Periimplant Soft Tissue Barrier at Experimental One-Piece Mini-implants with Different Surface Topography in Humans: A Light-Microscopic Overview and Histometric Analysis

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ABSTRACT

Background: Following connection to the oral cavity, osseointegrated dental implants and surrounding tissues are exposed to microbiologic and biomechanical challenges. The establishment of a firm functional periimplant soft tissue barrier (PSTB) is considered to be important to protect the implant's interface from invasion of bacteria. The current knowledge on the histologic architecture of the PSTB is mainly based on animal experiments.

Purpose: The aim of this study was to histologically characterize the PSTB formed in humans around experimental one-piece mini-implants with different surface topography.

Materials and Methods: Five patients received a total of 12 experimental titanium, one-piece mini-implants with an oxidized ($n = 4$), an acid-etched ($n = 4$), or a machined ($n = 4$) surface distal to therapeutic implants. Following transmucosal healing of 8 weeks and at abutment connection of the regular implants, the mini-implants were harvested with a layer of surrounding hard and soft tissue. The specimens were fixed and processed for histologic sectioning according to standard procedures. The most central bucco-oral section cut in the long axis was used for morphologic analyses of the PSTB. The vertical soft tissue morphology was quantified using histometric measurements.

Results: The overall height of the soft tissue, that is, the biologic width, was around 4 to 4.5 mm and consisted of an epithelial and a supracrestal connective tissue barrier. The junctional epithelium established the attachment to the implant surface, whereas the collagen fibers and fibroblasts of the connective tissue seal were oriented parallel to the implant. The epithelial attachment was shorter at the oxidized and acid-etched surfaces compared with the machined surfaces. Accordingly, the oxidized and acid-etched mini-implants exhibited a longer zone of connective tissue seal.

Conclusion: The periimplant soft tissue formed at the experimental one-piece mini-implants in humans was of a character similar to that described in animal studies. The oxidized and acid-etched implants revealed less epithelial downgrowth and longer connective tissue seal than machined implants.

KEY WORDS: acid-etched surface, biologic width, histometric analysis, machined surface, mini-implant, one-piece implant, oxidized surface, soft tissue barrier

During clinical function, osseointegrated dental implants and surrounding tissues are exposed to microbiologic and biomechanical challenges, which may lead to unwanted clinical complications, such as marginal bone resorption and soft tissue recession. The establishment of a firm functional soft tissue barrier is considered to be important to protect the implant's interface from invasion of bacteria from the oral cavity. Moreover, the periimplant soft tissue's resistance to mechanical forces during chewing and toothbrushing may influence the long-term aesthetic appearance of the implant-supported restoration.

The height of the soft tissue along the implant/abutment surface, that is, the distance from the alveolar bone to the mucosal margin, is often referred to as the

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biologic width. Histologic evaluations in dogs have demonstrated that a healthy and functional junctional epithelium (JE) will attach to a biocompatible abutment surface similar to the attachment to a tooth. More apically, between the JE and the bone, a zone of connective tissue is interposed. The fibroblasts and the connective tissue fibers are parallel aligned to the implant components, which is in contrast to the situation at teeth, where the connective tissue fibers are inserted into the cementum layer of the root surface in a perpendicular manner.[1–3]

Less information is available about the influence of surface topography on the development and maintenance of the soft tissue barrier. It has been found that a very smooth surface with irregularities below 0.2 μm of the abutment is negative, as indicated by deepened pockets.[4] However, it is not known what the corresponding optimal roughness factor should be.

The current knowledge of the soft tissue barrier formed around implants is mainly based on animal experiments. However, the results of animal studies do not always correspond to the biologic behavior of soft tissue in the human environment. Therefore, a model was developed for evaluating the human soft tissue response to experimental one-piece mini-implants.

The aim of this study was to histologically characterize the periimplant soft tissue barrier formed in humans around experimental one-piece mini-implants with different surface topography.

