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Influence of frequent clinical probing during the healing phase on healthy peri-implant soft tissue formed at different titanium implant surfaces: a histomorphometrical study in dogs

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Abstract

Objectives: To investigate (i) the impact of different titanium implant surfaces on soft tissue integration over 6 months, and (ii) the influence of frequent clinical probing during the healing phase on the established mucosal seal.

Material and Methods: Standardized clinical probing was randomly performed (12 dogs, probing *versus* control) at different transmucosal surfaces [machined (M), sand-blasted/acid-etched (SLA), and chemically modified acid-etched (modA), modSLA] at 2, 4, 8, and 12 weeks (i.e. 1 ×, 2 ×, 3 ×, and 4 ×). Histomorphometrical analysis (e.g. mucosal margin (PM) – apical extension of the junctional epithelium (aJE), PM – coronal level of bone-to-implant contact (CBI) was performed at 4, 8, 12, and 24 weeks.

Results: While M and SLA groups revealed a split formation, epithelial cells and connective tissue were in close contact to modA and modSLA surfaces. Frequent clinical probing (i.e. 3 × and 4 ×) increased mean pocket depths, PM-aJE, and aJE-CBI values in all groups and markedly disrupted the epithelial and connective tissue attachment.

Conclusions: It was concluded that irrespective of the surface characteristics, a frequent clinical probing at short intervals during the healing phase was associated with dimensional and structural changes of the mucosal seal.

Key words: animal study, biological width, clinical probing, histomorphometry, surface hydrophilicity

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Conflict of interest and source of funding

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The establishment of a proper soft tissue integration at the transmucosal part of an osseointegrated titanium implant is a prerequisite to separate the supporting alveolar bone from the oral environment (Berglundh et al. 1991, Lindhe & Berglundh 1998). Nowadays, there is substantial evidence supporting the view that poor oral hygiene is a risk indicator for peri-implant diseases, including peri-implant mucositis and peri-implantitis (Heitz-Mayfield 2008). Basically, the accumulation of bacterial plaque biofilms at submucosal aspects of the titanium

surface may escape oral hygiene procedures and favour inflammatory reactions in the adjacent soft and hard tissues. From a histological point of view, the transmucosal attachment at submerged and non-submerged implants consists of a junctional epithelium (JE) with a length of approximately 2 mm and a connective tissue zone with a height of approximately 1–2 mm, thus resulting in a 3–4 mm-wide zone of biological soft tissue coverage of the implant-supporting bone (Berglundh et al. 1991). While the outer zone of the subepithelial connective tissue was observed to be well vascularized and cell rich with fibres running in different directions (Buser et al. 1992), its inner zone appeared to be poorly vascularized and consisted of numerous dense collagen fibres, running close to the implant surface in a parallel direction (Gotfredsen et al. 1991, Buser et al. 1992, Berglundh et al. 1994, Abrahamsson et al. 1996, Cochran et al. 1997). Even though the inner zone of the connective tissue commonly revealed a close contact to machined (M), roughly sandblasted, and plasma-sprayed titanium implants, the collagen fibres were mostly oriented parallel to the respective surfaces (Berglundh et al. 1991, Gotfredsen et al. 1991, Listgarten et al. 1992, Abrahamsson et al. 1996, Cochran et al. 1997). Accordingly, the peri-implant mucosa is commonly recognized as scar tissue, exhibiting an impaired resistance to bacterial colonization (Buser et al. 1992, Berglundh et al. 1994). Recently, a new methodology was used with the goal to produce hydroxylated/hydrated titanium surfaces with identical microstructure to either acid-etched (A), or sand-blasted, large grit, and acid-etched (SLA) substrates, but with a hydrophilic character (modA and modSLA, respectively) (Buser et al. 2004, Rupp et al. 2006). The specific production process used for modA/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 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). Preliminary experimental studies performed in dogs have pointed out that hydrophilic surfaces may also improve early stages of soft tissue integration of either non-submerged or submerged titanium implants (Schwarz et al. 2009). While M, A, and SLA implants appeared to be clearly separated by a dense connective tissue zone with parallel-running collagen fibres and rare blood vessel formation, modA and modSLA implants revealed a well-vascularized subepithelial connective tissue exhibiting collagen fibres that had started to extend and attach partially perpendicular to the implant surface. However, these data were based on a short-term observation of 28 days, and therefore the stability of the peri-implant mucosa over time cannot be estimated (Schwarz et al. 2007a, b). As probing of the soft tissues around implants has nowadays become a routine procedure during clinical monitoring, the potential influence of a mechanical disruption of the mucosal seal formed at modA and modSLA implants should also be taken into consideration. So far, the influence of a single conventional probing has only been assessed for M surfaces and reported to be associated with a complete re-establishment of the mucosal seal after 5 days of healing (Etter et al. 2002). From a clinical point of view, monitoring of the peri-implant tissue conditions may require frequent probing procedures over a longer period of time. In addition, time-critical treatment protocols (i.e. immediate or early loading), as recommended for modSLA titanium implants (Zöllner et al. 2008), may also require to consider the potential influence of clinical probing during the healing period of the peri-implant tissues.

Therefore, the present experimental animal study aimed at investigating (i) the impact of M, SLA, modA, and modSLA titanium implant surfaces on soft tissue integration over a period of 6 months, and (ii) the influence of frequent clinical probing during the healing period on the mucosal seal.

Material and Methods

Animals

A total of 12 Foxhounds, aged 15–16 months (mean weight 32.7 ± 3.7 kg) were included in the study. All animals exhibited a fully erupted permanent dentition. During the experiment, the dogs were fed once per day with a 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 adaptation 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, and fourth pre-molar as well as first and second molar (P2-M2) was performed bilaterally. After 3 months of healing, surgical implantation of modSLA and SLA screw-type titanium implants with differently structured transmucosal surfaces (i.e. M, SLA, modA, modSLA) was performed in a non-submerged healing procedure during the second phase. Clinical probing was randomly allocated in a split-mouth design and initiated at 2 weeks following implant placement and repeated after 4, 8, and 12 weeks of healing.

Titanium implants

A total of 48 modSLA (referred to as SLActive®, Institut Straumann AG, Basel, Switzerland) and 48 SLA (Institut Straumann AG) screw-type titanium implants (RN, standard plus, ∅3.3 mm, length 8 mm) revealed the following surface modifications at the transmucosal part (height: 1.8 mm), including 24 each:

- SLA: M – machined surface (R_a: 0.10 ± 0.03 μm) SLA – standard SLA surface, sandblasted with large grits of 0.25–0.5 mm and acid etched with HCl/H_2SO_4 (R_a: 3.22 ± 0.88 μm)
- modSLA: ModA – same etching procedure as SLA but rinsed under N_2 protection and directly stored in an isotonic NaCl solution, again protected by N_2 filling modSLA – same sandblasting and etching procedure as SLA but rinsed under N_2 protection and directly stored in an isotonic NaCl solution, again protected by N_2 filling

Surface roughness was measured two dimensionally using a profilometer (Perthometer Concept, Mahr, Germany) equipped with a diamond-tracing stylus (point radius 2 μm; point angle: 90°) (Rupp et al. 2006).Each type of titanium implant was randomly assigned to each

hemimandible. Accordingly, each animal received a total of eight implants bilaterally in the lower jaw, including two M, two SLA, two modA, and two modSLA implants, respectively.

