Bacterial Adhesion on Commercially Pure Titanium and Zirconium Oxide Disks: An In Vivo Human Study

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Background: Little is known about the mechanisms of bacterial interaction with implant materials in the oral cavity. A correlation between plaque accumulation and progressive bone loss around implants has been reported. Bacterial adhesion shows a direct positive correlation with surface roughness. Other surface characteristics also seem to be extremely important with regard to plaque formation. Different adhesion affinities of bacteria have been reported for different materials. The aim of this study was to characterize the percentage of surface covered by bacteria on commercially pure titanium and zirconium oxide disks.

Methods: Ten patients participated in this study. A removable acrylic device was adapted to the molar-premolar region, and commercially pure titanium (control) and zirconium oxide (test) disks were glued to the buccal aspect of each device. The surface roughness of titanium and test specimens was similar. After 24 hours, all disks were removed and processed for scanning electron microscopy, for the evaluation of the portion of surface covered by bacteria.

Results: In control specimens, the area covered by bacteria was 19.3% ± 2.9; in test specimens, the area was 12.1% ± 1.96. The disk surface covered by bacteria on test specimens was significantly lower than that of control specimens ($P = 0.0001$).

Conclusion: Our results demonstrate that zirconium oxide may be a suitable material for manufacturing implant abutments with a low colonization potential. *J Periodontol 2004;75:292-296.*

KEY WORDS

Bacterial adhesion; dental abutments; dental implants; zirconium oxide.

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Little is known about the mechanisms of bacterial interactions with implant materials in the oral cavity.[1] The microflora around dental implants appear to be similar to that found around natural teeth and, thus, microbial pathogens associated with periodontitis may also contribute to implant failures.[2,3] A correlation between plaque accumulation and progressive bone loss around implants has been reported in experimental and clinical studies.[4] Plaque accumulation on implant surfaces or abutments induces an inflammatory reaction in the gingiva and alveolar mucosa just as around teeth.[5-7] In fact, bacterial infection has been reported to be one of the reasons for implant failure.[8-11]

The longevity of oral implants can be jeopardized by either peri-implantitis or occlusal overload.[4,12] In the partially edentulous patient, in whom pockets around teeth act as a reservoir for the colonization of the pockets around implants, the risk for inflammatory reactions of the peri-implant soft tissues seems higher than in the fully edentulous patient.[13] Bacterial adhesion shows a direct positive relationship with surface roughness.[14-21] It must, however, be borne in mind that surface roughness is only one of the parameters involved in plaque formation. Moreover, it has been clearly shown that the initial colonization of an intraoral hard surface starts from the surface irregularities (cracks, grooves, or abrasion defects) and subsequently spreads out.[21] The surface with a low Ra value strongly inhibits accumulation and maturation of plaque within 24 hours.[16]

The influence of surface roughness on bacterial adhesion and colonization of transgingival abutments and healing implant components has been demonstrated in in vitro and in vivo studies.[5,16] At the bottom of the surface irregularities, the bacteria are sheltered from removal forces, and this allows them to establish a strong and irreversible attachment to the surface.[6] After several days of undisturbed plaque formation, rough surfaces harbor a more mature plaque, comprising an increased proportion of motile organisms and spirochetes.[21] Other surface characteristics of the components of the transgingival element, in addition to surface roughness, also seem to be extremely important in plaque formation and may be helpful in the prevention of peri-implant soft-tissue pathology.[22]

Different adhesion affinities of bacteria have been reported for different materials, such as titanium and titanium alloys.[1] New ceramic abutments have been introduced to improve esthetic results.[5,22] Zirconium oxide implants are bioinert, and they have an excellent resistance to corrosion, good biocompatibility, and high resistance to wear. They have also been demonstrated to possess high values of bending strength and fracture toughness.[23-27] Zirconium oxide has no cytotoxic effect on fibroblasts; in vitro carcinogenicity tests and the teratogenicity test (cellular chromosome aberrations) are negative; and in genotoxicity tests, the absence of aberrations in chromosomic patterns in cells cultured on zirconium plates has been reported.[27] The purpose of this study was to characterize by scanning electron microscopy (SEM) the percentage of surface covered by bacteria on commercially pure (c.p.) titanium and zirconium oxide disks.

