Supra- and Subgingival Biofilm Formation on Implant Abutments with Different Surface Characteristics

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Purpose: *The aim of the present study was to establish a noninvasive method for quantitative analysis of supra- and subgingival biofilm formation on dental implants considering different surface modifications.* ***Materials and Methods:*** *Patients of both sexes were included. They had to be in generally good health, partially edentulous, and the recipient of at least 1 screw-type implant with an abutment possessing supra- and subgingival areas. Healing abutments were inserted for 14 days. The abutment surfaces were divided into quadrants that were sandblasted, ground, acid-etched, and untreated (with the latter surface as a control). Biofilm formation on the healing abutments was analyzed using scanning electron microscopy, including secondary-electron and Rutherford backscattering-detection methods. Calculation of biofilm-covered surfaces was performed depending on grey-values, considering supra- and subgingival areas. After calculating absolute and relative biofilm-covered surfaces depending on localization, the influence of surface modification on biofilm formation was analyzed.* ***Results:*** *Fifteen healing abutments were inserted in 11 patients. In all surface properties plaque adhesion in supragingival areas was significantly higher (17.3% ± 23.1%) than in subgingival areas (0.8% ± 1.0%). Biofilm accumulation in supragingival areas was significantly increasing by higher surface roughness, whereas this influence was not detected in subgingival areas.* ***Conclusion:*** *The described method is valuable for investigation of supra- and subgingival biofilm adhesion on surface-modified implant abutments. There was a significant influence of surface localization (supra- and subgingival) as well as surface modification on biofilm formation. (Case Control Study)* Int J Oral Maxillofac Implants 2008;23:327–334

Key words: *abutment, biofilm, implant, scanning electron microscopy, surface roughness*

Dental titanium implants have a wide variety of uses ranging from replacement of a single tooth to oral rehabilitation with a complete prosthesis. One requirement for long-term success of osseointegrated titanium implants is lack of inflammation of the peri-implant tissues.[1–3] When the implant is exposed to the oral cavity, its surface is colonized by micro-organisms. Depending on their pathogenesis, this can induce peri-implantitis. The subsequent inflammation of the peri-implant mucosa and destruction of the peri-implant bone can compromise the long-term success of implants.

It is assumed that gingiva surrounding natural teeth and mucosa around implants has the potential to prevent subgingival biofilm formation and a similar potential to respond to early plaque accumulation.[4–7] In early biofilm formation, crevicular fluid, junctional epithelium, and a network of circular collagen fibers establish a barrier that prevents intrusion by micro-organisms. A low rate of accumulation of biofilm and tight peri-implant mucosal attachment are, therefore, preconditions for healthy peri-implant mucosa.[8]

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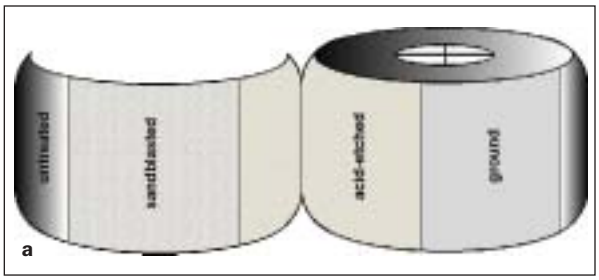

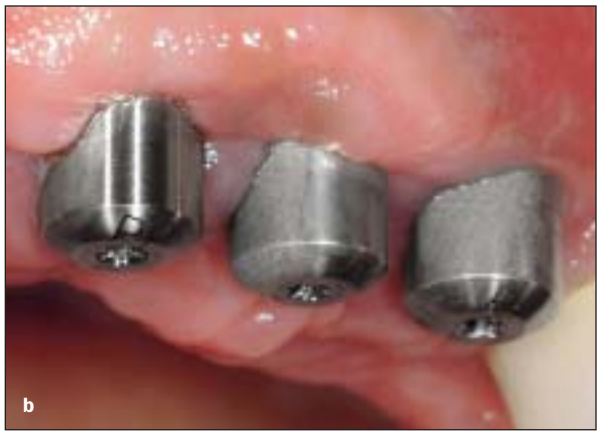

Fig 1 *(a)* Model of a surface modified abutment with 4 different surface treatments—standard machined titanium, sandblasted (Al_2O_3), acid-etched (hydrofluoric acid), and ground. *(b)* Modified abutments in situ.