Surgical procedure

Following intramuscular 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 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 anaesthetised. Intraoperative 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 in the same dose as described before. Additionally, a prophylactic administration of clindamycine (11.0 mg/kg body weight, Clerobe®, Pharmacia Tiergesundheit, Erlangen, Germany) was performed intra- and post-operatively for 3 days.

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.

In the second surgery, midcrestal incisions were made and mucoperiosteal flaps were reflected to expose the respective sites for implant insertion in the lower jaw. 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 (Schwarz et al. 2007a). All implants were inserted with good primary stability (i.e. lack of clinical mobility) in a way so that the implant shoulder (IS) exceeded the buccal aspect of the alveolar crest for 1.8 mm, as suggested in the surgical protocol of the manufacturer (Fig. 1a and b). In case of SLA, the implants were thoroughly rinsed with sterile saline before insertion. Following irrigation, mucoperiosteal flaps were repositioned with mattress sutures (Resorba®, Nuernberg, Germany), and implants were left to heal in a non-submerged position (Fig. 1c). In order to prevent a trauma to the peri-implant mucosa, oral hygiene procedures were omitted during the initial healing period of 7 days. Thereafter, a plaque control programme including tooth and implant cleaning just by the use of a toothbrush was initiated and performed twice per week without anaesthesia.

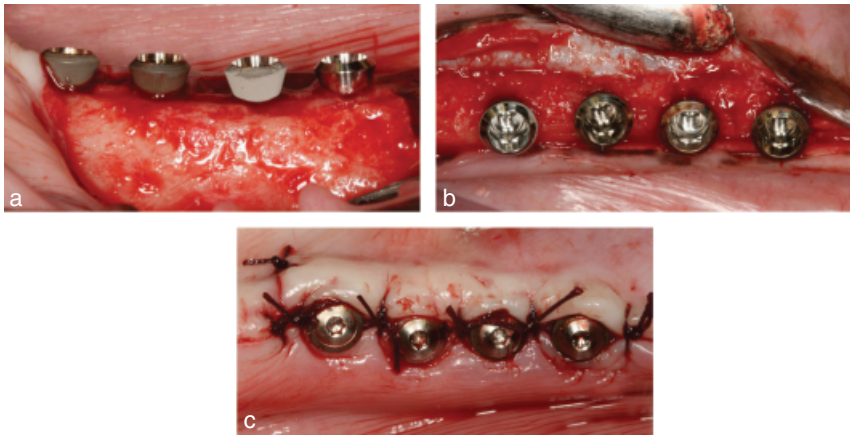

Fig. 1. (a) At 3 months after tooth extraction, screw-type titanium implants exhibiting the following surface modifications at the transmucosal (height: 1.8 mm) and endosseous parts were inserted bilaterally in the lower jaws (eight implants per animal):

Endosseous: SLA	Transmucosal: M	(M)
Endosseous: SLA	Transmucosal: SLA	(SLA)
Endosseous: modSLA	Transmucosal: modA	(modA)
Endosseous: modSLA	Transmucosal: modSLA	(modSLA)

The implants were inserted in a way so that the implant shoulder exceeded the buccal aspect of the alveolar crest for 1.8 mm. (b) Particular care was taken to preserve a residual thickness of the alveolar bone crest of at least 1 mm at both buccal and lingual aspects of each implant site. (c) All implants were left to heal in a non-submerged position.

Table 1. Outline of standardized clinical probing in different groups (three dogs per healing period)

Frequency of probing	Clinical probing*				Healing periods* (weeks)
	2 weeks	4 weeks	8 weeks	12 weeks	
1 ×	x	–	–	–	4
2 ×	x	x	–	–	8
3 ×	x	x	x	–	12
4 ×	x	x	x	x	24

*In relation to implant surgery. [x]A single clinical probing procedure was performed at respective time intervals.

Randomization and clinical probing

After 2 weeks of non-submerged healing, each side of the mandible was randomly allocated in a split-mouth design to either probing- or non-probing groups. All randomization procedures were performed according to a computer-generated list (RandList®, DatInfGmbH, Tübingen, Germany). A single standardized clinical probing was initiated at 2 weeks after implant placement and repeated after 4, 8, and 12 weeks of healing at respective sites. Three animals each were assigned to final healing periods of 4 (1 × probing), 8 (2 × probing), 12 (3 × probing), and 24 (4 × probing) weeks. Accordingly, the healing periods subsequent to the final probing procedure corresponded to 2 (1 × probing), 4 (2 × and 3 × probing), and 12 (4 × probing) weeks in the respective groups (Table 1).

Clinical probing was performed using a conventional periodontal probe (PCP 12, Hu-Friedy Europe, Leimen, Germany), measuring the probing depth (PD) from the mucosal margin

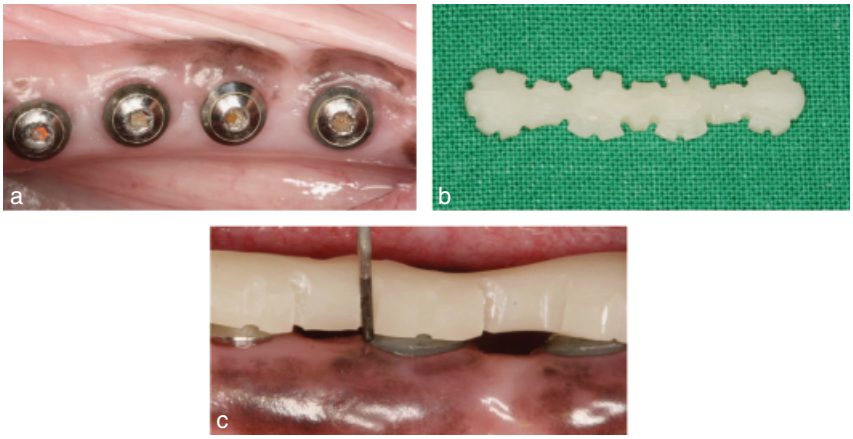

Fig. 2. (a) Clinical situation after 2 weeks of healing. Each side of the mandible was randomly allocated in a split-mouth design to either probing- or non-probing groups. (b) Acrylic stents exhibiting six vertical grooves per implant (i.e. mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual) were custom made for each probing site. (c) The grooves allowed a reproducible clinical probing (i.e. direction and parallel angulation along with the long axis of the implant), which was performed by the use of a conventional periodontal probe.

(PM) to the bottom of the probeable pocket. For all probing procedures, an intramuscular sedation with 0.17 mg/kg acepromazine (Vetranquil 1%, Ceva Tiergesundheit) was initiated and followed by a short-acting anaesthesia using 0.4 mg/10 kg medetomidin (Domitor®, Orion Corporation, Espoo, Finland). Individual acrylic stents (Bis-Acrylat-Composite, Luxatemp®, DMG, Hamburg, Germany) exhibiting six vertical grooves per implant (i.e. mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual), were assembled before the first probing procedure (Fig. 2a and b). The grooves allowed the exact reproducibility of both direction and parallel angulation of the probe along with the long axis of the implant at each specific site (Fig. 2c). All probing procedures were performed by one experienced, blinded investigator (D. F.).

