Nanoporous TiO_2 Thin Film on Titanium Oral Implants for Enhanced Human Soft Tissue Adhesion: A Light and Electron Microscopy Study

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ABSTRACT

Background: Previous experimental studies have demonstrated direct soft tissue attachment for nanoporous titanium dioxide (TiO_2) thin film on implants, while implants without TiO_2 thin film have not shown this capability.

Purpose: The aims were to evaluate and compare TiO_2 surface-modified experimental microimplants with unmodified microimplants with respect to tissue interaction of the human oral mucosa evaluated by light microscopy on ground sections and semithin sections and transmission electron microscopy on ultrathin sections, and to characterize the inflammatory response and the level of the marginal bone resorption.

Materials and Methods: The study was a single-center, randomized, comparative, clinical investigation with intrasubject comparison of implants with and without TiO_2 thin film in 15 patients.

Results: Two comparator microimplants showed mild erythema and expulsion of fluids. The surrounding tissues around all test implants were clinically healthy. The oral mucosa in contact with the abutment part of the microimplant was 72% for the test implants and 48% for the comparator implants, a statistically significant difference ($p = .0268$). No statistically significant difference was found in other histological variables. The marginal bone loss in 14 weeks was 0.5 mm for the stable test ($n = 11$) and 1.7 mm for the stable comparator implants ($n = 9$; $p = .0248$).

Conclusions: The nanoporous TiO_2 surface modification has potential clinical benefits because of increased adherence of soft tissue and possible reduced bone resorption.

KEY WORDS: bone resorption, inflammation, nanoporous TiO_2, oral implant, soft tissue attachment, surface modification, thin film

INTRODUCTION

Titanium, a biocompatible material, has been used in several innovative applications in implant therapy. The majority of research has been directed to find optimal solutions with respect to design and surface modifications to enhance implant incorporation in bone for dental, craniofacial, and orthopedic applications.[1–6]

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In the dental and craniofacial area, a proper soft tissue reaction may also be important for good and predictable clinical results.[7–10] The lack of direct adherence of soft tissues to the implant surface may, at least theoretically, be a problem because it presents an opportunity for microbes to enter the tissues and thereby cause peri-implant inflammation, infection, and bone resorption that may lead to implant failure.[11,12]

Nanoporous titanium dioxide (TiO_2) surface modification alters the pore structure, nanoscale topography and chemistry, as demonstrated by the works of Jokinen and colleagues[13] and Peltola and colleagues,[14] and may thereby change the reactivity of the implant surface. Studies in rats have shown decreased inflammatory reaction and negligible or decreased fibrous connective tissue capsule formation in subcutaneous tissues without signs of TiO_2 thin film resorption or adverse tissue reactions.[15] After 3 days of implantation in rats, direct attachment between the soft tissue components and the sol-gel derived TiO_2 was observed already, while the titanium control implants showed no evidence of soft tissue attachment.[15,16] Furthermore, in a study performed in dogs, TiO_2-modified transmucosal dental implants showed good epithelial attachment, decreased gingival inflammatory reaction, and less marginal bone resorption than the unmodified control titanium implants.[17]

Although several reports indicate an advantage of the TiO_2 thin film for soft tissue attachment, so far, no studies have been published where the TiO_2 thin film has been investigated in the human mucosa to verify its potential clinical advantages. Furthermore, no studies have investigated the soft tissue reactions in three different levels of resolutions. The aim of the present investigation was to evaluate and compare nanoporous TiO_2 surface-modified implants with unmodified implants with respect to interaction of the human oral mucosa and the implant surface, as evaluated with light microscopy on ground sections, on semithin and ultrathin sections for transmission electron microscopy (TEM). Another aims were to characterize the inflammatory response toward the surface modification in human oral soft tissue and to investigate if the test surface may decrease the level of the marginal bone resorption.

MATERIALS AND METHODS

Implants and Surface Modification

A total of 30 experimental implants were used in this study. The implants were made of cp Ti grade 4 and machined with a turning process. The experimental microimplants consisted of an oral mucosa-penetrating part (abutment part) and a threaded part intended for stabilization during bone incorporation (Figure 1). Two different lengths of the experimental microimplants were available according to the gingival thickness at the site of implantation: 10 and 13 mm long, with the oral mucosa-penetrating investigational part 3.4 and 6.4 mm long, respectively. The diameter of all microimplants was 2.2 mm. The surface of the oral mucosa-penetrating part of the microimplant was either a nanoporous TiO_2 thin film (15 test implants) or an unmodified turned surface (15 comparator implants). The surface roughness was measured with an interferometer (MicroXam, PhaseShift, AZ, USA). Nine three-dimensional measurements were performed on the cylindrical part of the two surfaces, respectively. In addition, nine measurements were performed on the threaded part to investigate the roughness relevant for bone incorporation. The measuring area for all measurements was 200×200 μm. A Gaussian filter was applied to remove waviness and errors of form. Four different parameters were used to characterize the surface: S_a (μm) = average height deviation; S_{sk} = skewness of the height frequency distribution; S_{ds} ($1/mm^2$) = density of summits, a spatial parameter; and S_{dr} (%) = surface enlargement, a hybrid parameter including variation in height and spatial direction. The test surface was manufactured by using the sol-gel

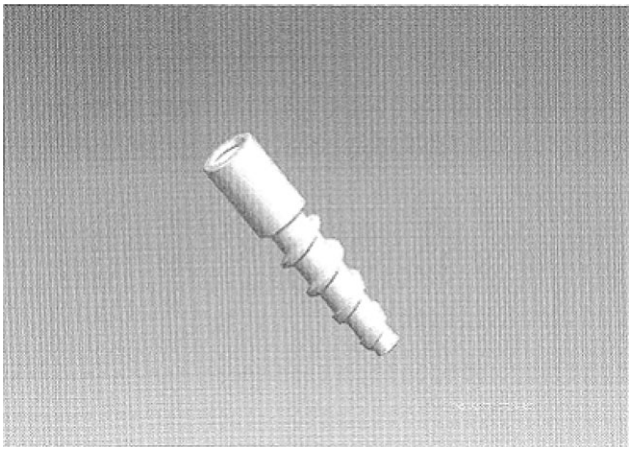

Figure 1 Experimental microimplant (length: 10 mm; diameter: 2.2 mm).

technique (MetAlive®, Vivoxid Ltd, Turku, Finland). Dipping into the sol-gel, heating, and washing were repeated five times to obtain five subsequent layers. This surface modification has been demonstrated to result in a final thickness of the pure TiO_2 surface of about 380 nm (five layers), a porosity of 21.0%, a mean nanoroughness, S_a, of 0.88 nm, and the crystalline phase mainly anatase.[13] The outermost surface has been demonstrated to contain "surface pores" between 15 and 50 nm.[14]

All experimental microimplants used in the investigation were packed in 2-mL Greiner polypropylene vials™ (Greiner Bio-One GmbH, Frickenhausen, Germany) and steam sterilized.

