Fibroblast growth on surface-modified dental implants: An *in vitro* study

Birte Groessner-Schreiber,[1] Anja Neubert,[2] Wolf-Dieter Müller,[2] Michael Hopp,[2] Michael Griepentrog,[3] Klaus-Peter Lange[2]

[1]*University of Kiel, School of Dental Medicine, Clinic for Restorative Dentistry and Periodontology, Kiel, Germany*
[2]*Humboldt-University of Berlin (Charité), School of Dental Medicine, Clinic for Prosthodontics, Berlin, Germany*
[3]*BAM Federal Institute for Materials Research and Testing, Berlin, Germany*

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Abstract: A major consideration in designing dental implants is the creation of a surface that provides strong attachment between the implant and bone, connective tissue, or epithelium. In addition, it is important to inhibit the adherence of oral bacteria on titanium surfaces exposed to the oral cavity to maintain plaque-free implants. Previous *in vitro* studies have shown that titanium implant surfaces coated with titanium nitride (TiN) reduced bacterial colonization compared to other clinically used implant surfaces. The aim of the present study was to examine the support of fibroblast growth by a TiN surface that has antimicrobial characteristics. Mouse fibroblasts were cultured on smooth titanium discs that were either magnetron-sputtered with a thin layer of titanium nitride, thermal oxidized, or modified with laser radiation (using a Nd-YAG laser). The resulting surface topography was examined by scanning electron microscopy (SEM), and surface roughness was estimated using a two-dimensional contact stylus profilometer. A protein assay (BCA assay) and a colorimetric assay to examine fibroblast metabolism (MTT) were used. Cellular morphology and cell spreading were analyzed using SEM and fluorescence microscopy. Fibroblasts on oxidized titanium surfaces showed a more spherical shape, whereas cells on laser-treated titanium and on TiN appeared intimately adherent to the surface. The MTT activity and total protein were significantly increased in fibroblasts cultured on titanium surfaces coated with TiN compared to all other surface modifications tested. This study suggests that a titanium nitride coating might be suitable to support tissue growth on implant surfaces. © 2003 Wiley Periodicals, Inc. J Biomed Mater Res 64A: 591–599, 2003

Key words: titanium implants; hard coatings; thermal oxidation; wettability; fibroblasts

INTRODUCTION

The long-term success of an implant is strongly dependent on good adhesion of the surrounding tissue to the biomaterial. Cellular behavior such as adhesion, morphologic change, functional alteration, and proliferation are greatly influenced by surface properties, including hydrophilicity, roughness, texture, and morphology. In extensive investigations of soft tissue responses to oral implant surfaces, it has been shown that surface treatment of implant materials significantly influences the attachment of oral fibroblasts as well as epithelial cells.[1–5] Furthermore, titanium implants exposed to the oral cavity require surface modification to inhibit the adherence of oral bacteria.[6] Additionally, these modified surfaces must resist mechanical wear such as brushing and professional cleaning.[7] It is therefore important to evaluate systematically the role of different surface properties (chemical composition as well as microstructure) and to assess the biological performance of different implant materials.[8]

In our previous study,[9] bacterial colonization was examined on titanium implant surfaces modified with a titanium nitride (TiN) or zirconium nitride (ZrN) coating, thermal oxidation (TiO_2), or laser radiation (Ti-laser). When compared with polished titanium (Ti-pol), the number of adhering bacterial colonies was significantly reduced on titanium surfaces coated with inherently stable titanium hard materials such as TiN and ZrN. Similar results were achieved by thermal oxidation. In contrast, laser radiation did not result in a significant reduction of bacterial colonization.

Correspondence to: B. Groessner-Schreiber, Klinik für Zahnerhaltungskunde und Parodontologie, Arnold-Heller-Str. 16, D-24105 Kiel, Germany; e-mail: groessner-schreiber@konspar.uni-kiel.de

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For an endosseous implant surface, Gaggl et al.[10] showed that laser treatment of implant surfaces led to an optimal surface structure free of contamination with foreign elements. Although a certain roughness was defined as a necessary surface requirement to promote soft tissue growth, the influence of laser-modified surfaces has to date remained largely unexplored.

