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Fatigue and Fluoride Corrosion on *Streptococcus mutans* Adherence to Titanium-Based Implant/Component Surfaces

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Abstract

Purpose: The influence of fatigue and the fluoride ion corrosion process on *Streptococcus mutans* adherence to commercially pure Titanium (Cp Ti) implant/component set surfaces were studied.
Materials and Methods: Thirty Nobel implants and 30 Neodent implants were used. Each commercial brand was divided into three groups. Group A: control, Group B: sets submitted to fatigue (10^5 cycles, 15 Hz, 150 N), and Group C: sets submitted to fluoride (1500 ppm, pH 5.5) and fatigue, simulating a mean use of 5 years in the oral medium. Afterward, the sets were contaminated with standard strains of *S. mutans* (NTCC 1023) and analyzed by scanning electronic microscopy (SEM) and colony-forming unit counts (CFU/mL).
Results: By SEM, bacterial adherence was verified only in group C in both brands. By CFU/mL counts, *S. mutans* was statistically higher in both brands in group C than in groups A and B ($p < 0.05$, ANOVA).
Conclusion: The process of corrosion by fluoride ions on Cp Ti implant/component sets allowed greater *S. mutans* adherence than in the absence of corrosion and with the fatigue process in isolation.

The long-term success of titanium implants is directly connected to their excellent mechanical properties, resistance to corrosion, and biocompatibility.[1] When titanium is in the oral environment, it is constantly submitted to mechanical and thermal forces and to the aggressiveness of the host oral environment.[2]

In addition to biocompatibility and biofunctionality, titanium-based biomaterials are highly ductile and resistant to cyclic forces in the oral environment. These cyclic forces, known as fatigue, are produced by masticatory movements in a range of force of up to 370 N and a frequency of 1.25 Hz;[3,4] however, Graves et al[5] relate that fatigue is capable of causing internal microcracks in titanium-based implants.

In the oral cavity, in addition to mechanical forces, implants are also exposed to aggressive agents such as bacterial biofilm and saliva. This environment is particularly favorable to the biodegradation of metals, due to its thermal, ionic, microbiologic, and enzymatic properties.[6]

The corrosion process results from electrochemical reactions that occur between a metal material and the surrounding environment. Titanium is known to present high resistance to corrosion against attack by solutions containing strong mineral acids, such as hydrochloric acid (HCl) or sulphuric acid (H_2SO_4), with titanium corrosion remaining extremely low in these situations;[7] however, when titanium is placed in contact with a fluorinated medium, its titanium oxide layer, TiO_2 is damaged, and is easily degraded.[7] This occurs due to the incorporation of fluoride ions into the oxide layer, considerably reducing its protective properties.[7-11] Furthermore, fluorides are capable of altering the titanium surface, increasing its roughness.[12]

Fatigue of the dental prosthetic implant/component set associated with exposure to fluoride ions may lead to slackening of the component, forming gaps, which serve as regions for plaque accumulation, between the implant/abutment. The fluorides may also cause corrosion, leaving the surfaces exposed to the oral cavity rougher, making it easier for microorganisms

Table 1 Division of the implant/component sets in their respective groups

Brands	Group A (control)	Group B (fatigue)	Group C (fatigue and fluoride)
Nobel (Nb)	10 (group NbA)	10 (group NbB)	10 (group NbC)
Neodent (N)	10 (group NA)	10 (group NB)	10 (group NC)

to become installed where they are protected from oral hygiene mechanisms (movements of brushing, swallowing, and flow of crevicular fluid).[13-15]

From this accumulation, an inflammatory reaction may originate, due to the host response to these microorganisms, developing into periimplantitis, which if left untreated will lead to bone destruction around the implant and its consequent loss.[13,15,16]

There are few studies on the behavior of dental implants in the face of fatigue and the corrosive action of chemical agents containing fluoride in the medium and long term, and it is necessary to study their causes and consequences more precisely.

