CLINICAL ORAL IMPLANTS RESEARCH

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The adhesion of oral bacteria to modified titanium surfaces: role of plasma proteins and electrostatic forces

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Abstract

Objectives: Modifications of titanium (Ti) implant surfaces have a significant effect on early biofilm formation and the outcome of implant procedures. The aim of this study was to examine the role of plasma proteins and electrostatic forces in the adhesion mechanism of oral bacteria to modified Ti surfaces.

Materials and methods: Ti discs with three different types of surface modifications, machined, acid-etched, and acid-etched and blasted, were examined for adhesion of oral bacteria: *Streptococcus mutans*, *Porphyromonas gingivalis,* and *Fusobacterium nucleatum*. Following pretreatment of the Ti with ion rich solutions or coating by human serum albumin or fibronectin, bacterial adhesion was examined by scanning electron microscopy and assessed quantitatively by DNA analysis. Ti coating by proteins as well as bacterial adhesion and their interrelationships were further investigated through confocal scanning laser microscopy.

Results: Acid-etched and blasted Ti surfaces exhibited significantly higher amounts of bacteria adhesion than the other two surfaces. Calcium was found to serve as a bridging agent in the adhesion process of *S. mutans* and *F. nucleatum* to Ti surfaces. Although albumin coating of the Ti reduced the adhesion of *S. mutans* to all surfaces, it had no influence on the adhesion of *P. gingivalis* or *F. nucleatum*. Coating the Ti with fibronectin enhanced *P. gingivalis* and *F. nucleatum* adhesion.

Conclusions: Bacterial adhesion to Ti surfaces is roughness-dependent, and the adhesion mechanism is influenced by ions and proteins of the initial coating derived from the blood.

The main biological factors for dental and orthopedic implant failure are infection and inflammation of the surrounding tissues (Esposito et al. 1998; Leonhardt et al. 2003).

Few studies have provided data on the prevalence of oral peri-implant diseases. Zitzmann & Berglundh (2008) reported that peri-implant mucositis occurred in 80% of the subjects and in 50% of the implant sites. Furthermore, peri-implantitis was identified in 28% – 56% of subjects – and in 12% – 43% of implant sites. It was also reported that mucositis is the most frequent biological complication of implant-supported single crowns, occurring adjacent to 9.7% of implants after 5 years (Jung et al. 2008). Moreover, Esposito et al. (2007) found through a Cochrane review that the estimated prevalence of peri-implantitis around implants with roughened surfaces is significantly higher than turned Brånemark implants. The development of inflammation around oral implants is linked to the accumulation of specific bacterial biofilms. While implants surrounded by healthy tissues display a microbiota associated with periodontal health, peri-implantitis is related to the presence of putative periodontal pathogens, such as *Porphyromonas gingivalis, Prevotella intermedia, Treponema denticola,* and *Aggregatibacter actinomycetemcomitans* (Leonhardt et al. 1999; Shibli et al. 2007). However, when implant materials were exposed to the oral flora *in vivo,* they were colonized predominantly by Streptococci, and the number of viable plaque-forming bacteria found on the implants was shown to depend on their surface properties (Nakazato et al. 1989).

In vivo and *in vitro* studies have demonstrated that the bacterial coating on Ti surfaces is roughness-dependent and that adhesion to rough surfaces is significantly higher than to smooth ones (Quirynen et al.

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1996; Rimondini et al. 1997; Pier-Francesco et al. 2006).

Other factors that may play a role in the mechanism of bacterial adhesion to implants are local host agents, e.g., plasma constituents, salivary ions, and proteins that adsorb to the implant surface. We have previously shown that the main salivary proteins that adsorb to Ti *in vivo* and *in vitro* are alpha amylase and albumin (Steinberg et al. 1995; Kohavi et al. 1995, 1997), and that one of the mechanisms for albumin adsorption to Ti is through calcium (Ca^{++}) bridges (Klinger et al. 1997). We have also found that the main plasma proteins that adsorb to Ti are albumin and fibronectin (Sela et al. 2007).

The commercial introduction of new Ti implants is currently based mainly on the development of specific physicochemical characteristics of the surface. Moreover, the "Biochemical Modification of Ti Surfaces" approach stands for the enhancement of bone healing at the implant interface through the immobilization of biomolecules to the surfaces of the Ti (Puleo & Nanci 1999; Morra 2007). It was also suggested that bone healing can be stimulated through modifications in the Ti implant surfaces by peptides or extracellular matrix proteins (Middleton et al. 2007). *In vitro* studies have shown that human plasma fibronectin coatings of Ti surfaces enhance fibroblast attachment and influence keratinocyte adhesion, pellicle formation, and thrombogenicity (Scheideler et al. 2007). Furthermore, coating of Ti implants with plasma fibronectin *in vivo* enhanced earlier osseo-integration in mice (Jimbo et al. 2007).