Titanium normally is covered with a thin protective oxide film, which largely determines the surface properties of an implant. The oxide film is presumably responsible for the excellent biocompatiblity of titanium implants as a result of a low level of electronic conductivity,[11] a high corrosion resistance, and a thermodynamically stable state at physiological pH values.[12] Several studies have shown that different surface properties of titanium oxides have a significant effect on biological response.[4,13–16]

The properties of hard-coated surfaces such as titanium nitride are presently a focus of interest, particularly with respect to their performance on the surfaces of cutting, punching, or shaping tools, as well as on machine parts and decorative coatings on consumer goods. Metallic dental prostheses and instruments are coated with TiN to improve corrosion resistance and mechanical resistance. Furthermore, it is preferred because of its golden color.[17,18] 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. If the sputtering parameters are optimized, sputtering can be used to produce dense, homogeneous corrosion-protective TiN coatings free of pinholes and cracks.[17,19] The physical vapor deposition (PVD) process can also be used to deposit multilayer coatings.[20,21]

The aim of the present study was to analyze the influence of different surface treatments of titanium implant surfaces (polished titanium, a TiN coating, thermal oxidation, laser radiation) on fibroblast growth and protein synthesis. Furthermore, the effect of the different surface treatments on cellular morphology was evaluated, on the assumption that in each case a lengthening of the cells can be expected to provide a more favorable adhesion behavior than a spherical cell shape. Special attention was focused onto the TiN coating to determine whether this antimicrobial surface treatment is as good as or better than other clinically used implant surfaces for supporting fibroblast growth.

MATERIAL AND METHODS

Four different surfaces were selected. Based on antimicrobial properties, TiN and TiO_2 were compared against uncoated polished titanium.[9] The influence of laser-modified surfaces has to date remained largely unexplored. Similar to techniques using physical vapor deposition or thermal oxidation, the laser enables implant surface treatment without direct contact and therefore without contamination.[10] Therefore, we used laser radiation as another surface treatment.

Preparation of titanium 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 polishing with 6- and 3-μm diamond suspension followed. Before coating the substrates were cleaned with ethanol (70%) in an ultrasonic bath.

After polishing, three different surface treatments were performed: Titanium discs were either coated with TiN by (PVD coating) or modified by thermal oxidation or laser radiation. PVD coating, performed in the Federal Institute for Materials Research and Testing (BAM Berlin, Germany) was performed in a HTC 625 Multilab ABS™ coating system (HAUZER Techno Coating) with unbalanced magnetron sputtering as described previously.[9] The medium thickness of the TiN coating was 1.8 μm ± 0.2 μm. The coating did not change or cover the original texture of the surface.

Thermal oxidation of the titanium discs was performed in a laboratory furnace at 700°C. For solvent-cleaned machined titanium surfaces, the oxide thicknesses were typically 2–3 nm.[13] Thermal oxidation in air resulted in an oxide composition that was mainly TiO_2 (rutile). The thickness of the thermal oxides was dependent on the time and temperature of oxidation. For example, the oxide thickness of titanium samples oxidized for 60 min at temperatures between 100 and 450°C was in the range of 6–40 nm. The amount of surface contamination, predominantly hydrocarbons and sometimes traces of Cl, decreased with increasing oxidation temperature. Therefore, in our study, thermal oxidation of titanium discs was performed at 700°C for 60 min so that they could grow a thick oxide layer.

Structuring with laser radiation was performed using a Nd-YAG Laser (pulse power: 50 Joule, pulse length: 8.4 ms; spot diameter: 0.8 mm; wave length 1.064 μm; Laser Star, BEGO, Bremen). Polished titanium discs and tissue culture polystyrene plastic were used as controls.

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 (24-well plates) until the beginning of experiments. Surface topography and wettability were analyzed.

Characterization of surface topography

To describe surface topography, a two-dimensional profilometer was used (2D contact stylus profilometer, Perthometer S6P, Perthen, Germany). Before topography modi-

fication, 10 polished discs were picked randomly and examined by scanning the surface profile over a distance of 1.25 mm total length.[22] 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).

Scanning electron microscopy of modified surfaces

Scanning electron microscopy (SEM) was used to examine the surface topography of the modified titanium discs before incubation with fibroblasts (Hitachi S 4100 with field emission gun; BAM, Berlin).

Surface wettability

Surface free energies (SFE) were determined from contact angle measurements with benzylethanol, dijodmethane, formamide, and water as wetting agents by using the concept of polar and dispersion components.[23]

Cell culture

Mouse fibroblasts (balb/3T3; ATCC; in accordance with DIN EN ISO 10993-5) were used as a cell model to investigate the effects of surface and material variations on soft tissue response. Preliminary experiments with fibroblasts cultured on polished titanium discs at different time periods (3, 24, 48 h, 3, 4, 6, and 7 days) were carried out to determine both the best cell passage number to use and plating time. The growth curve revealed an exponential growth of the fibroblasts between day 3 and day 6 after plating, and the growth plateau was reached at day 7. Based on these results, days 4 and 5 were chosen to carry out the experiments.

The fibroblast cultures were maintained in Dulbecco's Modified Essential Medium (Sigma) supplemented with 10 m*M* Hepes (4-(2-Hydroxyethyl)-1-piperazinethansulfonic acid), 10% fetal bovine serum (Sigma), and 1% penicillin/streptomycin at 37°C in humidified air and 5% CO_2.

