to CBI [modified sand-blasted/acid-etched (modSLA), 2 weeks, lateral aspect, Toluidine blue stain, original magnification $\times$ 25].

the implants were thoroughly rinsed with sterile saline before insertion. Following irrigation, primary wound closure was achieved with consecutive resorbable 5.0 polyglygolic acid sutures (Resorba®, Nürnberg, Germany), and implants were left to heal in a submerged position.

Animal sacrification and retrieval of specimens

The animals were sacrificed (overdose of sodium pentobarbital 3%) after a healing period of 2 and 12 weeks, respectively, including two animals each. 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 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 (MMA, Technovit 7200, Heraeus Kulzer, Wehrheim, Germay) for non-decalcified sectioning. 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 lateral and central parts of the respective defect areas, resulting in three sections approximately 500 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, Wehrheim, Germay) to opaque plexiglass and ground to a final thickness of approximately 30 μm. All sections were 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).

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). Digital images (original magnification $\times$ 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, 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), percent linear fill (PLF) was defined as NBH divided by DL, the amount of new BIC was measured as percentage of the distance from BD to BTB (BIC-D) and for the non-defect area of the implant surface as percentage of the distance from BD at the buccal aspect to BTB at the oral aspect (BIC-ND), 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 (Fig. 1b).

Intra-examiner reproducibility

Both microscopic observations and histomorphometrical measurements were performed by one experienced investigator masked to the specific experimental conditions. For histomorphometry, calibration was performed by means of five histological sections. The examiner evaluated the specimens on two separate occasions, 48 h apart. Calibration was accepted if measurements at baseline and at 48 h were similar at $>$90% level.

Statistical analysis

The statistical analysis was performed using a commercially available software program (SPSS 14.0, SPSS Inc., Chicago, IL, USA). Mean values and standard deviations were calculated for each implant in each dog. The data rows were examined with the Kolmogorow–Smirnow test for normal distribution. For the statistical evaluation of the

changes within groups (i.e. either central or lateral aspects), the paired *t*-test was used. For the 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 postoperative healing was uneventful in all dogs. No complications such as allergic reactions, abscesses or infections were observed throughout the study period. Furthermore, there were no signs of any wound dehiscence or exposure of the transmucosal part of the implant body in both groups.

Histological observations/ histomorphometrical analysis – defect area

The mean values and percentages of DL, NBH, PLF, BIC-D, BF and D-CBI for each group at 2 and 12 weeks are presented in Table 1. At 2 weeks, there were no statistically significant differences in mean DL values between modSLA and SLA implants at both central and lateral aspects of the defects ($p > 0.05$, respectively). Histological evaluation of SLA implants at 2 and 12 weeks revealed that wound healing in all dehiscence defects was predominantly characterized by the formation of a poorly vascularized, dense connective tissue (Fig. 2a). Minute amounts of new bone formation were observed only occasionally, and were limited to the most apical part of the defect (Fig. 2b). Accordingly, histomorphometrical and statistical analysis failed to demonstrate any significant increases of mean NBH, PLF, BIC-D or BF values in the respective defect areas ($p > 0.05$, respectively) (Table 1). In some specimens, the apically directed down-growth of the connective tissue even seemed to compromise osseointegration of SLA implants at the buccal aspects in an area underneath the dehiscence-type defect (Fig. 2a). Moreover, histomorphometrical analysis revealed a remodelling process, resulting in a horizontal and vertical loss of the alveolar bone at the buccal aspect of some specimens after 12 weeks (Fig. 2c). While mean D-CBI values appeared to be within the range of respective DL values at 2 weeks, statistical analysis revealed a significant increase of mean D-CBI values after 12 weeks at both central and lateral aspects ($p < 0.001$, $p < 0.05$, respectively) (Table 1).