Therefore, the aim of this study was to assess the influence of fatigue and the fluoride ion corrosion process on *Streptococcus mutans* adherence to commercially pure titanium-based implant/component set surfaces, simulating a mean use of 5 years in the oral environment.

Materials and methods

In this study, 60 commercially pure titanium-based (Cp Ti) implants, components (preparation stubs), and screws of two commercial brands were used: Nobel Biocare (MKII, Nobel Biocare AB, Goteborg, Sweden) and Neodent (Titamax Liso®, Neodent, Curitiba, Brazil). All the implants measured 15/3.75 mm in size, and were of the external hexagon type with machined surface.

Preparation of the sets

The dental prosthetic implant/component sets were assembled in accordance with the instructions of their manufacturers, and a torque of 32 N/cm^2 was applied with a manual controlled force prosthetic torque meter (Neodent). They were randomly distributed into three groups of ten components for each brand analyzed (Table 1).

Fluoride ion corrosion test

The implant/component sets belonging to group C were placed in a 1500 ppm pH 5.5 sodium fluoride solution for 184 hours, simulating contact with fluoride of 21 times a week for 5 years, with a mean of 2 minutes at a time (mean estimate of tooth brushing with a fluoride-containing dentifrice three times a day). The solution was changed every 12 hours, and the sets were washed under running water for an interval of 30 seconds each before being submerged in the replaced solution again.[17]

Mechanical fatigue test

The mechanical fatigue tests were performed in groups B and C, the latter having been previously submitted to immersion in a fluorinated solution (corrosion). The mechanical test was performed using a Material Test System machine (MTS 810, MTS System Corporation, Minneapolis, MN), equipped with Test Star II software.

The test was performed in accordance with the model proposed by Merz et al,[18] in which a metal sphere is placed on the implant/component set for the purpose of simulating the crown. The implant/component sets and spheres were adapted to a device at an angle of 30 degrees of inclination. About six implant threads had been left outside the grab support, simulating an extremely unfavorable clinical situation of loss of bone support.

In this test a load of 150 N was used, at a frequency of 15 Hz, and 100,000 cycles were set to simulate a 5-year period of mastication.[18,19] Group A was not submitted to any test.

Microbiologic test

After the corrosion and fatigue tests, the sets were sterilized in ultraviolet light for 20 minutes for microbiologic tests.[20] To contaminate the implant/component sets, aliquots of standard strains of *S. mutans* (NTCC 1023) were used. Before the sets were contaminated, approximately 1 mL [1.0×10^7 colony-forming unit counts (CFU/mL)] of the standard strain suspension was inoculated into 10 mL of Muller Hinton broth culture medium and incubated in a bacteriological incubator at 37°C for 24 hours under microaerophilia to obtain the concentration of 10^7 CFU/mL, according to the degree of turbidity on the McFarland scale.

Contamination of the implant/component sets

To contaminate the specimens, the implant/component sets were put into sterile Falcon tubes containing 5 mL of Brain Heart Infusion (BHI) broth. Next, each Falcon tube was individually inoculated with an aliquot corresponding to 10^7 CFU/mL (100 μL) of *S. mutans.* After inoculation, the tubes were incubated in a bacteriological incubator at 37°C for 24 hours in an orbital agitator (100 rpm) simulating the dynamic oral environment.

After incubation, the sets were divided into two subgroups, with eight implant/component sets of both brands in each group being used for analyzing bacterial adherence by means of CFU/mL counts. Two of the implant/component sets of both brands of each group were analyzed by scanning electronic microscopy (SEM).

It is important to note that one trained examiner, blind to the group of analysis performed and the CFU counts (colony-forming counts), and another trained examiner, blind to the analyzed sets, performed the microscopy analysis. For analysis by SEM, the implant/component sets were fixed in 2.5% glutaraldehyde for 15 minutes.

