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Plaque formation on surface modified dental implants

An *in vitro* study

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Abstract: Bacterial adhesion on titanium implant surfaces has a strong influence on healing and long-term outcome of dental implants. Parameters like surface roughness and chemical composition of the implant surface were found to have a significant impact on plaque formation. The purpose of this study was to evaluate the influence of two physical hard coatings on bacterial adhesion in comparison with control surfaces of equivalent roughness. Two members of the oral microflora, *Streptococcus mutans* and *Streptococcus sanguis* were used. Commercially pure titanium discs were modified using four different surface treatments: physical vapour deposition (PVD) with either titanium nitride (TiN) or zirconium nitride (ZrN), thermal oxidation and structuring with laser radiation. Polished titanium surfaces were used as controls. Surface topography was examined by SEM and estimation of surface roughness was done using a contact stylus profilometer. Contact angle measurements were carried out to calculate surface energy. Titanium discs were incubated in the respective bacterial cell suspension for one hour and single colonies formed by adhering bacteria were counted by fluorescence microscopy. Contact angle measurements showed no significant differences between the surface modifications. The surface roughness (R_a) of all surfaces examined was between 0.14 and 1.00 μm . A significant reduction of the number of adherent bacteria was observed on inherently stable titanium hard materials such as TiN and ZrN and thermally oxidated titanium surfaces compared to polished titanium. In conclusion, physical modification of titanium implant surfaces such as coating with TiN or ZrN may reduce bacterial adherence and hence improve clinical results.

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Long-term stability of dental implants depends on the integration of the artificial material into the surrounding bone and connective tissue. Problems in osseous healing of dental implants appear to be largely solved, whereas the sealing of the implant surface through soft tissue may be crucial for the therapeutic success (Berglundh et al. 1992; Abrahamsson et al. 1998). Bacterial adherence and colonization are considered to play a key role in the pathogenesis of infec-

tions related to biomaterials (Gristina 1987). Exposure of the implant in the oral cavity presents a unique surface that can interact with native host bacteria, leading to plaque formation.

The interaction of host flora with teeth involves a highly selective process related to specific interactions between tooth-bound salivary pellicles and bacterial surface adhesions (Gibbons & Van Houte 1980). Alterations in either salivary pellicles or the bacterial surface can

modify the initial bacterial attachment and therefore may alter the potential to develop plaque-derived periodontal disease (Absolom et al. 1987; Busscher et al. 1986; Pratt-Terpstra et al. 1991).

It has been reported that different implant materials promote selective adherence during early plaque formation (Ruona et al. 1991; Rasperini et al. 1998). An *in vivo* study exposing different implant materials on gingiva to the oral flora showed that streptococci were the predominant colonizing microorganisms and that the number of viable plaque-forming bacteria was dependent on material surface properties (Nakazato et al. 1989). On intra-oral hard tissues *Actinomyces* species and streptococci are considered early colonizers, preparing the environment for late colonizers that require more demanding growth conditions. Many of these bacteria like *Fusobacterium*, *Capnocytophaga* and *Prevotella* species that bind to streptococci are also known to be involved in periodontal infections. It is therefore most important to develop implant surfaces (around the transmucosal portion) that reduce the number of initially adhering bacteria, minimizing plaque formation and subsequent inflammation of the soft tissues.

Surface characteristics of implant materials appeared to influence plaque formation also *in vitro* (Wu-Yuan et al. 1995). Parameters like surface free energy and especially surface roughness were found to have significant impact on this process. Surface roughness was suggested to be more important than surface free energy (Nakazato et al. 1989; Quirynen et al. 1990; Quirynen et al. 1993). Therefore, an implant surface ideal to resist bacterial colonization should be mainly smooth to allow the formation of an epithelial seal that prevents plaque accumulation.

While a rough transmucosal part of an implant will enhance plaque formation, the bony and connective tissue interface requires a porous or microtextured surface to promote tissue ingrowth. In a clinical study on titanium abutments, it was concluded that a certain threshold roughness (around an R_a of 0.2 μm) might be most suitable to obtain a stable soft tissue sealing around transmucosal abutments. A titanium surface which is too smooth will therefore prevent cell

attachment. However, an increase in surface roughness of the transmucosal portion above the R_a of 0.2 μm will facilitate early plaque formation. A smoothening below a threshold R_a of 0.2 μm showed no further significant changes, either in the total amount or in the periodontal pathogenicity of adhering bacteria (Bollen et al. 1996; Quirynen et al. 1996). Therefore, an ideal transmucosal implant surface should not only minimize bacterial adhesion, but at the same time allow epithelial and connective tissue attachment.

In the past it was found that the biocompatibility of metal implants could be strongly enhanced by hard ceramic coatings separating body fluids from the metal (Sella et al. 1991). In several studies, hard coatings were used to reduce plaque formation on implants (Graf & Bärenklau 1993) or metal parts of partial dentures (Wisbey et al. 1987; Knotek & Löffler 1992; Gütschow 1994). Results of *in vivo* experiments using two different titanium hard coatings recommended the use of an osteophilic titanium-zirconium-oxide coating for the endosseous part of an implant. For the supragingival part a titanium-niobium-oxinitride coating was suggested which is extremely wear resistant and reduces bacterial adhesion (Thull 1993).