Cultures were subdivided by trypsinisation using Trypsin-EDTA solution (0.05% trypsin:0.1% EDTA). The modified titanium discs were placed in standard 24-well tissue culture plates (Nunc), and cells were then seeded. One milliliter of cell suspension containing 1×10^4 cells/mL was added to each well. As a control substrate for cell attachment and growth, fibroblasts were plated directly onto tissue culture polystyrene plastic. The culture medium was changed every 3 days and cells were incubated for 4 days at 37°C in humidified air and 5% CO_2. All subsequent experiments were performed in triplicate and were repeated three times.

Cell spreading and morphology

Cellular behavior of the fibroblasts (spreading and morphology) was analyzed using SEM (Cambridge 360 S, Leica, UK) and fluorescence microscopy.

SEM of fibroblasts

Cells were seeded at a density of 5×10^3 cells/mL onto modified titanium discs. At day 4, cells were rinsed 3 times with phosphate-buffered saline (PBS) and then fixed with 3% paraformaldehyde for 10 min. After a final rinse with PBS, a contrast treatment in 1% osmium tetroxide (Alfa) for 1 h was performed, followed by extensive rinsing in PBS and dehydration through a graded series of ethanol from 30%, 50%, 70%, 90%, and 100%. After air drying, surfaces were thinly sputter coated with platinum (Super-Cool SCD 050, Balzers; with 60 mA about 5 min). Fibroblasts on polished titanium discs and on glass cover slips were used as controls. Photographs of discs with different surfaces were obtained both before and after incubation with the fibroblasts.

Fluorescence microscopy of fibroblasts

Cells were seeded at a density of 1×10^4 cells/mL onto the test specimens. At day 4, fibroblasts were rinsed three times with PBS, and 500 μL of a fluorescein-diacetat solution was added (50 μL of 1 mg fluorescein-diacetat /1 mL acetone were subsequently diluted with 50 mL of PBS). After 10 min, five areas were chosen randomly, and cells were examined and photographed using a fluorescence microscope (Axioplan 2/Axiophot 2; Zeiss, Jena; Kodak Ektachrome P 1600 ASA). Fibroblasts on polished titanium discs and on tissue culture polystyrene plastic served as controls.

Growth and proliferation assays

Activity of the mitochondrial dehydrogenases (MTT test)

A quantitative colorimetric MTT test was performed after 4 days of culture to characterize cellular metabolism (vitality) and, by implication, proliferation.One-hundred ten microliters of MTT solution [5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide in PBS] was added to each well, and the cells were incubated at 37°C for 3 h. The medium was then removed, and the discs were placed in new wells. One milliliter of dimethylsulfoxide was added to each well, followed by a 10-min incubation at room temperature on a shaker. The optical density (OD) was measured at 560 nm with an ELISA Reader.

Protein determination (BCA protein assay)

The BCA protein assay estimates total protein in the cell layer. Estimation of protein content was performed after 4 days of culture using the Pierce BCA Protein Assay Kit (Pierce Chemicals). All discs were placed in new wells and washed extensively with PBS. One milliliter of working reagent was added to each well followed by 30 min of incubation. OD was measured at 540 nm.

Statistical analysis

The Student's *t* test was used for comparisons between the different surfaces. Data were found to follow a Gauss Normal distribution (Kolmogoroff-Smirnov test). The *p* values have been corrected for multiple testing with the Bonferoni method. Corrected *p* values below 0.05 were considered statistically significant. The analyses were performed using SPSS for Windows.

RESULTS

Material surface analysis

Surface topographical description

Results for surface roughness measurements evaluated with a 2D contact stylus profilometer are shown in Table I. With the exception of the laser-treated surface, R_a values were between 0.1 and 0.2 μm, indicating a comparable rough structure of the surfaces. Laser radiation resulted in melting of the upper surface layer followed by recrystallisation and solidification. The resulting R_a value for this rougher surface was approximately 1 μm. However, surface roughness was not altered by sputtering of a polished titanium surface with nitride or thermal oxidation.

SEM of modified surfaces

Micrographs of each surface modification are shown in Figure 1(a–d). With the exception of 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. 1(b)] or thermal oxidation of a polished titanium surface [Fig. 1(c)] did not change or cover the original texture of the surface; instead, the PVD layer showed crystallization and followed the underlying surface structure. The resulting medium thickness of the coating was approximately 2 μm. The irregular laser structured surfaces exhibited metal grains and lines that appeared after melting of the upper layer of the surface after recrystallisation and solidification [Fig. 1 (d)].