Initial adhesion of bacteria to intraoral surfaces depends on the physico-chemical properties of the surface, for example, the distance from the bacteria to the solid substratum; the surface free energy of the bacteria, solid surface, and surrounding liquid; as well as the surface integrity and composition of the solid substratum.[9–12] In vivo studies have shown that bacterial colonization of rough titanium surfaces is greater than that of smooth surfaces and that reduction of surface roughness below a threshold value of Ra = 0.2 µm seems to have no further effect on quantitative and qualitative bacterial adhesion and colonization.[13,14]

After osseointegration, dental implants are not accessible for noninvasive analysis of biofilm formation on the implant surface. Up to now, tissue reaction to biofilm accumulation has been researched by harvesting osseointegrated implants, including peri-implant bone and soft tissues.[15] Intraoral biofilm accumulation on modified titanium surfaces has also been examined by fixing metal disks on acrylic splints.[16] However, this method has local confounders, such as the activity of the tongue and cheek. Furthermore, biofilm formation on transgingival and subgingival areas cannot be detected by this method. In another study, different materials were kept for several days in periodontal pockets for detection of subgingival bacterial plaque. However, in this method the foreign body might have had an influence on the permeability of the gingival margin.[17] Transfer of these results to the clinical situation is therefore limited.

The literature contains no reports of atraumatic clinical studies of the effect of the peri-implant barrier on differential supra- and subgingival biofilm adherence on implant surfaces. The objective of this study was to analyze by a noninvasive method supra- and subgingival biofilm formation on dental implants using temporarily inserted abutments. The effect of different surface treatments on biofilm formation in supra- and subgingival areas was also investigated.

MATERIALS AND METHODS

This study included male and female patients in generally good health who were partially edentulous and had at least 1 screw-type implant. After healing abutment insertion, the peri-implant mucosa was allowed to heal for 2 weeks. The abutment had to have a supragingival part and a subgingival part. Antimicrobial therapy in the 3 months prior to the study was considered an exclusion criterion. The study was approved by the ethics committee of Hannover Medical School (no. 3791) and was undertaken with the understanding and written consent of each subject.

A total of 15 titanium healing abutments (regular platform, Nobel Biocare, Göteborg, Sweden) were modified with respect to surface roughness, which was measured at 5 positions using a profilometer (LV-50-E; Hommelwerke, Schwenningen, Germany). In modified abutments the surface was divided into quadrants, and 3 quadrants were sandblasted with 110 µm Al_2O_3 (Devesting and blasting station, EWL 5423, KaVo Elektrotechnisches Werk Vertriebsgesellschaft, Leutkirch, Germany), ground with a smooth diamond bur, and acid-etched for 60 seconds with hydrofluoric acid (Ceramics Etch; Vita, Bad Säckingen, Germany). The surface in the fourth quadrant remained untreated as a control (Fig 1). Addi-