Properties of hard coatings, such as titanium nitride (TiN), are presently in the focus of interest, particularly with respect to their performance on tools for cutting, punching or shaping, as well as on machine parts and decorative coatings on consumer goods. Coating of metallic dental prostheses and instruments with TiN is applied to improve corrosion resistance and shear strength. Furthermore, it is preferred because of its golden color (Jehn & Baumgärtner 1992; Griepentrog et al. 1995). The use of an appropriate coating technique allows universal control of the required surface properties, resulting in reproducible thin hard coatings on almost any part of an implant. Sputtering can be used to produce dense, homogeneous corrosion-protective TiN coatings free of pinholes and cracks, if the sputtering parameters are optimized (Jehn & Baumgärtner 1992; Milosev & Navinsek 1994). The physical vapour deposition (PVD) process can also be used to deposit multilayer coatings

(Knotek & Löffler 1992; Okuniya & Griepentrog 1999).

In summary, it appears that an optimization of implant surfaces is still necessary. It is therefore important to systematically evaluate the role of different surface properties (chemical composition as well as microstructure) and to assess the biological performance of different implant materials (Kasemo & Lausmaa 1988).

The aim of the present *in vitro* investigation was to compare bacterial colonization on 5 different implant surfaces with a similar surface roughness.

Material and methods

Preparation of surfaces

Commercially pure titanium discs (grade 2, Friadent GmbH, Mannheim, Germany) measuring 10 mm in diameter and 2 mm in thickness were hand finished by wet grinding to 1200 grit on SiC paper. A final treatment with 6 and 3 μm diamond suspension followed. Before coating, the substrates were cleaned with ethanol in an ultrasonic bath.

Discs were divided into 4 groups with different surface topographies: A. Physical vapour deposition (PVD coating) with either titanium nitride or B. zirconium nitride (ZrN). C. Discs were modified by thermal oxidation (TiO_2 , 700°C, 60 min), whereby an increased temperature resulted in an increased thickness of the oxide layer. D. Structuring with laser radiation (Nd-YAG Laser, pulse power: 50 W, pulse length: 8.4 ms; spot diameter: 0.8 mm; wavelength 1.06 μm ; Laser Star, BEGO, Bremen). Polished titanium discs were used as control.

PVD coating was carried out in a HTC 625 Multilab ABSTM coating system (HAUZER Techno Coating) with unbalanced magnetron sputtering using a pulsed bias voltage (60 V, source power: 6.5 kW; deposition rate: 2.5 $\mu\text{m}/\text{h}$; coil current: 5 A; coating temperature: 410°C). PVD coating was performed in the Federal Institute for Materials Research and Testing, BAM, Berlin, Germany. The medium thickness of the coatings was 1.8±0.2 μm (TiN) and 2.2±0.2 μm (ZrN). The coating did not change or cover the original texture of the surface. The critical load (at this load

the first coating failure takes place) to test adhesion strength of these coatings is greater than 50 N (scratch tester; Revetest from CSEM with a Rockwell C indenter and a diamond cone [tip radius = 0.2 mm]). The microhardness of a TiN coating on highly polished titanium surfaces is in the range of 23000 N/mm² to 24000 N/mm² (expressed as plastic hardness which is comparable with values for the Vickers hardness) and of 20000 N/mm² to 21000 N/mm² for the ZrN coating (Fischerscope H 100). The Young's modulus is in the range of 330 GPa to 360 GPa for both coatings.

In comparison to these values the microhardness (plastic hardness) of the polished (uncoated) titanium surface is 1800 to 2000 N/mm² and the value for the Young's modulus is between 100 and 104 GPa.

After surface preparation the discs were cleaned by ultrasonication in absolute ethanol for 15 min followed by several rinses with sterile sodium chloride and distilled water. Titanium discs were placed on the bottom of Nunc multiwell dishes (12-well plates) until the start of experiments.

Characterization of surface topography

For description of the surface topography a two-dimensional profilometer was used (2D contact stylus profilometer, Perthometer S6P, Perthen, Germany). Before modification of the topography, 10 polished discs were picked randomly and examined by scanning the surface profile over a distance of 1.25 mm total length (Putthamer 1983). Maximum roughness within the distance measured (R_{max}), mean value of five single measurements within the distance examined (R_z) and the arithmetical mean of surface roughness of every measurement within the total distance (roughness average = R_a) were assessed (in accordance with DIN EN ISO 3274, 4287, 4288).

Surface free energies (SFE) were determined from contact angle measurements with benzylethanol, diiodmethane, formamide and water as wetting agents by using the concept of polar and dispersion components (Busscher et al. 1984).

Scanning electron microscopy was used to examine the surface topography

of the modified titanium discs (Hitachi S 4100 with field emission gun).

Saliva coating of the modified titanium discs

Paraffin-stimulated saliva of healthy donors was used throughout this study. Saliva was collected in chilled test-tubes, pooled, heated at 60°C for 30 min (in a water bath) to inactivate endogenous enzymes and then centrifuged (20,000×g) for 25 min at 4°C. The supernatant was saved and stored in 10 ml aliquots at -20°C. Immediately before starting the bacterial culture, the amount of saliva required was thawed at 37°C for one hour. Fifty percent of the titanium discs (from all surface modifications) were coated by incubation with the saliva for 1 h at 37°C followed by a short rinse with distilled water.