In contrast, histological evaluation of modSLA implants revealed a complete defect fill at 12 weeks following implant placement. In particular, at 2 weeks, newly formed trabeculae of woven bone, originating from both the lateral walls as well as the bottom of the defects, have started to invade the dehiscence areas. The newly formed woven bone was characterized by an intense staining of the mineralized matrix and the numerous osteocytes located in large lacunae. Once the tiny trabeculae reached the implant, they also appeared to line larger parts of respective modSLA titanium surfaces (Figs 3a and b). However, the mean values of NBH, PLF, BIC-D and BF appeared to be the highest at the lateral aspects of the respective defects (Table 1). Histological examination failed to demonstrate any de novo bone formation originating from the implant surface.

After 12 weeks, wound healing was predominantly characterized by the ongoing formation of new bone within the defect areas. Histological observation revealed that modSLA implants seemed to be surrounded by a firmly attached mature, parallel-fibred woven bone (Figs 3c and d). Early signs of remodelling, replacing the primary bone by secondary osteons, were apparent (Figs 3a and b). Accordingly, histomorphometrical and statistical analysis revealed significant increases of mean NBH, PLF, BIC-D and BF values in the respective defect areas (Table 1). This was particularly true for central aspects of the defect areas, as respective lateral aspects merely exhibited slight increases in mean NBH, PLF, BIC-D and BF values. However, at both central and lateral aspects, mean D-CBI values decreased statistically significant at 12 weeks ($p < 0.001$, $p < 0.01$, respectively), outlining that the newly formed buccal aspects of the alveolar bone even reached the level of the respective oral aspects (Figs 3c and d).

Histological Observations/ Histomorphometrical Analysis – Non-Defect area

The mean values and percentages of BIC-ND for each group at 2 and 12 weeks are

Table 1. Histomorphometric results (± SD) in both groups at 2 and 12 weeks ($n = 24$ implants)

	modSLA		SLA	
	Central	Lateral	Central	Lateral
DL (mm)				
2 weeks	3.2 ± 0.2	3.3 ± 0.2	3.3 ± 0.2	3.4 ± 0.1
12 weeks	3.4 ± 0.1	3.3 ± 0.2	5.1 ± 1.9†	4.1 ± 0.6†
p value*	NS	NS	$p < 0.001$	$p < 0.01$
NBH (mm)				
2 weeks	1.1 ± 0.2	2.6 ± 0.6	0†	0†
12 weeks	3.3 ± 0.2	3.2 ± 0.2	0.4 ± 0.1†	0.3 ± 0.1†
p value*	$p < 0.001$	$p < 0.01$	NS	NS
PLF (%)				
2 weeks	34	80	0†	0†
12 weeks	97	98	10†	7†
p value*	$p < 0.001$	$p < 0.01$	NS	NS
BIC-D (%)				
2 weeks	27	61	0†	0†
12 weeks	80	85	5†	4†
p value*	$p < 0.001$	$p < 0.001$	NS	NS
BIC-ND (%)				
2 weeks	74	72	56†	54†
12 weeks	84	86	76	75
p value*	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
BF (mm2)				
2 weeks	0.4 ± 0.1	1.8 ± 0.4	0†	0†
12 weeks	2.4 ± 0.3	2.3 ± 0.4	0.07 ± 0.04†	0.08 ± 0.03†
p value*	$p < 0.001$	$p < 0.01$	NS	NS
D-CBI (mm)				
2 weeks	2.1 ± 0.2	0.6 ± 0.4	3.1 ± 0.1†	3.4 ± 0.2†
12 weeks	0.2 ± 0.1	0.1 ± 0.1	4.3 ± 1.4†	3.8 ± 0.6†
p value*	$p < 0.001$	$p < 0.01$	$p < 0.001$	$P < 0.05$

*Comparisons within groups (paired *t*-test).

†Comparisons between groups (unpaired *t*-test, $p < 0.001$, respectively).