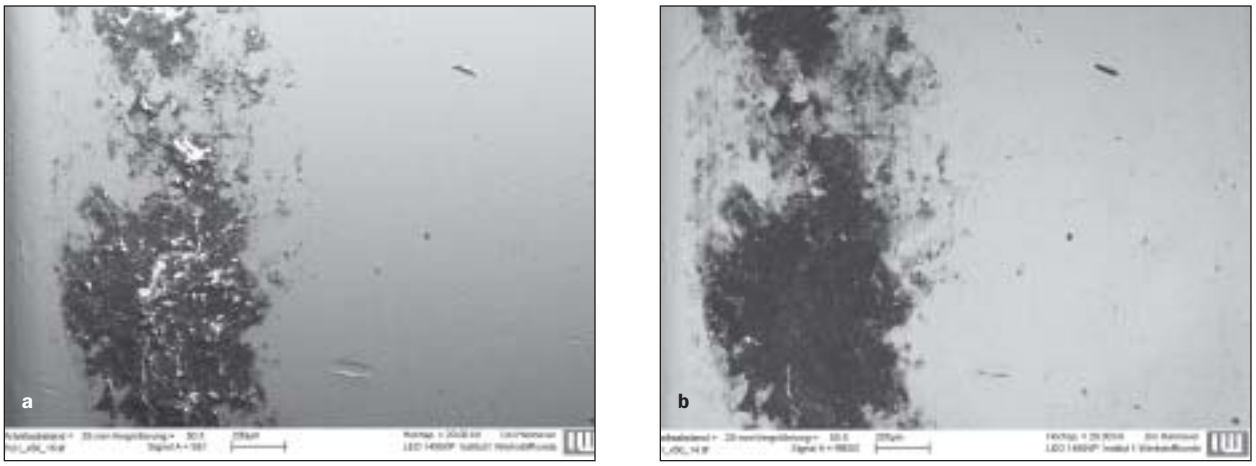

Fig 2 Abutment surface covered with biofilm in supragingival areas. *(a)* Secondary-electron micrograph; *(b)* RBSD micrograph.

tionally, in 2 healing abutments, glass-polyalkenoate-cement fillings (Ketac-Fil; 3M ESPE, Seefeld, Germany) were placed where subgingival areas were expected to investigate the effect of the filling material as well as the gap formation between filling and abutment on biofilm formation. After modification, all samples were cleaned in an ultrasonic bath (Sonorex RK 100 H; Bandelin Electronic, Berlin, Germany) and sterilized (134°C, 14 minutes, cassette autoclave, SciCan, Toronto, ON, Canada).

After abutment insertion, silicone impressions (Silagum-Putty/Silagum-Light, DMG; Chemisch Pharmazeutische Fabrik, Hamburg, Germany) of the abutments were made; these were used later to define supra- and subgingival areas. Patients were instructed not to use antibacterial mouth irrigation and to continue their oral hygiene with a medium-hard toothbrush even in the region of the abutments. After 14 days the abutments were removed, rinsed with sterile water, and dried by air.

Biofilm formation on the healing abutments was analyzed by scanning electron microscopy (SEM; LEO 1455 VP, Carl Zeiss SMT AG, Oberkochen, Germany). Secondary-electron method was used for topographical overview (Fig 2a), and the Rutherford backscattering-detection method (RBSD) was used for detection of biofilm-covered surfaces (Fig 2b).

Biofilm coverage of supra- and subgingival surfaces was measured separately by use of surface-analysis software (Image J 10.2 for Apple; National Institutes of Health, Bethesda, MD). The 32-bit RBSD micrographs were transformed into 8-bit pictures on which biofilm-covered surfaces appeared dark and noncovered titanium surfaces appeared bright. Biofilm-covered and non–biofilm-covered surfaces were subsequently differentiated by use of gray values. The line of demarcation between supra- and subgingival regions was obtained from previously made silicone impressions. In the impressions control healing abutments were placed, and subgingival surfaces were marked with high-dispersive color (Okkluspray; Omnident, Rodgau, Germany; Fig 3a). Supra- and subgingival areas were distinguished by superimposing the RBSD pictures of the sample and the marked control (Figs 3a to 3d).

Documentation and statistical analysis was performed using the data-processing program SPSS (PC version 13.0 for Windows; SPSS, Chicago, IL). After calculating the dependence of absolute and relative biofilm coverage related to supra- and subgingival surface areas, the effect of surface modification on biofilm formation was analyzed. Data were compared by use of the Friedman test and the Wilcoxon signed-rank test. *P* values less than .05 were regarded as statistically significant.