TABLE I
Description of the Surface Topography Using a 2D Contact Stylus Profilometer

Surface	R_a [μm] ± SD	R_{max} [μm] ± SD	R_z [μm] ± SD	*n* (discs)
Ti polished	0.14 ± 0.01	1.69 ± 0.21	1.33 ± 0.11	10
TiO_2	0.11 ± 0.02	0.98 ± 0.146	0.87 ± 0.07	3
TiN	0.19 ± 0.03	1.82 ± 0.24	1.43 ± 0.18	3
Ti-laser	1.00 ± 0.04*	8.17 ± 2.28*	5.61 ± 0.87*	3

Mean values of R_a, R_{max}, and R_z in μm (± SD).
TiO_2, oxidized titanium surface; TiN, titanium coated with titanium nitride.
*$p < 0.005$.

Surface wettability

Values for SFE were assessed using the sessile drop technique. Polished titanium, TiN, 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 II). Because of drop formation, it was not possible to use this technique for the TiO_2-modified surface.

SEM of fibroblasts

SEM showed mouse fibroblasts on day 4 after plating on four different titanium surfaces [Fig. (2a–d)]. Cells grown on all modified surfaces tested displayed good adhesion and spread well. However, cellular morphology varied. Fibroblasts cultured on polished titanium discs showed a wide variety of shapes. Spindle shaped, elongated cells as well as spread multipolar or round fibroblasts were seen. The majority of the fibroblasts on oxidized titanium surfaces showed a more spherical or triangular cell shape with fine and long filopodia. Filopodia of cells cultured on laser-treated titanium surfaces and on discs coated with TiN appeared to be stronger, cells were well spread and showed a polygonal cell shape. Many cells had a flat morphology and appeared intimately adherent to the surface. Cellular growth seemed to be denser, as on polished or oxidized titanium. After 4 days in culture, no clear orientation of cells could be noticed on any of the surfaces examined.

Fluorescence microscopy of fibroblasts

Cellular morphology and spreading revealed a similar behavior as observed with SEM. Fibroblasts on polished [Fig. 3(a)] and oxidized titanium discs were evenly distributed and showed a spherical or oval shape. Cells on laser-treated titanium surfaces revealed a similar shape. The discs coated with TiN showed both spindle-shaped fibroblasts with long filopodia or cells that were flatly stretched out. Many of them had strong filopodia [Fig. 3(b)]. Such processes are supposed to aid in anchoring the cell to the substrate during division.

Growth and proliferation assays

Activity of the mitochondrial dehydrogenases (MTT test)

The activity of mitochondrial dehydrogenases was significantly higher in fibroblasts cultured on discs

