Modified Implant Surface with Slower and Less Initial Biofilm Formation

Gordon John, Dr. med. dent.; Jürgen Becker, Prof. Dr. med. dent.; Frank Schwarz, Prof. Dr. med. dent.

ABSTRACT

Background: Peri-implant mucositis and peri-implantitis are a raising issue in dental implantology. Peri-implant infections are mainly caused by the formation of biofilm. Different surface textures exhibit various conditions for biofilm formation resulting in several speed of maturation and development.

Materials and Methods: On three different titanium implant surfaces, machined-surface (M), sandblasted large grit, and acid-etched surface (SLA) and machined-modified acid-etched surface (mod MA) initial biofilms were collected. Plaque formation was investigated by erythrosine staining and energy-dispersive X-ray spectroscopy (EDX). For testing the biocompatibility of these plaque-settled surfaces, autoclaved specimens were settled with human gingival fibroblasts, and cell viability was tested.

Results: The mean initial plaque surface was detected in the following descending order: M > SLA > mod MA. The differences between these groups were significant. The highest cell viability was detected in the M groups, whereas mod MA and SLA showed comparable results. The results of initial biofilm formation were proved by EDX.

Conclusions: Within the limitations of this study, conclusion can be made that mod MA surface shows significant slower initial biofilm formation which could be an advantage in initial transgingival healing process and also an easement for oral hygiene of patients because maturation of plaque is retarded, and immature biofilms are easier to remove.

KEY WORDS: implant, implant surface, peri-implantitis, titanium

INTRODUCTION

Dental implantology is a common treatment procedure in replacing missing teeth.[1] The widespread use of dental implants is also connected to some disadvantages and complications.[2] Peri-implant infections are an increasing focus in dental implantology,[3] additionally caused by the demographic change with the ever-increasing number of elder patients.[4] Peri-implant diseases are divided into two main groups. The term of *peri-implant mucositis* describes the reversible inflammation of the surrounding soft tissues of an implant, being comparable to gingivitis.[5] The other main group of peri-implant infections is peri-implantitis describing a nonreversible condition touching the implant surrounding tissues leading to a decrease of the bony foundation of the implant.[6,7] This state is comparable to periodontitis, caused by the same pathogenic bacteria and associated with biofilm formation.[8,9] The main causative factor for peri-implant diseases is the colonization of the implant surface with pathogenic microorganisms.[10] The process is associated with the presence of marker microorganisms like *Porphyromonas gingivalis, Prevotella intermedia, Treponema denticola*, and *Aggregatibacter actinomycetemcomitans*.[11,12] Previous studies investigated the bacterial colonization and biofilm formation on different implant surfaces after insertion in the oral cavity. Once being exposed to the oral environment, the implant surface is covered by a pellicle layer, a organic stratum mainly consisting of proteins, glycoproteins, and lipids, which initiates bacterial colonization and biofilm formation.[13,14] The initiation of bacterial colonization is done by *Streptococci* species,[15] being the basis for attachment of further

Oral surgeons, Department of Oral Surgery, Westdeutsche Kieferklinik, Heinrich-Heine-University, Düsseldorf, NW, Germany

Reprint requests: Dr. Gordon John, Oral Surgeon, Department of Oral Surgery, Westdeutsche Kieferklinik, Heinrich-Heine-University, Moorenstr. 5, 40225 Düsseldorf, NW, Germany; e-mail: gordon.john@med.uni-duesseldorf.de

DOI 10.1111/cid.12140

microorganisms like *Prevotella* or *Fusobacterium* species. Some studies reported about clinical prevalence of peri-implant infections. Peri-implant mucositis occurs in up to 80% in implant sites and 50% of patients.[3] The prevalence of peri-implantitis is stated at 56% in the implant sites and 43% of patients.[16]

It is known that different implant surfaces have various properties for adherence of bacteria during initial biofilm development.[17,18] Surface free energy and roughness are considered to be important factors for initial bacterial colonization and enable an ample scope for modifications on implant surfaces for deceleration of biofilm formation. The peri-implant attachment of soft tissue is characterized inconsistently. Because of the absence of a cement layer on the implant surface, it is mostly postulated that the connective tissue fibers are mainly oriented circumferentially, parallel to the implant surface.[19–21] There are also some studies describing connective tissue fibers running perpendicularly toward the surface of dental implants, which is comparable to the situation of soft tissue surrounding teeth. These periodontal condition mimicking structures are more frequently found on implant surfaces that are microstructured.[22,23] Thus, these surfaces could provide an advantage in a more stable and resistant peri-implant tissue. However, do these surfaces also have disadvantages regarding the plaque accumulation?

The aim of the present study was the evaluation of such a microstructured, modified titanium implant surface regarding speed of plaque formation in comparison with established implant surfaces.