MATERIALS AND METHODS

Patient Selection

This study was approved by the ethical committee of the University of Zürich. Patients scheduled for implant surgery in the posterior part of the mandible or the maxilla were consecutively included in the study provided that they fulfilled the following criteria:

- The patient's anatomy allowed for the placement and harvesting of one to two mini-implants distally to regular implants without interference to anatomic critical landmarks (mandibular canal, nervus lingualis, sinus maxillaris) or to the therapeutic implants.
- Prospective harvesting of the mini-implants was conducted at the time of second-stage surgery at the regular implants 8 weeks following implant placement.
- Prospective harvesting of the mini-implants, including a layer of surrounding bone, would leave a strict intrabony defect with no interference to critical anatomic landmarks or neighboring regular implants.
- Informed consent of participating in the study was signed by the patient.

Mini-implant

The experimental mini-implants were screw-shaped devices made of commercially pure titanium (diameter 2.3 mm; length 10 mm; Figure 1A). The implants had a threaded and slightly conical part (length 6 mm), to be inserted into bone, and a cylindrical abutment portion (length 4 mm), to be in contact with the soft tissue. Furthermore, the mini-implants exhibited either a machined surface (Figure 1B), an acid-etched surface (Figure 1C), or a surface with an oxidized and microporous TiO_2 layer (Figure 1D).

Surgical Procedures

Patients fulfilling the inclusion criteria received therapeutic implants in the posterior part of the mandible or maxilla according to standard surgical procedures. During the same surgical session, one to two mini-implants were placed distally to the regular implants (Figure 2A). A distance of 5 mm between the most distal regular implant and the mini-implant was always established to avoid any negative influence on the regular implant at the time of harvesting of the study implant. Bone quality was evaluated during the drilling sequence using the classification according to Lekholm and Zarb.[5]

The threaded part of the study implants was placed flush with the bone crest, leaving the 4 mm abutment portion exposed (see Figure 2A). Following implant placement, the margins of the buccal and lingual mucoperiosteal flaps opposing the study implants were contoured using soft tissue punches with the same diameter as the mini-implant to allow for a firm soft tissue adaptation (see Figure 2A). Thereafter, the flaps were repositioned and sutured to submerge the regular implants and to allow for transmucosal healing of the study implants (Figure 2B). Postoperative controls and maintenance were conducted on a weekly basis.

Following an undisturbed healing period of 8 weeks, the mini-implants were carefully harvested with a small layer of surrounding hard and soft tissue using