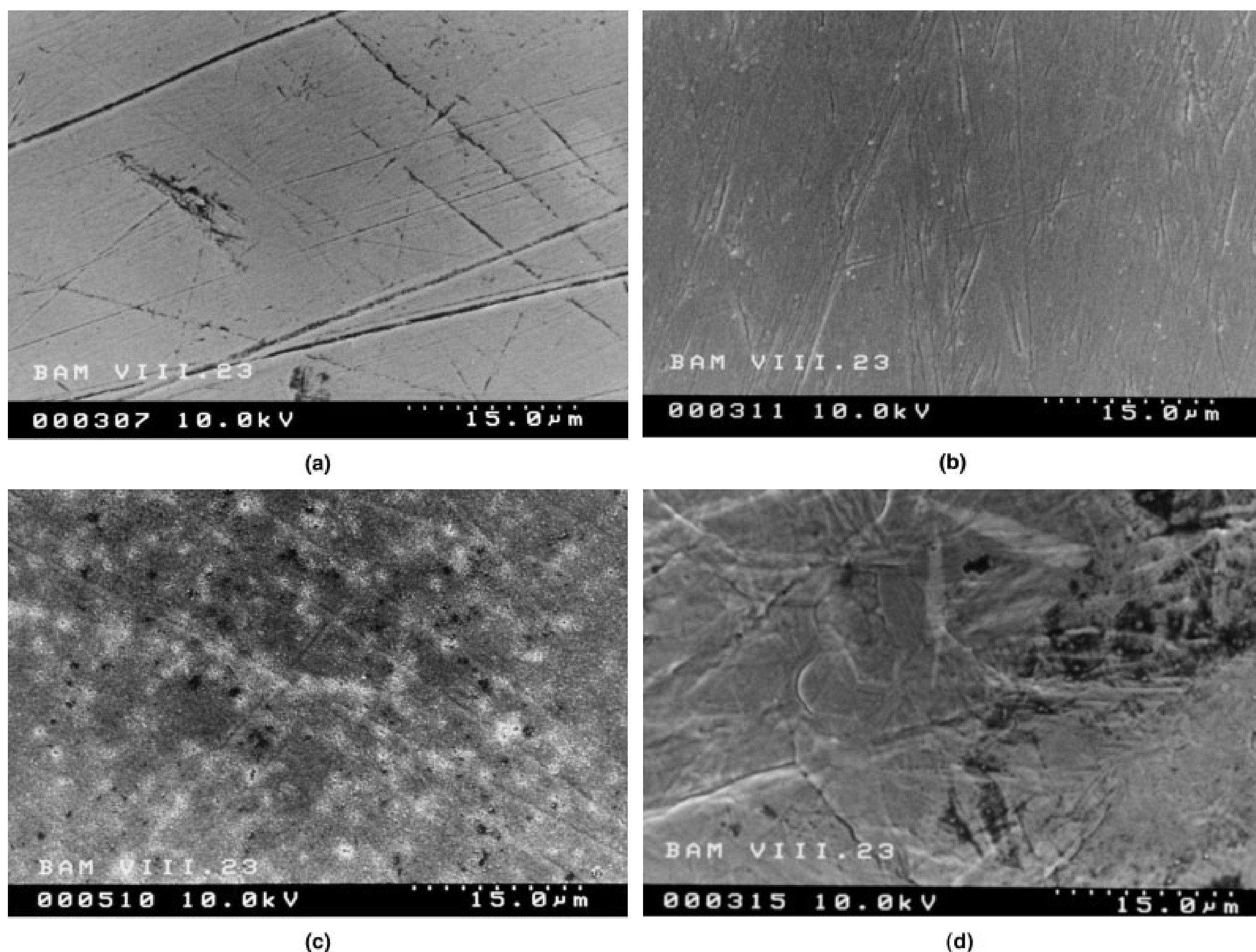

Figure 1. SEM of surface modified titanium discs. (a) polished titanium (control); (b) titanium discs coated with TiN; (c) thermal oxidation of a polished titanium surface; and (d) a laser structured titanium surface. Magnification 1:2000.

coated with TiN as compared to cells on all other surfaces tested (Fig. 4). Differences in mean values between cells cultured on polished, oxidized, or laser-treated titanium discs did not reach statistical significance.

Protein determination (BCA protein assay)

The amount of total protein in the cell layer was determined for all modified titanium surfaces at day 4 after plating. Because the surface areas on each disk were comparable results were expressed as μg protein/well (Fig. 5).

Fibroblasts on all surfaces tested showed a higher protein content than cells on tissue culture polystyrene plastic. The protein content in fibroblasts on discs coated with TiN was significantly higher compared to cells cultured on TiO_2 and polished titanium. The difference in mean values of total protein content between cells cultured on polished, oxidized, or laser-treated titanium discs was very low and not statistically significant.

DISCUSSION

The successful incorporation of dental implants strongly depends on a firm longstanding adhesion of the tissues surrounding the implant. Deeper periodontal structures need to be protected from bacterial invasion and subsequent infection. The cellular reaction is influenced by the properties of the bulk material as well as the properties of the surface, that is, the chemical composition and the topography.[24–28]

TABLE II
Contact Angle Measurements Using the Sessile Drop Technique

Titanium Discs (n = 5)	Surface Energy [mN/m]		
		Disperse (d)	Polar (p)
Ti-polished	35.6 ± 1.1	= 33.2 ± 0.4 (d)	+ 2.4 ± 0.7 (p)
TiN	38.7 + 1.2	= 35.2 ± 0.5 (d)	+ 3.5 ± 0.7 (p)
Ti-Laser	35.3 + 1.3	= 34.0 ± 0.5 (d)	+ 1.3 ± 0.7 (p)

The surface energy is shown with disperse (d) and polar (p) parts in [mN/m]. N = 5 discs examined.
TiN, titanium coated with titanium nitride.