RESULTS

Eleven patients, 4 female and 7 male, between 18 and 75 years old (mean 52 ± 21.2 years), participated in the study. All patients had at least 1 screw-type Brånemark implant (Nobel Biocare) inserted 3 to 6 months before the study began. Fifteen titanium healing abutments (RP; Nobel Biocare) were inserted for 14 days (Fig 1a). All possessed supra- and subgingival parts and were surrounded by attached gingiva. The localization (mesial, distal, buccal, lingual) of the 4 surface modifications resulted randomly from the process of abutment insertion.

The surface roughness measurements were carried out over a measuring length of 1.5 mm using a cutoff length of 0.25 mm. The sandblasted surfaces

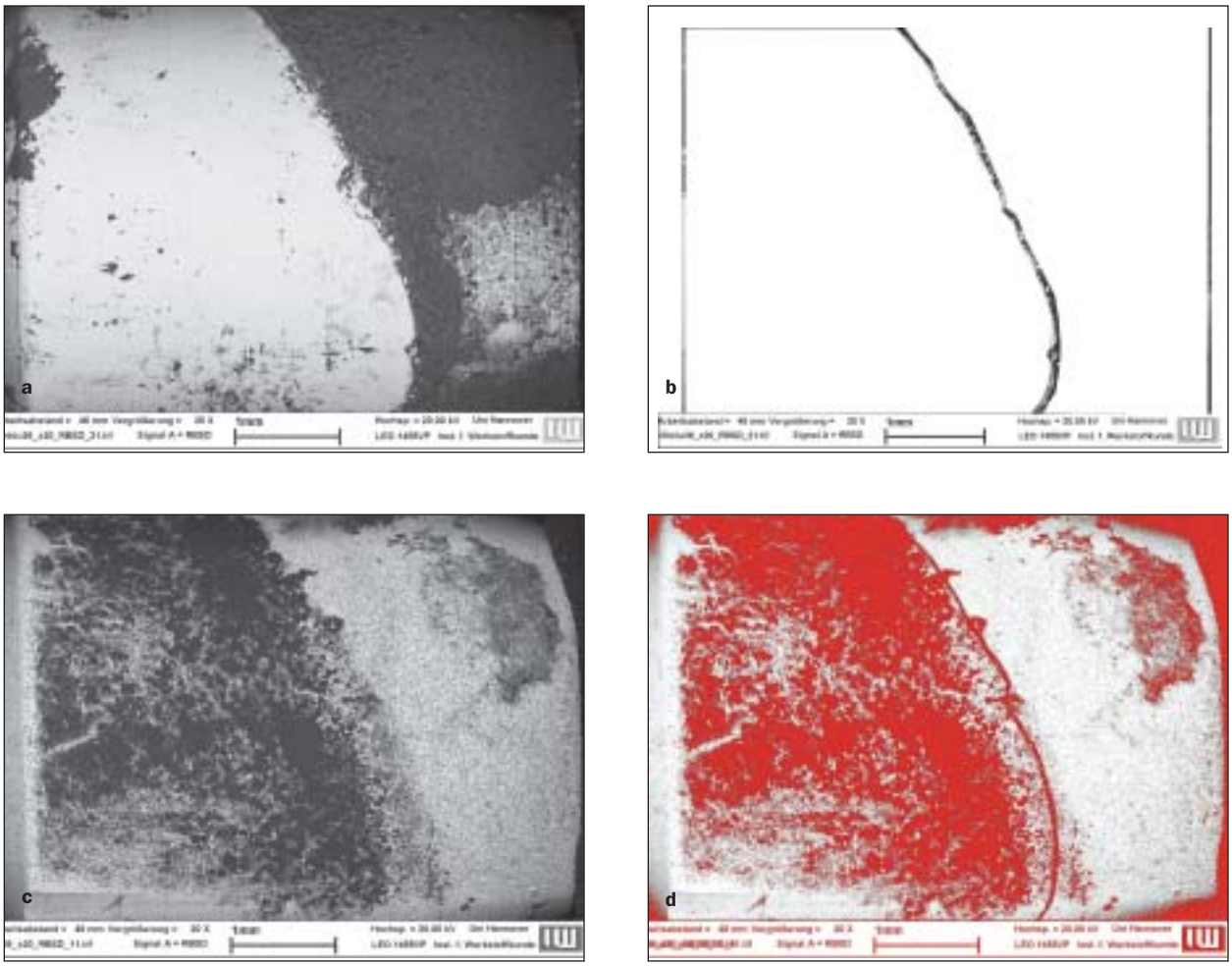

Fig 3 *(a)* RBSD micrograph of a control abutment; the subgingival area appears dark. *(b)* The same micrograph with cleared areas outside the line of demarcation. *(c)* RBSD micrograph of a sample abutment. *(d)* RBSD micrograph of a sample abutment superimposed over the micrograph of the control abutment in threshold mode.

had a mean Ra of 0.9 µm, the ground surfaces 0.4 µm, the acid-etched surfaces 0.3 µm, and the control surfaces 0.2 µm.

SEM analysis showed that 53% of all abutment surfaces were located supragingivally and 47% were located subgingivally. On all investigated healing abutments a biofilm was detected by RBSD micrographs after removal from the oral cavity (Fig 2). If even adherent cells of the peri-implant mucosa, which were structurally different from biofilm, were detected in subgingival areas, as was verified by the use of secondary-electron micrographs, the region of the image was "cut out" before analysis (Fig 4).