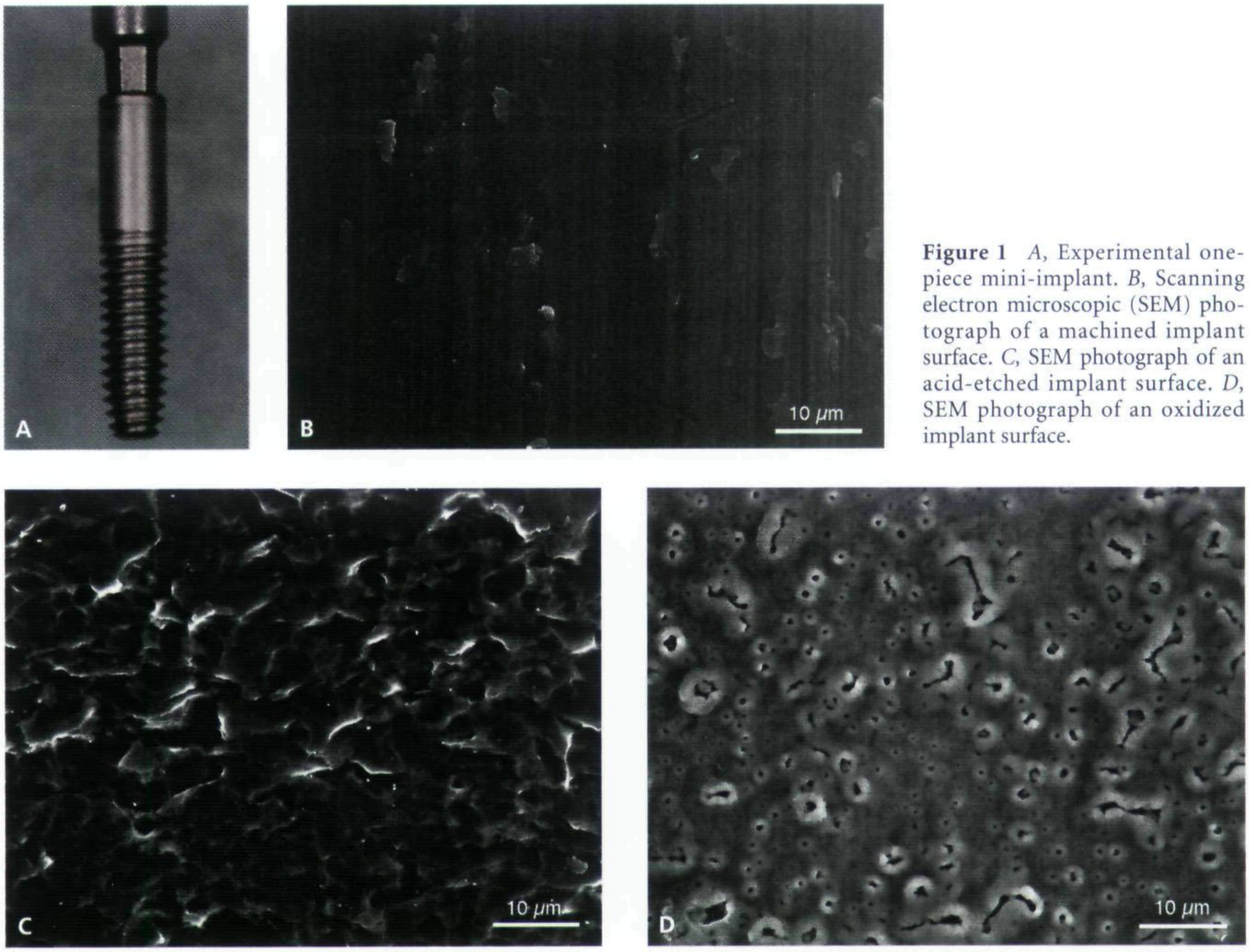

Figure 1 *A*, Experimental one-piece mini-implant. *B*, Scanning electron microscopic (SEM) photograph of a machined implant surface. *C*, SEM photograph of an acid-etched implant surface. *D*, SEM photograph of an oxidized implant surface.

soft tissue punches and trephine burs (Figure 3). Flaps were raised to allow for abutment connection procedures at regular implants. The flaps were extended distal to the area of tissue harvesting. The area was extensively rinsed with sterile saline solution and covered with a bioresorbable collagen membrane (Bio-Gide®, Geistlich AG, Wolhusen, Switzerland) to obtain complete healing of the resulting intrabony defect.

Specimen Preparation and Analysis

Immediately following harvesting, the biopsies were fixed in half-strength Karnovsky's solution at 4°C. Thereafter, the specimens were processed for histologic examination. Both nondemineralized ground sections, prepared according to the cutting and grinding technique described by Donath and Breuner,[6] and 1– to 2–μm-thick resin sections (Epon 812, Fluka, Buchs, Switzerland) of demineralized biopsies were produced. The most central section cut in the long axis and in a buccolingual direction was used for descriptive analyses and linear measurements of the periimplant soft tissue barrier (Figure 4A). The analyses were performed using either a stereomicroscope (Leica MZ16; Leica AG, Glattbrugg,

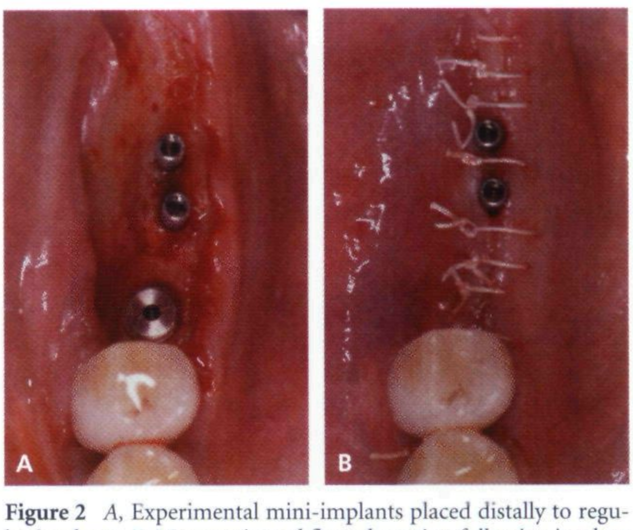

Figure 2 *A*, Experimental mini-implants placed distally to regular implants. *B*, Mucoperiosteal flap adaptation following implant insertion.