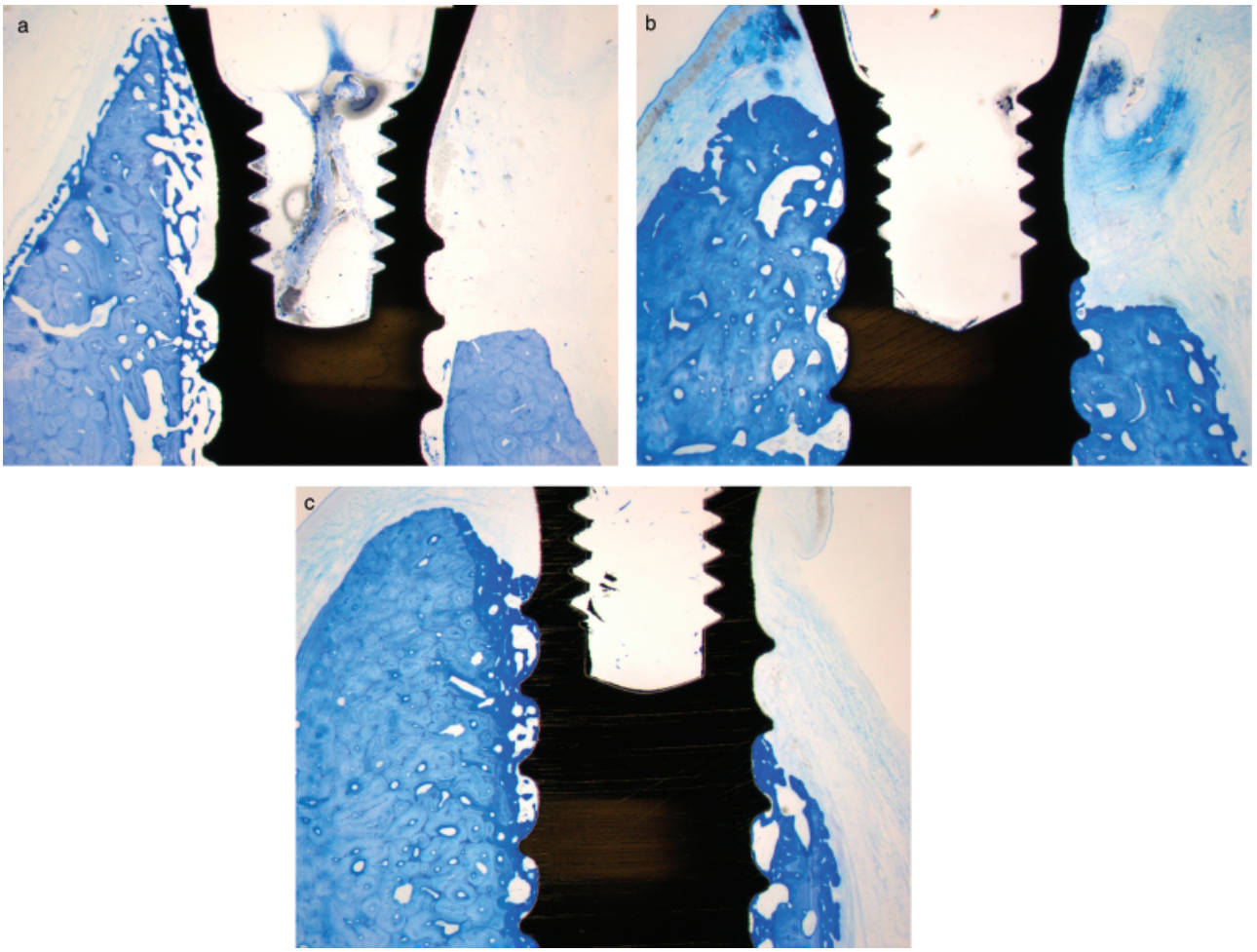

Fig. 2. (a, b) Histological evaluation of dehiscence defects at sand-blasted/acid-etched (SLA) implants after 2 and 12 weeks revealed that wound healing was predominantly characterized by the formation of a dense connective tissue (a). Minute amounts of new bone formation were observed only occasionally, and were limited to the most apical part of the defect (b) (Toluidine blue [TB] stain, original magnification × 25) (a) Two weeks – central aspect, (b) 12 weeks – central aspect.(c) In some specimens, histomorphometrical analysis revealed a remodelling, resulting in a horizontal and vertical loss of the alveolar bone at the buccal aspect after 12 weeks (SLA, 12 weeks, central aspect, Toluidine blue stain, original magnification × 25).