Studies on otologic Ti implants and tympanostomy tubes revealed that coating by human serum albumin (HSA) may inhibit the adherence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* to the implants (Kinnari et al. 2005). Furthermore, we have previously shown that coating of Ti implants with salivary albumin significantly reduced adhesion of oral periodontal pathogenic bacteria (Steinberg et al. 1998).

Although a considerable bulk of studies exists on the properties of different Ti surfaces, the role of oral microorganisms and the mechanisms of bacterial adhesion to implants have not yet been comprehensively examined. Moreover, the effects of plasma protein-coated Ti surfaces on the adhesion of oral periodontal pathogenic bacteria are not fully understood.

The aim of this study was to investigate the adhesion mechanisms of selected oral bacteria to modified Ti surfaces and to examine the role of the plasma proteins albumin and fibronectin, as well as electrostatic forces, in the adhesion mechanism.

Material and methods

Ti disks

Ti alloy ($TiAl_{(6)}V_{(4)}$) disks (6 mm in diameter) were manufactured for this research by Alpha-Bio Tec©, implant company, Israel, with three types of surface modifications: machined (turned), dual acid-etched, and acid-etched and sand blasted. The Ti disks were characterized physically and chemically as previously described (Sela et al. 2007).

Pretreatments: coating by plasma protein or ions

To determine the role of plasma proteins in the adhesion of bacteria to Ti surfaces, the disks were incubated in 1 mg/1 ml HSA or 0.1 mg/1 ml human serum fibronectin (HSF) (Sigma-Aldrich, Rehovot, Israel) for 2 h. The characteristics of adsorption have been described previously (Sela et al. 2007).

To examine the role of electrostatic forces in the mechanism of bacterial adhesion, the disks were suspended for 2 h at room temperature in 1 mM $CaCl_2$ or 1 mM KCl. The disks were then washed three times with double distilled water.

Bacteria

Streptococcus mutans ATCC 27351, *P. gingivalis* 381 and *Fusobacterium nucleatum* PK1594 were used in this study. *Streptococcus mutans* were cultured in brain heart infusion broth (Acumedia Manufacturers Inc., Baltimore, MD, USA) overnight at 37°C in an atmosphere enriched with 5% CO_2. *Porphyromonas gingivalis* and *F. nucleatum* were cultured in Wilkins anaerobe broth (Oxoid Ltd, Basingstoke, Hampshire, England) overnight at 37°C in an anaerobic chamber in an atmosphere of 85% N_2, 10% H_2, and 5% CO_2. The Ti disks with or without protein coating or ion pretreatment were suspended in bacterial cultures under sterile conditions in triplicate.

Ethylene glycol tetra-acetic acid removal treatment

To examine the role of Ca^{++} in the adsorption mechanism and the reversibility of the adhesion, the disks were treated with ethylene glycol tetra-acetic acid (EGTA), which has the ability of chelating Ca^{++}. Ti disks treated with 1 mM $CaCl_2$ and incubated with the three bacteria overnight were washed three times with sterile double distilled water and suspended in 0.1 mM EGTA solution for 60 min. Untreated disks served as controls.

Examination of bacterial adhesion to modified Ti surfaces by scanning electron microscopy

Following overnight incubation of the modified Ti disks with *S. mutans* and *P. gingivalis* as described above, adhesion of the bacteria to the Ti surfaces without protein coating was examined through a high-resolution scanning electron microscope (SEM) (Sirion, FEI Company, Netherlands). The disks were treated with 2.5% glutaraldehyde, washed in cacodylate buffer (0.1 M, pH 7.2), and post-fixed in 2% osmium tetroxide for 1 h. The samples were then dehydrated through a graded series of ethanol and mounted on stubs. Following air drying, the stubs were coated with gold (120 Å thickness) using a sputter coater (Polaron ES100, Polaron Equipment Ltd, Watford, UK).

Qualitative assessment of bacterial adhesion to protein-coated Ti surfaces by confocal scanning laser microscope

Ti disks coated with HSA conjugated with fluorescein label were incubated with *S. mutans* in culture broth. Following incubation, the disks were fixed by formaldehyde, and stained by propidium iodine to label the surface-adherent *S. mutans* cells. A confocal scanning laser microscope (CSLM) (LSM 410, Zeiss, Germany) was used to observe the albumin coat, the adhesion of *S. mutans,* and their interrelationships. A negative control was used to set the microscope parameters to avoid reflection of the Ti itself as much as possible.