MATERIALS AND METHODS

Five healthy volunteers (two women, three men; mean age 27.6 ± 2.9 years) were included in this study. Each volunteer was given a precise description of the procedure and had to sign a prior informed consent. The inclusion criteria for patients were (1) good level of oral hygiene (Plaque Index [PI] < 1); (2) no signs of inflammation of the surrounding soft tissues; (3) no systemic antibiotic therapy during the last 6 months; and (4) nonsmokers. Prior to the start of the study, participants obtained a professional tooth cleaning. The study protocol was approved by the ethical committee of the Heinrich Heine University of Düsseldorf.

In this study, three different surfaces were investigated: a hydrophobic machined surface (M, titanium grade 4, S_a: 0.171 ± 0.007 μm, contact angle [CA] 72.5°), a hydrophobic sandblasted and acid-etched surface (SLA, titanium grade 4, sandblasted with large grids of 0.25–0.5 mm and acid etched with HCl and H_2PO_4, S_a: 1.512 ± 0.051 μm, CA 87.0°), and a hydrophilic, chemically modified acid-etched surface (mod MA, titanium grade 4, acid etched with HCl and H_2PO_4 and stored in 0.9 M NaCl, S_a: 0.759 ± 0.004, CA <5°).

The volunteers received acrylic resin splints for the upper jaw wherein four titanium discs with a diameter of 15 mm and a thickness of 1 mm were fixed in impressions with cyanoacrylate glue (Loctide 496, Henkel, Düsseldorf, Germany) in a distance of 1 mm to the palate. In this way, a moist and nutritious environment was ensured, whereas the disturbance by soft tissues and the tongue was excluded. The allocation of the samples to the splints was randomly assigned according to a computer-generated list (Randlist, DatInf GmbH, Tübingen, Germany). Initial biofilm was collected for 24 and 48 hours. In this period, volunteers maintained their regular diet, the splints were kept intraorally for the whole time except during the procedure of mechanical tooth brushing that had to be done only with water and without the help of tooth paste or mouth-rinsing solutions. Immediately after plaque collection, period splints were removed from the oral cavity, and specimens were extracted from the splint and rinsed with water, and 72 samples were stained with erythrosine (Erythrosine B, Certistain®, Merck KGaA, Darmstadt, Germany). These samples were photographed at a ×8 magnification by the use of a stereo microscope (SZ61, Olympus Europa Holding GmbH, Hamburg, Germany) and a digital camera (ColorViewIII, Olympus Holding GmbH, Hamburg, Germany).

For analyzing the surfaces of samples, a professional image and documentation software (Cell D, Olympus Europa GmbH, Hamburg, Germany) was used. Ten measurements were taken per sample by random placing 16 mm^2 square fields on the sample surface. According to previous studies, initial plaque surface (IP) was measured as a percentage of the whole measuring field.[7,23] The evaluation was done by one experienced examiner being masked to the study conditions.

For analyzing surface morphology, three discs of both groups were gently rinsed with pure water, dehydrated in increasing concentrations of acetone (40–100% in steps of 10%). After drying in hexamethyldisilazane, samples were sputter coated with gold and examined using scanning electron microscopy (SEM; S-3000N,

Hitachi, Pleaston, CA, USA). The surface morphology was descriptively evaluated by one experienced examiner masked to the particular conditions of the study.

Thirty specimens were used for evaluating the biocompatibility of the biofilm-coated surfaces via cell viability measurement. Directly after being removed from the oral cavity, they were autoclaved and settled with human gingival fibroblasts (HGF, pass: 4, Provitro GmbH, Berlin, Germany). Additionally, five unworn specimens with M, SLA, and mod MA surfaces were taken as control groups; 10,000 HGF cells were cultured in 1 ml of Dulbecco's modified Eagle Medium (high glucose, Glutamax; Sigma-Aldrich, Schnelldorf, Germany) with the supplement of 10% fetal bovine serum (Sigma-Aldrich) and 1% penicillin/streptomycin per well in non-binding 24 well plates (Corning® Ultra-low attachment 24 well plate, Sigma-Aldrich). The cell culture conditions were set at a temperature of 37°C, a humified atmosphere of 95 and 5% CO_2. The change of nutrition medium was performed after 3 days. Two dates of measurement were set: on day 3 and day 6. Cell viability was measured by the use of a luminescence assay (CellTiter-Glo®, Promega, Mannheim, Germany) in a luminometer (Victor 2030, PerkinElmer, Rodgau, Germany). The signal was measured in counts per second (CPS).