Biofilm covered 17.3% ± 23.1% of all supragingival surfaces, in contrast to only 0.8% ± 1.0% of subgingival areas. This difference was statistically significant ($P < .05$).

A line separating supra- and subgingival areas was apparent from the presence of supragingival biofilm-covered surfaces and biofilm-free subgingival surfaces. This borderline was congruent with that of the colored abutments (Fig 3d).

Figure 5 shows the distribution of the several biofilm formations on differently modified surfaces. On sandblasted surfaces biofilm formation occurred on 47.4% ± 32.4% of supragingival areas and 1.3% ± 1.2% of subgingival areas. On ground areas biofilm was found on 18.6% ± 31.4% of supragingival surfaces and on 0.6% ± 0.8% of subgingival surfaces. On etched surfaces biofilm was present on 15.6% ± 18.7% of supragingival surfaces and 0.6% ± 1.3% of subgingival areas, whereas on untreated controls biofilm formation occurred only on 5.7% ± 14.4% of supragingival surfaces and on 0.6% ± 1.2% of subgingival surfaces (Fig 6). The differences between supragingival biofilm formation on the different surfaces were statistically significant. Only comparison of ground surfaces with acid-etched surfaces showed no significance in supragingival biofilm accumulation (Table 1). In subgingival areas, differ-