MATERIALS AND METHODS

Ten patients between the ages of 19 and 27 years and in excellent systemic health participated in the study. All patients gave their informed consent, and the protocol was approved by the Ethic Committee of Chieti University. The participants were selected on the basis of good periodontal health and no signs of mouth breathing. One week before the study, supragingival plaque and calculus were professionally removed, oral hygiene procedures were established, and ideal gingival health conditions were obtained in all volunteers (Löe and Silness gingival index = 0).[28] None of the subjects had used mouthrinses or had taken antibiotics during the previous 6 months. In each of the 10 participants, a removable acrylic device was adapted to the molar-premolar region of each quadrant of the jaws. The devices were self retaining and did not require etching or bonding to the teeth surfaces. C.p. titanium and zirconium oxide disks§ were glued to the buccal aspect of each device. Two zirconium disks (test) were glued to the devices inserted in the right quadrant and two titanium disks (control) were glued to the devices inserted in the left quadrant. A total of 40 disks (20 test and 20 control) were used in this study.

An additional 10 disks (five test and five control) were analyzed for the surface characterization, and the surface roughness was evaluated by SEM‖ and a profilometer:¶ an average of three readings were performed for each surface. The arithmetic mean of surface roughness of every measurement within the total distance (roughness average = R_a) was assessed. The surface roughness values were expressed as a mean ± standard deviation (SD). Two areas of 200 µm in diameter were evaluated for each disk surface. Neither cleaning procedures nor agents for chemical plaque control were applied to the disks for the entire test period. After 24 hours, all disks were removed and processed for SEM to evaluate the portion of the surface covered by bacteria.

Scanning Electron Microscopy

After removal, the disks were put in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.4 for 4 hours. They were then dehydrated with increasing concentrations of ethanol solutions (50%-70%-90%-100%) and left for 12 hours in 113 Freon (trichlorotrifluoroethane) as a transition fluid to critical point drying.# Finally, the chambers were glued to aluminum stubs and coated with 20 to 30 nm of gold. The disk surface was examined with a scanning electron microscope‖ operating at 20 to 30 KV, with tilt angles ranging from 10° to 45°. SEM evaluations were performed by three independent observers who expressed an estimate of bacterial amount on the disk surface. Five areas of 100 µm to 130 µm in diameter were evaluated for each disk and an image in JPEG format was created.

Quantitation of the percentage of the surface covered by bacteria was done on the JPEG images using a personal computer associated with a histometry software package with image-capturing capabilities.**

Statistical Evaluation

The differences in the percentages of surface covered by bacteria in the two groups were evaluated with the analysis of variance (ANOVA). The percentage of implant surface covered by bacteria was expressed as a mean ± SD. Statistically significant differences were set at $P < 0.05$.

RESULTS

Surface Characterization

Grooves and ridges, typically produced during the manufacturing, were present in both types of surfaces.

§ Cercon, Degussa, Hanau, Germany.
‖ LEO, Cambridge, U.K.
¶ Mitutoyo Corporation, Tokyo, Japan.
# Polaron CPD 7501 Bomb, Polaron Equipment, Watford, England.
** Image-Pro Plus 4.5, Media Cybernetics Inc., Immagini & Computer Snc, Milano, Italy.

The surface roughness (Ra) was 0.73 ± 0.05 µm for the titanium disks and 0.76 ± 0.06 µm for the zirconium oxide disks.

Titanium Surface

A plaque was observed, consisting of a few cocci and a higher proportion of rods and filamentous-shaped bacteria (Figs. 1 and 2). A thin, regular layer of cocci was found in many areas of the surface. Salivary proteins, in contact with the implant surface, were found in a large portion of the surface. The salivary proteins were identified by their irregular shape and their size, which was smaller than bacteria. At higher magnifications, salivary proteins, cocci, and many colonies of microorganisms were found. No mineralized plaque was noted on most specimens by 24 hours. The area covered by bacteria was 19.3% ± 2.9% (Table 1).