presented in Table 1. In particular, modSLA implants revealed significantly higher mean BIC-ND values at 2 weeks in comparison with SLA implants ($p < 0.001$, respectively). While SLA implants were surrounded by newly formed trabeculae of woven bone, covering most parts of the endosseous surfaces, modSLA implants appeared to be surrounded by a firmly attached mature, parallel-fibred woven bone. The maturity of the woven bone was also identifiable by the development of primary osteons. After 12 weeks, wound healing was predominantly characterized by the ongoing formation of new bone in both groups. In particular, SLA surfaces also exhibited a firmly attached mature, parallel-fibred woven bone. Moreover, in both groups, early signs of remodelling, replacing the primary bone by secondary osteons, were apparent. Histomorphometrical and statistical analysis revealed significant increases of mean BIC-ND values in both groups ($p < 0.001$, respectively); however the differences between modSLA and SLA implants were statistically non-significant ($p > 0.05$, respectively) (Figs 2 and 3).

Discussion

The present study was designed to investigate histomorphometrically in a beagle dog model bone regeneration in acute-type buccal dehiscence-type defects at submerged modSLA and SLA titanium implants. Within its limits, it was observed that modSLA implants exhibited a complete defect fill at 12 weeks following implant placement, while wound healing at SLA implants was merely characterized by the formation of a dense connective tissue at 2 and 12 weeks, without any increases in mean NBH, PLF, BIC-D or BF values. In this context, it must be emphasized that the surgical creation of standardized buccal dehiscence-type defects in dogs is a commonly used procedure to evaluate bone regeneration adjacent to titanium implants (Becker et al. 1990, Zablotsky et al. 1991, Stentz et al. 1997, Cho et al. 1998, Hockers et al. 1999, Kohal et al. 1999, Casati et al. 2002, Oh et al. 2003). There might be several aspects to explain the discrepancy with respect to initial bone formation at both types of implants. First of all, it must be emphasized that the hydrophilic surface properties noted for hydroxylated/hydrated modSLA resulted in a higher wettability when compared with conventional SLA