Bacterial cultures

Pure cultures of bacterial strains were prepared and frozen in aliquots as stocks. From frozen stock, cultures of *Streptococcus sanguis* (clinical isolate, Institut für Mikrobiologie und Hygiene, Universitätsklinikum Charité) and *Streptococcus mutans* (DSM 10664, Deutsche Sammlung Mikroorganismen) were plated onto Columbia blood agar plates (Columbia blood agar base, Oxoid CM 331 containing 5% sterile defibrinated blood) and grown aerobically at 35°C in an atmosphere containing 5% CO₂.

For further cultivation both bacterial strains collected from these plates were inoculated into tryptone soya broth (Oxoid CM 129) grown to reach the late stationary phase and harvested by centrifugation at 2,000×g for 15 min. The bacterial pellet was washed twice in 3 ml of 50 mmol/l Tris-HCL buffer (pH 7.2). Bacteria were then resuspended in the same buffer and the concentration was adjusted to a density of 1.5×10^7 cells/ml. Immediately before starting the experiments, the bacterial suspension was subjected to low-intensity ultrasonic radiation for 40 s on crushed ice to get single cells or pairs.

Titanium discs were incubated in 5 ml of a suspension containing 1.5×10^7 cells/ml of *S. mutans* or *S. sanguis*, re-

spectively (gentle rotation in a wet chamber for 1 h at 37°C).

Experiments with modified titanium surfaces were repeated three times using 3 discs for each run with and without saliva coating. After 1 h of incubation, discs were removed and rinsed 6 times each with 1 ml distilled water. Bacterial microcolonies were fixed in 2.5% glutaraldehyde at 4°C for 30 min followed by an additional fixation step in 1% Acridine Orange at room temperature for 30 min. Subsequently, discs were rinsed with distilled water to remove excess dye.

Quantitative analysis was performed using a fluorescence microscope (Olympus BH 2-RFCA ×1.25). Samples were examined at 400-fold magnification. The number of microcolonies was determined in five randomly selected representative fields on each disc. This part of the study was performed as a blinded investigation carried out by a person not aware of the experimental conditions.

Statistical analysis

Non-parametric tests, the Kruskal-Wallis test and the Wilcoxon-Mann-Whitney U-test, were used for comparisons between the different surfaces. The *P*-values have been corrected for multiple testing with the Bonferroni method. *P*-values below 0.05 were considered statistically significant. The analyses were performed using SPSS for Windows.

Results

Material surface analysis

Surface topographical description

The R_a (arithmetical mean of surface roughness of every measurement within the total distance = roughness average), R_{max} (maximum roughness within the distance measured) and R_z values (mean value of five single measurements within the distance examined) for surface roughness evaluated with a 2D contact stylus profilometer are shown in Table 1. Except for the laser modified surface, the R_a values were between 0.14 and 0.20 μm indicating a comparable rough structure of the surfaces. Laser radiation resulted in melting of the

Table 1. Description of the surface topography using a 2D contact stylus profilometer (mean values of R_a , R_{max} and R_z in μm (\pm SD))

Surface	R_a [μm] \pm SD	R_{max} [μm] \pm SD	R_z [μm] \pm SD	n (discs)
Ti polished	0.14 \pm 0.01	1.69 \pm 0.21	1.33 \pm 0.11	10
TiO ₂	0.19 \pm 0.03	1.78 \pm 0.39	1.38 \pm 0.23	3
Ti-TiN	0.19 \pm 0.03	1.82 \pm 0.24	1.43 \pm 0.18	3
Ti-ZrN	0.20 \pm 0.01	2.69 \pm 1.08	1.79 \pm 0.26	3
Ti-laser	1.00 \pm 0.04*	8.17 \pm 2.28	5.61 \pm 0.87	3

TiO₂=oxidated titanium surface
Ti-TiN=titanium coated with titanium nitride
Ti-ZrN=titanium coated with zirconium nitride
*= p <0.004

Table 2. Results of contact angle measurements using the sessile drop technique. The surface energy is shown with disperse (d) and polar (p) parts in [mN/m]. N=5 discs examined

Titanium discs n=5	Surface energy [mN/m]	
	disperse (d)	polar (p)
Ti polished	35.6 \pm 1.1=33.2 \pm 0.4 (d) + 2.4 \pm 0.7 (p)	
Ti-TiN	38.7 \pm 1.2=35.2 \pm 0.5 (d) + 3.5 \pm 0.7 (p)	
Ti-ZrN	34.3 \pm 1.2=33.3 \pm 0.5 (d) + 1.0 \pm 0.7 (p)	
Ti-Laser	35.3 \pm 1.3=34.0 \pm 0.5 (d) + 1.3 \pm 0.7 (p)	

Ti-TiN=titanium coated with titanium nitride
Ti-ZrN=titanium coated with zirconium nitride

upper surface layer followed by recrystallization and solidification. The resulting R_a value for this much rougher surface is $1 \mu\text{m}$. However, sputtering of a polished surface with nitride or thermal oxidation did not alter surface roughness.