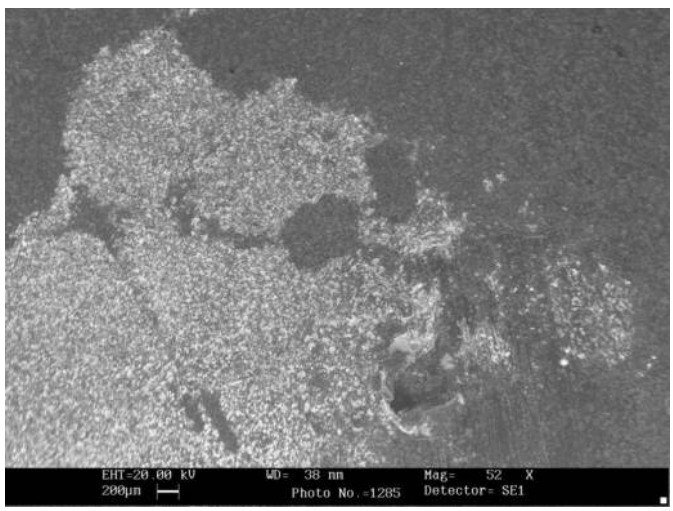

Figure 1.
Titanium. Empty spaces between the colonies of bacteria are present.

Zirconium Oxide Surface

In many areas, no bacteria or salivary proteins were observed (Fig. 3). In other areas, only small colonies of a few cocci were found. In the major portion of the disk surface, empty spaces between the colonies of bacteria were present. Polymorphous aggregates of microorganisms were present, consisting mainly in cocci and short rods. No calcification of the bacteria was observed. The area covered by bacteria was 12.1% ± 1.96% (Table 1).

Statistical Evaluation

The differences of the Ra between the two surfaces were not statistically significant ($P = 0.415$). The disk surface covered by bacteria on test specimens was significantly lower than that of control specimens ($P = 0.0001$) (Table 1).

Table 1.

Statistical Evaluation of the Percentage of Disk Surface Covered by Bacteria

	Mean	SD	Standard Error	*P* Value
Zirconium disk	12.1%	1.96	0.46	0.0001*
Titanium disk	19.3%	2.90	0.68	

* Significant at 95% (according to the ANOVA test).

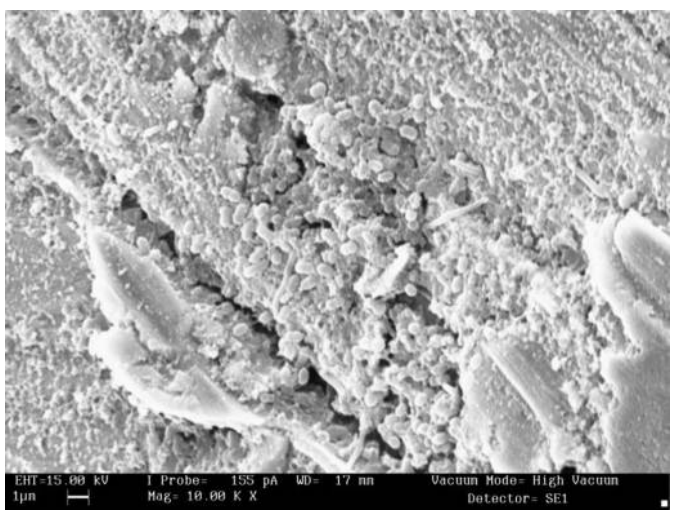

Figure 2.
Titanium. A homogeneous layer of cocci or filamentous bacteria covers the titanium surface.

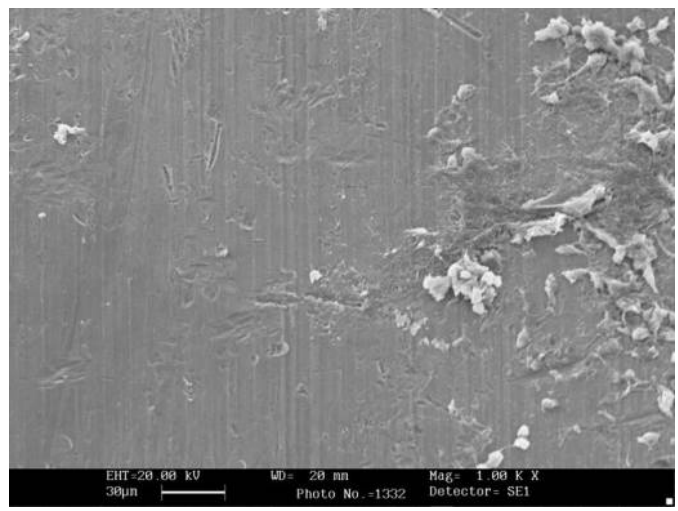

Figure 3.
Zirconium oxide. A small number of bacteria cover the zirconium oxide surface.