Quantitative evaluation of bacterial adhesion by DNA analysis

Quantitative evaluation of the bacterial adhesion to the modified Ti surfaces was performed as follows: disks with or without protein coat or ion pretreatment were incubated with the three selected oral bacteria in triplicate, as described above. The disks were then washed and suspended in TRI Reagent® solution (Molecular Research Center, Inc., Cincinnati, OH, USA) with glass beads (particle size $\leq$106 μm) (Sigma-Aldrich), and bacterial cells were disrupted using a FastPrep® instrument (BIO 101, Inc., Vista, CA, USA). The disks were then removed, and the remaining solutions, which contained the treated bacterial cells, were centrifuged. The supernatant was collected, and DNA precipitation was performed according to the TRI Reagent® manufacturer's protocol. Briefly, the collected supernatant fluid was supplemented with 1-bromo-3-chloropropane (BCP)

(Molecular Research Center) and centrifuged. The upper aqueous phase was carefully removed, and the DNA-containing supernatant was collected from the interphase, suspended in iso-propanol, and centrifuged again. The upper phase was carefully removed, and the resulting DNA pellet was washed with 75% ethanol, centrifuged, and air dried until total evaporation of the ethanol. The DNA pellet was resuspended in 0.025-ml double distilled H_2O.

Quantitative measurements were carried out to determine the DNA concentration using a Nanodrop instrument (ND-1000, Nanodrop Technologies, Wilmington, DE, USA).

The removed disks were examined by the CSLM to rule out the presence of bacterial traces (data not shown).

Statistical methods

The two-way analysis of variance (ANOVA) and all pairwise multiple comparison procedure (Tukey Test) were used to compare quantitative variables between the three Ti surfaces studied, the two protein coats, and the two ions pretreatments. All tests were two-tailed, and a *P*-value of 5% or less was considered statistically significant. In this model, the main effects and the interactions were assessed.

Results

The examination of bacterial attachment through SEM revealed that the acid-etched and blasted Ti surfaces displayed more adherent bacteria and a different pattern of bacterial coating compared to the other surfaces (Fig. 1). Although the acid-etched and blasted Ti surfaces exhibited numerous large groups of bacteria, only few scattered bacteria were found on the machined Ti surfaces, and small groups of bacteria could be seen on the acid-etched Ti surfaces.

The CSLM images demonstrated that albumin adsorption to the acid-etched and blasted Ti surface was irregular and thicker than on the machined and acid-etched surfaces. A similar pattern of adhesion could be seen in CSLM images of Ti incubated with *S. mutans*. Moreover, coating of the Ti surfaces by albumin reduced the amount of bacterial adhesion (Fig. 2) on all the surfaces examined.

The quantitative evaluation of bacterial adhesion to the modified Ti surfaces (through DNA analysis) is presented in Figs 3 and 4. As can be seen, the acid-etched and blasted surfaces exhibited the highest amounts of bacterial adhesion, followed by the acid-etched surfaces and the machined surfaces, which showed the lowest amounts of adherent bacteria. The bacterial adhesion to the acid-etched surfaces was 1.4–2.3 times higher than to the machined surfaces, for the various bacteria. The adhesion of the acid-etched and blasted surfaces was 3–6.4 times higher than the machined surfaces adhesion, for the various bacteria. The differences in the adhesion between all three modified Ti surfaces (machined vs. acid-etched, machined vs. acid-etched and blasted, and acid-etched vs. acid-etched and blasted) for each of the bacteria tested were found to be statistically significant by the ANOVA test and Tukey tests, with $P \leq 0.002$ in all cases.

Protein coating

Coating the tested Ti surfaces with HSA resulted in a significant decrease in the adhesion of *S. mutans*. The mean adhesion proportions were 44%, 45%, and 54% for the machined, acid-etched, and acid-etched and blasted surfaces, respectively, compared with the surfaces without HSA coating. This decrease was found to be statistically different according to ANOVA test ($P < 0.001$), normality test passed, and there were no interactions between the surface and the protein coat. However, there were no significant differences in the adhesion of *P. gingivalis* or *F. nucleatum* to the surfaces following coating by albumin (Fig. 3).

Coating the surfaces with HSF resulted in a significant elevation in *P. gingivalis* adhesion. Compared to surfaces without HSF coating, the mean proportions were 202%, 142%, and 169% for the machined, acid-etched, and acid-etched and blasted surfaces, respectively. Moreover, coating of the Ti surfaces by HSF caused a significant elevation in the adhesion of *F. nucleatum* to all the surfaces examined. Compared to surfaces without HSF coating, the mean proportions were 165%, 165%, and 140% for the machined, acid-etched, and acid-etched and blasted surfaces, respectively. The increase adhesion of *P. gingivalis* or *F. nucleatum* to the HSF coated surface was found to be statistically different according to ANOVA test ($P < 0.001$), normality test passed, and there were no interactions between the surface and the protein coat, for each of the tested bacteria. On the other hand, fibronectin coating did not significantly alter the adhesion of *S. mutans* to the Ti surfaces.