Intra-examiner reproducibility

Before the start of the experimental part of the study, a clinical calibration procedure was initiated. In particular, five patients attending the Department of Oral Surgery at the Heinrich Heine University Düsseldorf, each showing two implants with PDs $\geqslant$4 mm on at least one aspect, were used to calibrate the examiner. The examiner evaluated the patients on two separate occasions, 48 h apart. Calibration was accepted if measurements at baseline and at 48 h were within a millimetre at >90% of the time.

Retrieval of specimens

The animals were sacrificed (overdose of sodium pentobarbital 3%) after a healing period of 4, 8, 12, and 24 weeks (three dogs each), respectively and the oral tissues were fixed by a 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, Germany) for non-decalcified sectioning. After 20 h, the specimens were completely polymerized. Each implant site was cut in the bucco-lingual 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 specimens, resulting in three sections of approximately 500 μm in thickness each (Donath 1985). Subsequently, all specimens were glued with acrylic cement (Technovit 7210 VLC, Heraeus Kulzer) to opaque plexiglas and ground to a final thickness of approximately 30 μm. All sections were stained with Masson Goldner Trichrome.

Histological analysis

Histomorphometrical analyses as well as microscopic observations were performed by one experienced investigator (I. M.) 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 $\times$ 200) were evaluated using a software program (Cell D®, Soft Imaging System, Münster, Germany).

The following landmarks were identified in the stained sections (Schwarz et al. 2007a):

IS, PM, the marginal portion of the peri-implant mucosa; aJE, the apical extension of the long JE; and bone-to-implant contact (CBI), the most coronal level of bone in contact with the implant surface. Linear measurements were made by drawing a vertical line, following the long axis of the implant, from IS to PM (IS-PM), PM to aJE (PM-aJE), and aJE to CBI (aJE-CBI) at both buccal and lingual aspects (Figs 3–6).

Statistical analysis

The statistical analysis was performed using a commercially available software program (SPSS 17.0, SPSS Inc., Chicago, IL, USA). 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. Because of the small number of animals, only descriptive statistical analyses of the clinical and histomorphometrical parameters were applied.

Results

Clinical observations and PD measurements

The post-operative healing was considered as generally uneventful in all dogs. No complications such as allergic reactions, abscesses or infections were observed throughout the study period of 6 months.

Mean PD values as assessed in single and frequent probing groups after 2, 4, 8, and 12 weeks of healing are presented in Table 2. In all groups, frequent clinical probing during the healing phase (i.e. 3 $\times$ and 4 $\times$) was associated with an increase in mean PD values (Table 2).

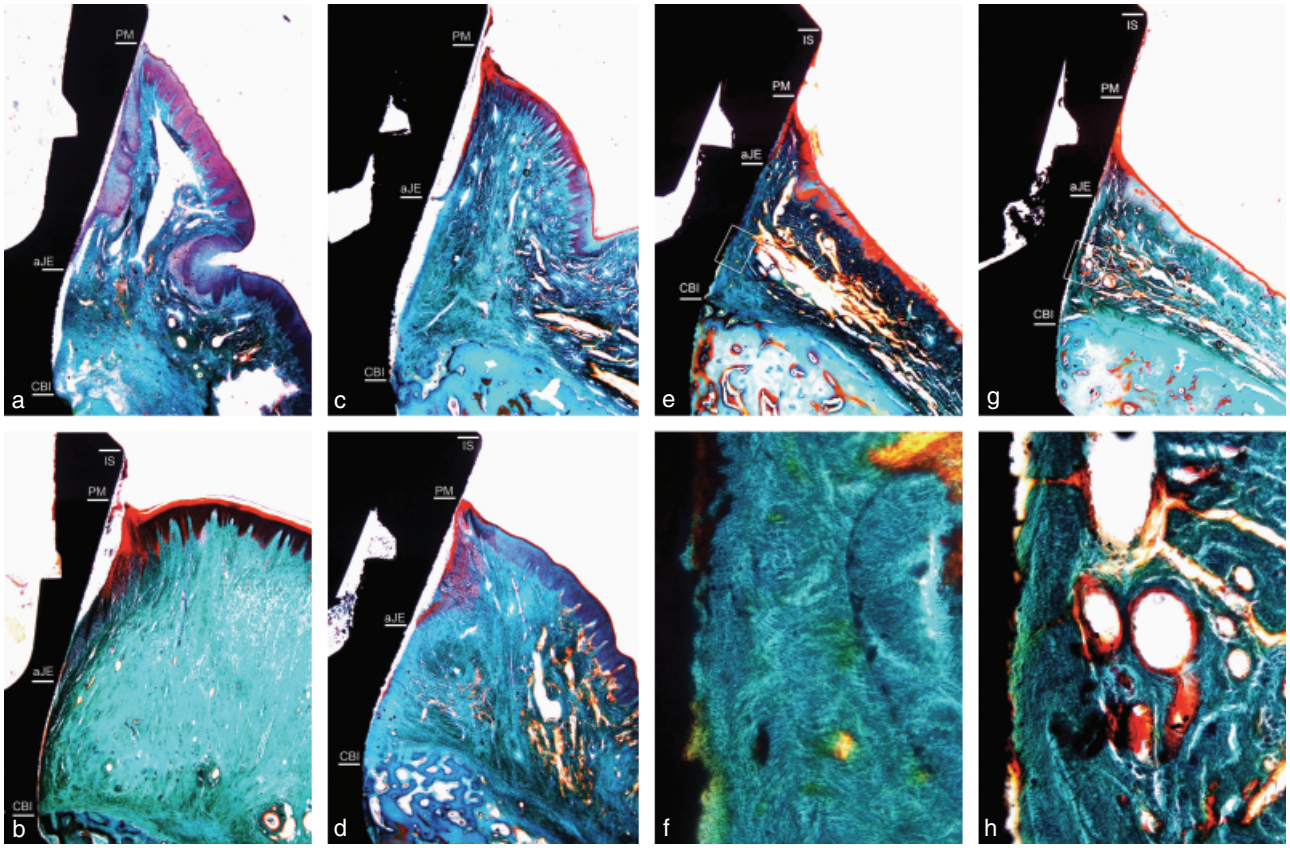

Fig. 3. Representative histological views (Masson Goldner stain) of wound healing at 4 weeks after implant placement. Unprobed M and SLA titanium implants were commonly characterized by a split formation, separating the transmucosal aspect from the junctional epithelium and the subepithelial connective tissue. In both groups, a single probing procedure resulted in an obvious enlargement of the transmucosal gap. (a) M (Control, buccal aspect, original magnification × 40). (b) SLA (Control, lingual aspect, original magnification × 40). (c) M (probing 1 × , lingual aspect, original magnification × 40). (d) SLA (probing 1 × , lingual aspect, original magnification × 40). The epithelial cells and a well-vascularized subepithelial connective tissue appeared to be in close contact to the transmucosal aspect of either unprobed or probed modA and modSLA surfaces. (e) modSLA (Control, buccal aspect, original magnification × 40). (f) Higher magnification (× 400) of box area shown in (e). (g) modA (probing 1 × , lingual aspect, original magnification × 40). (h) Higher magnification (× 400) of box area shown in (g). Landmarks for the histomorphometrical analysis: IS, implant shoulder; PM, marginal portion of the peri-implant mucosa; aJE, the apical extension of the long junctional epithelium; CBI, the most coronal level of bone in contact with the implant.