Investigation Design and Patient Selection

The study was designed as a single-center, randomized, controlled clinical investigation with intrasubject comparison of two different surfaces. Participants in the study were sought among subjects coming to the clinic for a standard dental implant treatment. The investigation was conducted in accordance with the ethical principles in the Declaration of Helsinki and with applicable regulatory requirements including standards of the International Organization for Standardization (ISO) ISO 14155-1 (general requirements for clinical investigations of medical devices), ISO 14155-2 (clinical investigation plans), and in adherence to Danish law and regulations (e.g., Act on Medical Devices 1046; Danish Medicines Act 1180; Act on Product Safety 364).

Prior to the initiation of the investigation, the local ethics committee (De Videnskabetiske Komitéer for Københavns og Frederiksberg kommuner, KF1 og KF2) reviewed and approved the investigational plan.

Number of Subjects Needed (Power Analysis)

No previous clinical data in humans using the new, modified surface on an experimental device were available. In preclinical data, the SD of the distance to marginal gingiva (from the reference point) and of the distance to marginal alveolar bone crest (from the reference point) was from 0.4 to 0.5 mm when a study was conducted with 6 beagles and with 16 implants.[17] If similar variation in humans in the target variables was assumed and if a decrease of 20% in SD as a result of intrasubject comparison was taken into account, the half length of the 95% CI of mean difference with 15 subjects would have been approximately 0.2 mm, which was considered as sufficient precision for estimation purposes in the exploratory analysis. This calculation was the reason for including 15 subjects in the study. They were consecutively enrolled in the study as they passed the inclusion and exclusion criteria.

The following were inclusion criteria:

1. Written informed consent
2. Age of 18 years or older
3. Eligible for having the regular dental implant system
4. Judged to have a bone quality that enabled the removing of the experimental microimplants within 12 to 16 weeks (criterion was clinically verified and documented by the investigator at the beginning of the implantation procedure)

The following were the exclusion criteria:

1. Concurrent disease or condition that, in the opinion of the investigator, was a contraindication for participation
2. Simultaneous participation in another medical device or investigational drug trial
3. The subject being pregnant or breast-feeding
4. The subject having an implanted stent and/or a heart valve

Eight men and seven women passed the inclusion criteria. The mean age was 55.7 years (max: 67 years; min: 40 years). Two patients smoked 10 to 20 cigarettes per day, one patient smoked 4 cigars each day, and one patient smoked pipe, approximately 10-g tobacco per day. Most of the patients were partially dentate. One patient had mild hypertension, and another had allergy, glaucoma, and hypertension, while the remaining 13 patients were considered healthy.

For the discomfort and inconvenience to the subjects to be minimized, the study operations (implantation and removal) were scheduled to the same visits as the regular dental treatment was carried out. The 15 patients were recruited during a 2-month period.

Randomization

A sealed randomization envelope was prepared for each subject. The envelope was opened only after the subject number was allocated to the subject.

At the implantation visit after the bone quality was assessed, the eligible subjects were consecutively given subject numbers. The subject number assigned the test

implant to be implanted either mesially or distally (in case of unilateral implantation) or, on the left or right (in case of bilateral implantation), according to a computer-generated randomization sequence using random permuted blocks. The used software was SAS PROC PLAN (seed number 2699; SAS Inc., Cary, NC, USA).

Implant Operations

Each subject received two microimplants penetrating the oral mucosa: one test and one comparator implant.

With this intrasubject comparison, each subject acted as his or her own control. At the time of operation of the regular dental implants, the two experimental microimplants were placed either in upper (nine patients) or in lower jaw (six patients) by using a flapless surgical technique. Microimplants were placed in each subject in anatomically similar locations in order to ensure comparable soft tissue anatomy for the intrasubject comparison. The majority of the implants were positioned in posterior location: 12 test and 10 comparator implants were placed in the molar region, 2 comparator implants were placed in the premolar region, and 3 implants from both groups were inserted in canine/incisive region. Operations were carried out by using good aseptic surgical praxis as in the regular dental implant surgery. Local anesthesia for the placement of regular dental implants was provided and supplemented if considered necessary. A 2-mm twist drill with low rotary speed and sterile saline were used to prepare the implantation sites for experimental implants. Microimplants were placed, and primary stability was clinically estimated and recorded (stability defined as no relative movement between implant and bone).

Routine X-Ray Imaging

As a part of the routine clinical procedure, radiographs were taken at the time of the implantation and at the time of the second-stage surgery (i.e., at the microimplant removal). The marginal bone level was determined from the routine radiographs by the investigator by measuring the distance from the top of the microimplant to the level of marginal alveolar bone crest, allowing for the evaluation of bone-level changes between the time of insertion and the time of removal of samples, within each implant. Measurements were carried out directly from digital radiographs by using computerized calibrations.

Sample Retrieval

Fourteen weeks (±2 weeks) after implantation, at the same visit as the prosthetic treatment with regular dental implants was commenced, the two experimental microimplants were removed after the clinical investigation.

Clinical signs and symptoms of inflammation or infection at the site of the implantation were subjectively evaluated by the investigator by using the following scale:

1. Erythema (0: none, 1: mild, 2: moderate, 3: severe)
2. Expulsion of tissue fluids at light pressure, including pus (yes/no)
3. Stability of the experimental microimplant (stable; yes/no)
4. Tenderness at the site of experimental implantation (yes/no)

Local anesthesia for the regular dental implant treatment was provided and supplemented if considered necessary. Before retrieval, a mark was made with a bur on the buccal (facial) side of each microimplant. In case the microimplant was mobile or had gingiva overgrown over its top, no cut could be made (Figure 2).