Ion pretreatments

Pretreatment of the Ti disks by ions was carried out to examine the role of electrostatic forces in the mechanism of bacterial adhesion. It can be seen in Fig. 4 that pretreatment of Ti by Ca^{++}, a divalent positively charged ion, caused a significant increase in *S. mutans* adhesion. Compared to surfaces without Ca^{++} pretreatment, the mean adhesion proportions were 224%, 199%, and 128% for the machined, acid-etched, and acid-etched and blasted surfaces, respectively. This increase was found to be statistically different according to ANOVA test ($P < 0.001$), normality test passed, and there were no interactions between the surface and the Ca^{++} pretreatment.

Removal of the Ca^{++} from the Ti surfaces by EGTA (a Ca^{++} chelator) (Fig. 5) caused a significant decrease in the adhesion of these bacteria. The average proportions of bacteria removed were 78% and 77% for the machined and acid-etched surfaces, respectively. However, EGTA treatment of the acid-etched and blasted surfaces was found to be less effective, with an average bacterial removal proportion of 57%. Without Ca^{++} pretreatment, the reductions in the adhesion of *S. mutans* were 20%, 17%, and 11% for the machined, acid-etched surface, and acid-etched plus blasted surfaces, respectively (not statistically significant).

The same pattern was observed when *F. nucleatum* adhesion was examined following ion pretreatment (Fig. 4). Ca^{++} pretreatment caused a significant enhancement of *F. nucleatum* adhesion, with means of 157%, 134%, and 137% for the machined, acid-etched, and acid-etched and blasted surfaces, respectively, compared with surfaces without Ca^{++} pretreatment.

The adhesion of *P. gingivalis* to Ti was not influenced by Ca^{++}, and pretreatment by K had no significant effect on the adhesion of all tested bacteria to the Ti surfaces.

Discussion

Bacterial infection is considered one of the main reasons for early and late failure of oral implants, as it may lead to the disruption of integration between the implants and their surrounding tissues. Furthermore, as bacterial biofilms are related to most oral infections, the formation of biofilms on implant surfaces may play an important role in the development of peri-implant infections.