Histological observations/ histomorphometrical analysis

The mean values of IS-PM, PM-aJE, and aJE-CBI for all implants in both groups at 4, 8, 12, and 24 weeks are presented in Tables 3–6.

4 weeks

Histological observation revealed that all type of surface modifications supported the establishment of a well-dimensioned and organized peri-implant mucosa including a barrier epithelium and a collagen-rich subepithelial connective tissue. Signs of an inflammation were only observed occasionally and appeared to be limited to the most coronal aspect of the epithelial cells facing the peri-implant sulcus. These areas were demarcated by a mixed chronic inflammatory cell infiltrate. Basically, variations in density, size, and extension of the cellular infiltrates were not observed between the different types of surfaces.

The transmucosal aspect of unprobed M and SLA implants was commonly separated from the JE and the subepithelial connective tissue by a gap. Histologically, the subepithelial connective tissue was mainly characterized by parallel running collagen fibres and a low density of vascular structures. However, towards the periphery, this inner zone passed into a well-vascularized outer zone, which was composed of collagen fibres running in different directions (Fig. 3a and b). A single probing procedure resulted in an obvious enlargement of the transmucosal gap at both M and SLA implants. While the mechanical disruption was most commonly limited to the epithelial component, some specimens also revealed an increased separation of the most coronal aspect of the subepithelial connective tissue (Fig. 3c and d).

Epithelial cells revealed a close contact to unprobed modA and modSLA implant surfaces (Fig. 3e). Similarly, the subepithelial connective tissue adjacent to modA and modSLA groups appeared to be firmly attached to the implant surfaces. This inner contact zone was composed of numerous blood vessels and well-organized tiny collagen fibres running in a direction perpendicular to the respective implant surfaces (Fig. 3f). A single clinical probing also resulted in a mechanical disruption of the epithelial and connective tissue attachment at both modA and modSLA implants (Fig. 3g). However, some of these areas exhibited

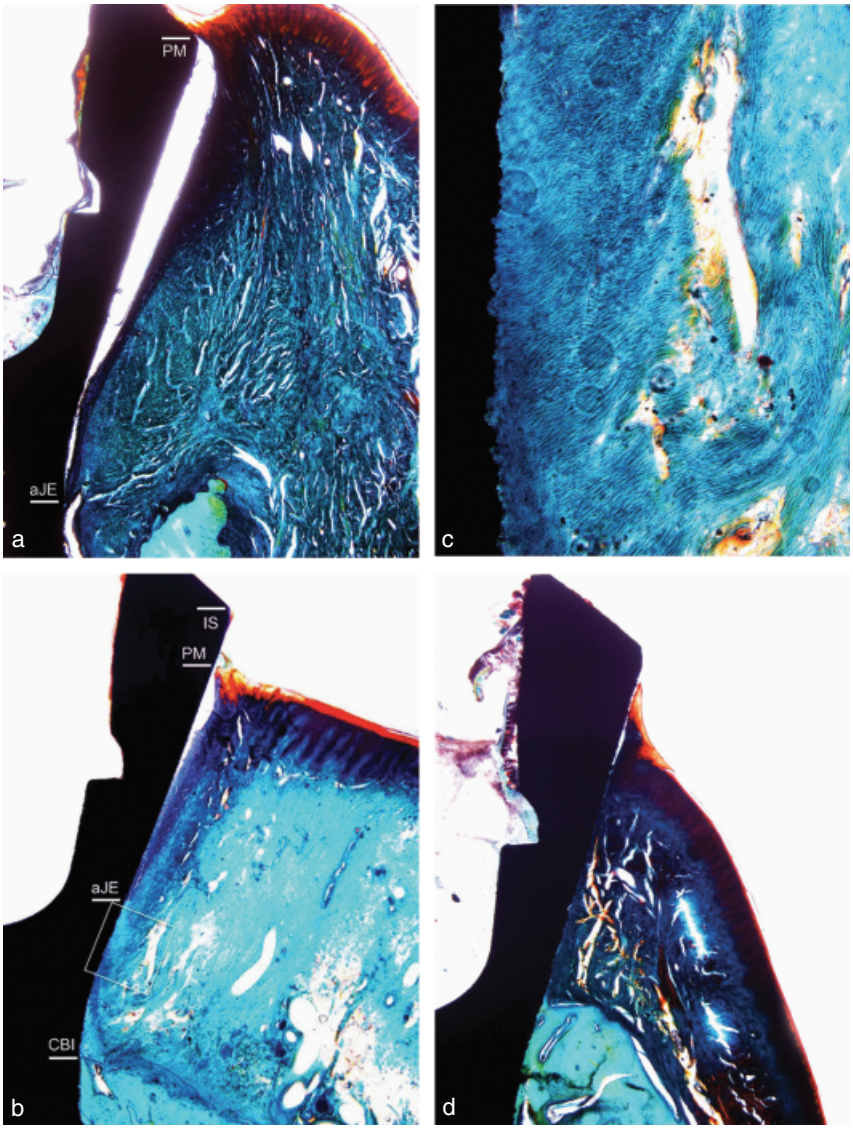

Fig. 4. Representative histological views (Masson Goldner stain) of wound healing at 8 weeks after implant placement. A frequent clinical probing procedure (2 ×) was associated with a clear split formation at M and SLA implants. In contrast, modA and modSLA implants commonly revealed an almost intact mucosal seal, which was characterized by a perpendicular alignment of collagen fibres to the transmucosal aspect of respective titanium surfaces. (a) SLA (probing 2 × , buccal aspect, original magnification × 40). (b) modA (probing 2 × , buccal aspect, original magnification × 40). (c) Higher magnification (× 400) of box area in (b). (d) modSLA (probing 2 × , buccal aspect, original magnification × 40). Landmarks for the histomorphometrical analysis: IS, implant shoulder; PM, marginal portion of the peri-implant mucosa; aJE, the apical extension of the long junctional epithelium; CBI, the most coronal level of bone in contact with the implant.

a slight residual adaptation of collagen fibres, thus decreasing the width of the transmucosal gap (Fig. 3h) (Table 3).

8 weeks

Histologically, the structural composition of both the epithelial and connective tissue zone at all unprobed control implants revealed the same characteristics as observed after 4 weeks (Fig. 3). When compared with the corresponding control groups, frequent probing (2 ×) at SLA and modA implants tended to be associated with an increase of mean aJE-CBI values at either buccal or lingual aspects, thus resulting in a slight resorption of the supporting alveolar bone (Fig. 4a and b, Table 4). While clinical probing obviously caused a clear split formation in the M and SLA groups, modA and modSLA implants commonly revealed an almost intact mucosal seal. In particular, the affected subepithelial zone of connective tissue was characterized by a perpendicular collagen fibre alignment on a level equivalent to the corresponding unprobed control sites (Fig. 4c and d).