For most of the subjects, a 5-mm trephine drill with sterile irrigation or a very small bone chisel was used to make a cut even around the microimplant through the mucosa and 2 to 3 mm into the bone (Figure 3). In a few cases, a biopsy punch (diameter: 5 mm) was used to make the cut. During the removal, care was taken to protect the microimplant-gingiva interface from any rupturing forces. The same procedure was performed to remove both microimplants from each subject.

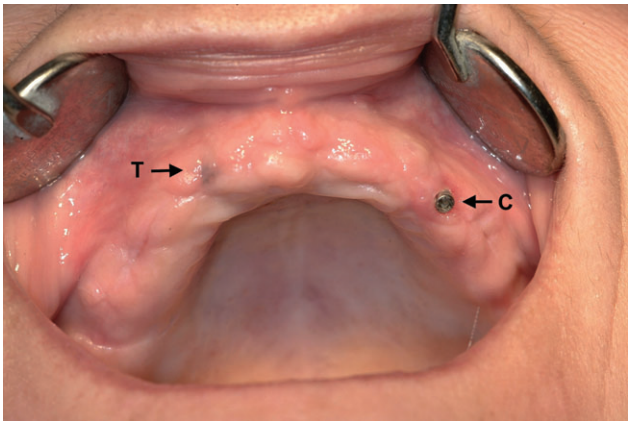

Figure 2 Photograph of the clinical situation immediately before implant retrieval. The comparator implant (C) is visible, but the test implant (T) is overgrown by soft tissue.

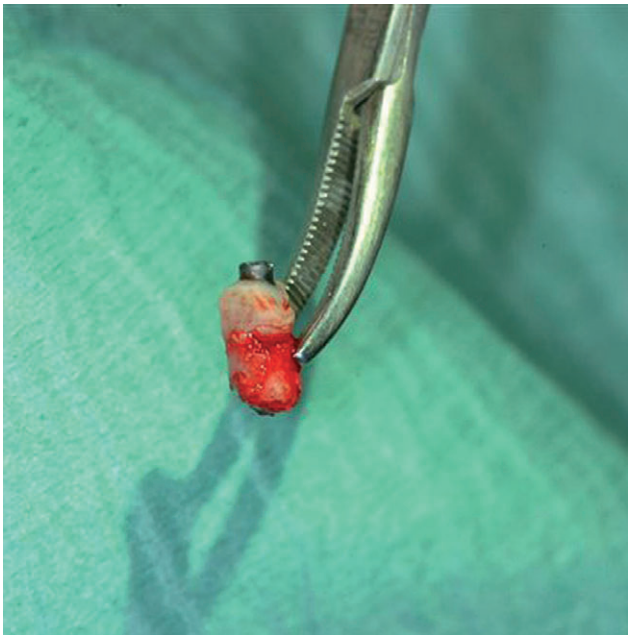

Figure 3 Photograph of an implant with surrounding tissue immediately after retrieval.

Immediately after the retrieval, the microimplants, together with the surrounding tissues, were put into test tubes containing 10 mL modified Karnovsky fixation (2% paraformaldehyde, 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer), placed in a refrigerator, and sent to the laboratory within 72 hours.

Two weeks after the microimplants were removed, a phone call was made to all patients to check and record if any adverse events, concomitant medication, or concomitant treatment had occurred.

Preparation of Sections for Histological Analyses

At the laboratory, the samples were dehydrated in increasing ethanol concentrations and subsequently infiltrated and polymerized in heat-curing resin (Agar 100 Resin, Agar Scientific Ltd., Stansted, Essex, England). Resulting blocks with the microimplant and the tissues were sawed in half along the long axis of the microimplant in bucco-palatinal direction (if no buccal mark was identified, the laboratory would select an area rich in tissue).

Light Microscopy Analysis of Ground Sections

One-half of each block was prepared for ground sectioning by using a cut and ground machine (Exakt Apparatebau, Hamburg, Germany), according to the technique described by Donath,[18] resulting in sections with a thickness of about 20 μm for light microscopic (LM) evaluations.

Prior to staining, all sections were pretreated for 10 minutes in 25% H_2O_2 during constant stirring and subsequently rinsed in tap water. All sections were then stained with toluidine blue.

The staining solution was prepared to a concentration of 1% toluidine blue dissolved in 1% borax, mixed in 4:1 proportion with 1% Pyronin-G.

In addition, for those samples with enough material left, a second section was prepared and stained with Richardson, 1% methylene blue dissolved in 1% borax mixed in proportion 1:1 with 1% Azur II. The slides from both staining methods were then let to air dry and subsequent cover slipped with Pertex mounting media.

A quantitative LM histological analysis provided an evaluation of the degree of soft tissue to metal contact as percentage of the distance along the entire abutment part of the microimplant. In addition, the depth of crevice or sulcus of the marginal gingiva, the area of the sulcus, the height of marginal gingiva,the total thickness of gingiva, and the length of the abutment part were measured. The histology measurements used in the data analyses are illustrated in Figure 4. Histological measurements and analysis were performed by an experienced personnel blinded to the protocol.

Light Microscopy Analysis of Semithin Sections

The other half of the block was prepared by using an electrolytical dissolution technique[19] whereby the bulk part of the metal was removed. After the electrolytical dissolution, the sample was cut horizontally to remove the bone. The sample was then reembedded, and semithin sections (about 1.5 μm) were prepared (staining with Richardson stain) (Figure 5). Total number of the inflammatory cells (lymphocytes, plasma cells, macrophages, and polymorphonuclear cells) and the number of fibroblasts in the region of interest (ROI) were calculated in LM by placing a grid over the histological sample and counting cells from three different areas inside a ROI, each site 100×150 μm, and by using the mean of the three values. The ROI was chosen from the tissue-implant contact area, in the abutment part of the microimplant. One ROI per sample side was chosen. Furthermore, a qualitative LM histological analysis provided an evaluation of inflammation, tissue