Figure 3 Experimental mini-implant harvested with a layer of hard and soft tissue.

Switzerland)) or a light microscope (Leica DM6000B) connected to a high-resolution video camera (Leica DFC 480) and interfaced to a monitor and personal computer (Dell XPS, Dell Inc., Round Rock, TX, USA). This optical system was associated with histometry software package with image-capture capabilities (ImageAccess, Imagic, Glattbrugg, Switzerland).

STATISTICAL ANALYSIS

Descriptive statistics (mean values and standard deviations) were used for evaluation of the data.

RESULTS

Five patients received a total of 12 experimental one-piece mini-implants exhibiting either an oxidized ($n = 4$), an acid-etched ($n = 4$), or a machined ($n = 4$) surface. The experimental implants were placed distal to regular implants.

Clinical Observations

All regular implants and all mini-implants healed uneventfully. Based on visual inspection, the periimplant soft tissues around the study implants were clinically free of inflammation. Harvesting of the study implants was successful and without adverse effects at all sites.

GROSS HISTOLOGIC OBSERVATIONS

The vertical dimension of the periimplant soft tissue barrier was composed at all study implants of an epithelial barrier with sulcular epithelium and JE and a supracrestal connective tissue barrier (see Figures 4 to 6). In general, the sulcular epithelium consisted of nonkeratinizing basal and suprabasal cells. The cells of the JE established the epithelial attachment to the implant surface. The width of the JE decreased with increasing distance from the sulcus. Furthermore, the orientation of the basal and suprabasal cells of the JE was more or less parallel to the implant surface. At rough surfaces, the apical end of the JE was clearly visible (Figure 7), whereas at machined surfaces, downgrowth of the JE toward the alveolar crest occurred (Figure 8). The implant surface between the most apical cells of the JE and the alveolar crest was in all ground sections in direct contact with the supracrestal connective tissue. In an approximately 100 to 150 μm–wide area adjacent to the implant surface, the connective tissue was, in general, free from blood vessels and was dominated by collagen fibers oriented parallel to the longitudinal axis of the implant (Figures 9 and 10). Adjacent to this area, the connective tissue was densely packed with collagen fibers oriented circumferentially around the implants (see Figure 10). Perpendicularly oriented collagen fibers, directly contacting the implant surface, were not observed in any of the sections.