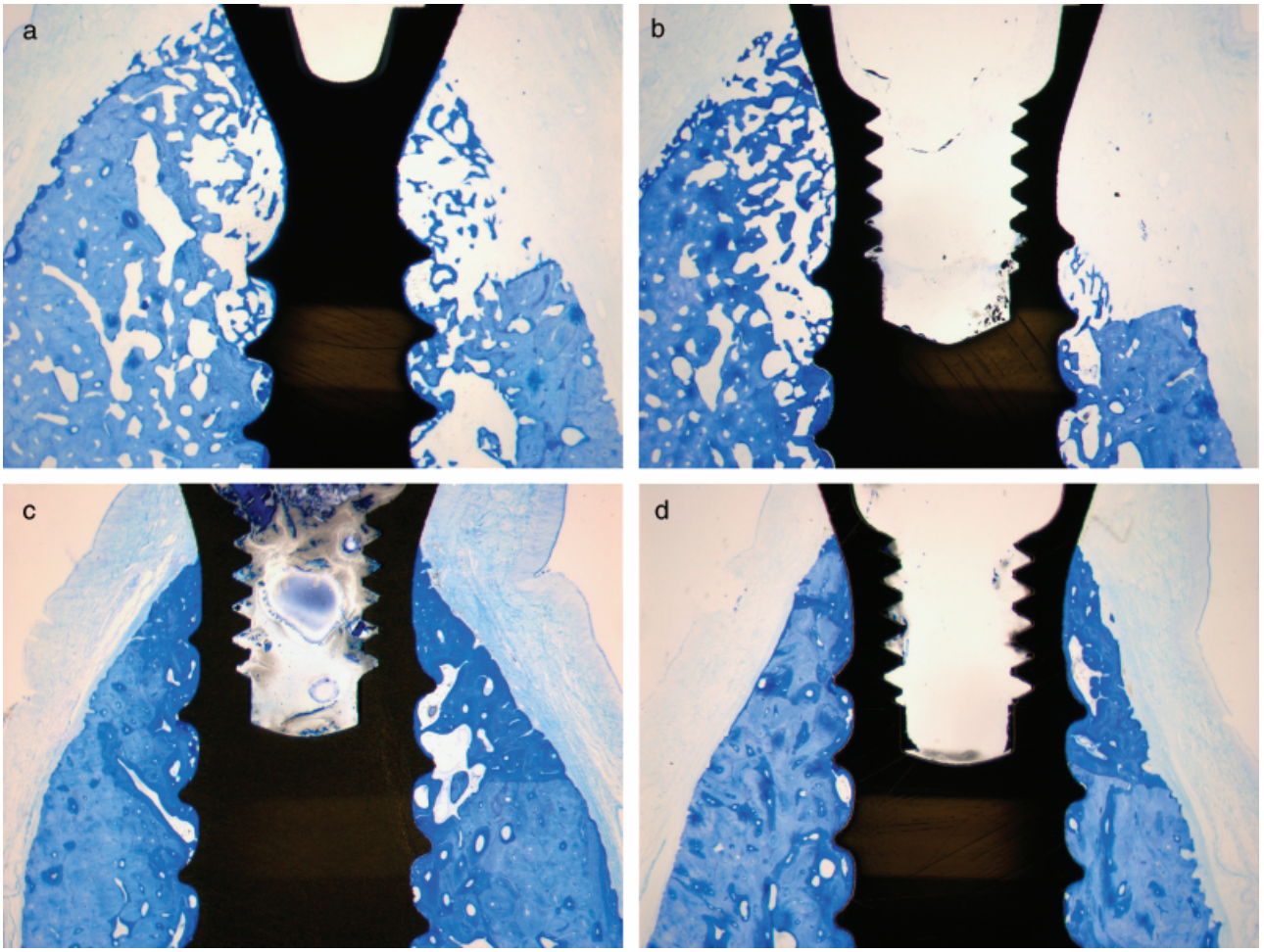

Fig. 3. (a–d) Histological evaluation of modified sand-blasted/acid-etched (modSLA) implants revealed that at 2 weeks, newly formed trabeculae of woven bone, originating from both the lateral walls as well as the bottom of the defects, have started to invade the dehiscence areas. However, mean values of new bone height (NBH), per cent linear fill, bone-to-implant contact and the area (mm^2) of new bone fill (BF) appeared to be the highest at the lateral aspects of the respective defects. After 12 weeks, histological observation exhibited that modSLA implants seemed to be surrounded by a firmly attached mature, parallel-fibred woven bone. The newly formed buccal aspects of the alveolar bone even reached the level of the respective oral aspects [Toluidine blue (TB) stain, original magnification $\times$ 25). (a) 2 weeks – lateral aspect (b) 2 weeks – central aspect, (c) 12 weeks – central aspect, (d) 12 weeks – central aspect.

surfaces (Rupp et al. 2006). In particular, the main outcome of dynamic wettability measurements was that modSLA implants revealed increased SFE and hydrophilicity with initial water contact angles of 0° compared with 139.9° for SLA implants. Accordingly, it might be hypothesized that SFE and hydroxylation/hydration of modSLA implant surfaces also improved the adhesion and subsequently the stabilization of the blood clot. Indeed, most recent results from a pilot study in dogs have pointed to a close adhesion of the blood clot to modSLA surfaces, while the coagulum was partially collapsed at SLA surfaces. Furthermore, immunohistochemical analysis of initial angiogenesis revealed that the organization of the blood clot seemed to be initiated within 24 h (Schwarz et al. 2007). Basically, the blood clot acts as a physical matrix that induces and amplifies the migration, proliferation and differentiation of various types of cells, subsequently leading to fibroplasia and angiogenesis (Lang et al. 2003). In this context, it must be pointed out that both stabilization of the blood clot and early angiogenesis were considered to be important factors strongly influencing wound healing following GBR procedures (Hardwick et al. 1994). Indeed, experimental studies in animals have indicated that formation of blood capillaries precedes the formation of new bone (Schmid et al. 1997, Schwarz et al. 2007). This observation may be explained by the fact that osteogenic cells were observed to arise from pericytes adjacent to the connective tissue of small blood vessels (Long et al. 1995, Rickard et al. 1996, Reilly et al. 1998). These findings are also in accordance with the results of the present study, as the lowest increases in mean NBH, PLF, BIC-D, or BF values at 2 weeks were observed at the central aspect of respective modSLA implants. In contrast, the mean values of NBH, PLF, BIC-D and BF appeared to be the highest at the lateral aspects of the respective defects, indicating that neovascularization of the blood clot and subsequently new bone formation appeared to start from open bone marrow spaces of the adjacent defect borders. Accordingly, the lack of new bone formation in the respective dehiscence-type defects at SLA implants might be explained by a partial or even full collapse of the blood clot within the