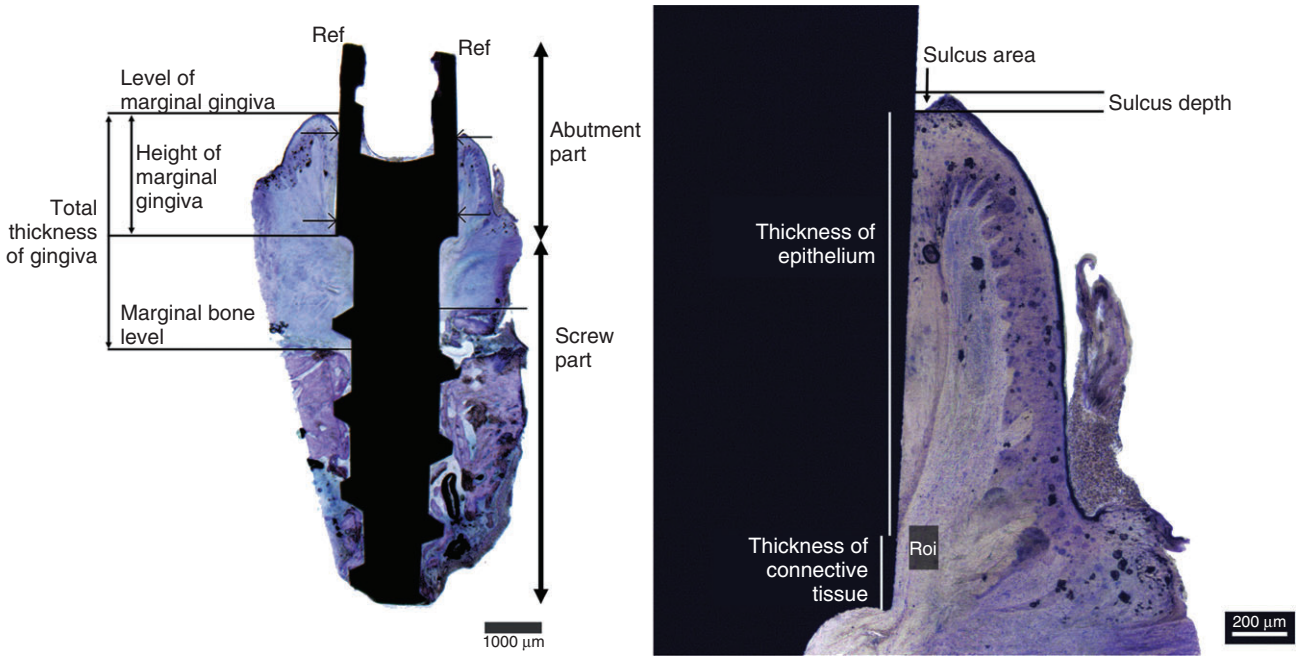

Figure 4 Microphotographs of a cut and ground section illustrating the histological measurements used for data analyses.

repair, and adherence of the oral mucosa to the surface of the device.

TEM Analysis

The ultrastructure of cells at the tissue-material interface was studied in TEM. The semithin sections (i.e., without the metal and the bone) were subjected to ultramicrotomy, providing ultrathin, i.e., 50-nm, sections for TEM of the metal oxide and associated tissues. All specimens were taken from the lower ⅓ of the abutment part of the implants, as judged from the semithin sections. Specimens were then further trimmed for ultrathin sectioning.

TEM was carried out for four test and five control samples. The following was evaluated: the cell membrane contact to material, the focal adhesion contact points, the presence of collagen attachment, and the interaction of inflammatory cells with the material surface or material fragments.

Statistical Analysis

All statistical analyses, including descriptive tables and listings, were produced by using SAS/STAT® software version 9.1 of the SAS System for Windows. No imputation procedures were applied on missing data. No interim analyses were performed.

Although no hypothesis was stated in the protocol, the significance level for statistical analyses was set to .05. In addition to 95% Cis, a paired *t*-test was applied to the primary and to the secondary endpoints. If assumptions of paired *t*-test were violated, then Wilcoxon signed rank test was used.

From routine radiographs, the difference in the distance from reference point (top of the microimplant) to marginal bone level at the time of implantation to the time of removal was calculated within subject and device. This difference was used as analysis variable when comparing devices. Additional analyses for x-ray data included Mann-Whitney *U* test to compare stable test devices to stable comparator devices.

Clinical signs and symptoms of inflammation or infection at the site of the implantation were tabulated by devices. Descriptive LM and TEM were listed by subject and discussed.

RESULTS

Surface Topography

The nanoporous TiO_2 test surface demonstrated a slightly smoother surface in terms of height deviation than the unmodified control. However, in the surface modified implants, the number of summits was larger, and this resulted in a greater surface area than in the control surface. The somewhat smaller height deviation in the test implants may be explained by the thin film filling some of the pits, thus reducing the average height