Initially, a microscopic analysis at 50× magnification was carried out to observe the general topography of the specimen. Next, microscopy was carried out in the region of possible standardized microorganism concentration, considered between the

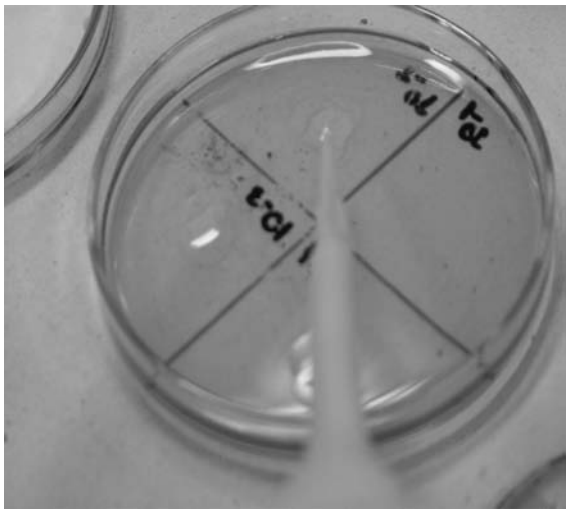

Figure 1 Plate seeded with 25 μL of dilutions of 10^{-1} to 10^{-4}.

third and fourth thread of the dental implant, at a 1000× magnification.

For the viable cell count, the implant/component sets after contamination were placed in test tubes containing 4.5 mL of sterile saline solution and submitted to sonication to release their microorganisms. These saline solutions containing the released microorganisms were diluted in sterile saline, in decimal series of 10^{-1} to 10^{-4} that would later be plated onto SB-20 plates (Fig 1) and incubated for 24 hours under microaerophilia in a bacteriological incubator at 37°C (Fig 2).

Figure 2 Seeded plates in jar of microaerophilia for incubation in bacteriological incubator at 37°C for 24 hours.

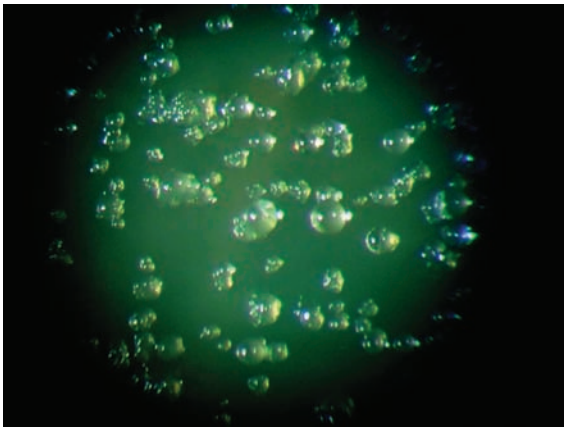

Figure 3 Aspects presented by *Streptococcus mutans*.

Seeding the material

After the colonies had grown, the CFU/mL counts for the inoculum were carried out. The colonial morphology characteristic of these microorganisms was confirmed with the aid of a stereoscopic loupe (Zeiss Optical, Oberkochen, Germany) (Fig 3).

Statistics

Analysis of variance (ANOVA)-Tukey's test was used, considered significant when $p < 0.05$.

Results

Macroscopically, loss of brightness on the surface could be observed in all the sets of group C that were exposed to fluorinated solution (1500 ppm, pH 5.5) when compared to sets in group A (control) (Fig 4). These alterations on the titanium surface are due to fluoride-titanium reactions on the oxide layer.

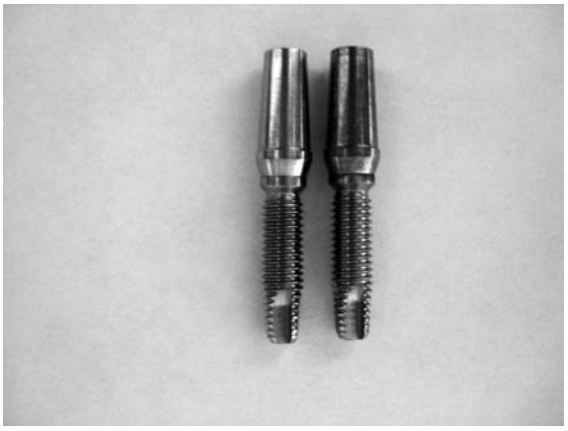

Figure 4 Loss of brightness observed macroscopically in sets exposed to fluorinated solution. Left-side set from group A (control); right-side set from group C (1500 ppm NaF, pH 5.5).