12 weeks

Histological observation of the unprobed control specimens in the M, SLA, modA, and modSLA groups revealed a stable and well-dimensioned, non-infiltrated epithelial and subepithelial connective tissue zone, exhibiting structural features comparable with the situation observed at 4 (Fig. 3) and 8 weeks. In comparison with the unprobed control implants, a frequent probing procedure (3 ×) tended to be associated with an increase of mean PM-aJE and aJE-CBI values as observed at both buccal and lingual aspects (Table 5). Histologically, the mechanical disruption was mainly localized to the epithelial component of the peri-implant mucosa, but occasionally also affected the most coronal zone of the subepithelial connective tissue. While the epithelial cells were commonly separated from all types of implant surfaces by a split, the subepithelial connective tissue also appeared to be loosely adapted in all groups. In particular, the specific orientation of the collagen fibres, as observed in both unprobed modA and modSLA groups was no longer evident after the frequent (3 ×) probing procedure. Accordingly, all groups investigated exhibited a dense subepithelial connective tissue with parallel running collagen fibres (Fig. 5a–d).

24 weeks

In all unprobed groups, the structural features of the non-infiltrated epithelial and subepithelial connective tissue zone were comparable with the situation observed at 4 (Fig. 3), 8, and 12 weeks. Basically, when compared with the unprobed control implants, the frequent mechanical disruption (4 ×) tended to be associated with ongoing increases of mean PM-aJE and aJE-CBI values (Table 6) as well as a clear split formation at the transmucosal aspect of all groups investigated. A direct attachment of collagen fibres from the subepithelial connective tissue to the respective titanium surfaces was only observed occasionally and limited to the most apical aspect of the instrumented area (Fig. 6a–c). In all

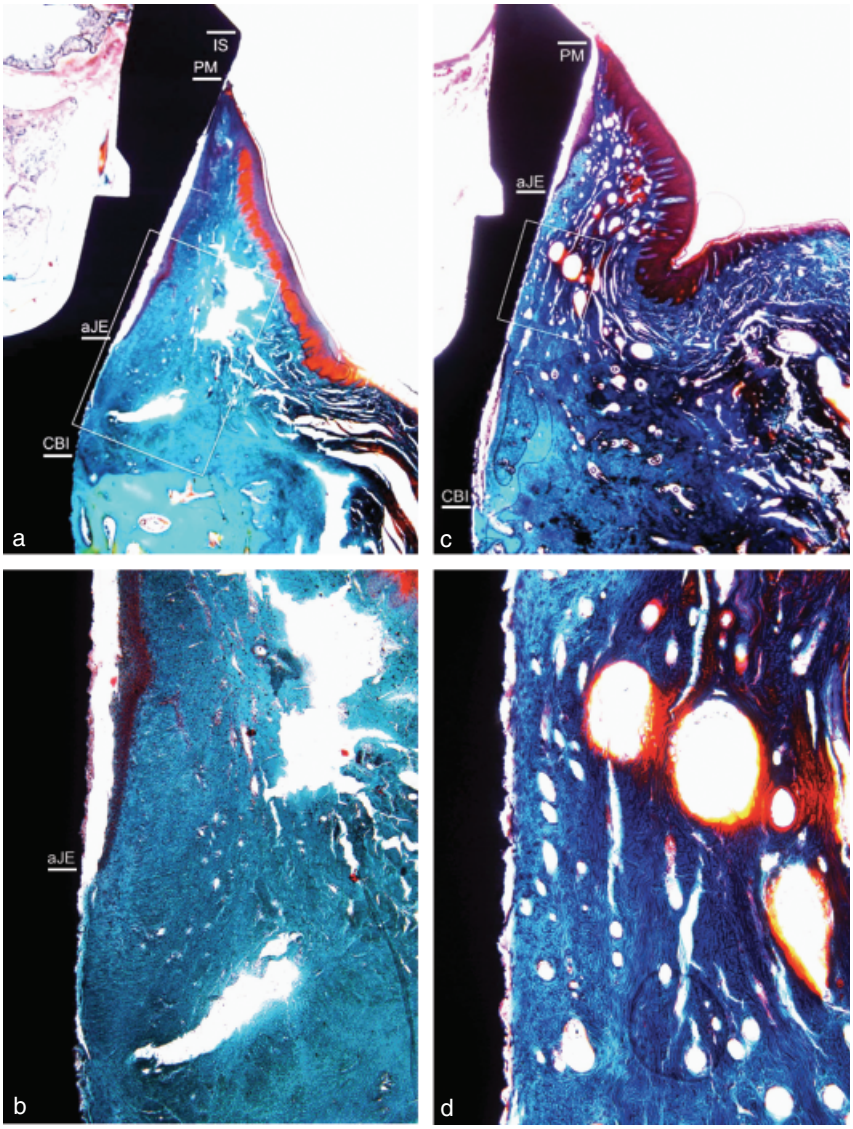

Fig. 5. Representative histological views (Masson Goldner stain) of wound healing at 12 weeks after implant placement. In all groups, the frequent clinical probing procedure (3 ×) resulted in a slight apical displacement of the barrier epithelium and the formation of a dense subepithelial connective tissue zone with parallel running collagen fibres. (a) SLA (probing 3 × , buccal aspect, original magnification × 40). (b) Higher magnification (× 100) of box area in (a). (c) modSLA (probing 3 × , buccal aspect, original magnification × 40). (d) Higher magnification (× 100) of box area in (c). Landmarks for the histomorphometrical analysis: IS, implant shoulder; PM, marginal portion of the peri-implant mucosa; aJE, the apical extension of the long junctional epithelium; CBI, the most coronal level of bone in contact with the implant.

probing groups, the apical displacement of the barrier epithelium was commonly associated with a slight resorption of the crestal alveolar bone (Fig. 6d).

Discussion

The present study was designed to investigate the impact of different surface characteristics on soft-tissue integration of titanium implants over a period of 6 months, and to assess the influence of a frequent clinical probing procedure during the healing phase on the mucosal seal in respective groups. Within its limitations, the histomorphometrical analysis has indicated that a single clinical probing was apparently not associated with potential changes of all parameters investigated. However, subsequent to a frequent clinical probing, all groups revealed obvious increases of mean PM-aJE values over time. While M and SLA groups exhibited obvious increases of mean PM-aJE values at 8 weeks (i.e. 2 × probing procedure), potential changes in the modA and modSLA groups were only observed at 24 weeks (i.e. 4 × probing procedure). In all groups, increases of mean PM-aJE values were also associated with an increase of mean aJE-CBI values, thus resulting in a slight resorption of the implant-supporting alevolar bone. When interpreting the present results, it must be emphasized that baseline probing of the peri-implant soft tissue conducted at short intervals of 2–4 weeks may not be of clinical relevance for commonly applied conventional loading procedures (i.e. healing period of 3–6 months). However, an earlier time point for clinical monitoring might be of potential relevance when considering more progressive protocols such as immediate (i.e. healing period <1 week) or early loading procedures (i.e. healing period of 1 week to 2 months) (Weber et al. 2009), which were recently recommended for modSLA surfaces (i.e. immediate or 28–34 days after surgery) (Zöllner et al. 2008). The observation that a single probing procedure may not have a detrimental effect on the soft-tissue seal is in agreement with the results of a previous similar experimental study performed in dogs (Etter et al. 2002). In this study, a single course of standardized clinical probing was performed at screw-shaped implants exhibiting a titanium-plasma coating at the endosseous aspect and a conventional M transmucosal part (1.8 mm). The histological analysis at day 0 revealed that the probe tip was located close to the most coronal level of the subepithelial connective tissue zone, thus mainly disrupting the epithelial attachment. A complete epithelial attachment was re-established after a healing period of 5 days. After 7 days of healing, both unprobed control and probed test sites revealed no significant differences of mean PM-aJE (1.77 ± 0.58 *versus* 1.89 ± 0.54 mm) and aJE-CBI (1.2 ± 0.60 *versus* 0.92 ± 0.52 mm) values. Basically, the mean PM-aJE and aJE-CBI values as observed for both probed and unprobed implants are in agreement with the present results noted for M implants at 4 weeks (Etter et al. 2002). In this context, it must be emphasized that Etter et al. (2002) allowed an initial healing period of 12 weeks to account for a maturation of the peri-implant soft tissue. This issue, how-