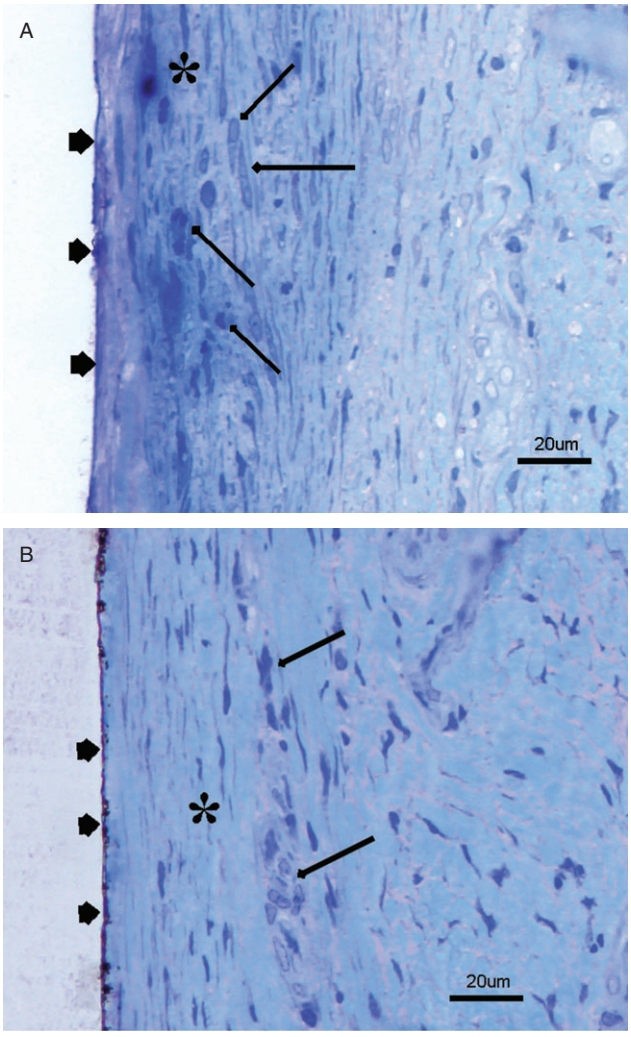

Figure 5 Light microscopy of semithin, resin-embedded specimen prepared by electrolytical dissolution of bulk titanium. The micrographs show part of a ROI from the tissue-implant contact area, at the lower one-third of the abutment part of the microimplants. *A,* Control implant. The implant surface is detected by the dense line (*arrowheads*) depicting the surface oxide remaining after the electrolytical dissolution. The tissue consists of a dense fibrous capsule (*) with elongated fibroblasts (some of which are depicted by *large arrows*) in parallel with the implant surface. The fibrous tissue consists also of a few scattered inflammatory cells, mainly macrophages (some of which are marked by *small arrows*). *B,* Test implant. A rather thick, dense line (*arrowheads*) depicts the remaining surface layer (titanium oxide) remaining after electrolytical dissolution. The remaining layer appears thicker than that detected for the control implants [see (A)]. A dense collagenous tissue (*) with slender fibroblasts is found close to the implant surface. Small aggregates of inflammatory cells are detected (some macrophages are indicated by *arrows*). This portion of the tissue is largely devoid of blood vessels [similar as for (A)].

deviation. This hypothesis is supported by the parameter S_{sk} that demonstrates fewer peaks than in the control surface. The threaded part was rougher than the cylindrical part, evidenced by parameters S_a, S_{ds}, and S_{dr}, but much smoother than standard turned implants[3] (Table 1).

Clinical Investigation

At the time of implant installation, five test implants had a compromised primary stability because of soft bone (four implants) or very soft bone (one implant). Four control implants were judged to have compromised primary stability because of soft bone (three implants) or very soft bone (one implant).

At the time of implant retrieval, neither erythema, expulsion of fluids, nor tenderness was reported for the test implants, while two controls demonstrated mild erythema and expulsion of fluids, and one of the two also demonstrated tenderness. The overall impression at implant retrieval was that the test implants demonstrated healthier and firmer attachment to soft tissue than the comparators. One-third of the microimplants, four test and six comparator implants, were assessed as clinically unstable at the time of removal. A total of eight subjects had either one or both microimplants unstable.

Marginal Bone Level from Routine X-Ray Images

Marginal bone level was determined from radiographs by measuring the distance from the top of the microimplant to the bone level. The intrasubject comparison of all stable and unstable implants revealed no statistically significant difference between the test and comparator implant in the change of the bone level from week 0 to week 14 (Table 2). When only stable implants were included in the intersubject statistical analysis, the median marginal bone loss was 0.5 mm for the tests and 1.7 mm for the controls, showing a statistically significant difference ($p = .025$).

Histological Investigation

Light Microscopy

Oral Mucosa in Contact with the Abutment Part of the Microimplant. The mean oral mucosa (epithelium and connective tissue) in contact with the abutment part of the microimplant was 72% (range: 27–97) for the test implants and 48% (range: 0–88) for the comparator implants (Table 3). The difference between the two implant surfaces was statistically significant ($p = .027$).

When epithelium and connective tissue was looked at separately, no statistically significant difference was

TABLE 1 Surface Roughness Measured with Interferometry

	S_a µm	S_{sk}	S_{ds} 1/mm^2	S_{dr} %
Test/Cylindrical part	0.156 (0.01)	0.583 (0.5)	124,769 (3,808)	3.606 (0.57)
Control/Cylindrical part	0.165 (0.01)	0.924 (0.41)	116,091 (8,567)	2.86 (0.5)
Threaded part (test and control)	0.212 (0.02)	0.984 (0.343)	156,774 (10,948)	8.603 (2.06)

The values are the mean of nine measurements for each surface, SD within parentheses.
S_a = average height deviation; S_{sk} = skewness of height distribution, negative value indicates more valleys than peaks; S_{ds} = density of summits; S_{dr} = surface enlargement compared with a totally flat reference area.

observed. The mean oral epithelium in contact with the abutment part of the microimplant was 63% (range: 27–95) for the test implants and 34% (range: 0–60) for the comparator implants.

The mean oral subepithelial connective tissue in contact with the abutment part of the microimplant was 79% (range: 42–100) for the test implants and 64% (range: 22–92) for the comparator implants.

TABLE 2 Bone Level (X-Ray); Change from Week 0 to Week 14 by Device Group

	Test *N* = 15	Comparator *N* = 15	Difference between Intrasubject Test and Comparator* *N* = 15
N	**15**	**15**	**15**
Mean	0.69	1.51	−0.83
STD	1.01	2.65	2.94
Min	−0.5	−2.0	−10.3
Median	0.5	1.4	−0.3
Max	3.5	9.8	2.8

*Calculation of intrasubject difference: test minus comparator.
Negative values = raised bone level. Positive values = reduced bone level.
N = number of samples.

TABLE 3 Percent of Oral Mucosa in Contact with the Abutment Part of the Microimplant

	Test *N* = 15	Comparator *N* = 15	Difference between Intrasubject Test and Comparator* *N* = 15
N	**13**	**12**	**10**
Mean	71.87	48.04	24.17
STD	20.03	22.56	28.93
Min	27.2	0	−24.2
Median	79.1	49.8	14.6
Max	97.4	87.8	83.9

*Calculation of intrasubject difference: test minus comparator.
N = number of samples.

Area of Sulcus of Marginal Gingiva. The median area of the sulcus next to the abutment part of the microimplant was 0.04 mm^2 (range: 0.004–0.2 mm^2) in the test group ($n = 9$) and 0.07 mm^2 (range: 0.005–0.4 mm^2) in the control group ($n = 7$). There was no statistically significant difference between the test and the comparator microimplant ($n = 5$).

Other Histology Measurements. The depth of crevice or sulcus of the marginal gingiva, the height of the marginal gingival, and the total thickness of the gingiva did not differ between test and control implants.