Additionally, two samples of native, unworn M, SLA, and mod MA surfaces and the corresponding surfaces with 48 hours biofilm collecting period were subjected to critical scrutiny via energy-dispersive X-ray spectroscopy (EDX; S-3000N, Hitachi). Compositions of the surface materials were detected, and elements were stated in weight percentage.

A professional analyzing software (SPSS® 21, SPSS, Munich, Germany) was used for determination of differences between both groups. Mean values and standard deviations were calculated for each group. Normal distribution was tested via Shapiro–Wilk testing. Differences in IP were investigated using Kruskal–Wallis testing. Differences in cell viability were detected via multiple comparisons with analysis of variance with post hoc testing using Bonferroni's correction. Results were considered to be statistically significant at a level of $p < .05\%$.

RESULTS

According to expectation, the mean IP of specimens with a 48-hour plaque settlement (79.7 ± 25.0%) was significantly higher than the IP observed in the 24-hour groups (64.7 ± 31.7%), $p < .05$. All groups with 48-hour biofilm formation showed higher mean IP (M 98.5 ± 2.3% > SLA 90.5 ± 5.8% > mod MA 50.1 ± 21.9%) than their corresponding groups with 24-hour plaque settlement (M 93.7 ± 4.4% > SLA 75.8 ± 17.4% > mod MA 24.6 ± 10.7%) (Figure 1). The differences between these comparative groups were significant ($p < .05$) contrary to the results detected in the M groups ($p = .25$). The differences between the several test groups were at both dates of measurement, 24 hours and 48 hours, significant ($p < .05$).

These results were confirmed by the scanning electron microscopy. The unworn specimens without any biofilm contamination showed their specific surface characteristics. The machined samples depicted cultrate and parallel grooves. The SLA surface showed its typical microporosity and macroporosity with the sharp edged peaks. The mod Ma surface exhibited microporosity that appeared smoother and more radiused than that one of the SLA surfaces. Because of the increasing thickness of the biofilm with prolonged wearing time, it was harder to focus the original surfaces. As expected on the surfaces with 48 hours plaque settlement, more plaque could be found than in the corresponding 24-hour groups. After 48 hours, an almost full coverage of all tested surfaces could be proved (Figure 2).

EDX analysis resulted in the highest percentage of titanium in the unworn M surface (98.9 ± 0.6%) followed by the unworn SLA surface (96.5 ± 0.4%), both

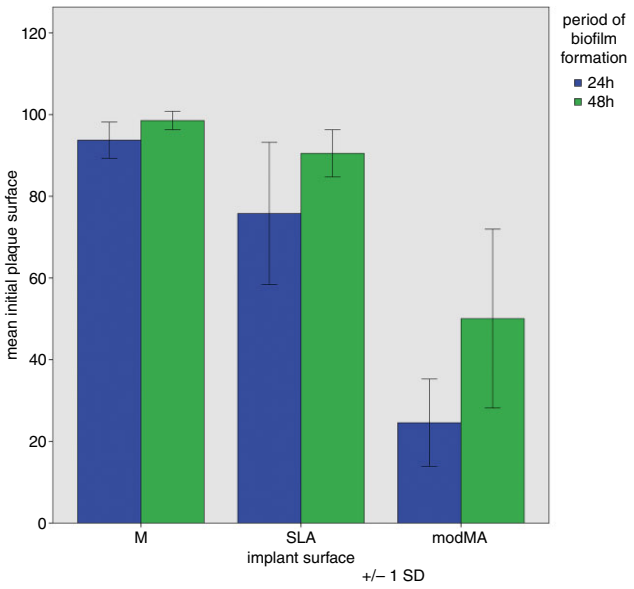

Figure 1 Overview on mean initial plaque surface on three different implant surfaces.