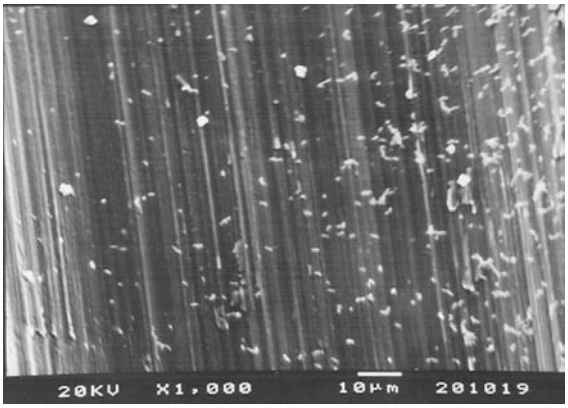

Figure 5 Surface of implants belonging to group NbC showing bacterial adherence.

In the fatigue test (100,000 cycles, 15 Hz, 150 N) no fractures or cracks were observed in the prosthetic Cp Ti implant/component sets belonging to groups B (fatigue) and C (fatigue + fluoride).

SEM was selected because it enables three-dimensional (3D) images of the samples to be observed. Adherence of *S. mutans* was observed on the Cp Ti dental prosthetic implant/component set surfaces exposed to fluoride and fatigue (group C) (Fig 5), and absence of *S. mutans* was observed in groups A and B (Figs 6 to 9) seen at 1000× magnification. When the microorganisms in question were found, microscopy at 5000× magnification (Figs 10 and 11) was performed to confirm the species under study.

The viable cell counts in CFU/mL found in the studied groups are shown in Table 2. It should be pointed out that each group was made up of eight specimens and that three repetitions of the experiment were made in each group, so that the results could be confirmed. Irrespective of the commercial brand, group C (fluoride + fatigue) presented statistically higher bacterial adherence than groups A and B.

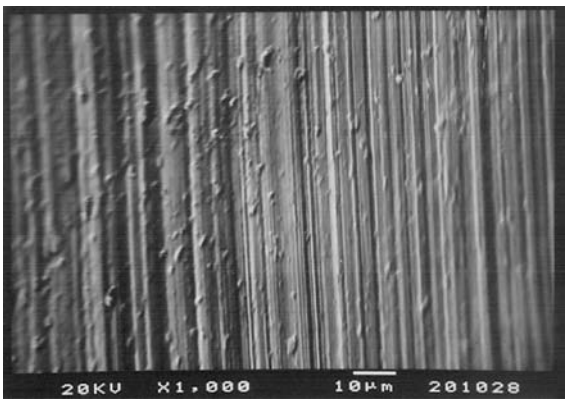

Figure 6 Surfaces of implants belonging to group NbA showing irregularities of the machining process of the implant but absence of bacterial adherence.

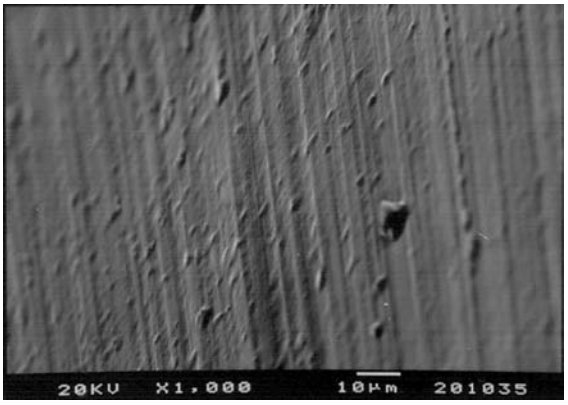

Figure 7 Surfaces of implants belonging to group NbB showing irregularities of the machining process of the implant but absence of bacterial adherence.