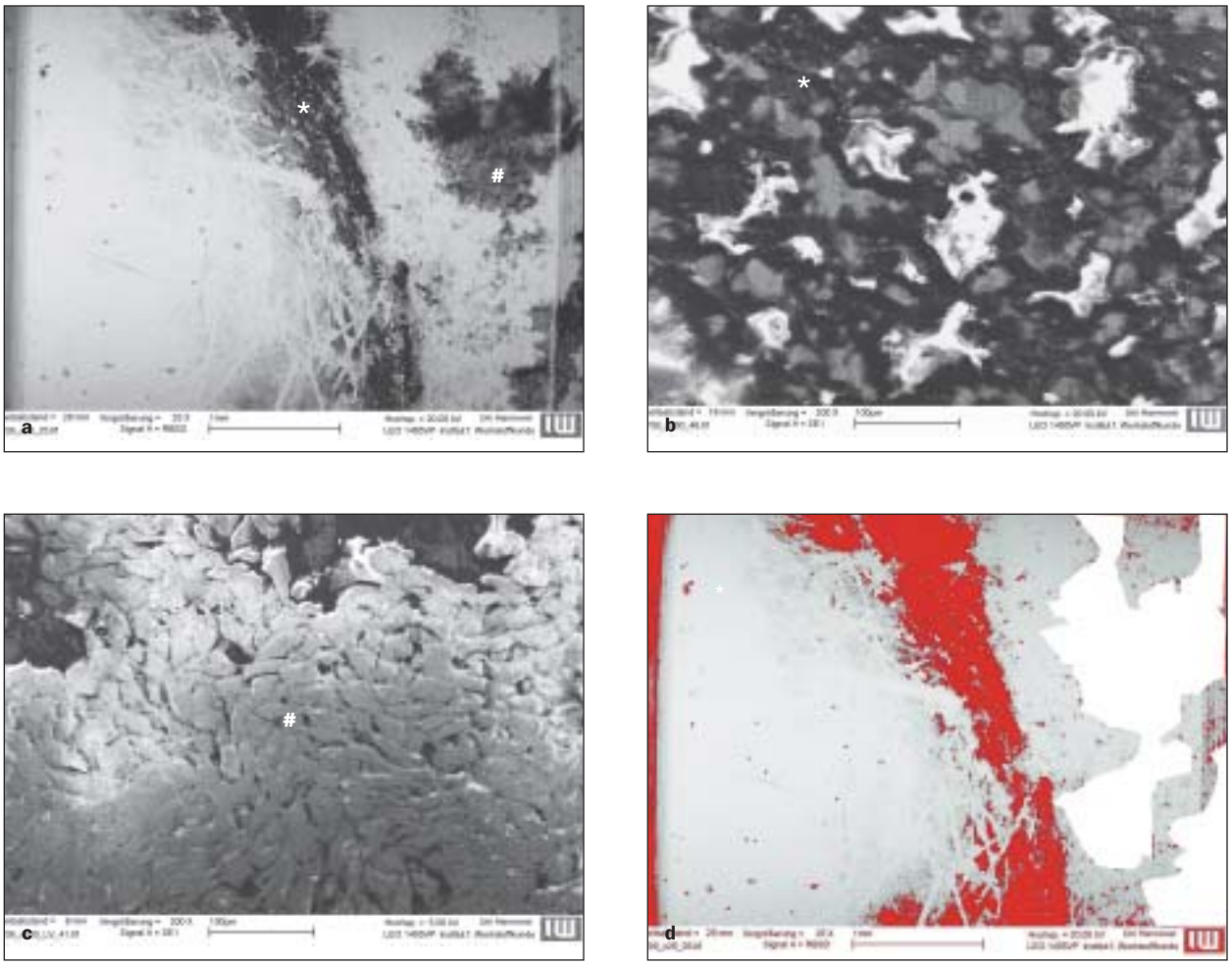

Fig 4 *(a)* RBSD picture of a sample abutment with peri-implant mucosal cells located subgingivally (#) and supragingival biofilm formation (*). Structural differences between *(b)* supragingival biofilm and *(c)* adherent peri-implant mucosal cells in subgingival areas can be clearly observed. *(d)* The image in 4a in threshold mode; identified peri-implant mucosal cells located subgingivally have been cut out.

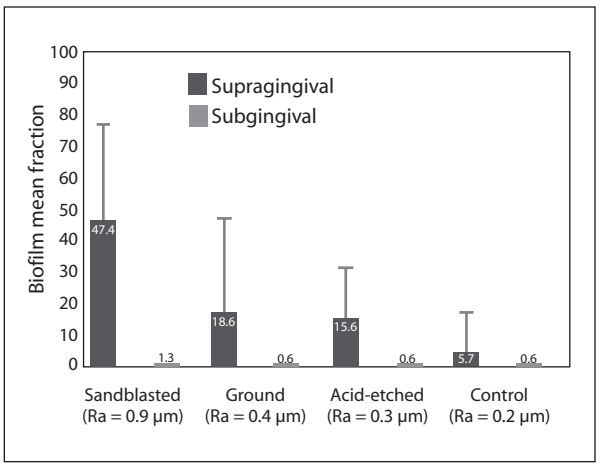

Fig 5 Percentage frequency of biofilm formation on surfaces with different treatments.