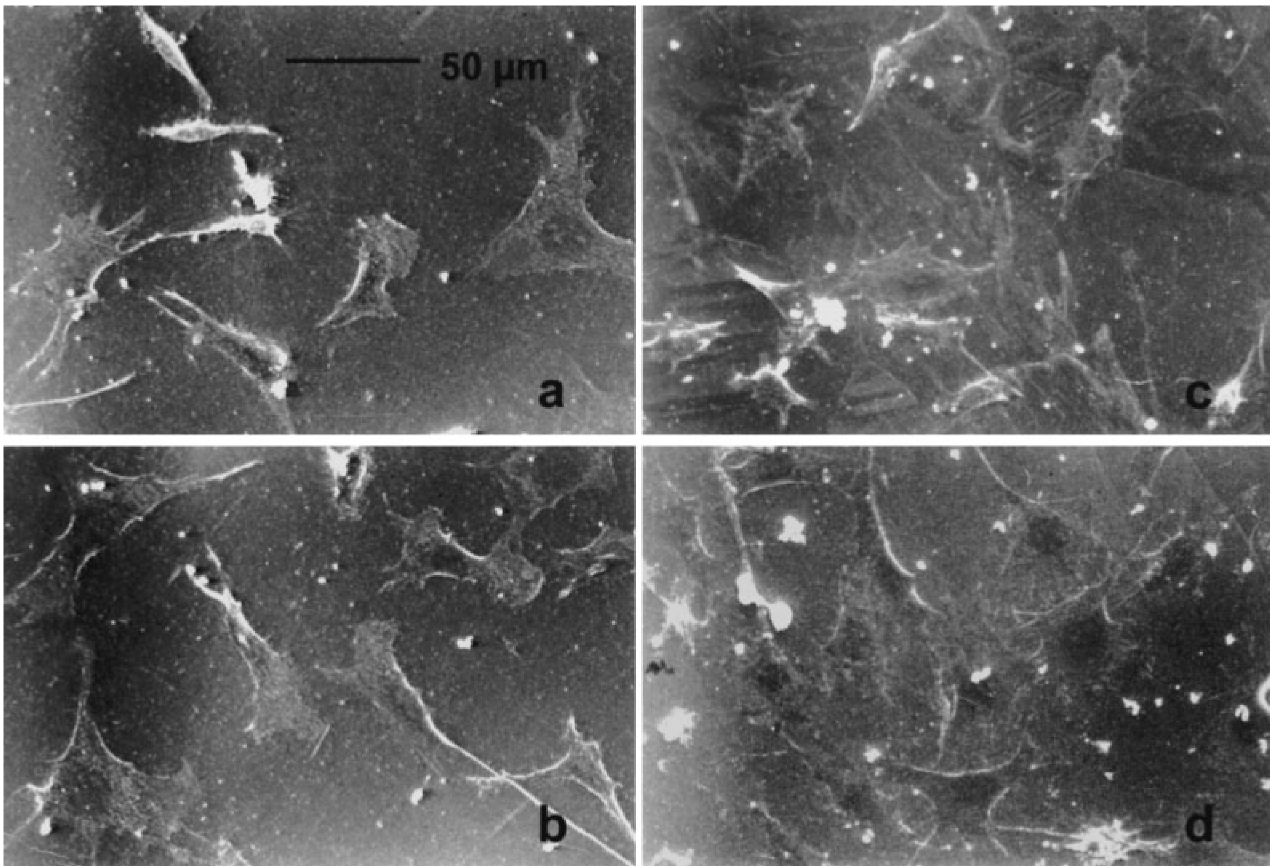

Figure 2. SEM of mouse fibroblasts on four different titanium surfaces after 4 days in culture. Fibroblasts on polished titanium surfaces were either spindle shaped and elongated or spread multipolar or round fibroblasts were seen (a). The majority of the fibroblasts on oxidized titanium surfaces showed a more spherical or triangular cell shape with fine and long filopodia (b). Filopodia of cells cultured on laser-treated titanium surfaces and on discs coated with TiN appeared to be stronger; cells were well spread and showed a polygonal cell shape (c,d).

In our study, surface topographical description of all modified titanium discs examined showed that, with the exception of the R_a of the laser structured surface that could not be reduced below 1 µm, other treatments had little effect on surface roughness (Table I). Determination of surface roughness for TiN, TiO_2, and polished titanium surfaces showed similar low R_a values between 0.11 µm and 0.20 µm.

SEM and fluorescence microscopy revealed that fibroblasts on all modified surfaces tested adhered and spread well but showed variation in cellular morphology. The majority of the fibroblasts cultured on laser-treated titanium surfaces and on discs coated with TiN had a flat morphology and appeared intimately adherent to the surface, indicating good attachment. Cellular growth seemed to be denser than on polished or oxidized titanium. The majority of fibroblasts on oxidized titanium surfaces showed a more spherical cell shape with fewer filopodia.

It is assumed that cell shape can regulate cell growth,[29] gene expression,[30] and extracellular matrix metabolism.[31] TiN, which appeared to have a higher density of cells by morphological analysis, also showed the highest MTT activity and total protein values. The simultaneous increase in both assay systems would be compatible with an increased number of fibroblasts in comparison to the other surfaces. However, this interpretation of the results is based on the assumption that the MTT test is not influenced by functional regulation. The MTT assay and total protein in the cell layer did not show significant differences between Ti-pol, Ti-laser, and TiO_2.