Discussion

Several materials used for oral hygiene, such as dentifrices, mouthwashes, and prophylactic agents, contain fluoride and can react with titanium surfaces. A majority of studies indicate that large concentrations of fluorides associated with acidic pH lead to a corrosive process in titanium.[8-11] In agreement, Bosker et al[21] observed that solutions containing more than 20 ppm of fluoride ions could destroy the titanium oxide layer.

Fluoride ions are effective components in the corrosion process,[22] because when titanium is placed in contact with a fluorinated medium, its oxide layer is damaged, and the titanium is easily degraded.[7] Some authors[7-11] suggest this occurs due to the incorporation of fluoride ions into the oxide layer, considerably reducing its protective properties. This could justify the loss of surface brightness observed macroscopically in the sets submitted to the action of fluoride (group C) in this study.

Studies, such as those of Campus et al,[23] showed that the concentration of fluorides in saliva after brushing with a dentifrice diminishes, but lower concentrations of fluoride are still found up to 24 hours after brushing.

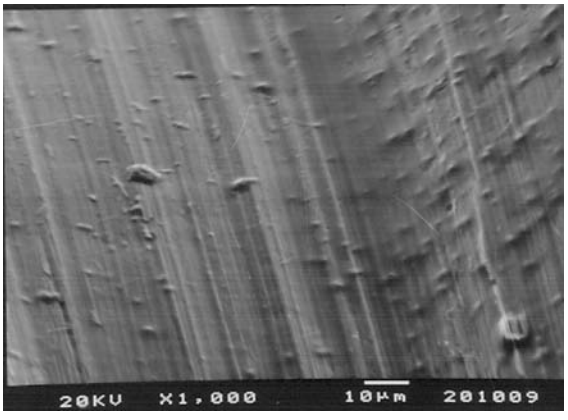

Figure 8 Surfaces of implants belonging to group NA showing irregularities of the machining process of the implant but absence of bacterial adherence.

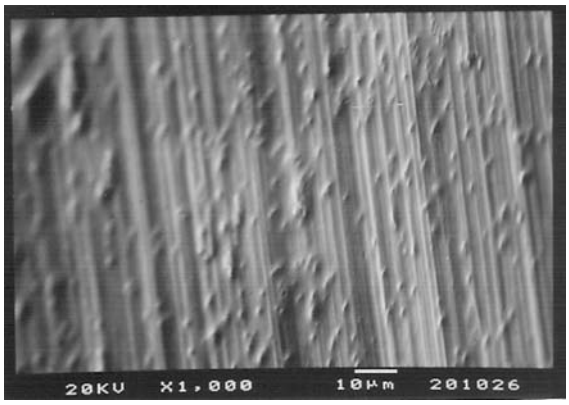

Figure 9 Surfaces of implants belonging to group NB showing irregularities of the machining process of the implant but absence of bacterial adherence.

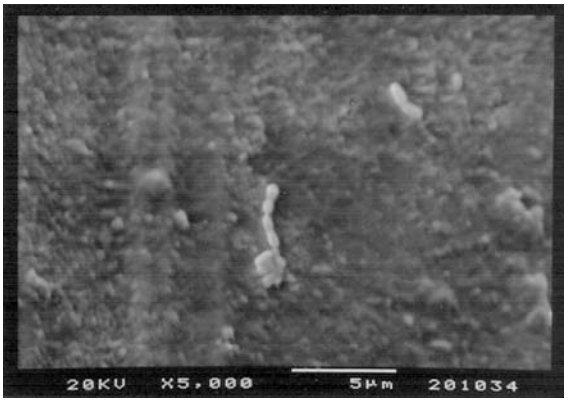

Figure 11 Surface of implants of group NbC showing *S. mutans* adhered to implant surface at 5000× magnification.

It is known that fluoride ions can cause corrosion of Cp Ti, and the result of this process is greater roughness of the surfaces exposed to the oral cavity, making it easier for microorganisms to become installed where they are protected from oral hygiene mechanisms (movements of brushing, swallowing, and flow of crevicular fluid).[13] This could justify the bacterial adhesion on the surfaces of the implant/component sets submitted to the corrosive action of fluoride (group C), as shown in this study.