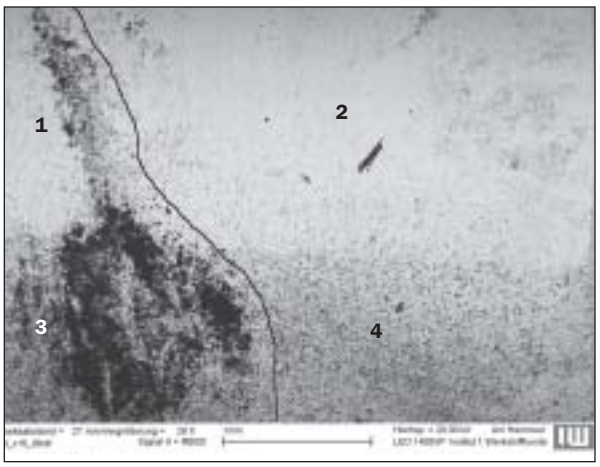

Fig 6 Supragingival rough surfaces show more plaque-covered areas than smooth surfaces; subgingivally no plaque was detected. *(1)* Supragingival control surface; *(2)* subgingival control surface; *(3)* supragingival sandblasted surface with plaque-covered areas; *(4)* subgingival sandblasted surface.

Table 1 *P* Values for Differences in Plaque Formation on Supragingival Surfaces

	Ground	Acid-etched	Control
Sandblasted	.018	.018	.012
Ground		.917	.034
Acid-etched			.028

ences in biofilm formation between areas with different surface treatments were not statistically significant. On subgingivally located glass-polyalcenoate-cement fillings, for which a marginal gap was apparent in the SEM images, no biofilm was detectable.

DISCUSSION

In the present study, differentiation between biofilm-covered and noncovered surfaces on titanium abutments was possible by use of the RBSD technique. In this technique primary electrons are used to scan the surface of a sample in a manner similar to secondary-electron microscopy. Electrons penetrating the sample and interacting with its atoms are diverted in their direction without losing energy. There is a certain probability that a few primary electrons will leave the sample as a consequence of an elastic scattering process. These high-energy Rutherford backscattering electrons are measured with the RBSD detector.

The probability that a primary electron will leave the sample depends on its atomic weight. A higher atomic number indicates a greater probability of backscattering. In RBSD, element-contrast pictures are produced that enable display of different elements. Surfaces of the sample covered with elements of low atomic weight (eg, carbon) appear darker than areas covered with elements of higher atomic weight (eg, titanium). Compared with the secondary-electron method, pictures generated by RBSD are less sharp, as a consequence of the effect of backscattering; less topographical information about the surface is obtained. However, pictures generated by RBSD method have a decisive advantage in software-assisted analysis; precise differentiation of biofilm-covered surfaces from noncovered surfaces is possible. In RBSD micrographs, biofilm is displayed as higher gray values (dark) because of the low atomic number of the elements of which it is composed (mostly carbon and hydrogen), while titanium surfaces appear as lower gray values (ie, they appear bright) because of the higher atomic number of titanium.[18] This computer-assisted analysis of biofilm formation is not possible using secondary-electron pictures.

To reduce measurement error during abutment analysis, 6 SEM pictures of each abutment were obtained, because in 2-dimensional pictorial representation of curved surfaces distortion can be a problem. Each picture was made after rotating the probe approximately 60 degrees. The clinical pictures were then superimposed over the marked control pictures.

Identification of supra- and subgingival-located areas of healing abutments using photographs, models, or clinical examination alone is problematic. In a previous study, this differentiation was performed at 6 sites around an abutment by measuring the distance from the gingival margin to the probe tip in the pocket.[14] In this study, control abutments were used to form silicone impressions. Subgingival areas were then marked with highly dispersive color (Okklufine FINO; Bad Bocklet, Germany) to reproduce the line of demarcation continuously. This method also avoided microtrauma caused by probing.