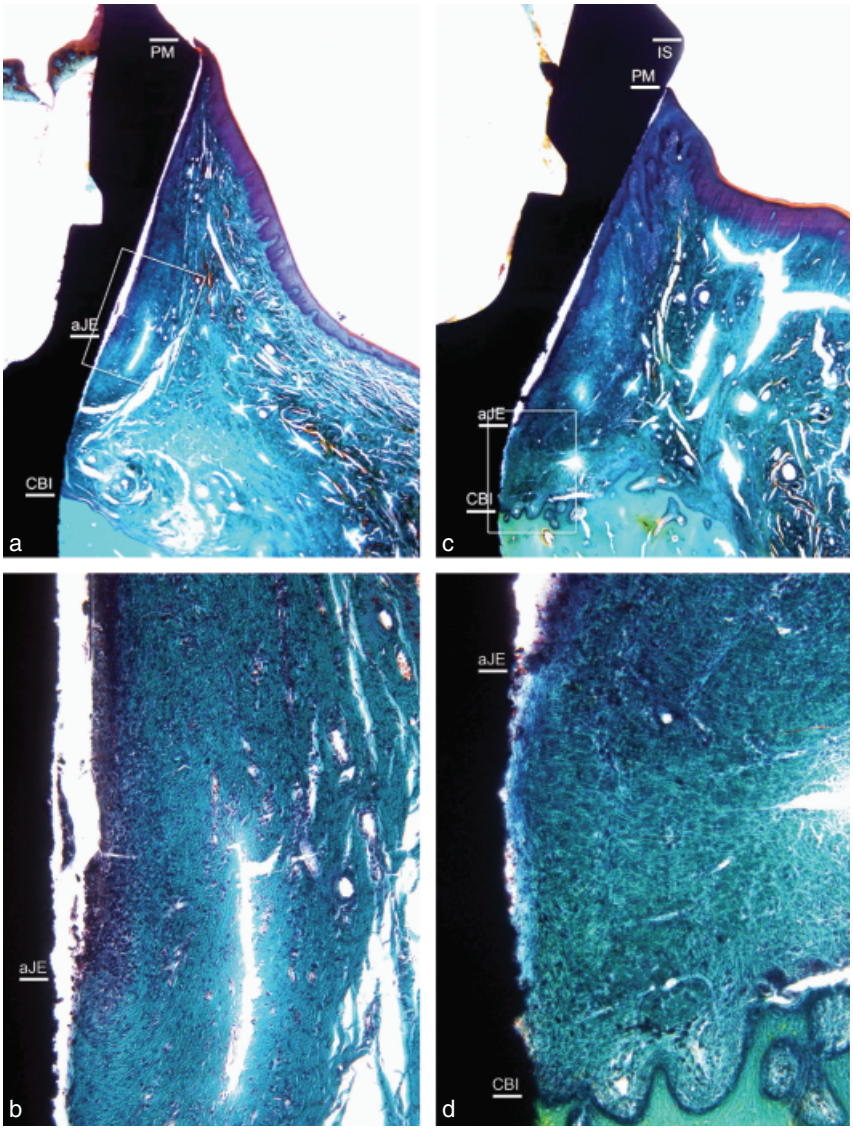

Fig. 6. Representative histological views (Masson Goldner stain) of wound healing at 24 weeks after implant placement. The apical displacement of the barrier epithelium was more pronounced after a frequent clinical probing procedure (4 ×) and commonly associated with a slight resorption of the implant-supporting alveolar bone in all groups. (a) M (probing 4 × , buccal aspect, original magnification × 40). (b) Higher magnification (× 100) of box area shown in (a). (c) modSLA (probing 4 × , buccal aspect, original magnification × 40). (d) Higher magnification (× 100) of box area in (c). Landmarks for the histomorphometrical analysis: IS, implant shoulder; PM, marginal portion of the peri-implant mucosa; aJE, the apical extension of the long junctional epithelium; CBI, the most coronal level of bone in contact with the implant.

Table 2. Mean probing depth (PD) values (± SD) as assessed at six aspects per implant after 2, 4, 8, and 12 weeks of healing

Group	M	SLA	modA	modSLA	Animals
Probing 1 ×	2.41 ± 0.62	2.34 ± 0.43	1.78 ± 0.34	1.42 ± 0.58	12
Probing 2 ×	2.49 ± 0.78	2.43 ± 0.52	1.40 ± 0.46	1.63 ± 0.31	9
Probing 3 ×	2.84 ± 0.69	2.94 ± 0.84	1.34 ± 0.76	1.64 ± 0.48	6
Probing 4 ×	3.13 ± 0.52	2.81 ± 0.73	2.10 ± 0.64	1.93 ± 0.48	3

ever, is controversially discussed in the current literature. In particular, Berglundh et al. (2007) have demonstrated that a maturation of the barrier epithelium and an organization of the collagen fibres in the subepithelial connective tissue at non-submerged SLA implants exhibiting a transmucosal M surface may require a healing period of at least 6–8 weeks. However, in terms of histomorphometrical assessment of mean IS-PM, PM-aJE, and aJE-CBI values, stable conditions at transmucosal M, SLA, modA, and modSLA titanium implant surfaces were observed after an initial healing period of 2 weeks (Becker et al. 2007, Schwarz et al. 2007a) and maintained over a period of 6 months (Becker et al. 2009). This observation is supported by the present results as obtained in the unprobed control groups, most extensively pointing to stable mean IS-PM, PM-aJE, and aJE-CBI values over time. From a biological point of view, however, it is impossible to estimate to what extent these histomorphometrical parameters may correlate with a maturation of the barrier epithelium and the subepithelial connective tissue. When interpreting the present results, it must also be emphasized that Etter et al. (2002) used a pressure-sensitive periodontal probe, allowing a light probing force of 0.2–0.25 N. The rationale for using a periodontal probe with a conventional readout in the present study was primarily based on the fact that this type of probe is widely used in the daily routine practice. Moreover, assessment of PD in periodontal maintenance patients using a manual probe was shown to be a reliable and reproducible procedure, which even appeared to be superior to several automated periodontal probes (Barendregt et al. 2006). However, one must realize that the probing pressure may be of crucial importance in the presence of inflammation in the peri-implant mucosa (Schou et al. 2002). While at healthy and mucositis sites, the probe penetration tented to stop at the histological level of connective tissue adhesion, it reached the base of the inflammatory lesion at peri-implantitis sites (Lang et al. 1994). The stringent oral hygiene procedures coupled with the histological evidence of healthy mucosal conditions in all groups may support the finding that probing in the present study also tended to stop at the most coronal level of the subepithelial connective tissue attachment. Moreover, clinical assessment of mean PD values appeared to correspond closely to the histological level of mean PM-aJE values in all groups investigated. Nevertheless, the use of a pressure-sensitive periodontal probe may have resulted in different outcomes of healing and needs to be further investigated. Despite the finding that mean PM-aJE and aJE-CBI values are in accordance with previous data (Berglundh et al. 1991, Abrahamsson et al. 1996,