Contact angle measurements

Values for surface free energy (SFE) were assessed by the sessile drop technique. Polished titanium, Ti-TiN, Ti-ZrN and laser-altered titanium surfaces did not show any significant difference. Values for SFE varied between 34.3 mN/m and 38.7 mN/m (Table 2). Due to drop formation it was not possible to use this technique for the TiO₂-modified surface.

Scanning electron microscopy

Micrographs of each surface modification are shown in Fig. 1(a–e). Except for the laser altered surface, all other surfaces revealed a similar picture with some unevenly distributed microgrooves showing occasional scratching and pitting. PVD-coating with TiN (Fig. 1b) or ZrN (Fig. 1c) or thermal oxidation of a

polished titanium surface (Fig. 1d) did not change or cover the original texture of the surface, instead the PVD layers showed oriented crystal growth and followed the underlying surface structure. The resulting medium thickness of the coatings was about $2 \mu\text{m}$. The irregular laser structured surfaces exhibited metal grains and lines that appeared after melting of the upper layer of the surface following recrystallization and solidification (Fig. 1e).

Bacterial cultures

Kinetics of bacterial colonization

Preliminary experiments were carried out to analyze the time course of bacterial colonization. *S. sanguis* and *S. mutans* were cultivated on titanium surfaces either untreated or coated with saliva for a period of 10, 30, 60 or 120 min. Under both conditions bacterial colonization followed a linear kinetic over a period of 2 h. From the kinetic data it was decided to count bacterial colonies after 60 min, a time within the linear range. On saliva uncoated discs, more single colonies of *S.*

mutans than of *S. sanguis* were observed. Saliva coating seemed to reduce the number of bacterial colonies favoring the colonization of *S. sanguis* as compared to *S. mutans*.

Bacterial colonization on different titanium surfaces

As compared to all other surfaces, the highest number of bacterial colonies was observed on polished titanium surfaces. However, while for *S. sanguis*, differences between polished Ti and Ti-laser surfaces were not significant, a significantly lower number of *S. sanguis* colonies was seen on Ti-ZrN-, Ti-TiN-coated and TiO₂-discs (Fig. 2A).

The results for the colonization of *S. mutans* on titanium surfaces are represented in Fig. 2B. The number of *S. mutans* colonies on modified titanium discs exceeded that of *S. sanguis* colonies. The highest number of *S. mutans* colonies was registered on polished titanium. A significantly lower number of colonies was counted on Ti-ZrN, TiO₂ and Ti-TiN. Differences for Ti-laser and polished Ti discs were not significant.

The same experiments done with saliva precoated titanium discs showed a distinctly lower number of bacterial colonies as compared to the uncoated surfaces (Table 3). The numbers of *S. mutans* and *S. sanguis* colonies on Ti-TiN, Ti-ZrN and the laser modified surface were lower when compared to polished titanium and TiO₂.

Bacterial counts for *S. sanguis* on Ti-ZrN and the laser modified surface were significantly lower when compared to polished titanium. However, high variation of experimental results could be noticed for all surfaces.

Discussion

The excellent biocompatibility of titanium surfaces mainly results from its surface properties. While problems in osseous healing of implants appear to be largely solved, biomolecular pellicle adsorption and subsequent accumulation and metabolism of bacteria on these surfaces is still a main reason for the induction of inflammatory processes. Many *in vitro* and *in vivo* studies showed that parameters like surface free energy and