In addition to surface topography, the properties of implant materials that affect cellular behavior include mechanical rigidity and wettability (SFE). The wettability of the surface plays an important role with respect to protein adsorption, cell attachment, and spreading.[28,32–34] Surfaces with a high surface free energy are reported to be more adhesive than those with a low surface free energy. In contrast to these findings, den Braber et al.[35] found that physicochemical parameters such as wettability and SFE play no measurable role in the shape and orientation of cells on microtextured surfaces.

In this study values for surface free energy did not reach significant differences between surface modifi-

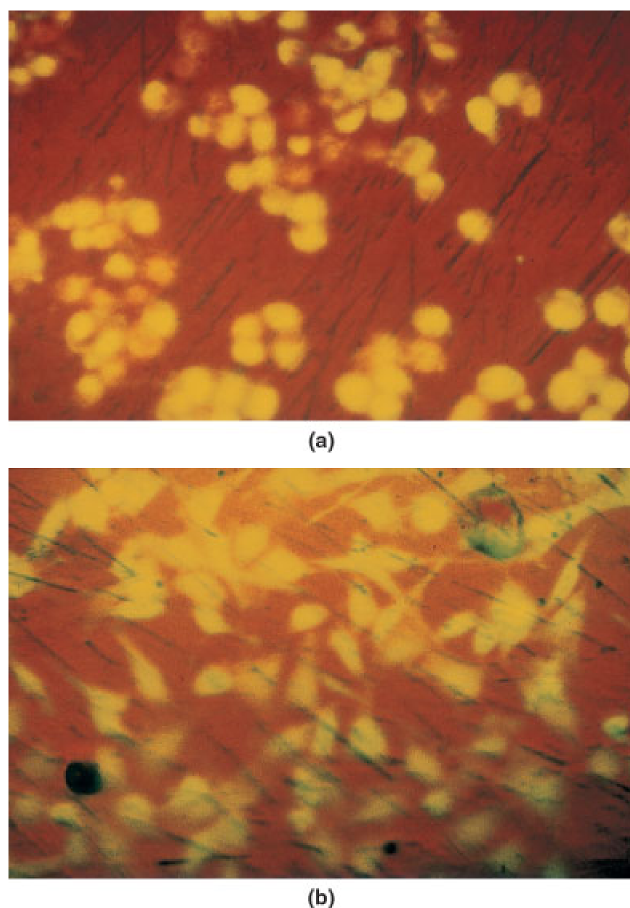

Figure 3. Fibroblasts on polished titanium discs were evenly distributed and showed a round or oval shape (a). The discs coated with TiN showed both spindle-shaped fibroblasts with long filopodia or cells that were flatly stretched out. Many of them had strong filopodia (b). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

cations (Table II). The polar and the dispersion component of the SFE showed a tendency towards higher values for TiN compared to Ti-pol and Ti-laser. However, the slight differences are unlikely to be a major factor inducing the observed differences in fibroblast growth.

In our study the actual properties of the oxide films on the various surfaces are not known. Normally titanium is covered with a thin protective oxide film that is rebuilt within milliseconds after any damaging. Polished titanium surfaces spontaneously oxidize in air and thus may be contaminated with carbon. However, the thick oxide layer that was intentionally produced to obtain a well-defined and reproducible surface (TiO_2 rutile) may be more hydrophobic than a polished titanium surface (as indicated by the drop formation in the wettability test). Apparently, the cell morphology on TiO_2 surfaces was different from all other cell surfaces. The spherical cell shape of the majority of fibroblasts on oxidized titanium could be an

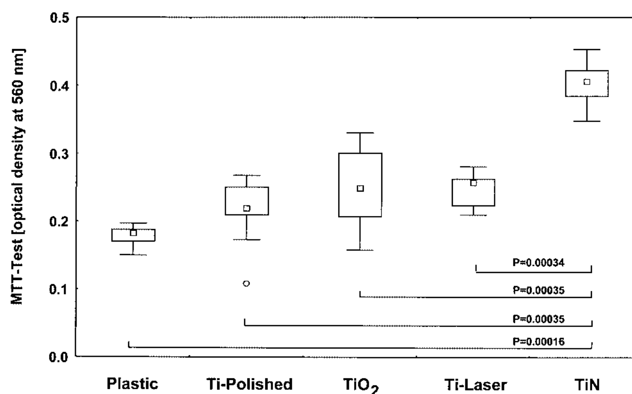

Figure 4. Box-plots of the activity of the mitochondrial dehydrogenases (MTT test) on modified titanium surfaces after 4 days of culture. Mean OD (white square in the box) and the 25th/75th percentiles as well as the non-outlier maximum and minimum are shown in box-plots. Outliers are denoted by circles. Control, fibroblasts on tissue culture polystyrene; Ti pol, polished titanium; TiN, titanium coated with titanium nitride; TiO_2, oxidized titanium surface. Statistically significant differences are indicated ($p < 0.05$).