Semithin Sections

Number of Inflammatory Cells and Fibroblasts. There was no statistically significant difference in the mean number of the inflammatory cells or the number of fibroblasts between the test and the comparator microimplants. Summaries of the inflammatory cells and fibroblasts are presented in Tables 4 and 5, respectively.

Qualitative Histological Description. A descriptive histological analysis of the semithin sections was performed

TABLE 4 Number of Inflammatory Cells in the Vicinity of the Implant Surface (cells/mm^2)

	Test *N* = 15	Comparator *N* = 15	Difference between Intrasubject Test and Comparator* *N* = 15
N	**11**	**10**	**7**
Mean	1,081	1,573	−409
STD	816	1,010	1,041
Min	233	443	−1,909
Median	880	1,335	−472
Max	2,790	3,340	1,145

*Calculation of intrasubject difference: test minus comparator.
N = number of samples.

TABLE 5 Number of Fibroblasts in the Vicinity of the Implant Surface (cells/mm²)

	Test $N = 15$	Comparator $N = 15$	Difference between Intrasubject Test and Comparator* $N = 15$
N	**11**	**10**	**7**
Mean	2,173	1,746	257
STD	670	640	958
Min	1,310	977	−1,210
Median	2,195	1,783	333
Max	3,254	2,665	1,590

*Calculation of intrasubject difference: test minus comparator. *N* = number of samples.

by using light microscopy. The quality of the sections allowed a detailed qualitative and quantitative analysis (see Figure 5). Nevertheless, some sections revealed either a lack of tissues or signs of trauma (such as fresh microfractures in bone and/or bleeding), which were most likely because of trauma during retrieval. The qualitative histological examination of the specimens could not reveal any differences between the test and the control specimens. A general observation was that the experimental dental microimplants were not well osseointegrated because contact between bone and implant was rarely noted. In contrast, close to implant threads, separate bone fragments undergoing resorption were often observed.

Oral epithelium showed some downgrowth in both test and control groups reaching the implant threads in two test implants and in 7seven comparator implants. A general and consistent finding was the observation that implant threads and part of the transgingival abutment part were surrounded by capsulelike, dense, fibrous connective tissue with elongated fibroblasts and parallel fiber orientation to the implant surface.

The degree of inflammatory reaction was low, both subepithelially and close to the transgingival part of the implant, as judged by semiquantitative assessment of the tissue around both test and control implants. Typically, chronic subepithelial inflammation with lymphocytes, plasma cells, and some macrophages and mast cells was observed. Polymorphonuclear leukocytes were rarely observed. In some of the cases, bacteria were detected in association with the part of the abutment that was exposed to the oral cavity. In four cases, two test and two comparator implants, metal fragments were observed in soft tissues.

TEM. In all specimens, both test and comparator, a remaining layer of probable Ti-oxide was recorded. From the specimens with the test surface, the thickness of this layer varied between 50 and 400 nm, whereas in the comparator specimens the variation was somewhat bigger (50 nm to 1 µm) (Figure 6).

Closest to this oxide layer, i.e., the interface, a layer of proteinacous material with a relatively dense appearance was seen. This layer varied in thickness from approximately 50 to 100 nm in most cases, but, in some cases, this layer even exceeded 5 µm (see Figure 6A).

At some instances, cells were seen close to the surface but without cellular attachment, pseudopodia, directly to the oxide layer. Some of these cells were of epithelial origin, and desmosomes were frequently seen in these sections. Also, fibroblasts close to the surface were seen and were showing signs of activation, i.e., an expanded rough endoplasmic reticulum and mostly euchromatin in the nuclei (see Figure 6, B–E).

In all sections, bundles of collagen fibers could be seen in all possible directions. In a few sections, the fibers were relatively close to the surface (see Figure 6F), but the most common finding was at a 1- to 5-µm distance from the surface.

A few inflammatory cells were seen in parts of the specimens, but at no instances were these cells recorded as active.

The ultrastructural examination of the specimens did not reveal any differences between the test and the control specimens.

DISCUSSION

The overall results of this clinical study gave a good indication that the nanoporous TiO_2 surface modification MetAlive may bring clinical benefits to the patients in terms of improved healing and reduced bone resorption after implantation. Although previous experimental research[15,16] of surface-modified implants versus controls demonstrated more prominent differences in soft tissue adaptation than was observed in the present investigation, the present results help to understand how the experimental surface would work on an actual commercial implant in a human. In the following discussion,