first 2 weeks of healing, allowing the ingrowth of a poorly vascularized connective tissue inside the wound area. As conventional TB staining may not be appropriate for the histological characterization of the non-filled part of the defect area after 2 weeks, further studies aimed at immunohistochemically evaluating tissue healing in dehiscence-type defects at both SLA and modSLA implant surfaces are needed in order to clarify this issue. However, previous histological observations in dogs have demonstrated that the blood clot adjacent to modSLA surfaces was generally substituted by a well-organized collagen-rich connective tissue at day 4. In contrast, due to the partial collapse of the blood clot at SLA implants, peri-implant wound healing at day 4 was predominantly characterized by the formation of granulation tissue (Schwarz et al. 2007). Another possible explanation for the significantly higher mean values of NBH, PLF, BIC-D, BIC-ND (2 weeks) and BF 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 osteocalcin synthesis and generated an osteogenic microenvironment through higher production of PGE2 and TGF-β1 (Zhao et al. 2005). Furthermore, Masaki et al. (2005) observed significant increases in ALP gene expression in osteoblasts grown on modSLA when compared with SLA implant surfaces. Indeed, the first signs of osteocalcin antigen reactivity in the provisional connective tissue adjacent to modSLA implants were observed at day 4 (Schwarz et al. 2007). In this context, it must be emphasized that the histological examinations in the present study failed to demonstrate any de novo bone formation originating from the implant surface, indicating that modSLA implants may not possess osteoinductive properties. This observation is also supported by a recent immunohistochemical characterization of early tissue reactions at modSLA implants in dogs (Schwarz et al. 2007). However, the results from a recent study in miniature pigs demonstrated significant differences in mean percentage of BIC between modSLA and SLA implants during the early stages of bone regeneration at 2 (49.30% *versus* 29.42%) and 4 weeks (81.91% *versus* 66.57%) of healing. At 12 weeks, both modSLA and SLA implants revealed a further increase in bone density as well as early signs of bone remodelling, resulting in a replacement of the primary bone by secondary osteons. However, the difference between both groups with respect to BIC was statistically non-significant (Buser et al. 2004). This observation is in agreement with the results of the present study, as modSLA revealed statistically significant higher mean BIC-ND values than SLA implants at 2 weeks, while the difference between both groups was non-significant at 12 weeks. Most recently, treatment of titanium with chromosulphuric acid has also been reported to result in ultra-hydrophilic bioadhesive surfaces, leading to improved BIC values compared with untreated control implants after 4 weeks of healing in dogs (Becker et al. 2006). When interpreting the present results, however, it was also observed that the newly formed buccal aspects of the alveolar bone at modSLA implants even reached the level of the respective oral aspects. Indeed, mean D-CBI values decreased statistically significant between 2 and 12 weeks, particularly at the central aspects of the respective defect areas. In contrast, histological observation of some specimens in the SLA group revealed a horizontal and vertical loss of the alveolar bone at the buccal aspect after 12 weeks, as indicated by a significant increase in mean D-CBI values over time. This might be explained by the compromised osseointegration underneath the defect area, caused by the down-growth of connective tissue, and subsequently a remodelling process leading to a resorption of the respective implant-supporting bone. Similar results were reported by Kohal et al. (1999), as non-submerged SLA implants also exhibited an increased DL from 4.8 ± 0.8 mm at baseline to 5.7 ± 1.1 mm after 6 months. These findings corroborate, to a certain extent, recent results from an experimental study in dogs, which have shown that particularly the buccal bone underwent marked remodelling and resorption following implant placement in fresh extraction sockets (Araújo et al. 2005). To the best of our knowledge, these are the first data evaluating bone regeneration in acute-type dehiscence defects at both modSLA and SLA titanium implants without the additional use of bone grafts or barrier membranes. However, previous experimental studies in dogs evaluated bone regeneration in acute-type dehiscence defects following GBR procedures, the application of enamel matrix derivative (EMD), or in untreated control defects at various types of submerged titanium implants (Becker et al. 1990, Zablotsky et al. 1991, Stentz et al. 1997, Cho et al. 1998, Hockers et al. 1999, Casati et al. 2002, Oh et al. 2003). The pattern of wound healing at SLA implants, as observed in the present study, is in agreement with most of the results obtained at untreated control defects, as 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, Hockers et al. 1999, Oh et al. 2003). In contrast, for titanium implants covered with a teflon membrane, Becker et al. (1990) reported a gain in mean NBH of 1.37 mm after a healing period of 18 weeks. In a similar study, Zablotsky et al. (1991) assessed the percentage of dehsicence repair after 8 weeks of healing at both hydroxyapatite-coated (HA) and grit-blasted titanium implants following application of a modified expanded polytetrafluoroethylene (ePTFE) membrane. After 8 weeks of healing, the HA-coated implants exhibited a mean defect fill of 95.17% and the grit-blasted implants a percent fill of 82.8%. Similar results were also reported by Stentz et al. (1997) for the combined application of demineralized freeze-dried bone allograft+ePTFE at both HA- and commercially pure titanium screw implants. Cho et al. (1998) also reported a nearly complete defect fill at 12 weeks of healing following the application of either ePTFE (81%; BIC: 49%) or ePTFE+xenogeneic derived freeze-dried demineralized bone matrix (84%; BIC: 67%) at both HA- and titanium plasma-sprayed implants. However, Hockers et al. (1999) merely observed a mean BIC of 17–20% for either autogenic bone (AB)+collagen membrane (BG), deproteinized bovine bone mineral+BG or BG alone at machined titanium implants, even though the vertical bone growth amounted to 45% of the defect height in the BG group, to 78% in the BO+BG group and to 69% in the AB+BG group. In contrast, following the application of two different collagen membranes at sand-blasted and acid-etched titanium implants, Oh et al. (2003) observed increased mean values of BIC (34.82–44.83%, respectively) and PLF (57.88–59.17%, respectively) after 16 weeks of