effect of the thicker oxide layer and an increased hydrophobicity, respectively. Many studies have shown that different surface properties of titanium oxides have a significant effect on biological response.[4,13–16,36] Williams and Williams[15] studied the adsorption of albumin to different metal surfaces. Among other metals, the study included titanium covered by a native oxide and bulk TiO_2. The authors found a significant difference in the albumin adsorption for the two different types of titanium oxide. Könönen et al.[4] studied the influence of titanium surfaces prepared by electropolishing, acid etching, and alumina grit blasting

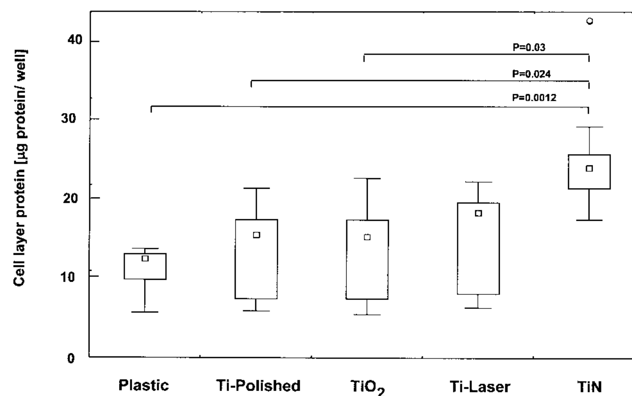

Figure 5. Box plots of protein content in fibroblasts on modified titanium surfaces after 4 days of culture (indicated as µg protein/well). Mean OD (white square in the box) and the 25th/75th percentiles as well as the non-outlier maximum and minimum are shown in box-plots. Outliers are denoted by circles. Control, fibroblasts on tissue culture polystyrene; Ti pol, polished titanium; TiN, titanium coated with titanium nitride; TiO_2, oxidized titanium surface. Statistically significant differences are indicated ($p < 0.005$).

on fibroblast behavior *in vitro*. Significant differences in cell shape, orientation, and proliferation were observed. These differences were attributed mainly to the different textures of the titanium surfaces, but differences in oxide composition and impurities were also considered to be likely contributing factors. The different surface treatments remove the native oxide layers and cause different charge distributions below the newly formed thin oxide layer. Looking at different pre-oxidized biomaterials, Oshida et al.[37] investigated the effect of surface texture on wettability, evaluated by measuring the surface contact angles. It was suggested that wettability might be related to the crystalline structure of the oxide films formed on these biomaterials. Based on our findings, it would be of interest to systematically investigate the role of the microstructure of the oxides (crystal structure and morphology), which both can be expected to be of importance.

The laser surface used in our study may also have been oxidized. As the laser technique enables implant surface treatment without direct contact and therefore without contamination,[10] the laser surface may have been cleaner and more hydrophilic (and more bioadhesive) compared to the polished titanium discs.

TiN-coated surfaces showed higher MTT values and total protein levels. Together with the morphological analyses, we suspect an enhanced ongrowth of fibroblasts. It is most likely that a significant percentage of the total protein synthesized by the fibroblasts represents type I collagen. It is possible that these differences are in part a result of the composition or the chemical properties of the TiN coating. The hard coating used in our study (TiN) could enhance fibroblast adherence and growth by masking the more reactive underlying titanium oxide surface. Cells cultured in medium containing serum proteins do not directly adhere to the substratum surface, but to an adsorbed layer of serum components,[38] which could form on TiN. These attachment proteins contain sites with peptides promoting cell attachment that are recognized by receptors on the cell surface (mostly by integrins). Even changes in protein conformation through interaction with the TiN surface might be possible. Additionally, *in vitro* and *in vivo* experiments are under way to examine these interactions.

TiN may be a suitable surface for implant coating because it also reduces bacterial adhesion. In previous studies, it was found that titanium discs coated with TiN or Zrn and a thermically oxidized titanium surface were able to reduce the number of adhering bacterial colonies compared to polished titanium.[9] This view is supported by earlier small clinical studies, which suggest that physical modifications (such as hard coatings) may have an influence on bacterial adherence.[39–41] Hard coatings were used to reduce plaque formation on implants[42] or metal parts of partial dentures.[20,40,42,43]

In conclusion, the findings of the present study revealed that all surface modifications examined allowed fibroblast attachment and growth. The property of hard coatings to reduce bacterial adherence (*in vitro*) and to support fibroblast growth might reduce the possibility, or at least the severity, of periimplantitis *in vivo*. Furthermore, the use of titanium hard coatings for implant abutments might prevent surface roughening because TiN or ZrN coatings are nonabrasive and have a high durability. The results from the present study provide valuable insights as to the suitability of TiN coating to support tissue growth on dental implant surfaces.

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