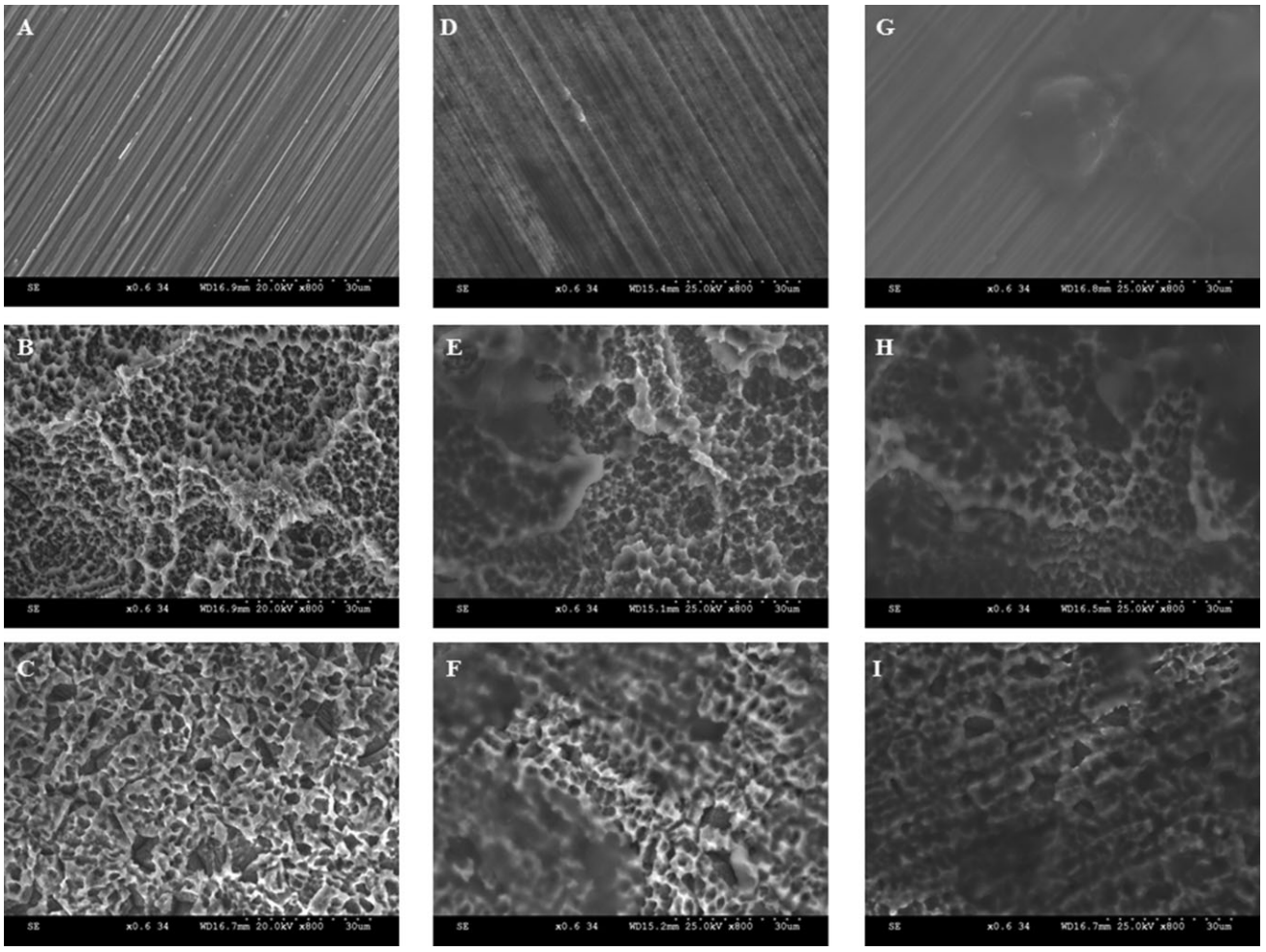

Figure 2 Representative pictures of scanning electron microscopy analysis, magnification of 800. *A*, M native. *B*, SLA native. *C*, mod MA native. *D*, M 24 hours. *E*, SLA 24 hours. *F*, mod MA 24 hours. *G*, M 48 hours. *H*, SLA 48 hours. *I*, mod MA 48 hours. (M = machined surface; mod MA = modified acid-etched surface; SLA = hydrophobic sandblasted and acid-etched surface.)

being significantly higher than all other groups. The unworn mod MA surface (77.0 ± 18.2%) and the mod MA surface with 48-hour plaque settlement (63.9 ± 5.4%) showed no significant differences, but both showing significantly more titanium than the 48-hour worn M surfaces (37.8 ± 7.2%) and the 48-hour plaque-settled SLA surfaces (35.0 ± 4.9%). The native mod MA surfaces were the only ones without detectable oxygen. Instead, sodium (14.2 ± 10.7%) and chloride (8.8 ± 7.5%) could be detected. Oxygen could be detected on surfaces in the following descending order: SLA 48 hours (15.0 ± 0.9%) > M 48 hours (13.7 ± 0.8%) > mod MA 48 hours (11.4 ± 1.3%) > unworn SLA (3.4 ± 0.4%) > unworn M (1.1 ± 0.6%). Only in the plaque-settled specimens carbon could be determined. The highest percentage was detected in the SLA 48-hour group (50.0 ± 4.1%) followed by the M 48-hour group (48.5 ± 7.1%) and the mod MA 48-hour group (24.7 ± 4.2 %) (Figure 3).