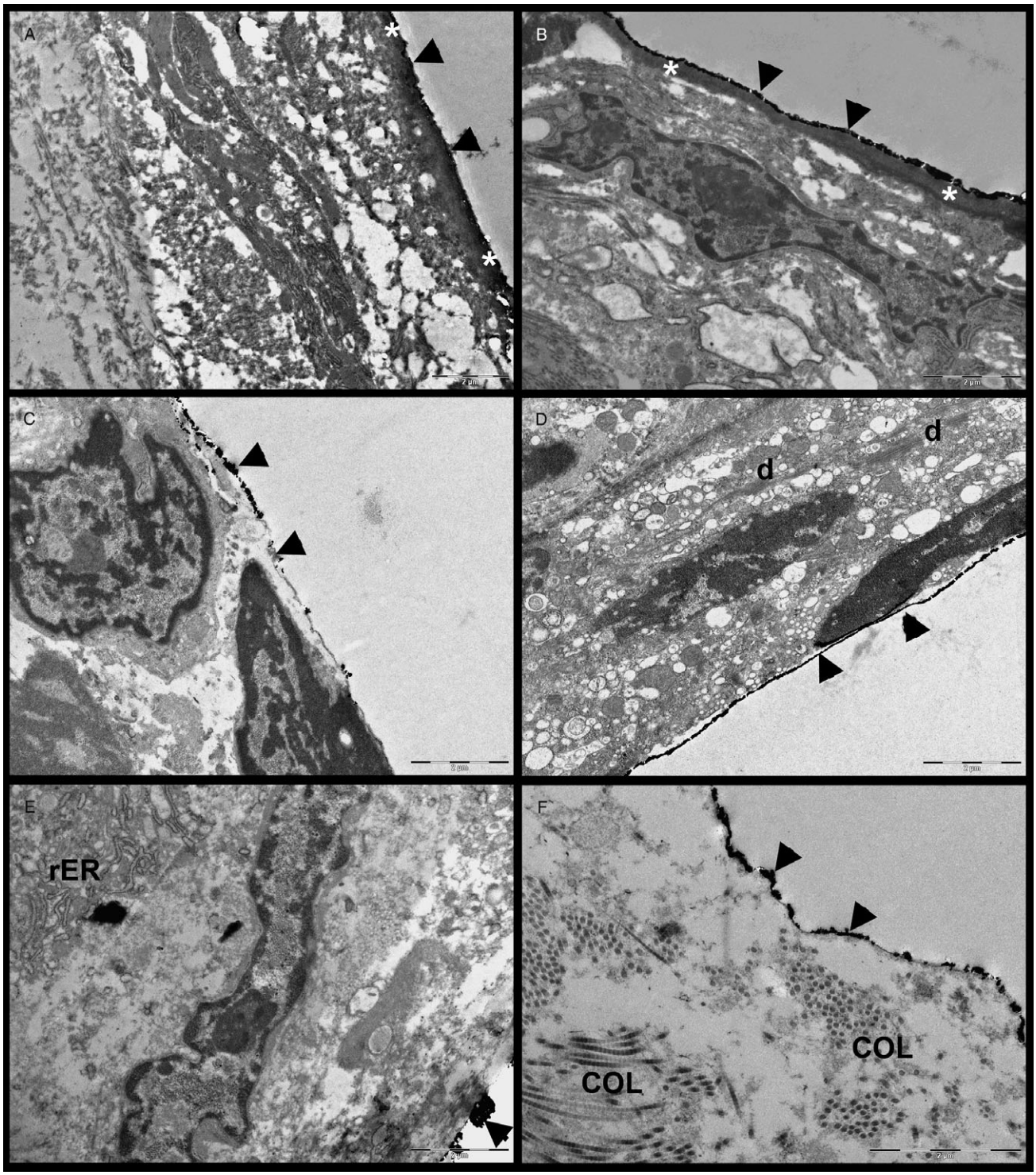

Figure 6 Transmission electron micrographs of the implant-tissue interface, *black arrowheads* (A–F) indicate remaining Ti-oxide layer of the implant surface. All sections are taken from the tissue-implant contact area, at the lower one-third of the abutment part of the microimplants. Scale bars 2 μm. *A,* Remaining Ti-oxide layer and a layer of proteinacous material (*white asterisk*) closest to it (test implant, facial side). *B,* Close to the remaining Ti-oxide a thin (400 nm) protein layer (*white asterisk*) can be seen. A fibroblast is attached to the protein layer (control implant, oral side). *C,* Two cells are closely related to the Ti-oxide, and only an extreme thin coat of protein is visible (test implant, facial side). *D,* Desmosomes (d) close to the surface (control implant). *E,* Fibroblast with rough endoplasmic reticulum (rER) showing signs of high activity (control implant). *F,* Collagen fibers (COL) are seen in all directions (test implant, facial side).

we wish to point out those findings that have lead us to conclude how the experimental surface may benefit the patients.

No clinical problems were observed among the test implants, and the clinician's overall impression of the test implants was very positive. At histological level, statistically significant difference was found for soft tissue contact when counting connective tissue and epithelial tissue together. Although epithelial contact, connective tissue contact, and sulcus area generally demonstrated more favorable mean values for the test implants, no statistically significant differences were obtained. Furthermore, no difference in histology was found for the inflammatory reaction. Both the test and the control implants had very low inflammatory reactions.

Histological evaluation was not able to explain in all respects the clinical findings. There are several plausible explanations for the lack of substantiation of the excellent clinical observations by all histological parameters. Firstly, it has to be emphasized that the most important observation, by far, related to implant performance is the clinical function. The clinical observation represents the full situation, while the histological findings emanate from small sections of the peri-implant tissue, thus only revealing a part of the tissue response at a given time point. Secondly, importantly, most beneficial biologic effects of the TiO_2 surface modification in previous experimental research have been observed in short term (1–2 weeks), whereas, in the long term, the differences have been smaller at the histological level. In a previous subcutaneous rat study, the biggest differences were observed at 3 and 11 days, but, at 12 weeks, the histological differences were already small.[15] An additional possible explanation for the absence of significant histological correlates is that the number of patients may have been too low. At the same time, it is known from cell culture tests with a number of cell types[20] and from mechanical pull out tests[21] that differences in the cell adherence and strength are seen very quickly, while mechanical pullout strength differences are still seen at time points (6 weeks) comparable with the time points when the histological differences are already small. From these observations, it can be concluded that the early events leading to initial cell adhesion may have a strong role in the overall performance of the implant.

In the present study, radiographic measurements demonstrated that less marginal bone resorption was observed on the test implants than on the control implants. This can be interpreted as a secondary effect of the improved soft tissue adherence because no surface treatment was applied in the threaded part of the implant. This finding is in accordance with the previously observed canine study result.[17] In addition, it may be speculated that also the recess of gingival tissue would therefore be reduced because the total thickness of the gingiva was not different on the two sides. Other way around, the minimal gingival recess may also prevent marginal bone resorption.

In the present study, osseointegration was not demonstrated, which is in contradiction to previous studies using microimplants.[22,23] A thorough analysis suggests that the absence of osseointegration was not a result of the TiO_2 surface modification because there were even more unstable implants in the control side. The most likely reasons were the changes in the design of the microimplant and the surface roughness of the threaded part. In the previous study, the microimplants did not have an abutment protruding the gingival tissue. Therefore, it can be argued that it is possible that the masticatory forces were able to disturb the bone-healing process. The threaded part of the experimental implants was much smoother than that of the standard turned implants. The S_a was 0.2 μm and the S_{dr} was 8.6%, while, typically, the S_a and S_{dr} value for standard turned implants are 0.7 μm and 20%,[3] respectively. It is therefore concluded that the smooth surface may have contributed to the instability and poor osseointegration found for the present implants. On the other hand, this may also have influenced the possibilities for soft tissue contact.

Finally, the nanoporous TiO_2 surface modification was not harmful and did not introduce more inflammatory response than the implants without the surface modification did. In a previous canine dental implant study,[17] less inflammation was observed among the test implants at 8 weeks. This difference from the present results may be explained by the longer follow-up time in the present human study. The inflammatory reactions could be expected to have subsided with or without the expected beneficial effect of the surface treatment. In the present study, no infections were observed in either group. Therefore, no conclusion could be drawn on the potential effect of the tissue contact in reduction of infections. The increased contact level of the tissue would indicate that such possibility exists. Typically, dental implants have a low occurrence of infections, and

to show differences at that level would have demanded larger number of patients.