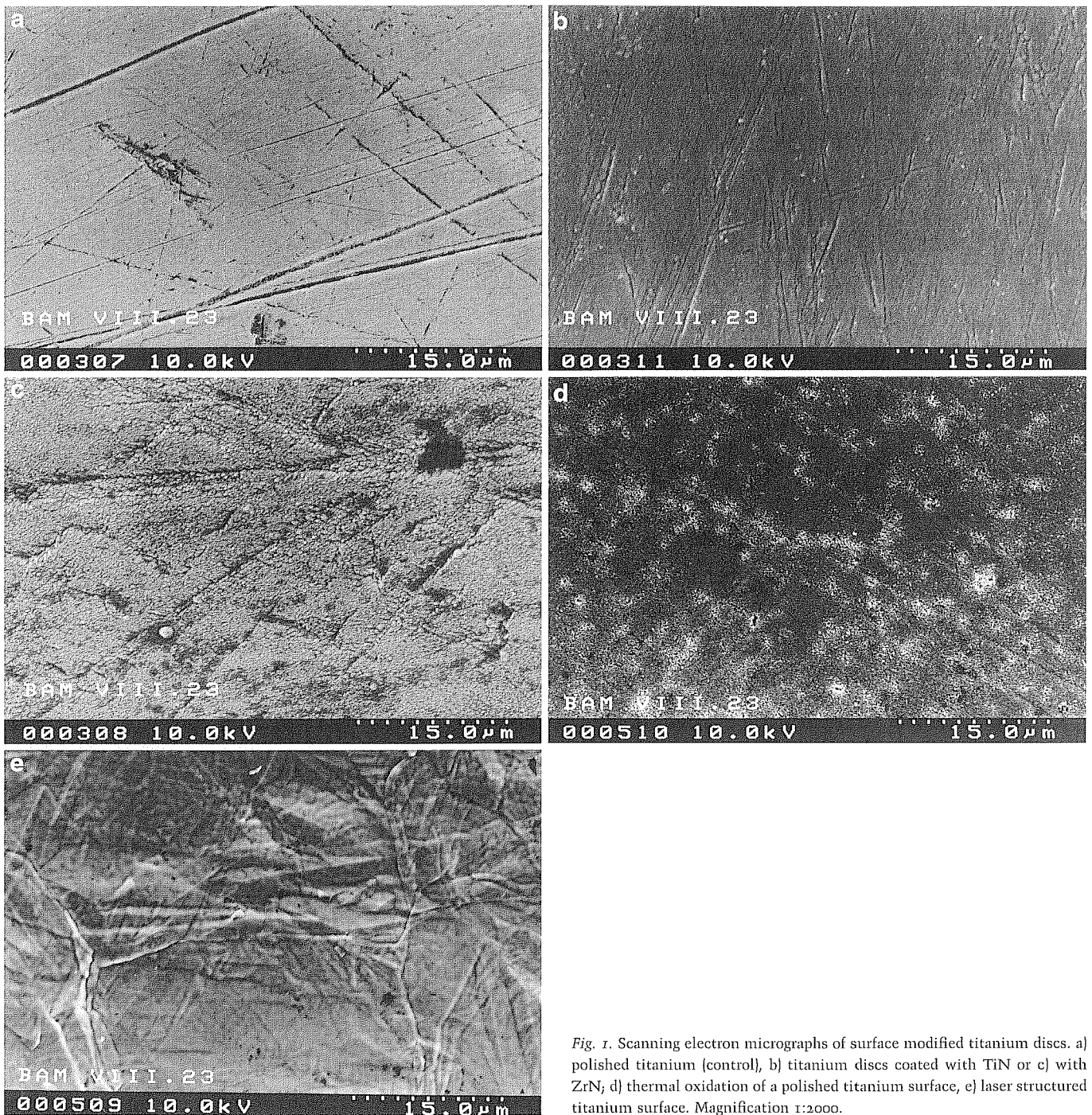


Fig. 1. Scanning electron micrographs of surface modified titanium discs. a) polished titanium [control], b) titanium discs coated with TiN or c) with ZrN; d) thermal oxidation of a polished titanium surface, e) laser structured titanium surface. Magnification 1:2000.

especially surface roughness have a significant impact on *de novo* plaque formation (Quirynen et al. 1990, 1993; Wu-Yuan et al. 1995; Rimondini et al. 1997).

Within this study, the influence of various surface modifications of titanium discs on the colonization of two plaque building bacterial species (*S. sanguis*, *S. mutans*) was tested *in vitro*. Except for the R_a of the laser structured

surface that could not be reduced below $1 \mu\text{m}$, other treatments had little effect on the surface roughness. Determination of surface roughness for Ti-TiN, Ti-ZrN and TiO_2 discs revealed similar low R_a values of around $0.2 \mu\text{m}$. Previous studies have demonstrated that a reduction of surface roughness is accompanied with reduced plaque formation until a threshold R_a of $0.2 \mu\text{m}$ (Quirynen et al.

1996; Bollen et al. 1996). In the present study values for surface free energy did not show significant differences between surface modifications (Table 2). In clinical applications the oral environment may alter surface free energies as shown by van Dijk et al. (1987). Salivary protein adsorption reduced differences originally present in surface free energies. Therefore, the extrapolation of our findings

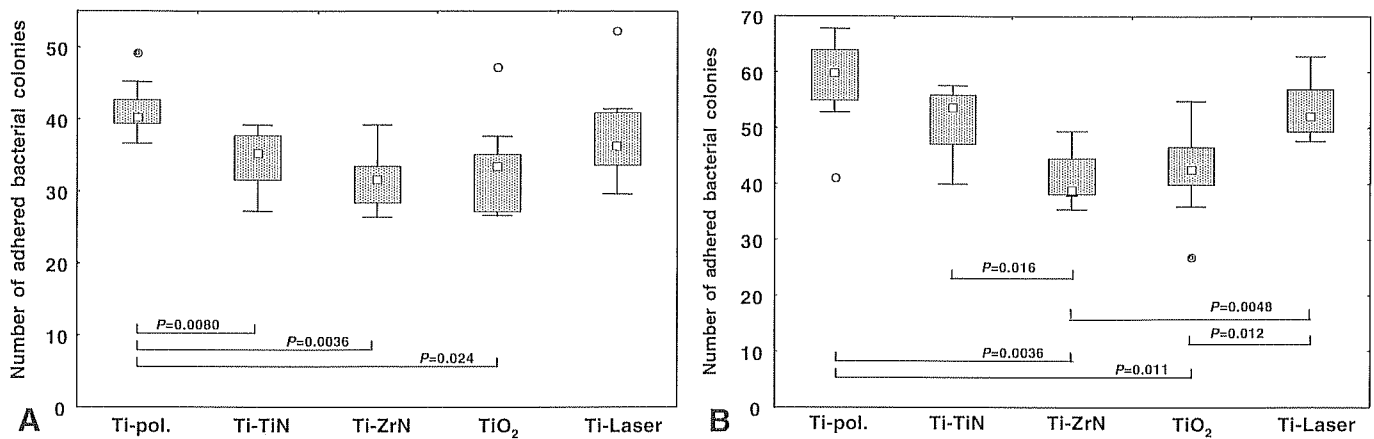


Fig. 2. A. Box-plots for the number of bacterial colonies (*S. sanguis*) on modified titanium surfaces after 1 h incubation. Mean number of colonies (white square in the box) and the 25th/75th percentiles as well as the non-outlier maximum and minimum are shown in box-plots. Outliers are denoted by circles. Ti pol.=polished titanium, Ti-TiN=titanium coated with titanium