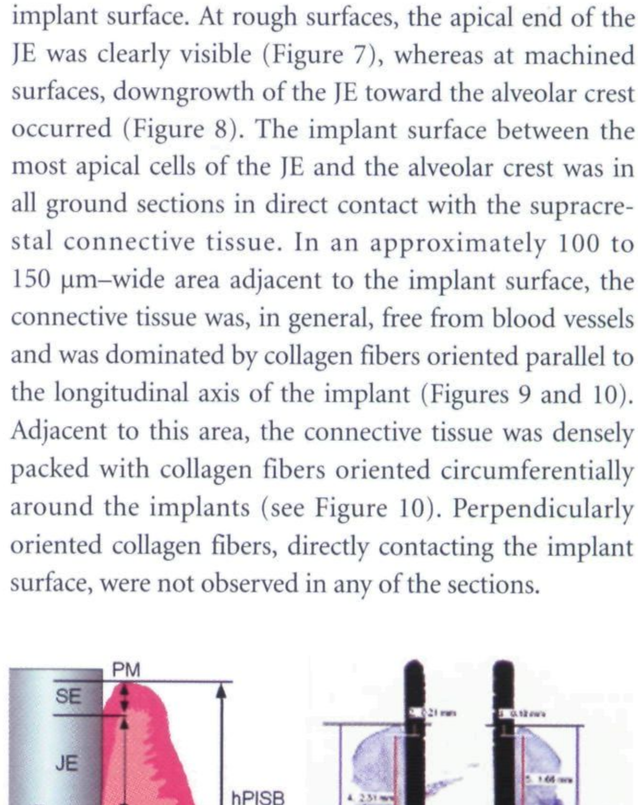

Figure 4 *A*, Schematic diagram illustrating the histometric measurements. aJE = apical termination of junctional epithelium; CT = supracrestal connective tissue; hPISB = height of the periimplant soft tissue barrier (ie, the biologic width); JE = junctional epithelium; PM = periimplant mucosal margin; SE = sulcular epithelium. *B*, Ground section with corresponding histometric measurements.

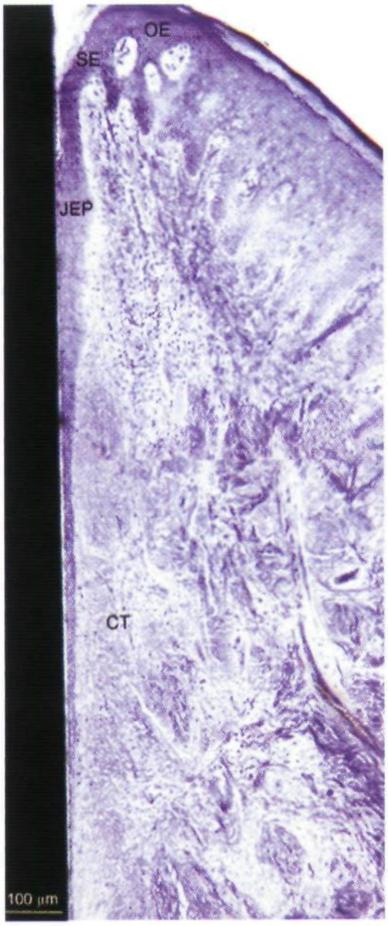

Figure 5 Coronal part of the periimplant soft tissue barrier. Note the oral (OE), sulcular (SE), and junctional epithelium (JE). CT = connective tissue.

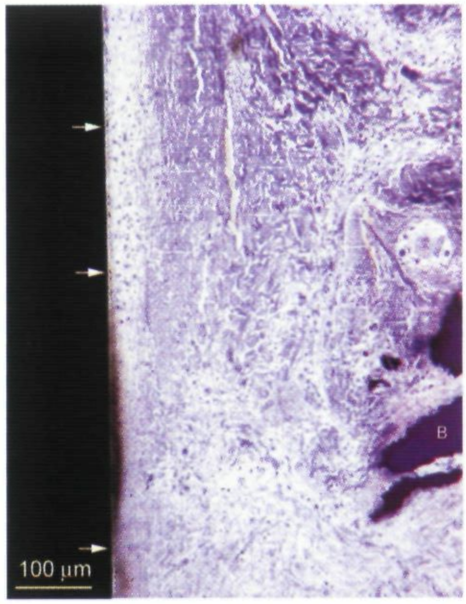

Figure 6 Apical part of the periimplant soft tissue barrier showing connective tissue contacting the implant surface (*arrows*).

Histometric Observations

The linear measurements are shown in Table 1. The overall height of the periimplant soft tissue barrier, that is, the biologic width, revealed a mean value of 4.0 mm for the oxidized surface, 4.5 mm for the acid-etched surface, and 4.1 mm for the machined surface. The epithelial barrier measured 1.8 mm for the oxidized surface, 1.9 mm for the acid-etched surface, and 3.4 mm for the machined surface. The corresponding height for the connective tissue seal amounted to 2.1, 2.6, and 0.6 mm, respectively.

DISCUSSION

The results of the present study revealed a slightly increased height of the periimplant soft tissue barrier, that is, the biologic width, compared with experimental data received from animal studies[1,3,7,8] and around natural teeth.[9] It is known from the dog model that the mucosa at mandibular postextraction sites, as used in the cited studies above, is of a very thin tissue type. The dimension of the biologic width established at implants placed in these experimental areas is consequently reduced compared with sites with thick tissue quality, as in the present human model.