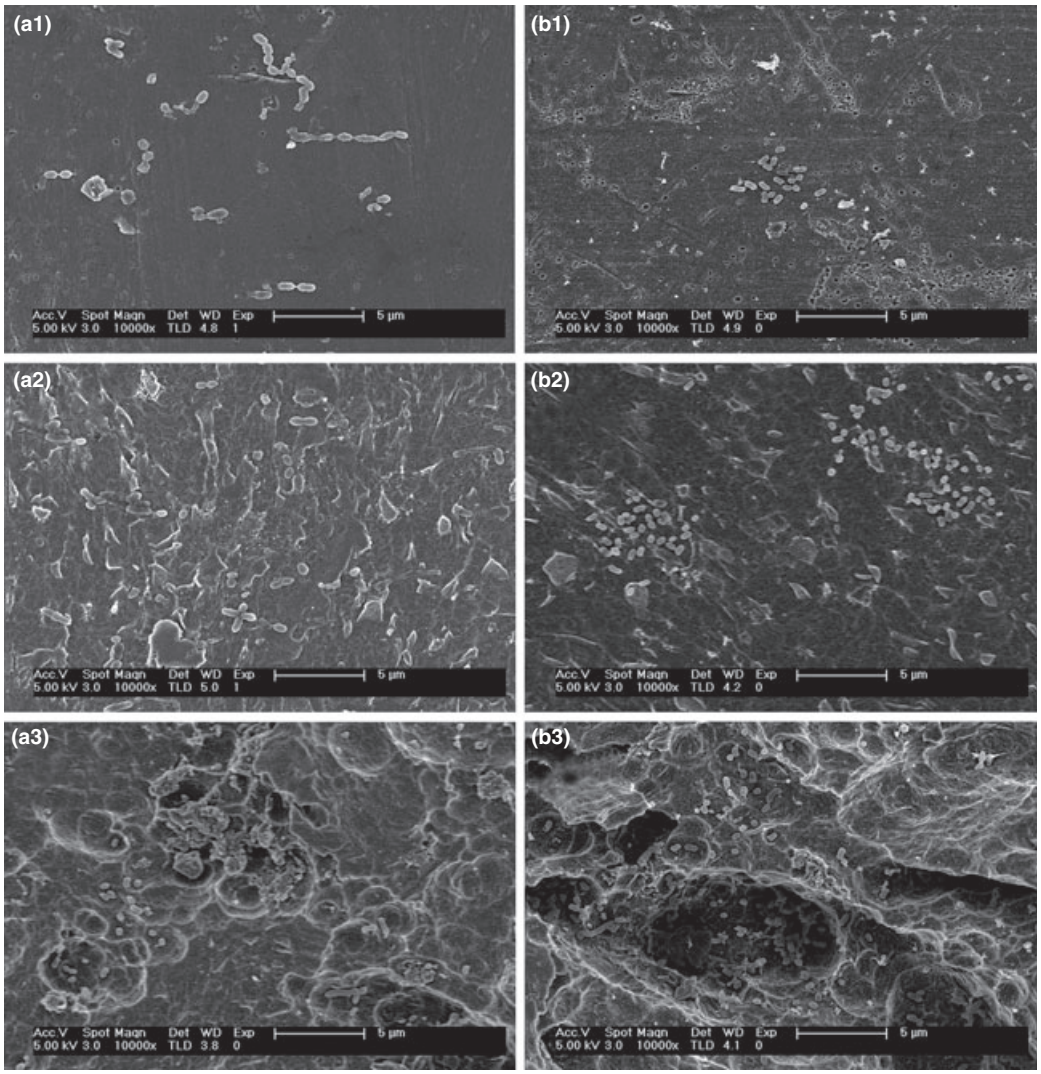

Fig. 1. Scanning electron microscope micrographs of *Streptococcus mutans* and *Porphyromonas gingivalis* adhesion to the Ti disks: A1-3 *S. mutans*, B1-3 *P. gingivalis*. In each serial, numbers represent the following surface modification: 1 = machined, 2 = acid-etched, 3 = acid-etched and blasted. Magnification: ×10, 000.

In previous studies, we demonstrated that the adsorption of plasma proteins to Ti surfaces is roughness dependent. It was also shown that the main plasma proteins adsorbed to Ti surfaces in the early stages of the implant process are albumin and fibronectin (Sela et al. 2007). The modified surfaces of the Ti alloy disks used in the present study have been characterized previously, both physically and chemically. Using atomic force microscopy, the acid-etched and blasted surfaces were shown to exhibit significantly higher roughness values.

The results of the present study demonstrate that bacterial adhesion to Ti surfaces, as for other biological materials, is roughness dependent. These findings are in accordance with those of other studies that pointed out the influence of surface roughness on bacterial adherence (Quirynen et al. 1996; Rimondini et al. 1997; Pier-Francesco et al. 2006). The increased adhesion onto very rough surfaces may be explained by their differentially enlarged surface areas related to irregularities created during the manufacture of these surfaces.

Unlike the widely studied mechanism of enamel biofilm formation, the mechanism of oral bacterial adhesion to dental Ti implant surfaces is not fully understood and needs to be further explored. Adhesion of bacteria to Ti surfaces *in vivo* may depend on the constituents of the conditioning film on the implant, e.g., proteins or ions. The primary pellicle that accumulates onto Ti surfaces exposed to the oral tissues is composed mostly of plasma ions and proteins. Moreover, the negatively charged Ti dioxide implant surface may attract positively charged ions, such as Ca^{++}.

Our results demonstrate that pretreatment of Ti surfaces with Ca^{++} ions increased the adhesion of *S. mutans* and *F. nucleatum* to the Ti surfaces, but had no influence on the adherence of *P. gingivalis*. The Ca^{++} chelator, EGTA, was found to remove *S. mutans* from the surface. Several well defined cell wall anchored adhesions are expressed on the surface of oral streptococci. These adhesions were shown to interact with molecules on other bacteria, on host cells surfaces, and salivary proteins. A divalent Ca^{++} ion was confirmed to be the binding cleft metal (Forsgren et al. 2009). *Fusobacterium nucleatum* possesses Ca^{++}-dependent binding proteins on the cell surface similar to those of *S. mutans* (Murray et al. 1988; Khemaleelakul et al. 2006). These findings indicate that the divalent ion Ca^{++} may serve as a bridging agent in the adhesion of bacteria to Ti surfaces.

Albumin was shown to be one of the first and main proteins that adsorbs to Ti surfaces exposed to plasma and saliva (Kohavi et al.