Table 3. Mean values (± SD) of IS-PM, PM-aJE, and aJE-CBI (in mm ± SD) after a single probing (1 ×) procedure at 4 weeks* (three dogs per healing period)

Group	Modification	Buccal			Lingual		
		IS-PM	PM-aJE	aJE-CBI	IS-PM	PM-aJE	aJE-CBI
Probing 1 ×	M	0.24 ± 0.61	1.79 ± 0.19	1.42 ± 0.86	0.31 ± 0.27	1.65 ± 0.35	1.98 ± 1.10
	SLA	0.29 ± 0.42	1.71 ± 0.58	1.46 ± 0.40	0.51 ± 0.38	1.96 ± 0.76	1.38 ± 0.44
	modA	0.32 ± 0.29	1.12 ± 0.33	1.12 ± 0.36	0.43 ± 0.38	1.21 ± 0.46	0.98 ± 0.22
	modSLA	0.24 ± 0.47	1.35 ± 0.47	1.19 ± 0.38	0.24 ± 0.42	1.20 ± 0.35	1.05 ± 0.16
Control	M	0.22 ± 0.61	1.92 ± 0.38	1.15 ± 0.72	0.29 ± 0.78	1.53 ± 0.26	1.37 ± 0.78
	SLA	0.31 ± 0.42	1.57 ± 0.55	1.49 ± 0.27	0.32 ± 0.28	1.59 ± 0.16	1.47 ± 0.52
	modA	0.22 ± 0.48	0.92 ± 0.39	1.08 ± 0.18	0.21 ± 0.41	1.27 ± 0.38	1.08 ± 0.36
	modSLA	0.33 ± 0.52	1.21 ± 0.49	1.13 ± 0.23	0.24 ± 0.31	1.13 ± 0.26	0.97 ± 0.34

*In relation to implant surgery.
IS, implant shoulder; PM, mucosal margin; aJE, apical extension of the junctional epithelium; CBI, bone-to-implant contact.

Table 4. Mean values (± SD) of IS-PM, PM-aJE, and aJE-CBI (in mm ± SD) after a frequent probing (2 ×) procedure at 8 weeks* (three dogs per healing period)

Group	Modification	Buccal			Lingual		
		IS-PM	PM-aJE	aJE-CBI	IS-PM	PM-aJE	aJE-CBI
Probing 2 ×	M	0.24 ± 0.43	1.96 ± 0.64	0.85 ± 0.68	0.29 ± 0.36	2.20 ± 0.27	0.82 ± 0.31
	SLA	0.19 ± 0.14	2.24 ± 0.57	0.86 ± 0.27	0.23 ± 0.24	1.93 ± 0.24	1.23 ± 0.57
	modA	0.34 ± 0.52	1.37 ± 0.33	0.95 ± 0.67	0.34 ± 0.18	1.01 ± 0.31	1.57 ± 0.35
	modSLA	0.33 ± 0.24	1.18 ± 0.34	1.46 ± 0.43	0.34 ± 0.26	1.09 ± 0.39	1.43 ± 0.40
Control	M	0.23 ± 0.48	1.80 ± 0.60	1.60 ± 0.33	0.24 ± 0.38	1.76 ± 0.17	1.06 ± 0.27
	SLA	0.32 ± 0.39	2.24 ± 0.29	1.06 ± 0.32	0.27 ± 0.29	1.85 ± 0.36	1.59 ± 0.53
	modA	0.24 ± 0.47	1.13 ± 0.22	1.22 ± 0.15	0.24 ± 0.21	1.15 ± 0.37	1.21 ± 0.31
	modSLA	0.34 ± 0.13	0.96 ± 0.15	1.48 ± 0.24	0.27 ± 0.22	1.21 ± 0.43	1.24 ± 0.45

*In relation to implant surgery.
IS, implant shoulder; PM, mucosal margin; aJE, apical extension of the junctional epithelium; CBI, bone-to-implant contact.

Table 5. Mean values (± SD) of IS-PM, PM-aJE, and aJE-CBI (in mm ± SD) after a frequent probing (3 ×) procedure at 12 weeks* (three dogs per healing period)

Group	Modification	Buccal			Lingual		
		IS-PM	PM-aJE	aJE-CBI	IS-PM	PM-aJE	aJE-CBI
Probing 3 ×	M	0.24 ± 0.46	2.03 ± 0.47	0.93 ± 0.32	0.35 ± 0.40	1.89 ± 0.96	1.35 ± 0.26
	SLA	0.38 ± 0.29	2.28 ± 1.28	1.25 ± 0.74	0.34 ± 0.56	2.18 ± 0.81	1.02 ± 0.45
	modA	0.35 ± 0.57	1.41 ± 0.34	1.38 ± 0.30	0.21 ± 0.30	1.18 ± 0.29	1.75 ± 0.16
	modSLA	0.24 ± 0.58	1.36 ± 0.47	1.25 ± 0.35	0.26 ± 0.58	1.37 ± 0.38	1.49 ± 0.29
Control	M	0.34 ± 0.14	1.60 ± 0.46	1.31 ± 0.46	0.24 ± 0.61	1.64 ± 0.22	1.68 ± 0.59
	SLA	0.24 ± 0.32	2.29 ± 0.77	1.09 ± 0.22	0.43 ± 0.38	1.72 ± 0.19	1.10 ± 0.48
	modA	0.29 ± 0.46	0.96 ± 0.22	1.46 ± 0.63	0.44 ± 0.57	1.25 ± 0.33	1.46 ± 0.22
	modSLA	0.24 ± 0.50	1.02 ± 0.36	1.56 ± 0.35	0.24 ± 0.40	0.93 ± 0.23	1.39 ± 0.32

*In relation to implant surgery.
IS, implant shoulder; PM, mucosal margin; aJE, apical extension of the junctional epithelium; CBI, bone-to-implant contact.