The architecture of the soft tissue barrier documented in the present study on human biopsies is well in line with those found in animal studies.[1,3,7,10] The soft

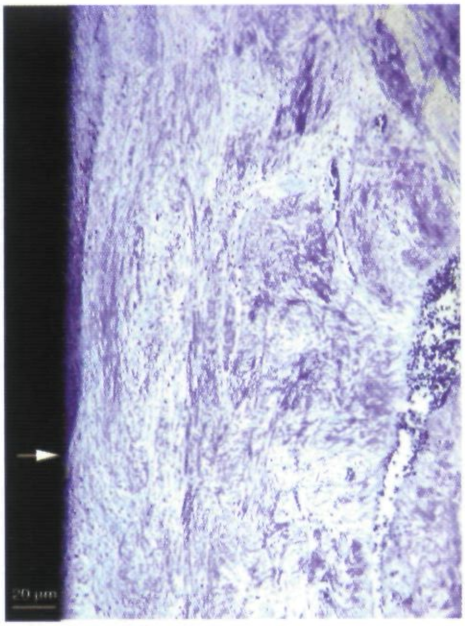

Figure 7 Apical end of the junctional epithelium (*arrow*) followed by the connective tissue compartment, as observed with rough surfaces.

Figure 8 Downgrowth of the junctional epithelium toward the alveolar bone crest, as observed with machined surfaces. aJEP = apical termination of the junctional epithelium; CT = connective tissue.

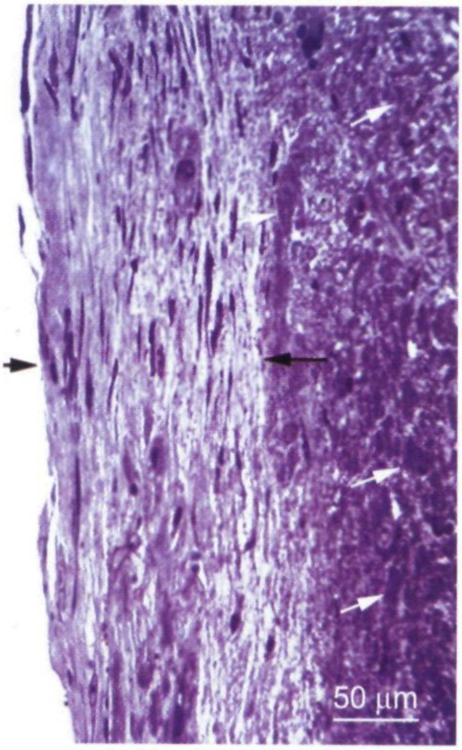

Figure 9 Avascular zone of the connective tissue adjacent to the implant surface (*between black arrows*) with an orientation of the collagen fibers parallel to the longitudinal axis of the implant. Note the cross-sections through circumferentially oriented collagen fiber bundles in the adjacent area (*white arrows*).

tissue healing at the mini-implants resulted in epithelial structures, including a periimplant sulcus and a JE, similar to those around natural teeth.[11] On the other hand, the connective tissue architecture observed close to the implant surface had no similarities with that around natural teeth because inserting perpendicular collagen fibers were not found. The absence of a real connective tissue attachment to the implant surface but a close connective tissue adaptation through a thin, avascular, and collagen fiber-rich, scar tissue–like layer has also been documented in several animal models.[1,12,13]

In the present study, the one-piece titanium mini-implants with an oxidized or an acid-etched surface

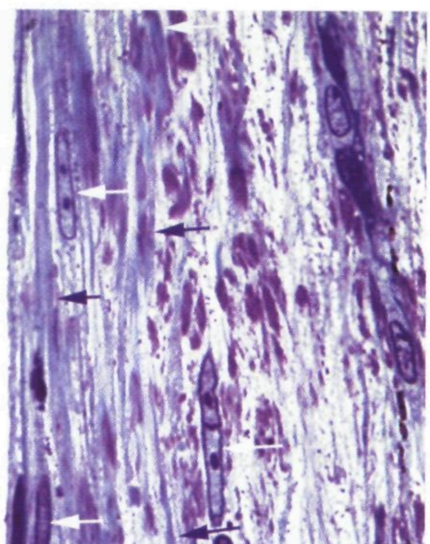

Figure 10 Higher magnification of the area adjacent to the implant surface. Note the orientation of the collagen fibers (*blue arrows*) and fibroblasts (*white arrows*) parallel to the longitudinal axis of the implant.