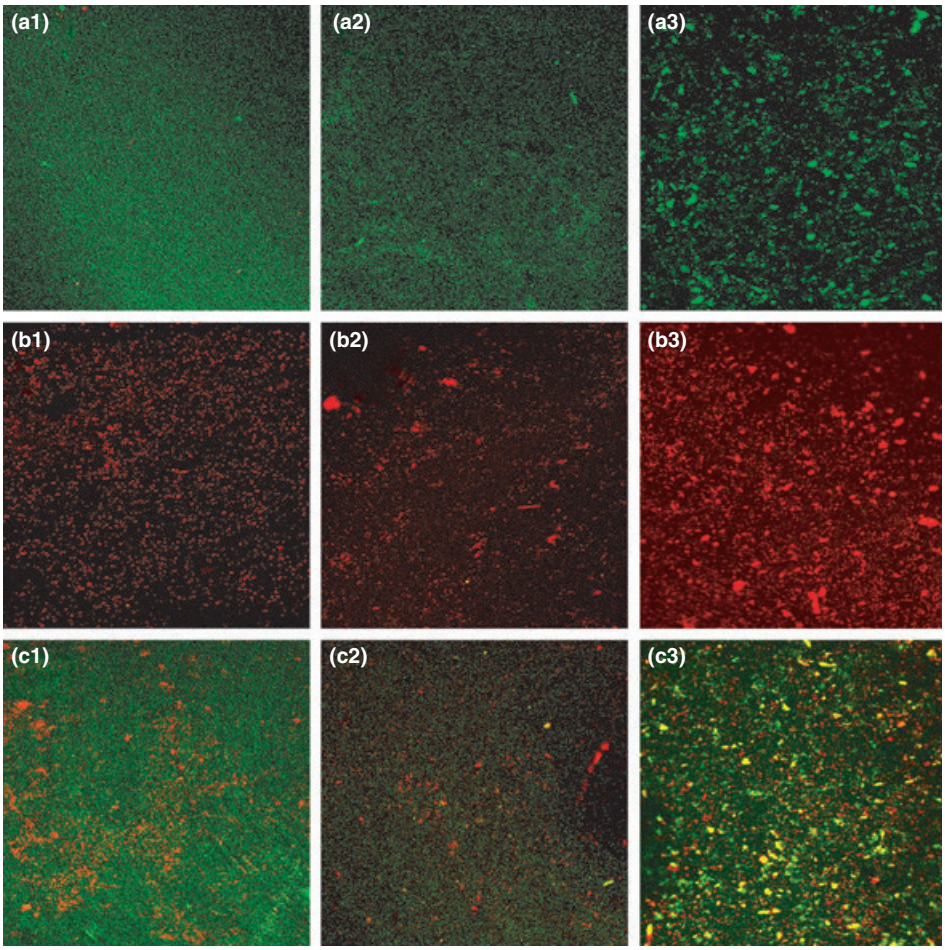

Fig. 2. Confocal scanning laser microscopy micrographs of the following coatings: A1-3 FITC labeled human serum albumin (green). B1-3 *Streptococcus mutans* with propidium iodine stain (red). C1-3 FITC labeled human serum albumin plus *S. mutans* with propidium iodine stain (yellow = green + red). In each coat, numbers represent the following surface modification: 1 = machined, 2 = acid-etched, 3 = acid-etched and blasted. Magnification: ×10.

1995, 1997; Steinberg et al. 1995; Sela et al. 2007). The beneficial properties of an albumin coating on the biological processes occurring in the interface between the implant and blood have been demonstrated (Tsai et al. 1990). Khan et al. (2007) showed that albumin coating on metallic surfaces may improve their biocompatibility in terms of thrombus formation, platelet adhesion, and hemolysis. Steinberg et al. (1995) showed that coating of Ti surfaces with human salivary albumin significantly reduced the adhesion of the periodontal pathogenic bacteria *Aggregatibacter actinomycetemcomitans* and *Actinomyces viscosus*. Furthermore, albumin coating of Ti surfaces was shown to reduce the adherence of *S. aureus* and *P. aeruginosa* and to decrease the contamination of tympanostomy tubes by exudates and bacteria (Kinnari et al. 2005; Kinnari & Jero 2005). An et al. (1996) also found that Ti surfaces coated with cross-linked bovine serum albumin showed reduced adherence of *Staphylococcus epidermidis in vitro.*

In the present study, serum albumin was shown to cause a significant reduction in the adherence of the Gram positive facultative *S. mutans* to modified Ti surfaces, but had no influence on the adherence of the Gram negative anaerobic *F. nucleatum* and *P. gingivalis*. The mechanism of the inhibition of *S. mutans* binding in the presence of HSA is not completely clear. It can be assumed that being an acidic protein, albumin may raise the net negative charge of the surface and reduce its hydrophobicity, thus reducing the affinity of the coated surfaces to bacterial adherence. As shown in this and earlier studies, these effects may depend on the bacterial strain and species, probably due to differences in bacterial cell surface properties (Reynolds & Wong 1983).