Examination of biocompatibility of the implant surfaces revealed highest cell viability in the native, unworn samples. On day 3, the highest cell viability was detected in the unworn M group (145802 ± 8476.6 CPS), showing significant higher results than all other groups, followed by the unworn SLA group (80912 ± 10991.4 CPS) and the mod MA group (80866 ± 19255.9 CPS), both depicting comparable results and being significantly higher than all plaque-settled groups. Cell viability within the plaque-settled groups could be detected in the following descending order: M 48 (3996 ± 3958.7 CPS) > M 24 (414 ± 176.3 CPS) > SLA 24 (320 ± 86.7 CPS) > mod MA 24 (300 ± 164.9 CPS) > mod MA 48 (128 ± 127.8 CPS) > SLA 48 (100 ± 52.9 CPS). Between these worn

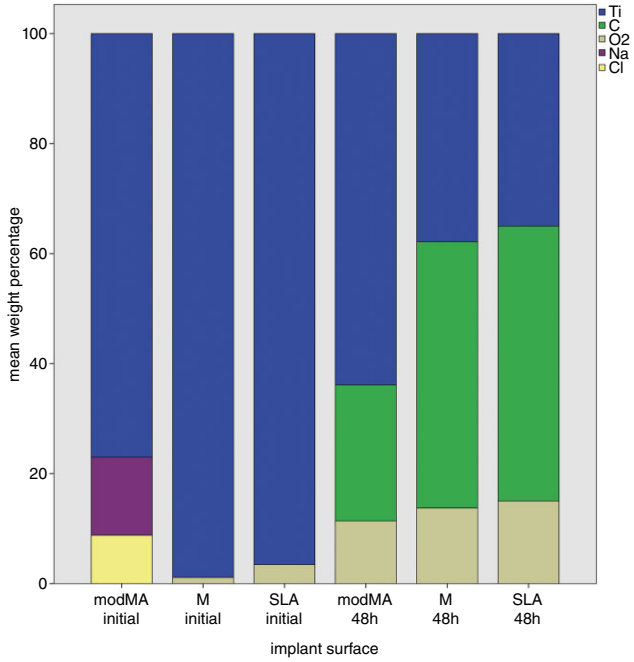

Figure 3 Results of energy-dispersive X-ray spectroscopy (EDX) analysis, overview on mean percentage of elements on the different implant surfaces of unworn (initial) samples and after 48 hours of plaque settlement.

groups, no significant differences could be determined. The examination on day 6 showed similar results; the highest cell viability was detected in the unworn M group (267384 ± 4623.8 CPS), being significant higher than all other groups, followed by the unworn mod MA group (161968 ± 22036.1 CPS) and the unworn SLA group (145936 ± 28235.5 CPS), both showing analogous results and being for their part significantly higher than all worn and biofilm-settled groups. Within these settled groups, cell viability was detected in the following descending order: M 24 (352 ± 411.2 CPS) > M 48 (104 ± 81.7 CPS) > SLA 24 (76 ± 53.7 CPS) > mod MA 24 (56 ± 47.8 CPS) > mod MA 48 (40 ± 28.3 CPS) > SLA 48 (24 ± 8.9 CPS) (Figure 4). Similar to the results of day 3, no significant differences could be determined between these worn groups. Expectably, the unworn groups showed higher results on day 6 than on day 3 with significant differences in the M and in the mod MA surfaces, whereas the cell viability decreased in the biofilm-covered specimens from day 3 to day 6.