healing. However, reduced BIC values were observed following the application of either EMD (PLF: 55.55%; BIC: 18.07), a synthetic membrane composed of polylactic/polyglycolic acid (PLA) (PLF: 53.89%; BIC: 24.42), or EMD+‴ PLA (PLF: 62.15%; BIC: 36.11) at sand-blasted and acid-etched titanium implants after 12 weeks of healing. 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 of barrier membranes. When interpreting these results, however, it must also kept in mind that possible differences noted between these studies might be related to different defect sizes as well as the number of animals used. Furthermore, it must be queried whether data obtained from a pre-clinical study performed in dogs can be applied to the clinical situation, as 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 modSLA titanium surfaces may promote bone regeneration in acute-type buccal dehiscence defects at submerged implants.

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

Clinical Relevance

Scientific rationale for the study: Hydrophilic surface properties of submerged modSLA titanium implants might ensure a stabilization of the blood clot and subsequently promote bone regeneration in dehiscence-type defects.

Principal findings: The present results have indicated that modSLA implants revealed a complete defect fill at 12 weeks following implant placement without the additional use of GBR procedures. In contrast, wound healing at SLA implants was predominantly characterized by the formation of a dense connective tissue at 2 and 12 weeks, without any signs of bone regeneration. Moreover, a remodelling process resulted in a horizontal and vertical bone loss at the buccal aspect of some specimens after 12 weeks.

Practical implications: modSLA surfaces might promote bone formation in acute-type buccal dehiscence defects at submerged titanium implants.