The fact that albumin coating had no significant effect on the adhesion of *P. gingivalis* cells to Ti may indicate that other factors are involved in the mechanism of their attachment to protein-coated surfaces, e.g., proteases, fimbriae, extracellular vesicles, or lipopolysaccharide.

Moreover, our results indicate that while coating by fibronectin significantly increased *P. gingivalis* and *F. nucleatum* adhesion to Ti surfaces, it had no significant effect on the adherence of *S. mutans*. These findings point to the specificity of the mechanism of adherence of the bacteria to fibronectin coated surfaces. Previous *in vivo* studies demonstrated that plasma proteins could mediate the adherence of *P. gingivalis* and *F. nucleatum* to oral tissues. Furthermore, these periodontal pathogenic bacteria were found to adhere significantly more to fibronectin and fibrinogen than to albumin (Carlén et al. 2003). *Porphyromonas gingivalis* fimbriae exhibit a wide variety of biological activities, including binding to various host proteins, specifically to salivary components, and to extracellular matrix proteins such as vitronectin and fibronectin (Nishiyama et al. 2007). The specific components by which *P. gingivalis* adheres to fibronectin were characterized and shown to be related to the bacterial fimbriae (Hamada et al. 1998; Nakagawa et al. 2005). The strong fibronectin-bind-

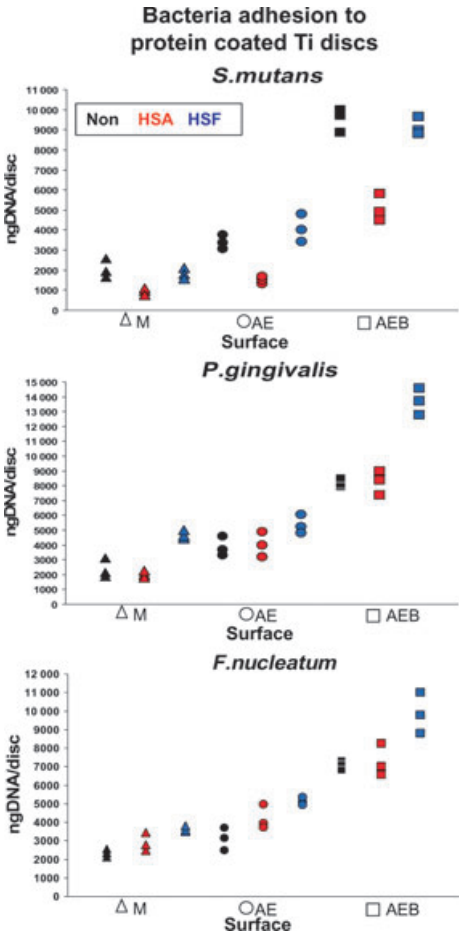

Fig. 3. Quantitative assessment by DNA analysis of *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum* adhesion to Ti surfaces with protein coat; HAS = albumin coat (red), HSF-fibronectin coat (blue), Non = surface without protein coat (black), for each bacterium. Data are presented as ng DNA per disk, as triplicates of each subgroup, for the machined (M = triangle), acid-etched (AE = circle), and acid-etched and blasted (AEB = square) surfaces.

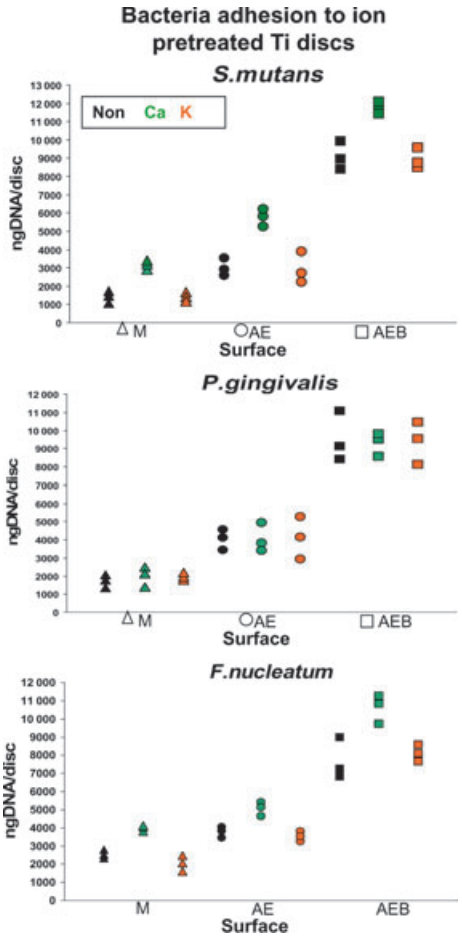

Fig. 4. Quantitative assessment by DNA analysis of *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum* adhesion to Ti surfaces with ion-pretreated; Ca = calcium pretreated (green), K = potassium pretreated (orange), and Non = without ion pretreatment (black) for each bacterium. Data are presented as ng DNA per disk, as triplicates of each subgroup, for the machined (M = triangle), acid-etched (AE = circle), and acid-etched and blasted (AEB = square) surfaces.