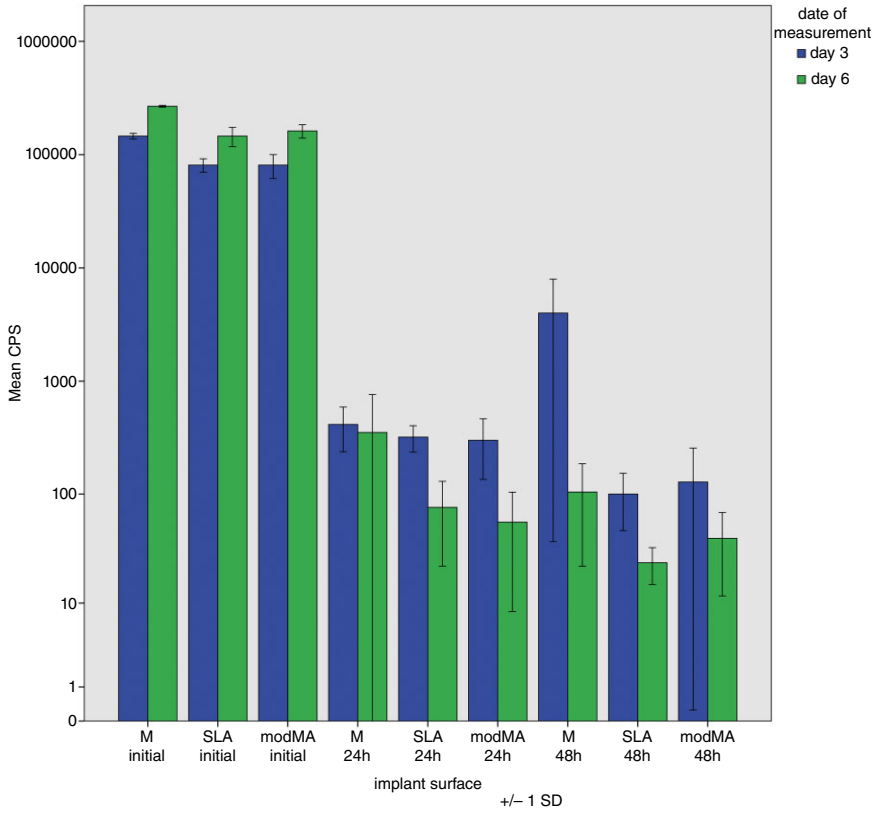

Figure 4 Overview on cell viability.

DISCUSSION

The present study was designed to evaluate in vivo initial biofilm formation on different implant surfaces. Knowing that actually because of the absence of shear forces, missing interaction between biofilm and peri-implant tissue and the deficiency of immunological defense on in vitro model is common knowledge to copy the situation of peri-implant mucosal tissue. This study was configured to imitate natural initial biofilm formation as well as possible. In contrast to biofilm models using selected pathogenic bacteria[24,25] for colonization assays in vitro, we wanted to simulate the natural initial biofilm formation. Therefore, we used a splint design described and successfully used in previous studies.[26–28] For undisturbed biofilm formation, the implant surfaces were turned to the palate in a distance of 1 mm. So, the manipulation because of soft tissue and tongue movement was excluded while a moist and nutritious environment was provided to the samples. Although the situation in vivo is more sophisticated, this model is more precise in detecting influences on biofilm formation.[29] Although in vitro plaque is adherent to almost every surface, plaque adhesion on hydrophobic supragingival surfaces is inferior to hydrophilic ones under in vivo conditions.[30] In the present study, it was also found that the super hydrophilic mod MA surface showed significantly less initial biofilm formation than the more hydrophobic SLA and M surfaces. These outcomes were proven by both histomorphometrical analysis and EDX analysis. The results of EDX analysis do not correspond to results of other studies, especially the absence of carbon in the original unworn samples. Other investigations detected up to 37.3 ± 3.4% carbon on unworn SLA surfaces.[31] Buser and colleagues used X-ray photoelectron spectroscopy for examination of the surface composition. It has been postulated that this method has a penetration in the region of some nanometers.[32] For the investigation of original samples, as Buser and colleagues did, this is a precise method, but for examination of thicker biofilms, which was the purpose of this current study, the EDX analysis with a penetration of several micrometers should be more effective. Also, this higher penetration appears to be accountable for the absence of carbon on the native samples in the current study. Former studies reported that the surface roughness has a higher impact on biofilm formation than the surface free energy.[25,33–35] Other studies also reported about the correlation between the surface roughness and the quantity of plaque accumulation.[36–38] The findings of the present study are in accordance to these outcomes as far as the rougher SLA surface (S_a: 1.022 μm) in the present study showed higher plaque accumulation than the mod MA surface (S_a: 0.186 μm). In contrast to that in the present study, the smooth M surface (S_a: 0.069 μm) showed the highest plaque accumulation in comparison to the SLA and mod MA surfaces. So, it is certain that the surface roughness is not the only factor ruling the amount of plaque and maybe also not the main factor. Schwarz and colleagues used a similar study design and described a significant higher plaque formation on a polished titan surface (R_a: 0.04) than on an SLA and mod MA, both with an R_a of 0.83 μm, after 24 and 48 hours of biofilm collection. In this investigation also, the smoother surface showed more initial biofilm formation than the rougher ones. After 48 hours, comparable results in form of an almost complete coverage by plaque of the polished surface and the SLA surfaces were detected.[39] It is questionable if the postulation for smooth implant surfaces ($R_a < 0.088$ μm) for transgingival implant components[40] is still of concern. Although the mod MA surfaces used in the present study (S_a: 0.186 μm) and the mod MA surface used in the study of Schwarz and colleagues[39] with an R_a of 0.83 μm, both being at the maximum limit respectively being higher, both surfaces showed less plaque formation than all other groups even in comparison to smoother surfaces, the M in the present study (S_a: 0.069 μm), or the polished surface (R_a: 0.04 μm) from the study of Schwarz and colleagues[39] However, it has to be declared that comparability of published surface topography values is limited as characterization methods are not accurately described. Additionally, it has to be pointed out that different methods of measurement can lead to varying results.[41] The results of this study indicate that the roughness seems not to be the sole factor influencing quantity and velocity of biofilm formation. Rather, the interaction of multiple factors of surface properties seems to promote or inhibit the plaque accumulation. The initial plaque formation on the mod MA surface seems to be slower, being associated with a slower maturation of plaque. Because the removal of immature plaque is easier than the elimination of mineralized, mature plaque,[23] the findings of the present study could indicate an advantage in preventing peri-implant diseases. Because of the slower maturation of plaque, it should be easier for the patients to remove