nitride, Ti-ZrN=titanium coated with zirconium nitride, TiO₂=oxidized titanium surface. Statistically significant differences are indicated ($P < 0.05$). B. Box-plots for the number of adhering bacterial colonies (*S. mutans*) on modified titanium surfaces after 1 h incubation. Mean number of colonies (white square in the box) and the 25th/75th percentiles as well as the non-outlier

maximum and minimum are shown in box-plots. Outliers are denoted by circles. Ti pol.=polished titanium, Ti-TiN=titanium coated with titanium nitride, Ti-ZrN=titanium coated with zirconium nitride, TiO₂=oxidized titanium surface. Statistically significant differences are indicated ($P < 0.05$).

into the clinical situation requires experimental validation. In addition, the colonization pattern of other oral bacteria might be different.

As compared with polished titanium, the number of adhering bacterial colonies was significantly reduced on titanium surfaces coated with inherently stable titanium hard materials such as zirconium nitride (ZrN) although roughness was not altered. Similar results were achieved by TiN coating and by a thermic oxidation. In contrast, laser radiation accompanied by an increase in surface roughness did not result in a significant reduction of bacterial colonization.

It appears that in our study the reduced number of adherent bacteria on TiN and ZrN coated titanium discs is not an effect of surface roughness or surface free energy. The hard coatings seem to have substantial influence on bacterial colonization. Several studies have shown that titanium surfaces are very reactive (Kasemo & Lausmaa 1988; Lausmaa 1991). Titanium is covered by a surface oxide approximately 2 to 5 nm thick. This oxide (mainly titanium dioxide) has amphoteric character and supports cationic and anionic exchange adsorption. At the interface between titanium oxides and saliva covalent, ionic

or hydrogen bonding can contribute to the adsorption of biopolymer molecules, thus providing a very reactive surface. The hard coatings used in our study (Ti-TiN and Ti-ZrN) seem to reduce the accumulation of plaque by masking the underlying more reactive titanium surface (independent of surface roughness).

It was already suggested earlier by smaller clinical studies that physical modifications (such as hard coatings) may have an influence on bacterial adherence (Graf & Bärenklau 1993; Thull 1993). Another clinical study demonstrated that coating of the metal parts of partial dentures with TiN resulted in a reduction of plaque formation

(Gütschow 1994). Yet another experimental clinical study evaluating plaque adhesion to titanium, ceramics and prosthetic materials showed that the highest plaque accumulation was found on polished titanium whereas the accumulation on zirconium oxide ceramic and aluminium oxide ceramic was almost fifty percent lower (Krämer et al. 1989). It appears that bacterial adherence on ceramic material or coatings with ceramic-like character (as hard coatings) is lower as compared to titanium alloys (Siegrist et al. 1991).

Within our study it was shown that reproducible surface coatings may have indeed a strong effect on bacterial colon-

Table 3. Counts of adhering bacterial colonies on saliva-coated titanium discs

	<i>S. mutans</i> [median]	Percentiles [25 th /75 th]	<i>S. sanguis</i> [median]	Percentiles [25 th /75 th]
Ti polished	7.8 (n=9)	2.6-8.2	17.6 (n=9)	11.4-19.6
Ti-TiN	3.4 (n=9)	3.0-10.8	7.4 (n=6)	2.0-8.8
Ti-ZrN	3.6 (n=9)	0.8-11.2	5.5 (n=6)*	2.4-7.0
TiO ₂	9.6 (n=9)	7.8-24.8	27.4 (n=9)	19.0-30.6
Ti-laser	2.8 (n=9)	1.8-14.8	2.7 (n=8) [#]	0.4-5.6

Ti-TiN=titanium coated with titanium nitride
 Ti-ZrN=titanium coated with zirconium nitride
 TiO₂=oxidized titanium surface
 P-values for comparisons between polished titanium and modified surfaces:
 * $P = 0.05$; [#] $P = 0.01$

ization. ZrN in particular appears highly suitable to reduce plaque formation. The thermally oxidized titanium surface used in our study as another modification is probably the most cost-effective surface treatment. Thermic oxidation resulted in reduction of bacterial colonization, although less effective than coating with ZrN.

In a study using controlled electrochemical oxidation, it could be demonstrated that a thicker oxide layer on titanium – which is also the case after using thermal oxidation as in our study – seems to reduce plaque adhesion (Krämer et al. 1989). The reduction of oxygen gaps at the titanium surface resulting in a more apolar surface structure was discussed as a possible reason. In our study, determination of the surface free energy was not possible for the oxidated Ti surface due to technical reasons. Therefore, we cannot exclude an influence of SFE on bacterial adhesion.