TABLE 1 Histometric Data of the Overall Height of the Periimplant Soft Tissue Barrier and the Corresponding Compartments

Surface	PSTB Height, mm	Sulcus Length, mm	JE Length, mm	CT Length, mm
Machined	4.1 ± 1.3	0.5 ± 0.1	2.9 ± 0.7	0.7 ± 0.2
Acid-etched	4.5 ± 0.5	0.5 ± 0.2	1.4 ± 0.6	2.6 ± 0.6
Oxidized	4.0 ± 0.8	0.2 ± 0.1	1.6 ± 0.3	2.2 ± 0.4

CT = connective tissue; JE = junctional epithelium; PSTB = periimplant soft tissue barrier.
Data are mean values ± SD.

revealed a lower JE height but a longer connective tissue seal compared with machined-surfaced mini-implants following a non-loaded, transmucosal healing of 8 weeks. The reason for this could just be speculated on. Although there is substantial evidence from in vivo studies that an increasing surface roughness of the intrabony part of implants results in increased resistance to compressive, tensile, and shear forces in bone[14–18] and increased bone-to-implant contact,[15,16,18] there is an obvious lack of knowledge with regard to a surface roughness effect on the initial establishment of the periimplant soft tissue barrier. The machined mini-implants used in the current study exhibited only minor surface irregularities, whereas the oxidized and acid-etched mini-implants showed pronounced surface irregularities. A possible hypothesis for explaining the reduced height in the JE at roughened-surface implants may be that a rough surface has a certain "conductive" effect on the connective tissue adhesion during healing, thereby inhibiting epithelial downgrowth. On the other hand, a smooth surface may allow for pronounced epithelial downgrowth compared with rougher surfaces. This effect has been described when comparing three different surfaces (sandblasted, fine sandblasted, polished) at the transmucosal level. A tendency for less epithelial downgrowth and more coronally ending connective tissue adaptation was found for rough surfaces when compared with polished and fine sandblasted implants.[12] In contrast, Abrahamsson and colleagues did not find any such differences when comparing soft tissue healing with acid-etched and machined abutments.[13]

In several studies conducted in the canine mandible using one- and two-piece implants, it has been stated that the apical migration of the JE was influenced by (1) the presence of an abutment/fixture junction (ie, "microgap") and its vertical positioning and/or (2) the presence of a transition zone between rough and smooth surfaces at the implant neck and its vertical position, that is, coronal to, apical to, or at the level of the bone crest.[10,19,20] Epithelial downgrowth has also been documented as a reaction toward multiple abutment shifts.[21] However, in the present study using one-piece implants with a transmucosal healing mode, none of these abovementioned effects can be responsible for differences in epithelial downgrowth.

The present study demonstrated that the use of mini-implants in adequate selected cases is a predictable, safe, and accurate technique for harvesting human periimplant tissues. Nevertheless, because of practical and ethical reasons, human biopsies are generally more subjected to differences in parameters influencing the tissue response, such as variations in local mucosa conditions, healing time, the age of the patient, smoking habits, and others. As a consequence, variations in tissue response between different human biopsies, as expressed by standard deviations, might be larger compared with more standardized conditions in animal experiments. Therefore, interpretations with regard to tissue response on only a small number of human biopsies should be made carefully. However, human histologic data are valuable to validate and confirm animal models.

CONCLUSIONS

The periimplant soft tissue formed at the experimental one-piece mini-implants in humans was of a character similar to that described in animal studies. The oxidized and acid-etched implants revealed less epithelial downgrowth and longer connective tissue seal than the machined implants.

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