the less and immature plaque from the mod MA surface in comparison with the established M or SLA surfaces. To our knowledge, this study is the first study using EDX analysis for investigation of plaque-settled implant surfaces. The results of EDX examination proved the results of erythrosine staining and histomorphometrical analysis. The detection of carbon in the 48-hour groups reflects the existence of organic components on the settled surfaces. The major benefit of EDX analysis in such investigations could be the detection of surface alterations resulting from surface treatment during mechanical or chemical plaque removal procedures. The investigation of cell attachment of HGF to the mod MA surface could not expose any disadvantage of clinical relevance in comparison to the SLA surface.

Within the limits of the present study, it might be concluded that the mod MA surface provides good conditions for fibroblast attachment. Also, the velocity of initial biofim formation was found to be significantly slower than on SLA or M surfaces. The combination of the high biocompatibility of the mod MA surface and the advantage of slower and less plaque formation in comparison to the established M could widen the time frame of undisturbed initial transgingival healing. More studies are needed to verify the findings of this study that could indicate that removal of plaque could be easier for patients, thus reducing the risk of peri-implant diseases like peri-implant mucositis or peri-implantitis.

REFERENCES

1. Klokkevold PR, Newman MG. Current status of dental implants: a periodontal prospective. J Oral Maxillo Implants 2000; 15:56–65.
2. Renvert S, Polyzois I, Maguire R. Re-osseointegration on previously contaminated surfaces: a systematic review. Clin Oral Implants Res 2009; 20(Suppl 4):216–227.
3. Zitzmann NU, Berglundh T. Definition and prevalence of peri-implant diseases. J Clin Periodontol 2008; 35(Suppl 8):286–291.
4. Karoussis IK, Muller S, Salvi GE, Heitz-Mayfield LJ, Bragger U, Lang NP. Association between periodontal and peri-implant conditions: a 10-year prospective study. Clin Oral Implants Res 2004; 15:1–7.
5. Berglundh T, Gotfredsen K, Zitzmann NU, Lang NP, Lindhe J. Spontaneous progression of ligature induced peri-implantitis at implants with different surface roughness: an experimental study in dogs. Clin Oral Implants Res 2007; 18:655–661.
6. Heitz-Mayfield LJ. Peri-implant diseases: diagnosis and risk indicators. J Clin Periodontol 2008; 35:292–304.
7. Schwarz F, Ferrari D, Popovski K, Hartig B, Becker J. Influence of different air-abrasive powders on cell viability at biologically contaminated titanium dental implants surfaces. J Biomed Mater Res B Appl Biomater 2009; 88:83–91.
8. Berglundh T, Lindhe J, Marinello C, Ericsson I, Liljenberg B. Soft tissue reaction to de novo plaque formation on implants and teeth. An experimental study in the dog. Clin Oral Implants Res 1992; 3:1–8.
9. Zitzmann NU, Berglundh T, Marinello CP, Lindhe J. Experimental peri-implant mucositis in man. J Clin Periodontol 2001; 28:517–523.
10. Quirynen M, De Soete M, van Steenberghe D. Infectious risks for oral implants: a review of the literature. Clin Oral Implants Res 2002; 13:1–19.
11. Leonhardt A, Renvert S, Dahlen G. Microbial findings at failing implants. Clin Oral Implants Res 1999; 10:339–345.
12. Shibli JA, Vitussi TR, Garcia RV, et al. Implant surface analysis and microbiologic evaluation of failed implants retrieved from smokers. J Oral Implantol 2007; 33:232–238.
13. Fürst M, Salvi GE, Lang NP, Persson GR. Bacterial colonization immediately after installation on oral titanium implants. Clin Oral Implants Res 2007; 18:501–508.
14. Salvi GE, Fürst MM, Lang NP, Persson GR. One-year bacterial colonization patterns of Staphylococcus aureus and other bacteria at implants and adjacent teeth. Clin Oral Implants Res 2008; 19:242–248.
15. Nakazato G, Tsuchiya H, Sato M, Yamauchi M. In vivo plaque formation on implant materials. Int J Oral Maxillofac Implants 1989; 4:321–326.
16. Lindhe J, Meyle J, Group D of European Workshop on Periodontology. Peri-implant diseases: consensus report of the Sixth European Workshop of Periodontology. J Clin Periodontol 2008; 35(Suppl 8):282–285.
17. Ruona K, Maxson BB, Syed S. In vitro adherence of bacteria to two implant materials. J Dent Res 1991; 70:477–483.
18. Rasperini G, Maglione M, Cocconcelli P, Simion M. In vivo early plaque formation on pure titanium and ceramic abutments: a comparative microbiological and SEM analysis. Clin Oral Implants Res 1998; 9:357–364.
19. Listgarten MA, Lang NP, Schroeder HE, Schroeder A. Periodontal tissues and their counterparts around endosseous implants. Clin Oral Implants Res 1991; 2:1–19.
20. Abrahamsson I, Berglundh T, Lindhe J. Soft tissue response to plaque formation at different implant systems. A comparative study in the dog. Clin Oral Implants Res 1998; 9: 73–79.
21. Listgarten MA, Lai CH. Ultrastructure of the intact interface between an endosseous epoxy resin dental implant and the host tissues. J Biol Buccale 1975; 3:13–28.
22. Buser D, Weber HP, Donath K, Fiorellini JP, Paquette DW, Williams RC. Soft tissue reactions to non-submerged