Even though thermic oxidation is a cost-saving method and resulted in reduction of bacterial adherence, the surface softness facilitates surface roughening on abutments during oral hygiene measures (Fox et al. 1990; Speelman et al. 1992). However, the use of titanium hard coatings for implant abutments might prevent surface roughening during professional oral hygiene procedures. Due to the hardness of the coatings used and the multilayer technique of the sputter process it appears unlikely that prophylactic measures (e.g. the use of scalers) or chemicals (e.g. fluoride) could alter surface characteristics.

Results of experiments performed on modified titanium discs coated with saliva revealed that the number of adherent *S. mutans* was much lower than for *S. sanguis*, which is in contrast to the uncoated discs. Compared to the uncoated titanium discs, the number of adhered bacteria on all modified and saliva coated discs was distinctly lower for both bacterial strains. These results are in correspondence with observations made by Weerkamp et al. (1988), Pratt-Terpstra et al. (1991) and Christersson & Glantz (1992). Pellicle coating results in a general reduction in the number of adhering bacteria, irrespective of the substratum free energy. Adsorption of salivary components to a surface, the princi-

pal part in pellicle formation, is likely to be specific to that surface.

At the moment we can not fully explain this aspect of our results. More bacterial colonies were counted for *S. sanguis* than for *S. mutans*, which is in contrast to bacterial counts on uncoated surfaces. One explanation is that *S. sanguis* has a very hydrophobic surface and there are many molecules in saliva, and thus in the pellicle, that could serve as hydrophobic receptors (Cowan et al. 1986). A higher number of available binding sites might be the reason for these findings. In addition, surface free energy is altered by saliva coating (van Dijk et al. 1987). However, this effect needs further investigation.

The composition of a titanium pellicle differs from enamel pellicle in that cystatins and low-molecular weight mucin were not detected but, in contrast to the enamel pellicle, a high-molecular weight proline-rich glycoprotein may be a prominent component (Edgerton et al. 1996). In consequence, these differences in pellicle composition might explain significant differences in the initial adhesion rate of some specific bacteria to the surfaces (Wolinsky et al. 1989; Kohavi et al. 1995; Edgerton et al. 1996).

In conclusion, TiN and ZrN-coating of titanium surfaces resulted in a clear reduction of bacterial adherence regardless of whether the discs were coated with saliva or not. Their use as a coating for the part of an implant penetrating the soft tissue and as implant abutments might reduce plaque formation and in this way mucosal inflammation.

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Résumé

L'adhésion bactérienne sur les surfaces implantaires en titane a une influence importante sur la guérison et l'état à long terme des implants dentaires. Des paramètres comme la rugosité de surface et la composition chimique de la surface implantaire ont un impact significatif sur la formation de la plaque dentaire. Le but de l'étude présente a été d'évaluer l'influence de deux recouvrements durs sur l'adhésion bactérienne en les

comparant à des surfaces de contrôle de rugosité équivalente. Deux membres de la flore buccale, le *Streptococcus mutans* et la *Streptococcus sanguis* ont été utilisés. Des disques en titane pur ont été modifiés en utilisant quatre types de traitement de surface: un dépôt par vapeur physique avec soit du TiN ou du ZrN, oxydation thermique et structuration par radiation laser. Des surfaces en titane polies ont servi de contrôle. La topographie de surface a été examinée par MEB et l'estimation de la rugosité de surface a été effectuée en utilisant un profilomètre de contact. Des mesures d'angle de contact ont été relevées pour calculer l'énergie de surface. Les disques de titane ont été incubés dans leur suspension cellulaire bactérienne respective pour une heure et des colonies simples formées par des bactéries adhérentes ont été comptées sous microscope à fluorescence. Les mesures d'angle de contact n'ont montré aucune différence significative entre les modifications de surface. La rugosité de surface de toutes les surfaces examinées se situait entre 0.14 et 1.00 µm. Une réduction significative du nombre de bactéries adhérentes a été observée sur des matériaux durs en titane simple comme le nitrite de titane (TiN) et le nitrite de zirconium (ZrN) et les surfaces en titane oxydées par la chaleur comparées au titane poli. En conclusion, la modification physique de surfaces implantaires en titane par recouvrement de TiN ou de ZrN peut réduire l'adhésion bactérienne et donc améliorer les résultats cliniques.