DISCUSSION

The present study has shown that statistically significant differences exist in bacterial adhesion between zirconium oxide and titanium surfaces. The first stage of plaque formation on different surfaces starts with the formation of an acquired pellicle;[1] this pellicle is usually composed of salivary proteins and bacterial cell-free enzymes and serves as a surface to which bacteria adhere while forming the biofilm.[1] Initial adhesion starts with the interaction of bacteria surface from a certain distance: at work are van der Waals attractive forces and electrostatic repulsive forces.[20] Other factors that may influence the initial bacterial adhesion are the distance of the bacterium from the surface, the ionic strength of the surrounding liquid medium, the surface-free energy of the bacterium and of the oral surface, and the roughness of the surface.[6]

Surface properties of transgingival implant components are important determinants in bacterial adhesion. In fact, the long-term survival of dental implants depends, in part, on control of bacterial infection in the peri-implant region.[29] The initial event in the pathogenesis of most bacterial diseases is the adhesion of bacteria to the host tissue.[30-32] Surface coverage by bacteria is not the critical measurement in plaque accumulation but only the initial adhesion event. The total amount of plaque that accumulates is the important factor relative to implant stability.

Bacterial adhesion onto implant or abutment surfaces is a critical issue.[33] A bacterial adhesion to implant surfaces is a first stage of peri-implant mucositis and peri-implantitis; in fact, a positive correlation has been found between oral hygiene and marginal bone loss around implants in the edentulous mandible.[4,12] The modification of the surfaces and the use of different materials have been shown to play a relevant role in the bacterial adhesion to implant surfaces.

In a previous study from our laboratory, we found lower amounts of bacteria on nitride-coated titanium implants than on titanium implants,[34] and Grossner-Schreiber et al.[35] found lower amounts of bacteria on zirconium nitride-coated materials. Rimondini et al.[22] also found that tetragonal zirconia, stabilized with yttrium surfaces, accumulated significantly fewer bacteria than titanium. These authors found that titanium surfaces appeared to be coated in a more uniform way with a structured biofilm, whereas the zirconium surface appeared colonized by clusters of bacteria.[22] Zirconium oxide has an ivory color, similar to the color of the natural tooth, and this fact renders it extremely useful in esthetically critical areas of the mouth.[26] In addition, its ability to transmit light makes it an ideal candidate for use in esthetic restorations.[26]

The most plausible explanation for our results probably lies in the superficial structure of zirconium oxide, particularly in its electric conductivity. Surface characterization, in fact, showed that the Ra values for the surface roughness of both control and test specimens were similar. Poortinga et al.[36] showed that the change in substratum potential as a function of the number of adhering bacteria is a measure of the amount of charge transferred between the substratum and the bacteria during adhesion. Poortinga et al., in another study,[37] showed that, during adhesion, depending on the specific resistivity of the substratum, bacteria either donated to or accepted electrons from the substratum, and that bacteria that had donated electrons to the substratum adhered more strongly than bacteria that had accepted electrons from the substratum. These results demonstrate that electron transfer plays a role in bacterial adhesion to surfaces.

CONCLUSIONS

Zirconium oxide surfaces showed a significant reduction of the presence of bacteria, and this fact is probably important for the health of the peri-implant soft tissues. These results justify the search for optimal physico-chemical parameters of abutment or the transgingival portion of implant surfaces for reduction of bacterial colonization by periodontal pathogens. Our results support previous findings[22] that zirconium oxide may be a suitable material for manufacturing implant abutments with a low colonization potential. It should, on the other hand, be stressed that our measurements are only a part of the events concerning bacteria–peri-implant soft-tissue interactions.

ACKNOWLEDGMENTS

This work was partially supported by the Ministry of Education, University, and Research (M.I.U.R.), Rome, Italy, and by the National Research Council (C.N.R.), Rome, Italy.

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Accepted for publication July 4, 2003.