unloaded titanium implants in beagle dogs. J Periodontol 1992; 63:225–235.
23. Schupbach P, Hurzeler M, Grunder U. Implant-tissue interfaces following treatment of peri-implantitis using guided tissue regeneration: a light and electron microscopic study. Clin Oral Implants Res 1994; 5:55–65.
24. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol 1998; 25:134–144.
25. Socransky SS, Haffajee AD. Periodontal microbial ecology. Periodontol 2000 2005; 38:135–187.
26. Hahn R, Netuschil L, Löst C. Initiale Plaquebesiedlung auf keramischen Restaurationsmaterialien. Dtsch Zahnarztl Z 1992; 47:330–334.
27. Schwarz F, Papanicolau P, Rothamel D, Beck B, Herten M, Becker J. Influence of plaque biofilm removal on reestablishment of the biocompatibility of contaminated titanium surfaces. J Biomed Mater Res A 2006; 77:437–444.
28. Schwarz F, Sculean A, Romanos G, et al. Influence of different treatment approaches on the removal of early plaque biofilms and the viability of SAOS2 osteoblasts grown on titanium implants. Clin Oral Investig 2005; 9:111–117.
29. Glantz PO. On wettability and adhesiveness. Odontol Revy 1969; 20(Suppl 17):1–132.
30. Quirynen M, Marechal M, Busscher HJ, et al. The influence of surface free-energy on planimetric plaque growth in man. J Dent Res 1989; 68:796–799.
31. Buser D, Broggini N, Wieland M, et al. Enhanced bone apposition to a chemically modified SLA titanium surface. J Dent Res 2004; 83:529–533.
32. Millard MM, Foy CD, Coradetti CA, Reinsel MD. X-ray photoelectron spectroscopy surface analysis of aluminum ion stress in barley roots. Plant Physiol 1990; 93:578–583.
33. Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Darius PL, van Steenberghe D. The influence of surface free energy and surface roughness on early plaque formation. An in vivo study in man. J Clin Periodontol 1990; 17:138–144.
34. Pier-Francesco A, Adams RJ, Waters MG, Williams DW. Titanium surface modification and its effect on the adherence of Porphyromonas gingivalis: an in vitro study. Clin Oral Implants Res 2006; 17:633–637.
35. Teughels W, Van AN, Sliepen I, Quirynen M. Effect of material characteristics and/or surface topography on biofilmdevelopment. Clin Oral Implants Res 2006; 17(Suppl 2):68–81.
36. Bollen CM, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature. Dent Mater 1997; 13:258–269.
37. Quirynen M, van der Mei HC, Bollen CM, et al. An in vivo study of the influence of the surface roughness of implants on the microbiology of supra- and subgingival plaque. J Dent Res 1993; 72:1304–1309.
38. Zitzmann NU, Abrahamsson I, Berglundh T, Lindhe J. Soft tissue reactions to plaque formation at implant abutments with different surface topography. An experimental study in dogs. J Clin Periodontol 2002; 29:456–461.
39. Schwarz F, Sculean A, Wieland M, et al. Effects of hydrophilicity and microtopography of titanium implant surfaces on Initial Supragingival Plaque Biofilm Formation. A Pilot Study. Mund Kiefer Gesichtschir 2007; 11:333–338.
40. Rimondini L, Fare S, Brambilla E, et al. The effect of surface roughness on early in vivo plaque colonization on titanium. J Periodontol 1997; 68:556–562.
41. Wennerberg A, Albrektsson T. Effects of titanium surface topography on bone integration: a systematic review. Clin Oral Implants Res 2009; 20(Suppl 4):172–184.