No apparent differences were observed between the test and control implants in the semithin sections or TEM analysis. This underlines previous observations that titanium with its outermost titania surface possesses suitable surface properties in terms of biocompatibility (e.g., soft tissues as reviewed by Holgers and colleagues[7]).

CONCLUSIONS

In conclusion, the results of this human dental clinical study in three different levels of resolution support previous promising results of TiO_2 surface modification in animal experiments. With the TiO_2 surface modification, clinical benefits can be expected in early healing of soft tissue, increased adherence of soft tissue, and reduced marginal bone resorption. In addition, there is reason to speculate that recess of gingival tissue and number of infections would be reduced, and these aspects should be topics of future studies.

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REFERENCES

1. Wennerberg A, Hallgren C, Johansson CB, Danelli SA. Histomorphometric evaluation of screw-shaped implants, each prepared with two surface roughnesses. Clin Oral Implants Res 1998; 9:11–19.
2. Hansson S. The implant neck: smooth or provided with retention elements. A biomechanical approach. Clin Oral Implants Res 1999; 10:394–405.
3. Albrektsson T, Wennerberg A. Oral implant surfaces. Part 1. A review focusing on topographical and chemical properties of different surfaces and in vivo responses to them. Int J Prosthodont 2004; 17:536–543.
4. Ferguson SJ, Broggini N, Wieland M, et al. Biomechanical evaluation of the interfacial strength of a chemically modified sandblasted and acid-etched titanium surface. J Biomed Mater Res A 2006; 78:291–297.
5. Meirelles L, Arvidsson A, Wennerberg A, Albrektsson T. Increased bone formation to unstable nano rough titanium implants. Clin Oral Implants Res 2007; 18:326–332.
6. Reigstad O, Siewers P, Rokkum M, Espehaug B. Excellent long-term survival of an uncemented press-fit stem and screw cup in young patients: follow-up of 75 hips for 15–18 years. Acta Orthop 2008; 79:194–202.
7. Holgers K-M, Esposito M, Källtorp M, Thomsen P. Titanium in soft tissues. In: Brunette DM, Tengvall P, Textor M, Thomsen P, eds. Titanium in medicine. Ch. 16. Berlin: Springer Verlag, 2001:513–560.
8. Klein M, Hohlfeld T, Moormann P, Menneking H. Improvement of epidermal adhesion by surface modification of craniofacial abutments. Int J Oral Maxillofac Implants 2000; 15:247–251.
9. Abrahamsson I, Zitzmann NU, Berglundh T, Wennerberg A, Lindhe J. Bone and soft tissue integration to titanium implants with different surface topography. An experimental study in the dog. Int J Oral Maxillofac Implants 2001; 16:323–332.
10. Abrahamsson I, Zitzmann NU, Berglundh T, Linder E, Wennerberg A, Lindhe J. The mucosal attachment to titanium implants with different surface characteristics: an experimental study in dogs. J Clin Periodontol 2002; 29:448–455.
11. Rutar A, Lang NP, Buser D, Bürgin W, Mombelli A. Retrospective assessment of clinical and microbiological factors affecting periimplant tissue condition. Clin Oral Implants Res 2001; 12:189–195.
12. Åstrand P, Engquist B, Anzén B, et al. A three-year follow-up report of a comparative study of ITI Dental Implants and Brånemark System Implants in the treatment of the partially edentulous maxilla. Clin Implant Dent Relat Res 2004; 6:130–141.
13. Jokinen M, Pätsi M, Rahiala H, Peltola T, Ritala M, Rosenholm JB. Influence of sol and surface properties on in vitro bioactivity of sol-gel-derived TiO_2 and TiO2-SiO2 films deposited by dip-coating method. J Biomed Mater Res 1998; 42:295–302.
14. Peltola T, Jokinen M, Rahiala H, et al. Effect of aging time on sol on structure and in vivo calcium phosphate formation of sol-gel-derived titania films. J Biomed Mater Res 2000; 51:200–208.
15. Paldan H, Areva S, Tirri T, et al. Soft tissue attachment on sol-gel-treated titanium implants in vivo. J Mater Sci Mater Med 2008; 19:1283–1290.
16. Areva S, Paldan H, Peltola T, Närhi T, Jokinen M, Linden M. The use of sol-gel-derived titania coating for direct soft tissue attachment. J Biomedical Materials Res 2004; 70A:169–178.
17. Rossi S, Tirri T, Paldan H, Kuntsi-Vaattovaara H, Tulamo R, Närhi T. Peri-implant tissue response to TiO2 surface modified implants. Clin Oral Impl Res 2008; 19:348–355.
18. Donath K. Die trenn-dünnschliff-technik zur herstellung histologischer präparate von nicht schneidbaren geweben und Materialien. Der Präparator 1988; 34:197–206.
19. Bjursten LM, Emanuelsson L, Ericson LE, et al. Method for ultrastructural studies of the intact tissue-metal interface. Biomaterials 1990; 11:596–601.
20. Peltola T, Vaahtio M, Meretoja V et al. Periodontal ligament and endothelial cell attachment to MetAlive™ surface

(abstract). Presented at the 8th World Biomaterials Conference, May 28–June 1, 2008, Amsterdam, The Netherlands.

21. Kangasniemi I, Areva S, Moritz N. Mechanical measurement of subcutaneous tissue attachment on MetAlive™ surface treated implants (abstract). Presented at the 8th World Biomaterials Conference, May 28–June 1, 2008, Amsterdam, The Netherlands.
22. Ivanoff C-J, Hallgren C, Widmark G, Sennerby L, Wennerberg A. Histologic evaluation of the bone integration of TiO2 blasted and turned titanium microimplants in humans. Clin Oral Implant Res 2001; 12:128–134.
23. Ivanoff C-J, Widmark G, Johansson C, Wennerberg A. Histological evaluation of bone response to oxidised and turned titanium micro-implants in human jawbone. Int J Oral Maxillofac Implants 2003; 18:341–348.