Many factors influence bacterial adhesion, including free energy on the surface and the mechanisms of bacteria themselves for fixation to surfaces; however, the roughness of biomaterials is the most relevant factor in the bacterial adhesion process.[13]

Because roughness is an important factor in the adhesion and extension of bacterial colonization,[14] the titanium corrosion caused by the action of fluorides[7-11] changes the surface roughness of exposed implants in the oral cavity. This may lead to a greater bacterial biofilm accumulation and the onset of periimplantitis.[13,15,16] In this study, bacterial adherence in group C (fluoride + fatigue) can be observed by means of SEM.

The study of bacterial adhesion on titanium surfaces is of fundamental importance, as a high correlation between the increase in bacterial biofilm formation and bone loss around implants in humans has been reported.[24,25] Bacterial adherence and colonization are the main factors in the pathogenesis of infections in biomaterials. Without initial bacterial adherence to the implant surface, subsequent accumulation and colonization will not occur.[26]

S. mutans are the first biofilm colonizers. Hydrophobic interactions are important in *S. mutans* adherence to uncovered Cp Ti surfaces.[27]

In this study, SEM was used to observe the adhered *S. mutans* to the Cp Ti dental prosthetic implant/component set surfaces. No *S. mutans* adherence to the Cp Ti dental prosthetic implant/component set surfaces in groups A (without exposure to fluoride and fatigue) and B (exposed to fatigue) was observed by SEM. Therefore, these species were present in the microorganism recovery and count tests. This event may have occurred due to the presence of microorganisms on the surface (but not adhered) of the Cp Ti dental prosthetic implant/component set surfaces.

Due to the ductility of titanium, it is not possible for a Cp Ti implant to fracture with one cycle only. With the application of cyclic load, the material starts to develop internal microcracks that may increase in number and size according to the number of cycles. Resistance to fatigue is given by the capacity to resist these repeated loads.[28] In this study, low cycle fatigue (up to 10^5 cycles) was performed, and no microcrack formation was observed on the implant surface and Cp Ti dental prosthetic implant/component sets. The fatigue process had no influence on bacterial adhesion.

For a better analysis of the effect of fatigue and fluorinated medium on the surfaces of Cp Ti dental implants and their

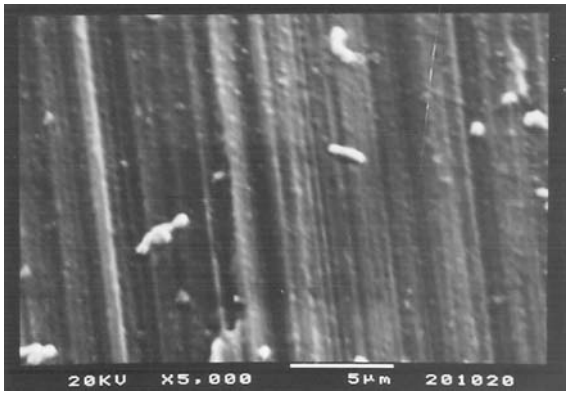

Figure 10 Surface of implants of group NbC showing *S. mutans* adhered to implant surface at 5000× magnification.

Table 2 Mean of microbiologic results in CFU/mL of each studied group

Brands	Group A	Group B	Group C
Nobel	1.71×10^{8a}	1.78×10^{8a}	4.23×10^{8b}
Neodent	1.41×10^{8a}	1.33×10^{8a}	3.0×10^{8b}

Means followed by different letters in rows represent statistically significant difference among the groups, by the ANOVA-Tukey test at the level of 5%.

respective prosthetic components, research that assesses the action of these processes (fatigue and action in a fluorinated medium) in various geometries and types of surfaces available on the market is required.

Conclusions

From the results obtained, it was concluded that when in prolonged contact with implant/component sets, a fluorinated medium whose pH and fluoride ion concentration are similar to those found in the oral cavity, leads to corrosion, which could then lead to a significant increase in bacterial adhesion on the Cp Ti implant/component surface.

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