Quantitative analysis of supragingival biofilm formation showed biofilm accumulated on 5.7% of untreated surfaces. In roughness-modified healing abutments, biofilm accumulated on 27.2% of the surface. The results of the biofilm formation showed a high standard deviation for all supragingival areas. This represents interindividual differences in the amount of biofilm-covered surface and can be explained by factors such as nutrition, tongue activity, and oral hygiene.

It has been postulated that rough surfaces above a threshold Ra value of 0.2 µm harbor more biofilm in supragingival areas.[10,13,14] The findings of the current study are in agreement with this postulation, because more supragingival biofilm was observed on abutment surfaces of higher roughness than on smoother surfaces. In an in vitro study it was shown that acid-passivated titanium surfaces inhibited bacterial adhesion of *Streptococcus sanguis* because of increased wettability.[19] In the present in vivo study, acid-etched surfaces showed no decrease in biofilm adhesion in comparison to control surfaces. The influence of surface passivation does not seem to play a key role for bacterial colonization under in vivo conditions. The increased roughness on the acid-etched surfaces apparently covers the effect of passivation.

Quantitative analysis of subgingival biofilm formation was performed for both untreated surfaces and those with modified surface roughness. Hardly any biofilm was observed on subgingival areas. Even on the abutments with gap formation around subgingival fillings no biofilm was detected. The differences between supra- and subgingival biofilm adherence were statistically significant. In the presence of

supragingival biofilm, the absence of subgingival biofilm led to a recognizable line of demarcation. A possible explanation for this might be a tight peri-implant barrier that inhibited subgingival biofilm accumulation. Such a barrier could be formed by circular collagen fibers and adherent hemidesmosoma, actin filaments, and microvilli.[8,20] In an animal study it was shown that this mucosal attachment does not depend on surface roughness,[21] which could explain the lack of subgingival biofilm, even on rough surfaces, in the present study. Immunologic reactions might also play a key role in the regulation of subgingival bacteria. Clinical studies showed the presence of T- and B-lymphocytes in the peri-implant mucosa after 3 weeks without oral hygiene.[22] The immunologic response to biofilm accumulation in the peri-implant mucosa and the gingiva of natural teeth seemed to be similar.[23]

In contrast with the results of this study, Quirynen et al found 25 times more subgingival biofilm formation on rough surfaces than on unmodified controls after a period of 3 months.[24] However, the aforementioned study, the planktonic bacteria in the crevicular fluid together with the bacterial biofilm adherent to the surface were analyzed rather than the adherent biofilm on the surfaces exclusively. In another study, it was shown that bacteria associated with periodontitis could colonize peri-implant pockets within 2 weeks.[25] However, unlike the present study, in that investigation only the planktonic bacteria in the crevicular fluid were quantitatively assessed. In the present study, the presence of the supragingival biofilm did not lead to accumulation of bacteria on the surface located in subgingival areas. An explanation for the absence of adherent subgingival biofilm might be a tight mucosal barrier and host defense mechanisms (crevicular fluid), which may have inhibited bacterial colonization of subgingival surfaces.

The results of the present study represent the clinical situation 14 days after insertion of the healing abutments. Long-term observations must be conducted now to show whether the presence of periodontal pathogens and consecutive destructive inflammation of the peri-implant mucosa lead to subgingival biofilm formation on titanium surfaces. Such long-term observations can be performed by use of the described method for atraumatic analysis of implant surfaces.

CONCLUSIONS

The described method allowed a quantitative analysis of biofilm-covered surfaces on titanium healing abutments. The results of this study show that there is a significant influence of surface roughness on biofilm accumulation in supragingival areas. However, the presence of supragingival plaque did not lead to a significant increase of biofilm accumulation in subgingival areas over a period of 14 days. Furthermore, surface roughness had no effect on biofilm accumulation on subgingival surfaces.

ACKNOWLEDGMENT

This study was supported in part by the HOMFOR research program of Saarland University.

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