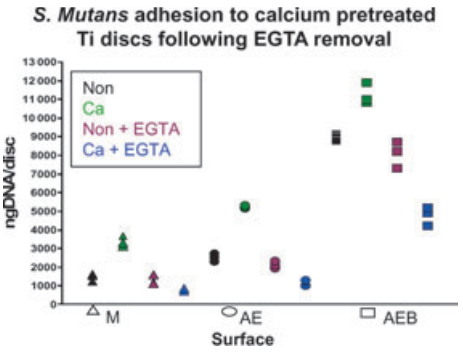

Fig. 5. Quantitative assessment by DNA analysis of *Streptococcus mutans* adhesion to calcium pretreated Ti surfaces with or without EGTA removal. Non = surface without calcium pretreatment (black), Ca = surface with calcium pretreatment previous to bacteria adhesion (green). Non EGTA = surface without calcium pretreatment, and with EGTA removal treatment after bacteria adhesion (purple) Ca EGTA = surface with calcium pretreatment previous to bacteria adhesion, and with EGTA removal treatment after bacteria adhesion (blue). Data are presented as ng DNA per disk, as triplicates of each subgroup, for the machined (M = triangle), acid-etched (AE = circle), and acid-etched and blasted (AEB = square) surfaces.

ing capacity of *F. nucleatum* was also demonstrated (Bolstad et al. 1996), and the fbpA gene of *F. nucleatum* was defined as a fibronectin-binding protein (Karpathy et al. 2007).

As fibronectin is one of the main plasma proteins that adsorbs to Ti surfaces (Sela et al. 2007), it may be concluded that plasma fibronectin serves as a mediator in the mechanism of adhesion of *P. gingivalis* and *F. nucleatum* to Ti surfaces.

Biomolecular modification of implant surfaces for the control of events that include cells and matrix apposition at the bone implant interface may be a logical and successful approach (Morra 2007). The goal of this approach is to immobilize proteins, enzymes, or peptides on the surfaces of Ti implants to induce favorable cell and tissue responses. This may control the tissue implant perimeter with molecules delivered directly to the interface. Plasma fibronectin is thought to have an essential role in the biological sequence of events that takes place in the implant tissue interface. The enhanced adsorption of blood proteins, such as plasma fibronectin, onto implant surfaces is speculated to be one of the reasons for improved osseointegration of Ti surfaces modified by techniques such as sandblasting. Meyer et al. (2004) demonstrated the formation of a fibronectin network on the Ti surface immediately following the interaction of the implant with the surrounding bone *in vivo*, which seems to mediate the bond between osteoblasts and the implant. It was suggested that plasma fibronectin activates signaling pathways directing osteoblast survival, cell-cycle progression, gene expression, and matrix mineralization (Garcia & Reyes 2005). It was further indicated (Jimbo et al. 2007) that coating Ti implants with plasma fibronectin enhanced earlier osseointegration in the mouse femur.

It is known that fibronectin matrix assembly and its cells binding capacity are associated with specific recognition of integrins and Arg-Gly-Asp (RGD) sequence (Wierzbicka-Patynowski & Schwarzbauer 2003). Recently, a chimeric construct in which the integrin-binding, RGD loop is inserted to recombinant fibronectin fragments that mimic the effects of ECM fibronectin on cell function was presented. This recombinant fibronectin fragments was found to be more efficient in wound healing parameters (Roy et al. 2011).

Previously, it was reported that fibronectin in the saliva can bind to *P. gingivalis* fimbriae and that may interfere with fimbria-mediates binding to eukaryotic cells (Lamont & Jenkinson 2000). For future research, it can be interesting to examine whether these bacteria, which bound to fibronectin, interfere with the fibronectin networking and could endanger the bond between osteoblasts and the implant.

Conclusions

Bacterial adhesion to Ti surfaces was found to be roughness-dependent, and the mecha-

nism of adhesion of oral bacteria to Ti surfaces was shown to be influenced by the constituents of the conditioning film, i.e., ions and proteins derived from the blood. The fact that these components act differently on specific oral bacteria may point to the complexity of the interactions between serum constituents, oral bacteria, and Ti surface properties. Coating Ti implant surfaces with plasma fibronectin seems to be a promising biochemical modification. However, the biocompatibility of this procedure should be further examined, as there is a possibility that such modifications may promote the accumulation of pathogenic bacteria, leading to infection and inflammation and thus risking the success of the implant procedure.

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