1998, Berglundh & Lindhe 1996), it must be emphasized that this is the first experimental study using either SLA, modA, or modSLA surfaces for the transmucosal aspect of SLA and modSLA titanium implants over a period of 6 months. In a previous study using the same animal model, these types of surface modifications were also compared with conventional M implants, and soft-tissue healing at unprobed sites was assessed at days 7, 14, and 28 (Schwarz et al. 2007a). Similarly to the present results, mean PM-aJE values after 28 days of healing also appeared to be lower in both modA (1.5 ± 0.7 mm) and modSLA (1.4 ± 0.3 mm) groups when compared with either M (1.7 ± 0.5 mm) or SLA (1.8 ± 0.6 mm) groups. While these differences did not reach statistical significance, mean aJE-CBI values were significantly higher at modSLA implants when compared with either M or SLA implants. Moreover, the specific histological features of both epithelial and subepithelial connective tissue attachment at 2 and 4 weeks in different groups is in agreement with the present results as obtained at unprobed control sites after 4, 8, 12, and 24 weeks of healing (Schwarz et al. 2007a). Accordingly, it might be suggested that the stability of both dimension and structure of the periimplant mucosa at plaque-controlled healthy M, SLA, modA, and modSLA implants can basically be maintained over

Table 6. Mean values (± SD) of IS-PM, PM-aJE, and aJE-CBI (in mm ± SD) after a frequent probing (4 ×) procedure at 24 weeks* (three dogs per healing period)

Group	Modification	Buccal			Lingual		
		IS-PM	PM-aJE	aJE-CBI	IS-PM	PM-aJE	aJE-CBI
Probing 4 ×	M	0.32 ± 0.37	2.11 ± 0.66	0.84 ± 0.29	0.30 ± 0.46	2.18 ± 0.51	0.93 ± 0.18
	SLA	0.37 ± 0.46	1.99 ± 0.43	0.98 ± 0.38	0.22 ± 0.22	2.38 ± 0.45	0.89 ± 0.31
	modA	0.30 ± 0.48	1.64 ± 0.36	1.58 ± 0.27	0.31 ± 0.48	1.57 ± 0.22	1.04 ± 0.35
	modSLA	0.21 ± 0.27	1.54 ± 0.23	1.47 ± 0.38	0.34 ± 0.42	1.60 ± 0.46	1.23 ± 0.25
Control	M	0.23 ± 0.41	2.00 ± 0.38	1.26 ± 0.42	0.52 ± 0.45	1.79 ± 0.64	1.03 ± 0.57
	SLA	0.42 ± 0.36	1.61 ± 0.36	1.67 ± 0.27	0.41 ± 0.50	1.81 ± 0.49	1.46 ± 0.34
	modA	0.25 ± 0.40	0.93 ± 0.36	1.64 ± 0.27	0.33 ± 0.38	0.95 ± 0.33	1.36 ± 0.68
	modSLA	0.34 ± 0.29	1.08 ± 0.22	1.36 ± 0.34	0.40 ± 0.42	1.09 ± 0.41	1.33 ± 0.34

*In relation to implant surgery.
IS, implant shoulder; PM, mucosal margin; aJE, apical extension of the junctional epithelium; CBI, bone-to-implant contact.

a period of 6 months. The soft-tissue stability at conventional M implants has even been proven under loaded conditions for up to 12 months (Hermann et al. 2000). For the time being, the cellular response involved in soft-tissue healing at either modA, modSLA, or commercially pure titanium implant surfaces still remains unknown and may require further investigations. However, a recent immunohistochemical analysis has indicated that the subepithelial connective tissue adjacent to modSLA implants was characterized by an intense fibronectin and proliferating cell nuclear antigen reactivity (Schwarz et al. 2007b). While fibronectin is involved in many cellular processes such as tissue repair, blood clotting, and cell adhesion by anchoring cells to collagen or proteoglycan substrates (Valenick et al. 2005, Napper et al. 2006), immunohistochemical localization of proliferating cell nuclear antigen reactivity can be used as a reliable marker of cells undergoing active proliferation (Paunesku et al. 2001, Murata et al. 2006). Apart from the detrimental effects of a frequent probing procedure on the dimension of the peri-implant mucosa (i.e. increases of mean PM-aJE and aJE-CBI values), mechanical disruption obviously impaired the structure of both epithelial and subepithelial connective tissue attachment. This was particularly true for modA and modSLA groups, because the initial orientation of collagen fibres in a direction partially perpendicular to the implant surface could no longer be observed after the frequent (3 ×) probing procedure at 12 weeks. In all groups, frequent clinical probing resulted in a clear split formation and separation of the peri-implant mucosa from the transmucosal aspect of the implant surface, thus potentially facilitating bacterial colonization of this area. In this context, it is important to point to the results from a previous study, indicating that surface hydrophilicity had no apparent effect, while microtopography had a highly uneven and unpredictable influence on plaque biofilm formation. In particular, in vivo supragingival plaque biofilm formation at 12, 24, and 48 h revealed the following mean scores: 12 h: SLA = modSLA > M > A = modA ($p < 0.001$; respectively); 24 h: SLA > modSLA = M > A = modA ($p < 0.001$; respectively); and 48 h: SLA = modSLA = M > A = modA ($p < 0.001$; respectively) (Schwarz et al. 2007c). The influence of both parameters has been reported to be less significant on subgingival plaque formation, as this environment may probably offer more niches for bacterial adhesion and survival (Quirynen et al. 1993). However, previous studies provide some evidence that plaque biofilms may alter the surface characteristics of titanium surfaces (Mouhyi et al. 2000, Schwarz et al. 2006). It was presumed that bacterial contamination of a titanium surface may affect its dioxide layer resulting in a lower surface free energy and subsequently reduced tissue integration (Baier & Meyer 1988, Sennerby & Lekholm 1993). These findings may explain, at least in part, the decreasing capacity of modA and modSLA implant surfaces to re-establish a proper mucosal seal subsequent to frequent probing procedures. Accordingly, biological contamination may probably render modA and modSLA surfaces to conventional A and SLA surfaces. As oxidized and A implants revealed less epithelial downgrowth and longer connective tissue seal than M implants in a human histological study (Glauser et al. 2005), the potential clinical benefit of modA and modSLA surfaces has to be determined in further investigations. However, with respect to mechanical bacterial plaque biofilm removal, both types of surface modifications must be classified as less accessible when compared with M surfaces (Schwarz et al. 2006).

Within the limits of the present study, it was concluded that irrespective of the surface characteristics investigated, a frequent clinical probing at short intervals during the healing phase was associated with dimensional and structural changes of the mucosal seal.

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Clinical Relevance

Scientific rationale for the study: Clinical probing is an essential procedure for the diagnosis and monitoring of peri-implant diseases. However, the influence of a frequent disruption of the mucosal seal during the healing phase at different titanium implant surface modifications has not been investigated.

Principal findings: In comparison with conventional M and SLA surfaces, the transmucosal aspect of unprobed chemically modified hydrophilic SLA titanium implants (i.e. modA and modSLA) was characterized by an improved epithelial and connective tissue attachment. In all groups, however, a frequent clinical probing (i.e. 3 × and 4 ×) was associated with a disruption of the soft-tissue attachment and resulted in increased PM-aJE and aJE-CBI values over time.

Practical implications: Frequent clinical probing at short intervals (2–4 weeks) during the healing period should be avoided as it might be associated with dimensional and structural changes of the peri-implant mucosal seal formed at different titanium implant surfaces.