Zusammenfassung

Die Adhäsion von Bakterien auf der Oberfläche von Titanimplantaten hat einen grossen Einfluss auf die Heilung und den Langzeiterfolg von Zahnimplantaten. Parameter wie Oberflächenrauheit und chemische Beschaffenheit der Implantatoberfläche scheinen einen signifikanten Einfluss auf die Plaquebildung zu haben. Das Ziel dieser Studie war es den Einfluss zweier physikalisch gesehen stabiler Hartstoffbeschichtungen auf die bakterielle Adhäsion mit einer Kontrolloberfläche von identischer Rauheit zu vergleichen. Für den Versuch wurden zwei Vertreter der oralen Mikroflora, *Streptococcus mutans* und *Streptococcus sanguis* verwendet. Im Handel erhältliche Reintitanscheiben wurden mit vier verschiedenen Oberflächenbehandlungen modifiziert: Die Bedampfung mit TiN oder ZrN (PVD), die thermische Oxidation und die Strukturveränderung der Oberfläche mit einem Hardlaser. Polierte Titanoberflächen dienten als Kontrolle. Die Oberflächentopographie wurde mit einem REM untersucht und gleichzeitig wurde die Oberflächenrauheit durch Abtasten mit einem Profilometer festgestellt. Ebenso erfolgten Berechnungen der Oberflächenenergie. Die Titanplättchen wurden in der jeweiligen Bakterienzellsuspension für eine Stunde inkubiert und die einzelnen durch adhären- deren Bakterien gebildeten Zellkolonien mittels Fluoreszenzmikroskopie ausgezählt. Messungen der Kontaktwinkel zeigten zwischen den verschiedenen Oberflächenmodifikationen keine signifikanten Unterschiede. Die Oberflächenrauheit (R_a) aller untersuchten Oberflächen bewegte sich zwischen 0.14 und 1.00 µm. Eine signifikant geringere Anzahl adhären- derer Bakterien, verglichen mit polierten Titanoberflächen, wurde auf Hartstoffschichten wie dem Titanitrid [TiN], und dem Zirkoniumnitrid [ZrN], sowie den thermisch oxidierten Titanoberflächen

ausgezählt. Zusammenfassend kann man sagen, dass eine physikalische Veränderung der Titanimplantatoberfläche wie zum Beispiel durch Beschichtung mit TiN oder ZrN eine verminderte bakterielle Adhärenz und somit auch eine Verbesserung der klinischen Resultate bewirken kann.

Resumen

La adhesión bacteriana en las superficies de los implantes de titanio tiene una fuerte influencia en la cicatrización y los resultados a largo plazo de los implantes dentales. Se encontró que parámetros como rugosidad de la superficie y composición química de la superficie del implante tenían un impacto significativo en la formación de placa. El propósito de este estudio fue evaluar la influencia de dos cubiertas físicamente duras en la adhesión bacteriana en comparación con superficies de control de rugosidad equivalente. Se usaron dos miembros de la microflora oral, el *Streptococo mutans* y *Streptococo sanguis*. Se modificaron discos comerciales de titanio puro usando

cuatro tratamientos de superficie: se realizó deposición física de vapor (PVD) con TiN o ZrN, oxidación térmica y estructuración con radiación láser. Se usaron superficies de titanio pulidas como control. La topografía de la superficie se examinó con SEM y la estimación de la rugosidad se realizó usando un profilómetro de aguja. Se llevaron a cabo mediciones de los ángulos para calcular la energía de superficie. Se incubaron los discos de titanio en las suspensiones de la células bacterianas respectivas durante una hora y se contaron las colonias bacterianas formadas por adhesión de bacterias con microscopio de fluorescencia. Las mediciones de los ángulos de contacto no mostraron diferencias significativas entre las modificaciones de superficie. La rugosidad de la superficie (R_a) de todas las superficies examinadas estuvo entre 0.14 y 1.00 μm . Se observó una reducción significativa del número de bacterias adherentes en materiales duros de titanio estable tales como nitrato de titanio (TiN) y nitrato de circonio (ZrN) y superficies de titanio oxidadas térmicamente comparadas con titanio pulido. En conclusión la modificación física de las superficies de implantes de titanio como cubiertas con TiN o ZrN pueden reducir la adherencia bacteriana y por ello mejorar los resultados clínicos.

要旨

チタン製インプラント表面の細菌付着は、歯牙インプラントの治療及び長期結果に強い影響を及ぼす。インプラント表面の粗性や化学的組成などのパラメータは、プラーク形成に有意の影響を及ぼすとされている。本研究は、同等の粗さを持つ対照表面と比較して、2種類の物理的ハード・コーティングが細菌付着に及ぼす影響を評価した。口腔菌叢の2つの細菌、*Streptococcus mutans* (S. mutans)と *Streptococcus sanguis* (S. sanguis)を用いた。

4つの異なる表面処理によって商用純チタン製ディスクを修飾した：すなわち TiN または ZrN による物理的蒸着 (PVD)、熱酸化及びレーザー照射を行った。チタン研磨表面を対照として用いた。表面の形状を SEM によって分析し、表面の粗性を、接触描記針プロフィロメータを用いて測定した。接触角度測定を行って、表面エネルギーを計算した。チタン製ディスクを個々の細菌細胞懸濁液中で1時間培養し、付着細菌によって形成された単独コロニーを蛍光顕微鏡によって数えた。接触角度の測定値には、表面性状間で有意差はなかった。分析した全ての表面の粗性 (R_a) は、0.14 μm から 1.00 μm の間であった。研磨チタンと比較して、窒化チタン (Tin) や窒化ジルコニウム (ZrN) のような化学的に安定したチタン硬材料及び熱酸化したチタン表面上では、付着細菌数の有意な減少が観察された。結論として、TiN や ZrN のコーティングなどのチタン製インプラント表面の物理的修飾は、細菌付着を減少し、さらには臨床結果を改善する可能性がある。

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